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VOL. XIV.

THE

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ROYAL DUBLIN SOCIETY.

Vol. XIV. (N.S.), No. 1. MAY, 1913.

THE EXTRACTION OF ZYMASE BY MEANS OF LIQUID AIR.

(Preliminary Note.)

BY

HENRY H. DIXON, Sc.D., F.R.S.,
UNIVERSITY PROFESSOR OF BOTANY, TRINITY COLLEGE, DUBLIN;

AND

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ASSISTANT TO THE PROFESSOR OF BOTANY, TRINITY COLLEGE, DUBLIN.

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THE

SCIENTIFIC PROCEEDINGS

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THE ROYAL DUBLIN SOCIETY.

I.

THE EXTRACTION OF ZYMASE BY MEANS OF LIQUID AIR.

(Preliminary Note.)

By HENRY H. DIXON, Sc.D., F.R.S.,
University Professor of Botany, Trinity College, Dublin;

AND

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Assistant to the Professor of Botany, Trinity College, Dublin.

[Read April 15. Published May 23, 1913.]

Recently we found it possible to obtain the sap from various plant-organs without change in concentration, by pressure after the organ was immersed for a few minutes in liquid air (Dixon and Atkins, 1). It seems that the exposure to the intense cold renders the protoplasm permeable, and the pressure forces out the solution from the vacuoles unchanged. This suggested the probability that similar exposure of the yeast-cell would render its protoplasm permeable, and that the zymase and other endo-enzymes would then be free to escape. Experiment has confirmed this surmise.[1]

Our experiments up to the present have been made with "liquid yeast," supplied to us from Guinness's Brewery, through the kindness of Mr. A. McMullen. This is the top yeast skimmed off the vat after the

---

[1] It is remarkable that Pasteur failed to extract zymase by freezing yeast (Harden, 3). Probably the temperature he employed was not below the eutectic point of the cell-solutes. That this point may have to be exceeded to kill a cell has been shown by Maximow (5).

fermentation of the wort is almost complete. From this the beer is removed by centrifuging, or by pressing through a fine linen cloth. The yeast, then a plastic mass, is made up into cylinders 10–15 cm. long, and 1·5 cm. diameter, and wrapped in paper. In this form it is lowered by means of a thread into a Dewar flask containing liquid air.[1] It is left immersed for ten or fifteen minutes till the cessation of active ebullition of the air indicates that the mass has fallen to the temperature of the liquid air. The cylinder is then withdrawn from the flask, the paper is removed from its lower end, and it is transferred to the tube of the centrifuge. As it thaws, the yeast runs down out of its paper covering into the tube. It is remarkable that the yeast, which before the application of liquid air was a sticky, plastic mass, has now, after exposure to the intense cold, become quite fluid. This change of consistency is observed even when condensation of atmospheric moisture on the cold and thawing mass is precluded.

Centrifuging for ten minutes, at 9000 revolutions per minute, causes the yeast-cells to sink in this fluid mass, and to leave a faintly opalescent brown liquid above. The volume of this liquid is about 30 per cent. of the volume of the plastic yeast frozen, and represents the juice or sap of the treated yeast-cells. This liquid has powerful fermentative properties, and contains zymase.

In one of the first experiments carried out in this manner, we took 160 c.c. of pressed yeast, froze, thawed, and centrifuged it in the manner described, thus obtaining 60 c.c. yeast sap. To 50 c.c. of this, 20 grammes cane-sugar was added, and the mixture was introduced into an Erlenmeyer flask in a thermostat at about 33° C. The flask was connected by means of a rubber tube leading out of the thermostat to two gas-burettes connected in series. The CO_2 evolved displaced mercury from the first burette into the second. When reading the volume, the mercury in the two was adjusted so as to stand at the same level. This arrangement, which was found quite convenient, was adopted in all the experiments.

Before each reading the fermenting fluid was thoroughly shaken.

Experiment 1.

Time. hr. min.	Volume of CO_2 in c.c.
24 25	32·0.
26 40	36·5.

No further evolution of gas.

---

[1] As in our previous work, we are indebted to Professor W. H. Thompson for the liquid air used in these experiments.

No antiseptic was added in this experiment, but it is evident that the high sugar concentration checks bacterial action. A similar experiment gave the following figures, the same quantities being used, with the addition of about 0·5 c.c. of toluene:—

EXPERIMENT 2.

Time.	Volume of CO_2 in c.c.
6 hours,	39·2.
18 hours,	65·2.

No further evolution of gas.

In this experiment the liquid at air-temperature was saturated with CO_2, so that some of the gas evolved and caught in the burette was CO_2 driven off by the initial rise of temperature due to the transference into the thermostat at 31° C.

In another experiment with similar quantities, to which toluene was also added, the liquid was not saturated with CO_2, and the following figures were obtained:—

EXPERIMENT 3.

Time. hr.	Time. min.	Volume of CO_2 in c.c.
0	40,	8·6.
1	5,	12·2.
13	40,	20·2.

No further evolution of gas.

In this experiment some of the same sample of sap was used as in the previous experiment (No. 2). In the meantime (14 hours) the sap was standing at ordinary room-temperature, and was presumably losing some of its fermentative efficiency. Notwithstanding this, it exhibited a very fair activity.

It was also noticed that while standing no evolution of CO_2 took place. This indicates that the sap obtained by the liquid-air method is practically free from glycogen or other fermentable carbohydrates.

The absence of fermentation from the supernatant liquid is in marked contrast to the behaviour of the solid sediment. When the liquid is poured off, the sediment froths actively, and in this respect behaves like the thawed frozen yeast before centrifuging. Presumably the glycogen retained within the cells is hydrolysed by the action of glycogenase which now has access to it, giving fermentable sugars. The zymase present is thus provided with a substrate.

It is of interest to compare the activity of the fluid extracted by means of the liquid air in the manner described with that extracted from yeast-cells by other methods.

In 1912 Lebedeff (4) described a method of extracting zymase from yeast by simple maceration. His method consists essentially in drying the yeast at 30°, and in macerating it for two hours at 35°C. with three times its weight of water, or for a longer period at a lower temperature. To compare the two methods the following experiment was made :—the beer was pressed off some "liquid yeast" and two samples of the solid yeast (*A* and *B* each about 100 g.) were made. *A* was dried at 30°C. for twenty-four hours, and weighed 25 g. The temperature was kept for three days at 35°C., and the yeast lost 0·4 g. To the dried solid 75 c.c. of water and a little toluene were added, and it was left to macerate for two hours at 35°C. It was then centrifuged and yielded 41 c.c. of liquid. 30 c.c. of this was put in the conical flask with 12 g. sugar and a little toluene. The production of CO_2 is given below :—

A (13)

By Maceration.

Time hr. min.	Volume of CO_2
0 10	0·8
6 40	13·3
54 25	25·8

B (12)

By Liquid Air.

Time hr. min.	Volume of CO_2
0 50	8·7
1 30	11·0
3 25	19·0
9 40	35·0

In column *B* are given the volumes of CO_2 produced by 30 c.c. of yeast-sap extracted by means of liquid air under similar conditions.

The 100 g. of the solid yeast in this case yielded a little over 32 c.c. sap. Calculation from the foregoing data shows that each gramme of fresh yeast afforded a total fermentation-volume of 0·35 c.c. CO_2 by Lebedeff's method, and 0·37 c.c. by liquid-air extraction.

From this comparison it appears that the fluid extracted from the sample of yeast by means of liquid air is quite as active as that obtained by the maceration method.

This experience was confirmed by another experiment where the maceration was effected at 20°–25°C. and a larger volume of water added, viz. :—to 21 g. of dried yeast 100 c.c. of water was added and maceration proceeded for twenty-one hours. As before, 30 c.c. of the fluid with 12 g. of sugar and a little toluene was used. Under *B* and *C* the activity of the same sample of yeast extracted by liquid air is given.

A (8) *By Maceration.*		*B* (6) *By Liquid Air.*		*C* (5) *By Liquid Air.*	
Time. hr. min.	Volume of CO_2.	Time. hr. min.	Volume of CO_2.	Time. hr. min.	Volume of CO_2.
0 25	4·0	1 5	8·0	0 30	3·4
1 45	8·6	3 5	14·6	0 40	8·6
3 15	9·8	4 15	17·8	3 45	15·4
29 0	11·4	4 55	20·0	10 45	31·0
		18 40	34·8		

Correction for dilution shows a total evolution of CO_2 amounting to 0·33 c.c., 0·38 c.c., 0·34 c.c. per gramme of undried yeast in *A*, *B*, and *C* respectively.

In this case, allowing for the dilution of the liquid obtained by maceration, its activity is again practically identical with that obtained by the liquid air method.

Some of the same yeast furnished a comparison of the method described by Giglioli (2); 100 g. of the solid yeast was mixed with 5 c.c. of chloroform. The mass became semi-fluid and frothed so vigorously that some 2 c.c. of the fluid was lost. After six hours' standing 100 c.c. of the fluid was centrifuged, and yielded 36 c.c. liquid, including chloroform. To 30 c.c. of this 12 g. of sugar was added, and it was set to ferment. Its activity, which was great at first, rapidly decayed.

EXPERIMENT 4.

Time. hr. min.	Volume of CO_2.
1 0	20·2

No further fermentation.

This is equivalent to 0·22 c.c. of CO_2 per gramme of yeast. It is possible that maceration for a shorter period would yield a more active liquid, or perhaps the same result might be attained by using a smaller amount of chloroform.

The generally low activity of the juice extracted by all these methods from the yeast at our disposal suggested that possibly the enzyme was impaired during the extraction. In the following experiment it was sought to render the zymase more stable by furnishing it with fermentable material immediately on its exit from the cells. With this object acccordingly, to 67 grammes of this solid yeast, while still frozen, was added a solution containing 23 c.c. of water, 18 g. of sugar, and a little toluene. Experience had showed that 67 g. of yeast would yield about 23 c.c. of yeast-juice;

therefore, the juice was diluted with about its own volume of water ; 40 c.c. of the mixture was allowed to ferment. The volumes of CO_2 evolved are recorded under *A*. For comparison under *B* is recorded the evolution of CO_2 from 40 c.c. of a mixture made up of 20 c.c. of yeast-juice extracted by the liquid-air method without a substrate, and 20 c.c. of water and 15 g. of sugar under similar conditions.

A (9) Time. hr. min.	*A* (9) Volume of CO_2.	*B* (11) Time. hr. min.	*B* (11) Volume of CO_2.
0 20	8·0		
0 50	10·8		
1 15	12·2	2 5	3·6
4 50	23·6	4 5	5·8
5 10	23·6	4 25	6·0
18 10	26·6	10 45	10·6
20 45	27·0	12 20	11·0

No further evolution of gas.

After correcting for volume-changes, owing to the addition of sugar, the above results correspond to an evolution of 0·57 c.c. for *A* and 0·19 c.c. CO_2 for *B* per gramme of yeast.

This comparison strongly supports the idea that the small activity of the yeast juice, and its rapid decay, are in part due to the action of a proteoclastic enzyme, the action of which is partly inhibited by the combination of the enzyme with the sugar. How far the absence of suitable phosphate and co-enzyme are limiting factors must be examined later.

In order to see if much zymase was retained in the sedimented yeast-cells after centrifuging, 50 g., the solid residue of Experiment 6, was mixed with 50 c.c. of water, and extracted for twenty-four hours at room-temperature. The semi-fluid mixture on being centrifuged yielded 37 c.c. liquid. 30 c.c. of this, with 12 g. of sugar and a small quantity of toluene, gave volumes of CO_2 as follows :—

EXPERIMENT 7.

Time. hr. min.	Volume of CO_2.
0 10	5·9.
1 40	18·5.
13 20	22·9.

No further evolution of gas.

The above figures correspond to 0·57 cc. CO_2 per gramme of residue or 0·29 c.c per gramme of fresh yeast, as against 0·38 c.c. given by the juice from the fresh yeast.

These figures clearly show that a large amount of zymase is retained by the cells.

It is also remarkable that the cells, after exposure to liquid air, are capable of taking up and retaining water, indicating that a condition of equilibrium is not yet attained, although the membranes are permeable. Culture experiments with the sediment showed that the exposure to the liquid air had killed the yeast.

In the hopes of obtaining part of the zymase remaining behind in the cells we allowed a sample of frozen yeast to thaw in five times its weight of water, and to macerate for twenty-four hours at room-temperature. After centrifuging, 30 c.cs. of the supernatant liquid, with 12 g. sugar, were set to ferment under the usual conditions.

EXPERIMENT 10.

Time. hr. min.	Volume of CO_2.
0 10	4·8.
0 50	9·6.
1 45	12·6.
3 0	12·8.
4 35	13·0.

No further evolution of gas.

From this table it is clear that the actual amount of zymase extracted may be much increased by dilution and maceration, as the yield was 2·2 c.c. of CO_2 per gramme of yeast. Of course the concentration of the enzyme is greatly reduced.

It appears then that the liquid-air method is as efficient as Lebedeff's method. It has the advantage of being very rapid. It requires only 30–40 minutes to prepare the zymase-containing fluid from the solid yeast. Also the time for changes taking place in the enzyme is reduced to a minimum.

The ease of the extraction of zymase by liquid air suggests its application to the extraction of other endo-cellular bodies from bacteria, &c. This point one of us is at present investigating along with Dr. A. Stokes.

Finally, another possible use of this method may here be suggested. It is generally held that sterilized food-stuffs are less assimilable owing to the destruction of the enzymes of the tissues. By our experiments it has been shown that, by means of liquid air, sap containing enzymes may be extracted from cells without serious alteration. This sap, frozen immediately after extraction, might be evaporated to dryness as ice under reduced pressure, as described by L. F. Shakell (5), and the resulting powder stored and added to the food as desired, to replace the enzymes lost by sterilization.

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OSMOTIC PRESSURES IN PLANT-ORGANS.

III.—The Osmotic Pressure and Electrical Conductivity of Yeast, Beer, and Wort.

BY

HENRY H. DIXON, Sc.D., F.R.S.,
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II.

OSMOTIC PRESSURES IN PLANT-ORGANS.

III.—The Osmotic Pressure and Electrical Conductivity of Yeast, Beer, and Wort.

By HENRY H. DIXON, Sc.D., F.R.S.,
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AND

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[Read April 15. Published May 24, 1913.]

In view of the rapid metabolism of the yeast-cell as regards carbohydrates a study of the osmotic equilibrium between it and the solution which it ferments seemed to be of interest.

It has recently been demonstrated by Paine (5) that alcohol penetrates the yeast cell readily, a state of equilibrium being soon reached in which the ratio of alcohol in the cell to that outside is a constant, deviating only slightly from 0·85. Salts, on the other hand, penetrate to a small extent, the ratio of the internal and external concentrations being no more than 0·1–0·25, except in the case of poisonous substances. Indeed, it is an open question how much of this apparent absorption is really due to adsorption on the surface.

To determine the osmotic pressure the method of thermo-electric cryoscopy (1) was employed. The unaltered yeast-juice was obtained by freezing the dry material in liquid air and centrifuging the resulting fluid mass as described in detail in our account of zymase extraction (3).

The electrical conductivity of the juice, beer, and wort was also determined, to give an idea of the relative proportions of electrolytes and non-electrolytes concerned in the production of osmotic pressures. The apparatus was the usual one, which we employed in previous work (2). All specific conductivity measurements were carried out at 0°, and are recorded as reciprocals of the resistance in ohms, not in Siemens' units.

Through the kindness of Mr. A. McMullen of the Guinness Research Laboratory, we were supplied with both pressed yeast and that skimmed from the vats with adhering beer. The beer was removed by centrifuging or by pressing through a linen cloth by hand.

In Table I are recorded the results thus obtained, Δ being the depression of freezing-point, *P* the osmotic pressure in atmospheres calculated from Δ, and *c* the specific electrical conductivity at 0°.

TABLE I.

No. of Expt.	Liquid.	Δ	P	$C \times 10^5$
582	Sap of washed Bakers' Yeast, .	1·064	12·80	780
583	,, pressed Brewers' Yeast, .	4·082	49·10	596
593	,, ,, ,, ,, .	3·370	40·53	671
598	,, ,, ,, ,, .	4·600	55·34	607
585	Wort, ...	1·177	14·16	149
594	,, ...	1·247	15·00	150
597	No. 594 fermented 7 days in open vessel in Laboratory, . .	1·545	18·58	207

From the above figures it may be seen that, both in osmotic pressure and electrical conductivity, pressed yeast gives values which are much higher than those of wort. Baker's yeast, however, gives a low osmotic pressure, but a high conductivity even after washing.

The figures afforded by the sap of yeast and by the surrounding nutritive fluid may be seen in Table II, p. 11.

On comparing the results given by beer with those of wort it is at once apparent that while the electrical conductivity remains much the same, the osmotic pressure becomes approximately three times as great during fermentation, when interrupted at the usual stage in the commercial process. Very complete fermentation, however, judging from the single experiment we performed, occasions a fall in osmotic pressure after the initial rise, and is accompanied by a marked increase in the conductivity (see Table I, No. 597). It is, however, possible that the conditions of this fermentation were abnormal, and there was probably considerable loss of liquid by evaporation. The above-mentioned experiment is substantiated by No. 609, which is the beer of No. 606 allowed to stand at air-temperature in a closed vessel with a little yeast. It will be noted that there is a fall in pressure, but a slight rise in conductivity.

Turning now to the yeast in Nos. 595, 598, 612, the osmotic pressure of the juice is much higher than that of the beer, corresponding in two cases to a difference in freezing-point of about 0·5°. In these cases the yeast was separated from the beer by centrifuging to remove adherent liquid as completely as possible, and was then frozen. This process occupies some time. In No. 612, where it was effected as rapidly as possible, less than one hour elapsed between the separation and the freezing in liquid air. In this case the divergence between the osmotic pressure of the yeast and of the beer was the greatest observed. In Nos. 607 and 610 yeast was allowed to stand for six hours and twenty-four hours respectively after separation before freezing. The results here show a diminution in pressure, owing most probably to respiratory changes, so that it has fallen slightly below that of the beer from which it was removed. From Nos. 610 and 611 it appears that this decrease in osmotic pressure takes place whether the yeast is kept dry or suspended in water. This rapid falling off shows very likely the normal rate of consumption of carbohydrate with the resulting increase in conductivity.

TABLE II.

No. of Expt.	Liquid.	Δ	P	$C \times 10^5$
595	Sap of Yeast,	3·907°	47·00	518
598	,, ,,	3·815°	45·88	558
607	,, ,, which was kept separated from beer for 6 hours before freezing,	3·367°	40·51	608
610	Sap of Yeast separated from beer kept 24 hours before freezing,	3·243	39·00	742
611	Sap of Yeast (same sample as in 610) suspended 24 hours in running water,	3·166	38·09	653
612	Sap of Yeast,	3·730	44·85	562
592	Beer of No. 595,	3·417	41·10	123
596	,, of No. 598,	3·655	43·96	125
606	,, of No. 607,	3·417	41·10	132
609	No. 606 kept 6 days,	2·996	35·22	145
615	No. 606 kept 7 days,	—	—	146
608	Beer of No. 610,	3·460	41·62	146
614	No. 608 kept 24 hours,	—	—	146
613	Beer of No. 612,	3·188	38·34	147

Such a lessening of pressure is under ordinary circumstances made good by the diffusion inwards of sugar from the wort, hence this carbohydrate must be able to pass freely into the cell, while the alcohol produced passes out, maintaining a constant ratio, as shown by Paine (*loc. cit.*). A well-marked but relatively small extra fall in pressure was observed in No. 611, where the yeast, after separation from the beer, was suspended in a linen cloth in a large vessel of water with a delivery tap and overflow.

The small degree of permeability of the yeast as regards electrolytes is clearly brought out by the conductivity of the juice being from four to five times that of the beer. Even allowing for fluctuations from sample to sample there is a well-marked rise in conductivity in yeast after its separation. While this may be due in part to decreasing viscosity of the sap owing to sugars having been used up, yet, quantitatively considered, this explanation seems insufficient, and Nos. 610 and 611 make it more probable that such a result is partly due to the retention of an acid produced in fermentation, which in the normal course would diffuse very slowly outwards. Succinic acid, for instance, and its more highly ionised ammonium salt have been shown by Ehrlich (4) to arise during fermentation from glutamic acid.

To avoid the possibility of error in the comparison of yeast-juice and beer owing to the expulsion of gases by freezing the former solid, measurements were made of both freezing-point and conductivity of beer as separated from yeast and after freezing solid. No appreciable difference was observed between the two sets of figures.

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THE
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ON THE BUOYANCY OF THE SEEDS OF SOME BRITANNIC PLANTS.

BY

R. LLOYD PRAEGER.

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III.

ON THE BUOYANCY OF THE SEEDS OF SOME BRITANNIC PLANTS.

By R. LLOYD PRAEGER.

[Read April 15. Published May 31, 1913.]

In the present paper the term "seed" is used in its original and familiar sense, namely, that which is sown; in other words, the natural unit of dispersal. This may consist of a seed proper, or of one or more seeds enclosed in a dry or fleshy envelope, varying greatly in different species as regards size and shape.

The capacity of seeds for remaining afloat in water is one which has a very important bearing on the subject of the dispersal and distribution of plants. Seeds which can float for weeks or months, or even in certain circumstances for a few days, may become widely spread by the agency of rivers and of lakes. If, in addition, they are capable of resisting the injurious effects of salt water, the power of floating becomes more important, conferring on the species possessing such seeds the possibility of dissemination across stretches of sea of greater or less extent.

Historical Summary.

The importance of the floating power of seeds in relation to the geographical distribution of plants has been long recognized. The large and buoyant seeds and seed-vessels which strew the shores of tropical islands, with their suggestion of rapid and easy colonization by the aid of sea-currents, have been familiar to botanical travellers since the days of the earlier voyagers. So long ago as 1695, Sir Hans Sloane drew attention to foreign seeds thrown up by the sea on the shores of Scotland and Ireland[1]; these were the seeds of tropical plants, brought by the Gulf Stream from the West Indies.

Darwin was the first to show that but a small proportion of flowering plants have seeds which float, although a large variety of seeds are not injured by even prolonged immersion in sea-water. He also found that by thoroughly drying certain seeds and seed-vessels, their floating power was materially

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[1] Phil. Trans., xix., pp. 398-400. 1696.

increased; the same result was obtained in a few instances by drying fruiting branches of certain species, though such cases were exceptional. His conclusion is that, allowing for dried seeds and branches, about one-tenth of a flora might be taken as capable of transport across a considerable stretch of sea, and of subsequently germinating.[1]

Other early observations on the subject are well summed up by Martins,[2] who himself performed a valuable series of experiments on seeds placed in sea-water. He selected 98 kinds of seeds, giving preference to large seeds with a thick coat, and to those of littoral plants. His results showed that the majority of these seeds floated on sea-water, about one-third sinking at once; also that one-third of the total were capable of germination after six weeks' immersion, and one-eleventh after three months' immersion. He concludes that the transport of seeds by currents plays an insignificant part in the dispersal of species between countries separated by sea.

In 1873 Thuret[3] published the results of several seasons' experiments on the same subject. He used seeds of 251 species, belonging to seventy-seven different orders. He demonstrated that the conclusion of Martins, that the seeds capable of floating in sea-water were twice as numerous as those incapable of the same, was incorrect as a generalization. Thuret attributed the error to want of thorough wetting of the seeds experimented on; but in a foot-note to the paper, Alphonse de Candolle points out that Martins, as has been stated above, selected seeds of high presumptive buoyancy—large seeds with thick coats, and seeds of littoral plants. Thuret's detailed list shows that more than half of his 251 species sank at once; most of the remainder had sunk at the end of one or two days; only a very few floated for a week or more. This result has been amply confirmed by subsequent observers. But just as Martins selected plants which gave a buoyancy percentage too high for general application, so Thuret erred on the other side. His list included very few littoral species, which, as a group, are now known to possess an especially high index of seed-buoyancy, and in consequence the 2 per cent. or so, which may be deduced from his tables as representing the seed-buoyancy of his plants, is too low to give an average figure for the seed-buoyancy of a flora.

Many detailed observations followed on the dispersal of seeds by water, those of Scandinavian and Danish botanists, Kolpin Ravn[4] and Sernander[5] for instance, being especially valuable. As regards our native flora, the

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[1] Origin of Species, ed. 6, pp. 506–509.

[2] Bull. Soc. Bot. de France, iv, p. 324. 1857.

[3] Bibl. Univ. et Revue Suisse. Arch. des Sciences Phys. et Nat., N.P., xlvii, 179–194. 1873.

[4] F. Kolpin Ravn: Om Flydeevnen hos Froene af vore Vand- og Sump-planter. Bot. Tidsskrift, xix. 1894.

[5] R. Sernander: Den Skandinaviska Vegetationens Spridningsbiologie. Upsala and Berlin, 1901.

most important series of observations are those of Guppy, who, in his exhaustive work on seed-dispersal in the Tropics,[1] furnishes much information relative to our native plants as well, including a buoyancy-table for 273 species. Those portions of his book which deal with the British flora constitute the main source of information relative to the buoyancy of the seeds of our native plants. His conclusion is that the seeds of 90 per cent. of the British flora sink either at once or within a few days, leaving 10 per cent. which alone possess buoyancy sufficient to render them capable of transference across any but a very narrow stretch of water. It will be noticed that this figure is identical with that arrived at by Darwin just half a century earlier, as representing the proportion of a flora capable of crossing, by means of currents, a considerable stretch of sea. His study of the buoyancy of Fijian and Hawaiian seeds led Guppy to adopt the same percentage as representing the seed-buoyancy of the flora of those regions.

THE PRESENT EXPERIMENTS.

The experiments of which the results are given below were undertaken in order to extend our knowledge of the buoyancy of seeds of plants which inhabit the British Islands, especially with a view of obtaining information useful in the study of the immigration and dispersal of our native flora. Until Guppy's time, no special attention had been given to our native plants as regards their seed-buoyancy. Guppy, as stated above, tested the seeds of some 273 of these ; and he added to his table results for about 60 additional species, the seed-buoyancy of which could be obtained from the writings of Darwin, Martins, Thuret, Kolpin Ravn, and Sernander. In the present list, results are given for 786 species. The number of species in Guppy's list which were not tested by me is 114. Adding these figures, we have now buoyancy-results for just 900 species—a number which, though falling far short of the total for the British Isles, still represents a proportion sufficiently large to permit of a generalization regarding the whole flora. While Martins and Guppy experimented mainly with seeds known or believed to have a high average of buoyancy, and thus obtained slightly abnormal results, I endeavoured to have all kinds of seeds equally represented in my experiments, whether the nature of the seeds themselves, the phylogenetic relationships of the plants which bore them, or their habitats, be taken into account.

Since power of dispersal is to be measured by its *maximum* in the case of any species, special care was taken to test the seeds, so far as was possible, in the condition of maximum efficiency which may occur in nature.

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[1] H. B. Guppy. Observations of a Naturalist in the Pacific . . . vol. ii : Plant-Dispersal. 1906.

Thoroughly dry seed was used in preference to seed just taken from a fresh, moist seed-vessel; though the latter is the condition in which the majority of seeds commence their dispersal-adventures, the former condition must frequently occur in nature. Seed gathered fresh and stored in a dry room for some months was used in preference to any other. Fleshy fruits were tested in a thoroughly dried as well as in a fresh condition. Drying has often an important effect in the case of fleshy fruits, while in the case of hard seeds its effect is usually inappreciable.

The experiments of Guppy[1] showed that between fresh and salt water a very slight difference exists as regards their effect on the buoyancy of seeds. After a number of tests to satisfy myself of this, I fell back on the more easily obtainable medium, and used fresh water throughout the experiments, resorting to salt water occasionally as a check. There is hardly an exception to the rule that seeds which sink in the one medium sink also in the other. The only effect of the salt water is to slightly increase the period of flotation ; and since, as stated above, care was taken to obtain a maximum period by using dried seed (and also, as appears below, by taking the period of the most efficient seed of each batch as representing its buoyancy-period), I believe my figures are already quite as high as we have any right to accept as a buoyancy-index even for sea-water.

The seeds, twenty to a hundred in number, whenever so great a number was available, were first cursorily examined for soundness, and then thoroughly shaken up with water in test-tubes, care being taken to remove adherent air-bubbles. The minority which did not sink were kept, and examined and shaken up twice a day, and transferred to fresh water occasionally until all the seeds—excepting occasionally a few whose soundness there was reason to doubt—had sunk. This maximum period was then entered opposite the name of the species in a copy of the "London Catalogue." Thus, for those seeds which did not all sink within a minute or so, 12 hours is the unit of time, and signifies anything up to 12 hours ; and so on for greater periods. The *maximum* efficiency is thus not alone entered, but in many cases somewhat exaggerated; however, the exaggeration is unimportant except for short periods, and even then has little significance, as seeds of short flotation-periods are ineffective for oversea dispersal.

After a flotation-period of a month or so, the seeds were examined and shaken up only once a day, and after about three months only once a week.

In the case of a considerable number of species, the dispersal-unit may be either the whole fruit or part of it. In the majority of cases it is the seed itself. In many others it is a dry indehiscent fruit. In cases like the

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[1] *loc. cit.*, p. 89.

species of Raphanus, it may be a portion of the fruit, with a seed enclosed. In the case of fleshy fruits, several conditions of dispersal are possible. A fresh berry may fall directly into a stream; or it may fall and get dried, and subsequently become immersed; or it may be eaten by a bird, and the wet seeds dropped into water, or dropped on a dry place and carried into a stream after dessication. So far as material served, buoyancy was tested under several or all of these conditions.

Where material was obtainable, two or even three batches of seed of one species from different sources were tested. Sometimes the results obtained from different batches of seed were uniform; occasionally, they differed widely. To this point I shall return presently. Where more than one figure is given in the table opposite the name of a species, whether in the same column or in different columns, it represents the flotation-periods of the most buoyant seeds of different batches.

EXPLANATION OF THE TABLE.

In order that the varying buoyancy of the seeds of different species may be more readily discerned, the results obtained are arranged in five columns in the table which follows:—The cross in the first column shows the species whose seeds sink at once; the second column gives (in hours) periods up to one day; the third gives (in days) periods up to one week; the fourth (in weeks) up to one month; the fifth gives (in months) periods over one month, up to 15 months, when observations ceased. The seeds which were still floating at the conclusion of the months' observations are shown thus— 15 +.

In a sixth column are added flotation-periods as given by Guppy. Guppy's tabulated results are less detailed than my own, and a full comparison between the two cannot therefore be made. The form in which his observations are given, the symbols which he employs, and the symbols by which his results are shown in the sixth column of my table, are as follows:—

	Guppy's symbol.	Symbol in following list.
Float for less than one week, .	(blank)	*
,, 1–4 weeks .	+	1–4 w.
,, 1–6 months, .	++	1–6 m.
,, 6–12 ,, .	vi ++	6–12 m.
,, over 12 ,, .	xii ++	12 + m.
Variable in floating power, .	var.	var.

Guppy's symbols, it will be seen, are comparable only to those given in the fourth and fifth columns of my table; results corresponding to those of my first three columns being shown by a blank in his table.

TABLE OF SEED-BUOYANCY.

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
RANUNCULACEAE.						
Clematis Vitalba *L.*,	—	12	—	—	—	—
Thalictrum alpinum *L.*,	—	—	2	—	—	—
minus *L.*,	—	—	3	—	—	—
majus *Crantz*,	—	—	5	—	—	—
Anemone nemorosa *L.*,	—	—	1	—	—	—
Adonis annua *L.*,	—	3	—	—	—	—
Ranunculus trichophyllus *Chaix*,	—	12	—	—	—	—
heterophyllus *Weber*,	—	12	—	—	—	—
Baudotii *Godron*,	—	12	—	—	—	—
Lenormandi *F. Schultz*,	—	—	1½	—	—	—
sceleratus *L.*,	—	—	3½	—	—	6–12 m. var.
Flammula *L.*,	—	—	5	—	—	*
Lingua *L.*,	—	12	—	—	—	—
Ficaria *L.*,	—	—	2½	—	—	*
auricomus *L.*,	—	—	2	—	—	—
repens *L.*,	—	—	3½	—	—	6–12 m. var.
bulbosus *L.*	—	—	3½	—	—	—
parviflorus *L.*,	×, ×	—	—	—	—	—
arvensis *L.*,	—	—	2½	—	—	—
Caltha palustris *L.*,	—	—	—	1⅐	—	1–4 W.
radicans *T. F. Forst*,	—	—	—	2	—	—
Trollius europaeus *L.*,	×	—	—	—	—	—
Eranthis hyemalis *Salisb.*,	×	—	—	—	—	—
Aquilegia vulgaris *L.*,	×	—	—	—	—	—
Delphinium Ajacis *L.*,	×	—	—	—	—	—
Actaea spicata *L.* (fresh fruit),	—	—	—	1	—	—
(dry fruit),	—	—	—	—	2¼	—
BERBERIDACEAE.						
Berberis vulgaris *L.* (fresh fruit),	—	—	4	—	—	*
,, (dry fruit),	—	1	—	—	—	
,, (seed),	×	—	—	—	—	

Table of Seed-buoyancy—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Nymphaeaceae.						
Nymphaea lutea *L.* (dry seeds), .	—	—	—	$1\frac{3}{4}$	—	*
Papaveraceae.						
Papaver Argemone *L.*, . .	×, ×	—	—	—	—	—
hybridum *L.*, . . .	×, ×	—	—	—	—	—
Rhoeas, *L.*, . . .	×	—	—	—	—	*
dubium *L.*, . . .	×	—	—	—	—	*
somniferum *L.*, . . .	×	—	—	—	—	—
Meconopsis cambrica *Vig.*, .	×	—	—	—	—	—
Glaucium flavum *Crantz*, .	×	—	—	—	—	*
Chelidonium majus *L.*, .	×, ×	—	—	—	—	*
Fumariaceae.						
Corydalis lutea *DC.*, . .	—	—	2	—	—	—
claviculata *DC.*, . .	—	12	—	—	—	—
Fumaria Bastardi *Bor.* . .	—	12	—	—	—	—
officinalis *L.*, . . .	—	18	4	—	—	—
Cruciferae.						
Matthiola incana *R. Br.*, .	—	—	2	—	—	—
sinuata *R. Br.*, . .	×	—	—	—	—	—
Cheiranthus Cheiri *L.*, . .	×, ×	—	—	—	—	—
Radicula Nasturtium-aquaticum, *R & B.*, ...	×	12	—	—	—	*
palustris *Moench*, . .	—	3	—	$1\frac{3}{4}$	—	—
Barbarea lyrata *Asch.*, . .	×	3	—	—	—	*
Arabis hirsuta *Scop.* . .	×	—	—	—	—	*
Cardamine flexuosa *With.*, .	×	—	—	—	—	—
hirsuta *L.*, . . .	×	—	—	—	—	*
Hesperis matronalis *L.*, . .	×	—	—	—	—	—
Sisymbrium officinale *Scop.*, .	×	—	—	—	—	—
Irio *L.*, ...	×, ×	—	—	—	—	—
Sophia *L.*, ...	×	—	—	—	—	—
Thalianum *J. Gay*, . .	×	—	—	—	—	*
orientale *L.*, . . .	×	—	—	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Alliaria alliacea *R. & B.*,	×	—	—	—	—	*
Erysimum cheiranthoides *L.*,	×, ×	—	—	—	—	—
Camelina sativa *Crantz*,	×, ×	—	—	—	—	—
Brassica Napus *L.*,	×	—	—	—	—	—
Rutabaga *DC.*,	×	—	—	—	—	—
campestris *L.*,	×	—	—	—	—	*
Sinapis nigra *L.*,	×, ×	—	—	—	—	—
arvensis *L.*,	×	—	—	—	—	—
alba *L.*,	×	—	—	—	—	*
Diplotaxis muralis *DC.*,	×, ×	—	—	—	—	—
Alyssum alyssoides *L.*,	×	—	—	—	—	—
Draba incana *L.*,	×	—	—	—	—	—
muralis *L.*,	×	—	—	—	—	—
verna *L.*,	×	—	—	—	—	*
Cochlearia officinalis *L.*,	×	—	—	—	—	*
danica *L.*,	×	—	—	—	—	—
anglica *L.*,	×	—	—	—	—	—
Thlaspi arvense *L.*,	×	—	—	—	—	*
alpestre *L.*,	×	—	—	—	—	—
Teesdalia nudicaulis *R. Br.*,	×	—	—	—	—	—
Lepidium Draba *L.*,	×	—	—	—	—	—
campestre *R. Br.*	×, ×	—	—	—	—	—
heterophyllum *Benth.*,	×	—	—	—	—	—
ruderale *L.*,	×	—	—	—	—	—
sativum *L.*	×	—	—	—	—	—
perfoliatum *L.*,	×	—	—	—	—	—
Capsella Bursa-pastoris *Med.*,	×	—	—	—	—	*
Subularia aquatica *L.*,	×	—	—	—	—	—
Coronopus didymus *Sm.*	×	—	—	—	—	—
Isatis tinctoria *L.*,	×	—	—	—	—	—
Cakile maritima *Scop.* (seed),	×	—	—	—	—	1–4 w.
Crambe maritima *L.* (dry fruit),	—	—	—	—	2	1–4 w.
,, (seed),	×	—	—	—	—	

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Raphanus Raphanistrum *L.* (dry fruit).	—	—	5	—	4	—
,, (seed),	×	—	—	—	—	
maritimus *Sm.* (dry fruit),	—	—	—	3	—	1–4 w.
,, (seed),	×	—	—	—	—	
RESEDACEAE.						
Reseda alba *L.*,	—	½	—	—	—	—
lutea *L.*,	×	—	—	—	—	—
Luteola *L.*,	×	—	—	—	—	*
CISTACEAE.						
Helianthemum Chamaecistus *Mill.*,	×	—	—	—	—	*
Marifolium *Mill.*,	×	—	—	—	—	—
VIOLACEAE.						
Viola palustris *L.*,	—	—	2½	—	—	*
odorata *L.*,	—	—	1	—	—	—
hirta *L.*,	—	12	—	—	—	—
Riviniana *Reich.*,	—	12	—	—	—	—
canina *L.*,	—	12	3	—	—	*
arvensis *Murr.*,	—	12	—	—	—	—
lutea *Huds.*,	—	—	1½	—	—	—
Curtisii *Forster*,	—	18	—	—	—	—
POLYGALACEAE.						
Polygala vulgaris *L.*,	×, ×	—	—	—	—	*
serpyllacea *Weihe*,	×	—	—	—	—	—
CARYOPHYLLACEAE.						
Dianthus deltoides *L.*,	×	—	—	—	—	—
plumarius *L.*,	×	—	—	—	—	—
Caryophyllus *L.*,	×	—	—	—	—	—
Saponaria Vaccaria *L.*,	×, ×	—	—	—	—	—
officinalis *L.*,	×	—	—	—	—	—
Silene latifolia *R. & B.*,	×	—	—	—	—	*
maritima *With.*,	×	—	—	—	—	*
anglica *L.*,	×, ×	—	—	—	—	—
noctiflora *L.*,	×, ×	—	—	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Silene acaulis *L.*,	×, ×	—	—	—	—	—
Armeria *L.*,	×	—	—	—	—	—
Lychnis Viscaria *L.*,	×	—	—	—	—	—
alba *Mill.*,	×, ×	—	—	—	—	—
Flos-cuculi *L.*,	×	—	—	—	—	—
dioica *L.*,	×	—	—	—	—	*
Githago *Scop.*,	×	—	—	—	—	—
Sagina procumbens *L.*,	×, ×	—	—	—	—	*
apetala *Ard.*,	×, ×	—	—	—	—	—
maritima *G. Don.*	×, ×	—	—	—	—	—
subulata *Presl*,	×	—	—	—	—	—
nodosa *Fenzl.*,	×	—	—	—	—	—
Honkenya peploides *Ehr.*,	—	—	2	—	—	12 + m.
Minuartia verna *Hiern*,	×	—	—	—	—	—
tenuifolia *Hiern*,	×	—	—	—	—	—
Arenaria trinervia *L.*,	×, ×	—	—	—	—	—
serpyllifolia *L.*,	×	—	—	—	—	—
ciliata *L.*,	×	—	—	—	—	—
norvegica *Gunn.*,	×	—	—	—	—	—
Stellaria media *Vill.*,	×	—	—	—	—	*
palustris *Retz.*,	×	—	—	—	—	—
graminea *L.*,	×	—	—	—	—	*
Cerastium viscosum *L.*,	×	—	—	—	—	—
vulgatum *L.*,	×	—	—	—	—	*
semidecandrum *L.*,	×	—	—	—	—	—
tetrandrum *Curt.*,	×, ×	—	—	—	—	—
arvense *L.*,	×	—	—	—	—	—
Alsine rubra *Crantz*,	×	—	—	—	—	*
rupicola *Hiern*,	×, ×	—	—	—	—	—
media *Crantz*,	×	—	—	—	—	—
marginata *Reich.*,	×	—	—	—	—	*
Spergula arvensis *L.*,	×, ×	—	—	—	—	*

Table of Seed-buoyancy—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
PORTULACEAE.						
Montia fontana *L.*,	×	—	—	—	—	*
ELATINACEAE.						
Elatine Hydropiper *L.*,	×	—	—	—	—	*
MALVACEAE.						
Malva sylvestris *L.*,	—	—	1½	—	—	*
moschata *L.*,	—	—	2½	—	—	—
rotundifolia *L.*,	—	12	—	—	—	*
Althaea officinalis *L.*,	×	—	—	—	—	—
Lavatera arborea *L.*,	—	12	1½	—	—	—
HYPERICACEAE.						
Hypericum Androsaemum *L.*,	×	—	—	—	—	—
calycinum *L.*,	—	12	—	—	—	—
elatum *Ait.*,	—	—	1	—	—	—
quadrangulum *L.*,	—	—	1	—	—	*
perforatum *L.*,	—	12	—	—	—	*
maculatum *Crantz*,	×	—	—	—	—	—
humifusum *L.*,	×	—	—	—	—	—
linariifolium *Vahl.*,	×	—	—	—	—	—
hirsutum *L.*,	×, ×	—	—	—	—	—
pulchrum *L.*,	×	—	—	—	—	—
elodes *L.*,	—	12	—	—	—	*
GERANIACEAE.						
Geranium sylvaticum *L.*,	×	—	—	—	—	—
pratense *L.*,	×	—	—	—	—	—
sanguineum *L.*,	×, ×	—	—	—	—	—
pyrenaicum *Burm. fil.*,	×	—	—	—	—	—
molle *L.*,	×, ×	—	—	—	—	—
rotundifolium *L.*,	×, ×	—	—	—	—	—
pusillum *L.*,	×	—	—	—	—	—
dissectum *L.*,	×, ×	—	—	—	—	—
columbinum *L.*,	×	—	—	—	—	—
lucidum *L.*,	×, ×	—	—	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Geranium Robertianum *L.*,	×	—	—	—	—	—
Erodium cicutarium *L'Hérit.*,	×	—	—	—	—	—
moschatum *L'Hérit.*,	×	—	—	—	—	—
maritimum *L'Hérit.*,	×	—	—	—	—	—
Oxalis Acetosella *L.*,	×	—	—	—	—	*
LINACEAE.						
Linum usitatissimum *Huds.*,	×, ×	—	—	—	—	*
angustifolium *Huds.*,	×, ×	—	—	—	—	*
perenne *L.*,	×	—	—	—	—	—
catharticum *L.*,	×	1	—	—	—	—
Radiola linoides *Roth.*,	×	—	—	—	—	—
AQUIFOLIACEAE.						
Ilex Aquifolium *L.* (fresh fruit),	—	—	1½, 2	—	—	*
,, (dry seed),	—	—	—	1½	—	—
CELASTRACEAE.						
Euonymus europaeus *L.* (fresh fruit),	×	—	—	—	—	1–4 w.
,, (dry fruit),	—	—	—	—	2	
,, (seed).	—	—	2½	—	—	
RHAMNACEAE.						
Rhamnus catharticus *L.* (dry fruit).	—	—	6	—	—	—
,, (seed),	—	—	3½	—	—	
Frangula *L.* (dry fruit),	—	—	—	1½	—	—
,, (fresh fruit),	—	—	—	$2\frac{2}{7}$, $2\frac{5}{7}$	—	
,, (dry seed),	—	12	—	1	—	
,, (fresh seed),	—	—	1½	—	—	
LEGUMINOSAE.						
Ulex europaeus *L.*,	×, ×	—	—	—	—	*
Gallii *Planch.*,	×	—	—	—	—	—
Genista tinctoria *L.*,	×, ×	—	—	—	—	—
Sarothamnus scoparius *Wimm.*,	×	—	—	—	—	*
Ononis repens *L.*,	×	—	—	—	—	*
Medicago sativa *L.*,	×, ×, ×	—	—	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Medicago sylvestris *Fries*,	×	—	—	—	—	—
falcata *L.*,	×	—	—	—	—	—
lupulina *L.*, (fruit),	—	—	1½	—	—	*
,, (seed),	×	—	—	—	—	
arabica *Huds.*, (fruit),	×	—	2½	—	—	—
,, (seed),	×	—	—	—	—	—
denticulata *L.* (fruit),	×	—	1	—	—	*
,, (seed),	×	—	—	—	—	
minima *Willd.* (fruit),	—	—	1½	—	—	—
,, (seed),	×	—	—	—	—	—
Melilotus officinalis *Lam.*	×	—	—	—	—	—
Petitpierreana *Hayne*,	×	—	—	—	—	—
Trifolium pratense *L.*,	×, ×	—	—	—	—	—
medium *L.*,	×	—	—	—	—	—
incarnatum *L.*,	×, ×	—	—	—	—	*
arvense *L.* (seed),	×	—	—	—	—	—
,, (fruit),	—	3½	—	—	—	—
striatum *L.*,	×, ×	—	—	—	—	—
scabrum *L.*,	×	—	—	—	—	—
subterraneum *L.*,	×, ×	—	—	—	—	—
glomeratum *L.*,	×	—	—	—	—	—
suffocatum *L.*,	×	—	—	—	—	—
repens *L.*,	×	—	—	—	—	—
hybridum *L.*,	×	—	—	—	—	—
fragiferum *L.*,	×, ×	—	—	—	—	—
procumbens *L.*,	×, ×	—	—	—	—	—
agrarium *L.*,	×, ×	—	—	—	—	—
dubium *Sibth.*,	×, ×	—	—	—	—	—
filiforme *L.*,	×, ×	—	—	—	—	—
Trigonella ornithopodioides *DC.*,	×	—	—	—	—	—
Lotus corniculatus *L.*,	×	—	—	—	—	*
uliginosus *Schkuhr*,	×	—	—	—	—	—
Anthyllis Vulneraria *L.*,	—	—	1	—	—	*

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Astragalus glycyphyllos *L.*,	×, ×	—	—	—	—	—
Onobrychis viciaefolia *Scop.*,	—	—	2½	—	—	—
Vicia hirsuta *S. F. Gray*,	×	—	—	—	—	—
sylvatica *L.*,	×	—	—	—	—	—
Orobus *DC.*,	×	—	—	—	—	—
Cracca *L.*,	×	—	—	—	—	—
sepium *L.*,	×	—	—	—	—	—
sativa *L.*,	×	—	—	—	—	*
angustifolia *L.*,	×, ×	—	—	—	—	—
lathyroides *L.*,	×	—	—	—	—	—
Lathyrus pratensis *L.*,	×	—	—	—	—	*
latifolius *L.*,	×	—	—	—	—	—
sylvestris *L.*,	×, ×	—	—	—	—	—
montanus *Bernh.*,	×	—	—	—	—	—
niger *Bernh.*,	×	—	—	—	—	—
ROSACEAE.						
Prunus spinosa *L.* (fresh fruit),	×	—	—	—	—	—
(stone),	×	—	—	—	—	—
Padus *L.* (stone),	—	12	—	—	—	—
Spiraea Ulmaria *L.*,	—	—	—	1½	—	1–4 w.
Filipendula *L.*,	—	—	1½	—	—	—
Sanguisorba officinalis *L.*,	—	—	2	—	—	—
Poterium Sanguisorba *L.*,	—	—	2, 2½	—	—	—
Agrimonia Eupatoria *L.*,	—	18	—	1	—	—
odorata *Mill.*,	—	—	3	—	—	—
Alchemilla vulgaris *L.*,	—	—	1½	—	—	—
alpina *L.*,	—	—	1, 2½	—	—	—
arvensis *Scop.*,	×	12	1	—	—	*
Potentilla rupestris *L.*,	—	12	—	—	—	—
Anserina *L.*,	—	—	—	—	15 +	—
argentea *L.*,	×	—	—	—	—	—
reptans *L.*,	×, ×	—	—	—	—	—
procumbens *Sibth.*,	×	—	—	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Potentilla sylvestris *Necker*, .	×	—	—	—	—	*
fruticosa *L.*, . . .	—	12	—	—	—	—
Comarum palustre *L.*, . .	—	—	—	—	2, 15 +	12 + m.
Rubus Idaeus *L.* (fresh fruit), .	×	—	—	—	—	—
,, (seed), . .	×	—	—	—	—	—
fruticosus *L.* (fresh fruit), .	×	—	—	—	—	—
,, (dry fruit), .	—	—	2	—	—	—
,, (seed), . .	×	—	1	—	—	—
saxatilis *L.* (seed), . .	—	12	—	—	—	—
Dryas octopetala *L.*, . . .	—	—	1	—	—	—
Geum Urbanum *L.*, . . .	×	—	—	—	—	—
rivale *L.*, ...	—	—	1½	—	—	—
Rosa spinosissima *L.* (fresh fruit),	—	—	1½	—	1½	—
,, (seed) .	×	—	—	—	—	—
hibernica *Temp.* (fresh fruit),	—	—	5	—	—	—
,, (fresh seed),	—	—	2	—	—	—
mollis *Sm.* (seed), .	—	—	1½	—	—	—
tomentosa *Sm.* (fresh fruit), .	×	—	—	—	—	—
,, (seed), .	×, ×	—	—	—	—	—
,, (dry fruit), .	—	—	—	2	—	—
eglanteria *L.* (seed), . .	—	—	5	—	—	—
obtusifolia *Desv.* (seed), .	×	—	—	—	—	—
canina *L.* (fresh fruit), .	×	—	—	—	—	—
,, (dry seed), . .	—	12	—	—	—	—
arvensis *Huds.* (fresh fruit),	—	1	—	—	—	*
,, (dry fruit), .	—	—	—	—	1½	—
,, (seed), .	×	—	—	—	—	—
Crataegus Oxyacantha *L.* (fresh fruit),	—	—	—	2	—	1–4 w.
,, (dry fruit), .	—	—	—	3	—	
,, (dry stone) .	×, ×	—	—	—	—	
Pyrus Aucuparia *L.* (fresh fruit),	×	—	—	—	—	—
,, (dry fruit), .	—	—	—	2	—	—
,, (seed), .	×	—	—	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Pyrus latifolia *Syme* (fresh fruit),	×	—	—	—	—	—
Malus *L.* (seed), . . .	×	—	—	—	—	—
LYTHRACEAE.						
Lythrum Salicaria *L.*, . .	×	—	—	—	—	*
Peplis Portula *L.*, . . .	×	—	—	—	—	*
ONAGRACEAE.						
Epilobium hirsutum *L.*, . .	—	—	1	—	—	*
parviflorum *Schreb.*, . .	—	—	1	—	—	*
montanum *L.*, . .	—	—	1	—	—	—
roseum *Schreb.*, . .	—	12	—	—	—	—
palustre *L.* (with pappus), .	—	1	1	—	—	—
,, (without pappus),	×	—	—	—	—	—
Oenothera biennis *L.*, . .	—	—	1½	1¾	—	—
odorata *Jacq.*, . . .	—	—	1	—	—	—
Circaea lutetiana *L.*, . . .	—	—	1	—	—	—
CUCURBITACEAE.						
Bryonia dioica *Jacq.*, . .	—	18	3	—	—	*
CRASSULACEAE.						
Sedum roseum *Scop.*, . .	×, ×	—	—	—	—	—
Telephium *L.*, . .	—	—	2	—	—	—
album *L.*, . . .	×	—	—	—	—	—
anglicum *Huds.*, . .	×	—	—	—	—	—
acre *L.*, ...	×	—	—	—	—	—
reflexum *L.*, . . .	×	—	—	—	—	—
rupestre *L.*, . . .	×	—	—	—	—	—
Cotyledon Umbilicus *L.*, . .	×	12	—	—	—	*
RIBESIACEAE.						
Ribes Grossularia *L.*, . .	×	—	—	—	—	—
SAXIFRAGACEAE.						
Saxifraga umbrosa *L.*, . .	×, ×	—	—	—	—	—
Geum *L.*, . . .	×	—	—	—	—	—
stellaris *L.*, . . .	×	—	1	—	—	—
aizoides *L.*, . . .	×	—	—	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Saxifraga hypnoides *L.*,	×	—	—	—	—	—
tridactylites *L.*,	×, ×	—	—	—	—	*
Chrysosplenium oppositifolium *L.*,	×	—	—	—	—	*
Parnassia palustris *L.*,	—	—	—	1, 3½	—	—
DROSERACEAE.						
Drosera rotundifolia *L.*	—	—	—	3	—	1–4 w.
longifolia *L.*,	—	—	—	—	1	—
anglica *Huds.*,	—	—	5½	—	—	—
HALORAGACEAE.						
Myriophyllum verticillatum *L.*,	—	—	1	—	—	—
Hippuris vulgaris *L.*,	—	—	—	—	15 + m.	—
UMBELLIFERAE.						
Hydrocotyle vulgaris *L.*,	—	—	—	2	—	12 + m.
Sanicula europaea *L.*,	×	—	—	—	—	—
Eryngium maritimum *L.*,	—	—	2	—	—	*
Petroselinum segetum *Koch*,	×	—	—	—	—	—
Carum Carvi *L.*,	×	—	—	—	—	—
Pimpinella Saxifraga *L.*,	×	—	—	—	—	—
Bupleurum falcatum *L.*,	×	—	—	—	—	—
Oenanthe fistulosa *L.*,	—	—	2½	—	—	—
pimpinelloides *L.*,	—	—	4	—	—	—
Lachenalii *C. Gmelin*,	—	—	1½	—	—	—
aquatica *Poir.*,	—	—	—	—	15 +	1–4 w.
fluviatilis *Colem.*,	—	—	—	1	—	—
Aethusa Cynapium *L.*,	—	—	3	—	—	*
Foeniculum vulgare *Mill.*,	—	12	—	—	—	—
Haloscias scoticum *Fries*,	—	—	5½	—	2½	—
Meum athamanticum *Jacq.*,	×	—	—	—	—	—
Crithmum maritimum *L.*,	—	—	—	—	5	1–6 m.
Angelica sylvestris *L.*,	—	—	—	—	15 +	12 + m.
Peucedanum officinale *L.*,	×	—	—	—	—	—
Pastinaca sativa *L.*,	—	—	1, 1	—	—	*
Heracleum Sphondylium *L.*,	—	—	1	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Daucus Carota *L.*,	—	—	2½, 2½	—	—	—
Coriandrum sativum *L.*,	—	—	1	—	—	—
Torilis Anthriscus *Bernh.*,	×	—	—	—	—	—
arvensis *Link*,	—	12	—	—	—	—
nodosa *Gaertn.*,	×, ×	—	—	—	—	—
Scandix Pecten-Veneris *L.*,	×	—	—	—	—	—
Chaerophyllum sylvestre *L.*,	×	—	—	—	—	*
Anthriscus *Lam.*,	×	—	—	—	—	—
temulum *L.*,	×	—	—	—	—	—
Myrrhis Odorata *Scop.*,	—	—	2½	—	—	—
Conium maculatum *L.*,	×	—	—	—	—	—
Physospermum cornubiense *DC.*,	—	—	1½	—	—	—
Smyrnium Olusatrum *L.*,	—	—	3, 3	—	—	*
CORNACEAE.						
Cornus suecica *L.* (seed),	—	—	—	1	—	—
LORANTHACEAE.						
Viscum album *L.* (fresh fruit),	×	—	—	—	—	*
,, (dry fruit),	—	6	—	—	—	
,, (seed),	×	—	—	—	—	
CAPRIFOLIACEAE.						
Sambucus nigra *L.* (fresh fruit),	×	—	—	—	—	*
,, (seed),	×	—	—	—	—	
Viburnum Opulus *L.* (fresh fruit),	×	—	—	—	—	—
,, (seed),	×	—	—	—	—	—
lantana *L.* (dry fruit),	—	—	2	—	—	—
,, (seed),	—	—	2	—	—	—
Lonicera Periclymenum *L.* (fresh fruit),	×	—	—	—	—	—
,, (dry fruit),	×	—	—	—	—	—
,, (seed),	×	—	—	—	—	—
RUBIACEAE.						
Sherardia arvensis *L.*,	×	—	—	—	—	—
Asperula odorata *L.*,	×	—	—	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours 1 to 24.	Days (1 to 7).	Weeks (1 to 4).	Months.	
Galium Cruciata *Scop.*,	—	—	1	—	—	—
Aparine *L.*,	—	12	—	—	—	*
Mollugo *L.*,	—	—	1	—	—	*
saxatile *L.*,	—	—	1½	—	—	—
umbellatum *Lam.*,	×, ×	—	—	—	—	—
palustre *L.*,	—	—	—	—	2⅓	6–12 m.
Rubia peregrina *L.*,	—	—	—	1	—	—
VALERIANACEAE.						
Valeriana officinalis *L.*,	—	—	3	—	—	—
Valerianella olitoria *Poll.*,	—	—	2½, 5	—	—	*
rimosa *Bast.*,	—	—	—	1½, 2	—	—
dentata *Poll.*,	—	—	1, 2½	—	—	—
eriocarpa *Desv.*,	—	—	2½	—	—	—
Kentranthus ruber *DC.*,	—	—	1½	—	—	*
DIPSACACEAE.						
Dipsacus sylvestris *Huds.*,	—	—	1	—	—	—
Knautia arvensis *Coulter*,	—	—	2½	—	—	—
Scabiosa Succisa *L.*,	—	—	—	—	1, 15 +	—
Columbaria *L.*,	—	—	2½	—	—	—
COMPOSITAE.						
Eupatorium cannabinum *L.*,	—	1	—	—	—	*
Aster Tripolium *L.*,	—	—	5	—	—	*
Erigeron acris *L.*,	—	12	—	—	—	—
Bellis perennis *L.*,	×	—	—	—	—	—
Solidago Virgaurea *L.*,	—	—	2	—	—	—
Inula Helenium *L.*,	—	—	2	—	—	—
salicina *L.*,	—	—	1½	—	—	—
vulgaris *Trev.*,	—	12	—	—	—	—
crithmoides *L.*,	—	—	1½	—	—	—
Pulicaria dysenterica *S. F. Gray*,	—	—	2½	—	—	—
Filago germanica *Huds.*,	×	—	—	—	—	—
apiculata *G. E. Smith*,	×	—	—	—	—	—
minima *Fries*,	×	—	—	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Gnaphalium uliginosum *L.*, .	×	—	—	—	—	—
sylvaticum *L.*, . . .	—	12	—	—	—	—
Achillea Millefolium *L.*, . .	—	12	1	—	—	*
Ptarmica *L.*, . . .	—	—	1	—	—	—
Anthemis arvensis *L.*, . .	—	—	1½	—	—	—
Cotula *L.*, ...	—	—	2, 2	—	—	—
Matricaria inodora *L.*, . .	—	12	—	—	—	*
discoidea *DC.*, . . .	—	12	3	—	—	—
Parthenium *L.*, . .	—	12	—	—	—	—
Chrysanthemum segetum *L.*, .	—	12	1½	—	—	*
Artemisia Absinthium *L.*, .	×	—	—	—	—	*
vulgaris *L.*, . . .	×	—	—	—	—	*
maritima *L.*, . . .	—	—	—	1¼	—	—
Tanacetum vulgare *L.*, . .	—	2	—	—	—	—
Doronicum plantagineum *L.*, .	—	12	—	—	—	—
Senecio vulgaris *L.*, . . .	—	—	5½	—	—	*
sylvaticus *L.*, . . .	—	18	—	—	—	—
viscosus *L.*, . . .	—	—	1½	—	—	—
squalidus *L.*, . . .	—	—	2	—	—	—
erucifolius *L.*, . . .	—	12	3	—	—	—
Jacobaea *L.*, . . .	—	18	—	—	—	—
aquaticus *Huds.*, . .	—	—	1	—	—	*
Bidens cernua *L.*, . . .	—	—	—	3	—	6–12 m.
tripartita *L.*, . . .	—	—	5	—	—	6–12 m.
Carlina vulgaris *L.*, . . .	—	—	2	—	—	—
Arctium majus *Bernh.*, . .	×	—	—	—	—	—
minus *Bernh.*, . .	×, ×	—	—	—	—	—
intermedium *Lange*, . .	×	—	—	—	—	—
Newbouldii *Ar. Benn.*, .	×	—	—	—	—	—
Centaurea nigra *L.*, . . .	—	12	—	—	—	—
Cyanus *L.*, . . .	×	—	—	—	—	—
Scabiosa *L.*, . . .	—	—	2	—	—	—
Onopordon Acanthium *L.*, . .	—	—	2½	—	—	—

Table of Seed-buoyancy—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks. (1 to 4).	Months.	
Carduus nutans *L.* (without pappus),	—	—	4	—	—	*
crispus *L.* (without pappus)	—	1½	—	—	—	—
lanceolatus *L.*,	—	—	3½	—	—	*
pratensis *Huds.*,	—	—	4	1½	—	—
Silybum Marianum *Gaertn.*,	—	18	—	—	—	—
Lapsana communis *L.*,	×	—	—	—	—	*
Cichorum Intybus *L.*,	—	1	2	—	—	—
Thrincia nudicaulis *Britten*,	—	—	1½	—	—	—
Leontodon autumnalis *L.*,	—	—	1	—	—	*
Tragopogon pratensis *L.*,	—	12	—	—	—	*
Picris hieracioides *L.* (without pappus)	×	—	—	—	—	—
Helminthia echioides *Gaertn.*,	—	12	1	—	15 +	*
Lactuca Serriola *L.*,	—	1½	—	—	—	—
virosa *L.*,	—	18	—	—	—	—
muralis *Gaertn.*,	—	2	—	—	—	—
Taraxacum officinale *Weber*,	—	—	4	—	—	*
Sonchus oleraceus *L.*,	—	—	3½	—	—	*
asper *Hill*,	—	—	3	—	—	—
arvensis *L.*,	—	—	4	—	—	—
Crepis capillaris *Wallr.* (without pappus)	×	—	—	—	—	*
biennis *L.*,	—	—	1, 1½	—	—	—
paludosa *Moench*,	—	2	—	—	—	—
Hieracium Pilosella *L.*,	—	18	—	—	—	—
aurantiacum *L.*,	—	—	6	—	—	—
anglicum *Fr.*,	—	—	2	—	—	—
vulgatum *Fr.*,	—	2	2	—	—	—
boreale *Fr.*,	—	—	1½, 2	—	—	—
Campanulaceae.						
Lobelia Dortmanna *L.*,	×	—	—	—	—	*
Jasione montana *L.*,	×	—	—	—	—	—
Campanula glomerata *L.*,	—	12	—	—	—	—
latifolia *L.*,	×	—	—	—	—	—

Table of Seed-buoyancy—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Campanula rapunculoides *L.*,	×	—	—	—	—	—
rotundifolia *L.*,	—	—	1	—	—	—
ERICACEAE.						
Arbutus Unedo *L.* (fresh fruit).	×	—	—	—	—	—
,, (dry fruit),	×	—	—	—	—	—
,, (seed),	×	—	—	—	—	—
Arctostaphylos Uva-ursi *Spreng.* (dry fruit	—	—	—	1, 1½	—	—
Calluna vulgaris *Hull,*	×	—	—	—	—	—
Erica Tetralix *L.*,	×	—	—	—	—	—
cinerea *L.*,	×	—	—	—	—	—
mediterranea *L.*,	—	1, $\frac{1}{10}$	—	—	—	—
vagans *L.*,	—	—	3½	—	—	—
Daboecia cantabrica *R. & B.*,	—	1	—	—	—	—
Azalea procumbens *L.*,	—	12	—	—	—	—
Pyrola minor *L.*,	—	—	2½	—	—	—
PRIMULACEAE.						
Primula veris *L.*,	—	1	—	—	—	—
elatior *Jacq.*,	×	—	—	—	—	—
scotica *Hook.*,	×, ×	—	—	—	—	—
Lysimachia vulgaris *L.*,	—	—	1	—	—	1–4 w.
nemorum *L.*,	×	—	—	—	—	—
Glaux maritima *L.*,	×	—	—	—	—	*
Anagallis arvensis *L.*,	×, ×	—	—	—	—	*
Centunculus minimus *L.*,	×	—	—	—	—	—
Samolus Valerandi *L.*,	×	—	—	—	—	*
LENTIBULARIACEAE.						
Pinguicula vulgaris *L.*,	—	3	—	—	—	—
OLEACEAE.						
Ligustrum vulgare *L.* (fresh fruit),	×	—	—	—	—	*
,, (dry fruit),	—	—	—	4	—	
,, (dry seed),	×	—	—	—	—	
Fraxinus excelsior *L.*,	—	—	3	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours 1 to 24.	Days (1 to 7).	Weeks (1 to 4).	Months.	
GENTIANACEAE.						
Blackstonia perfoliata *Huds.*,	×	—	—	—	—	—
Centaurion minus *Moench.*,	×	—	—	—	—	—
Cicendia filiformis *Delarbre*,	×	—	—	—	—	—
Gentiana Amarella *L.*,	—	1	—	—	—	—
campestris *L.*,	×	—	—	—	—	—
verna *L.*,	×	—	—	—	—	—
POLEMONIACEAE.						
Polemonium coeruleum *L.*,	×	—	—	—	—	—
CONVOLVULACEAE.						
Convolvulus arvensis *L.*,	—	12	—	—	—	*
Cuscuta Trifolii *Bab.*,	×	—	—	—	—	—
BORAGINACEAE.						
Cynoglossum officinale *L.*,	×	—	—	—	—	—
Anchusa sempervirens L.,	—	12	—	—	—	—
Lycopsis arvensis *L.*,	—	12	—	—	—	*
Symphytum officinale *L.*,	—	—	2½	—	—	*
Echium vulgare *L.*,	—	2	—	—	—	—
Lithospermum officinale *L.*,	×	—	—	—	—	*
Amsinckia lycopsoides *Lehm.*,	—	12	—	—	—	—
Myosotis scorpiodes *L.*,	—	12	1½	—	—	*
caespitosa *F. Schultz*,	—	—	4½	—	—	—
arvensis *Hill*,	—	—	1	—	—	*
SOLANACEAE.						
Solanum nigrum *L.* (fresh fruit),	×, ×	—	—	—	—	*
,, (dry seed),	×	—	—	—	—	
Dulcamara *L.* (fresh fruit),	×, ×	—	—	—	—	*
,, (dry seed),	×	—	—	—	—	—
Atropa Belladonna *L.* (fresh fruit),	—	—	1, 1	—	—	—
,, (dry seed),	×	—	—	—	—	—
Hyoscyamus niger *L.*,	×	—	1½	—	—	—
Datura Stramonium *L.*,	×	12	—	—	—	*

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
OROBANCHACEAE.						
Orobanche major *L.*,	—	3	—	—	—	—
rubra *Smith*,	—	12	$2\frac{1}{2}$	—	—	—
caryophyllacea *Smith*,	—	—	4	—	—	—
minor *Smith*,	—	—	$1\frac{1}{2}$, $2\frac{1}{2}$	—	—	—
Picridis *F. Schultz*,	—	—	$2\frac{1}{2}$	—	—	—
Hederae *Duby*,	—	1	3	—	—	—
Lathraea Squamaria *L.*,	×, ×	—	—	—	—	—
SCROPHULARIACEAE.						
Verbascum Thapsus *L.*,	×	—	—	—	—	—
Lychnitis *L.*,	×	—	—	—	—	—
nigrum *L.*,	—	12	—	—	—	—
Erinus alpinus *L.*,	×	—	—	—	—	—
Digitalis purpurea *L.*,	×	—	—	—	—	—
Antirrhinum majus *L.*,	×	—	—	—	—	—
Linaria Cymbalaria *L.*,	—	12	—	—	—	*
Elatine *Mill*,	×	3	—	—	—	—
spuria *Mill.*,	×	—	—	—	—	—
minor *Desf.*,	—	1, 12	—	—	—	—
repens *Mill.*,	—	3	—	—	—	—
purpurea *L.*,	—	12	—	—	—	—
Scrophularia nodosa *L.*,	×	—	—	—	—	*
aquatica *L.*,	×	—	—	—	—	*
umbrosa *Dum.*,	×, ×	—	—	—	—	—
Limosella aquatica *L.*,	×	—	—	—	—	—
Melampyrum pratense *L.*,	×, ×	—	—	—	—	—
sylvaticum *L.*,	×	—	—	—	—	—
Pedicularis palustris *L.*,	—	—	—	—	$1\frac{1}{8}$	1–6 m.
sylvatica *L.*,	×, ×	—	—	—	—	—
Rhinanthus Crista-galli *L.*,	—	—	—	2	—	6 + m. (var.)
Lasiopera viscosa *Hoffm.*,	×	—	—	—	—	—
Euphrasia officinalis *L.*,	×	—	—	—	—	—
salisburgensis *Funck*,	×	—	—	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

—	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Odontites rubra *Gilib.*,	—	12, 12	—	—	—	*
Veronica scutellata *L.*,	×	—	—	—	—	—
Chamaedrys *L.*,	×	—	—	—	—	—
montana *L.*,	×	—	—	—	—	—
officinalis *L.*,	×	—	—	—	—	—
serpyllifolia *L.*,	×	—	—	—	—	—
arvensis *L.*,	×	—	—	—	—	*
peregrina *L.*,	×	—	—	—	—	—
agrestis *L.*,	×, ×	—	—	—	—	*
Buxbaumii *Ten.*,	×	—	—	—	—	—
hederifolia *L.*,	×	—	—	—	—	—
LABIATAE.						
Mentha longifolia *Huds.*,	×	—	—	—	—	—
pubescens *Willd.*,	—	—	6½	—	—	6–12 m.
gentilis *L.*,	—	—	5½	—	—	—
arvensis *L.*,	—	—	1½	—	—	—
Pulegium *L.*,	×	—	—	—	—	—
Lycopus europaeus *L.*,	—	—	2½	—	15 +	12 + m.
Salvia Verbenaca *L.*,	×	—	—	—	—	*
pratensis *L.*,	—	—	6	—	—	—
Origanum vulgare *L.*,	—	—	1	—	—	—
Thymus Serpyllum *L.*,	×	—	—	—	—	—
Chamaedrys *Fr.*,	—	12	—	—	—	—
Clinopodium Acinos *O. Kuntze* (fruit),	—	—	2½	—	—	—
,, (seed),	—	12	—	—	—	—
vulgare *L.*,	×, ×	—	—	—	—	—
Melissa officinalis *L.*,	×	—	—	—	—	—
Scutellaria minor *Huds.*	×	—	—	—	—	—
Prunella vulgaris *L.*,	×	—	—	—	—	*
Nepeta hederacea *Trev.*,	×	—	—	—	—	*
Lamium amplexicule *L.*,	×, ×	—	—	—	—	—
molucellifolium *Fr.*,	×	—	—	—	—	—
hybridum *Vill.*,	×	—	—	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours. (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Galeopsis Ladanum *L.*,	×	—	—	—	—	—
Tetrahit *L.*,	—	—	2	—	—	*
Stachys officinalis *Franch.*,	—	—	1	—	—	*
sylvatica *L.*,	—	12	—	—	—	*
palustris *L.*,	—	—	—	—	15 +	1–6 m.
arvensis *L.*,	—	6	—	—	—	*
Marrubium vulgare *L.*,	×	—	—	—	—	—
Teucrium Scorodonia *L.*,	—	12	—	—	—	*
Ajuga Chamaepitys *Schreb.*,	—	12	—	—	—	—
VERBENACEAE.						
Verbena officinalis *L.*,	—	—	3½	—	—	*
PLUMBAGINACEAE.						
Statice Armeria *L.*,	—	—	2½	—	—	*
PLANTAGINACEAE.						
Plantago Coronopus *L.*,	×, ×	—	—	—	—	—
maritima *L.*,	×	—	—	—	—	*
lanceolata *L.*,	×, ×	—	—	—	—	*
media *L.*,	×	—	—	—	—	*
major *L.*,	×, ×	—	—	—	—	*
ILLECEBRACEAE.						
Corrigiola littoralis *L.*,	—	—	1	—	—	—
CHENOPODIACEAE.						
Suaeda maritima *Dum.*,	—	—	2½	—	—	*
Salsola Kali *L.* (seed),	×	—	—	—	—	1–4 w.
,, (dry fruit),	—	—	5½	—	—	
Chenopodium album *L.*,	—	—	4½	—	—	*
murale *L.*,	—	—	2½	1	—	—
hybridum *L.*,	—	—	1½	—	—	—
rubrum *L.*,	—	—	3½, 4	—	—	—
Bonus-Henricus *L.*,	×	—	—	—	—	—
Salicornia radicans *Sm.*,	×	—	—	—	—	—
Atriplex patula *L.*,	×	—	—	—	—	6–12 m.

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
POLYGONACEAE.						
Rumex conglomeratus *Murr.*,	—	—	—	—	15 +	12 + m.
sanguineus *L.*,	—	—	—	—	2	—
pulcher *L.*,	—	12	—	—	—	—
obtusifolius *L.*,	—	—	2½	—	—	*
crispus *L.*,	—	—	—	—	15 +	1–6 m.
Acetosa *L.*,	×	6	—	—	—	—
Acetosella *L.*,	×	—	—	—	—	—
Oxyria digyna *Hill*,	—	—	3½	—	—	—
Polygonum viviparum *L.* (bulbils),	×	—	—	—	—	—
lapathifolium *L.*,	—	—	1	—	—	*
maculatum *Bab.*,	—	—	3	—	—	—
Persicaria *L.*,	—	—	1	—	—	*
Hydropiper *L.*,	—	—	—	1	—	*
minus *Huds.*,	—	—	1½	—	—	—
aviculare *L.*,	×	—	—	—	—	*
Roberti *Lois.*,	—	—	—	—	1⅓	—
Convolvulus *L.*,	×	—	—	—	—	*
Fagopyrum esculentum *Moench*,	×	—	—	—	—	—
ELAEAGNACEAE.						
Hippophae Rhamnoides *L.* (seeds),	×	—	—	—	—	—
EMPETRACEAE.						
Empetrum nigrum *L.* (fresh fruit),	×	—	—	—	—	—
,, (dry fruit),	—	—	—	1	—	—
,, (seed),	—	—	—	1	—	—
EUPHORBIACEAE.						
Buxus sempervivens *L.*,	—	—	6	—	—	—
Euphorbia Helioscopia *L.*,	×	12	—	—	—	*
stricta *L.*,	—	—	1	—	—	—
hiberna *L.*,	—	—	1, 5	—	—	—
Esula *L.*,	—	12	—	—	—	—
Paralias *L.*,	—	—	4	—	—	1–6 m.
portlandica *L.*,	—	—	2½, 2½	—	—	*

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Euphorbia Peplus *L.*,	×	—	—	—	—	? *
exigua *L.*,	—	6	—	—	—	—
Lathyrus *L.*,	×	—	—	—	—	—
Mercurialis perennis *L.*,	—	—	1	—	—	—
annua *L.*,	×	—	—	—	—	—
CALLITRICHACEAE.						
Callitriche autumnalis *L.*,	×	—	—	—	—	—
URTICACEAE.						
Parietaria ramiflora *Moench*,	—	—	3	—	—	—
Urtica urens *L.*,	×	—	—	—	—	—
Humulus Lupulus *L.*,	—	—	3	—	—	—
ULMACEAE.						
Ulmus campestris *L.* (dry fruit),	—	—	5½	—	—	*
AMENTACEAE.						
Salix pentandra *L.*,	—	—	1½	—	—	—
repens *L.*,	—	6	—	—	—	—
reticulata *L.*,	—	—	2½	—	—	—
Myrica Gale *L.*,	—	—	—	—	14 +, 15 +	—
Betula verrucosa *Ehrh.*,	—	—	1, 2½	—	—	—
alba *L.*,	—	—	—	2	—	*
CONIFERAE.						
Taxus baccata *L.* (fresh fruit),	×, ×	—	—	—	—	*
,, (seed),	×	—	—	—	—	
Juniperus communis *L.* (dry fruit),	—	—	—	3, 4	—	—
Pinus sylvestris *L.*,	—	—	5	—	—	1–4 w.
TYPHACEAE.						
Typha latifolia *L.*,	—	—	4	—	—	*
angustifolia *L.*,	—	—	—	1½	—	*
Sparganium erectum *L.*,	—	—	—	1	15 +	12 + m.
minimum *Fr.*,	—	—	—	—	15 +	1–6 m.
ARACEAE.						
Arum maculatum *L.* (seed),	×	—	—	—	—	*

Table of Seed-buoyancy—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Naiadaceae.						
Potamogeton polygonifolius *Pourr.*,	—	—	1½	—	—	6–12 m.
coloratus *Hornem.*, . .	—	—	1½	—	—	—
alpinus *Balb.*, . . .	—	—	1½	—	—	—
pusillus *L.*, . . .	—	—	1	—	—	*
interruptus *Kit.*, . .	—	—	2½	—	—	—
filiformis *Pers.*, . . .	—	—	2½	—	—	—
Ruppia rostellata *Koch*, . .	—	—	1	—	—	—
Triglochin maritimum *L.*, . .	—	—	4½	—	—	*
palustre *L.*, . . .	—	—	6	—	—	*
Alismaceae.						
Alisma Plantago *L.*, . . .	—	—	—	—	15 +	6–12 m. (var.)
Sagittaria sagittifolia *L.* (fresh fruit-head),	×	—	—	—	—	6–12 m. (var.)
,, (dry seed), . .	—	—	—	1	—	
Orchidaceae.						
Orchis maculata *L.*, . . .	—	—	—	—	1½	—
incarnata *L.*, . . .	—	—	—	—	2½	—
Gymnadenia conopsea *Brown*, .	—	—	2	—	—	—
albida *Rich.*, . . .	—	—	3½	—	—	—
Neotinea intacta *Reich.*, . .	×	—	—	—	—	—
Ophrys apifera *Huds.*, . .	—	—	2½	—	—	—
Neottia Nidus-avis *Rich.*, . .	—	—	2½	—	—	—
Epipactis longifolia *Allioni*, .	—	—	—	—	1, 15 +	—
Cephalanthera longifolia *Fritsch*,	—	—	5	—	—	—
Iridaceae.						
Sisyrinchium angustifolium *Mill.*,	×	—	—	—	—	—
californicum *Ait.*, . .	×	—	—	—	—	—
Iris Pseudacorus *L.*, . .	—	—	—	3½	—	12 + m.
Romulea Columnae *S. & M.*, .	×	—	—	—	—	—
Amaryllidaceae.						
Leucojum aestivum *L.*, . .	×	—	—	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 24).	Weeks (1 to 4).	Months.	
LILIACEAE.						
Asparagus officinalis *L.* (seeds), .	×	—	—	—	—	—
Paris quadrifolia *L.*, . .	×	—	—	—	—	—
Convallaria majalis *L.* (fresh fruit),	×	—	—	—	—	—
,, (dry seed),	×	—	—	—	—	—
Polygonatum multiflorum *All.* (fresh fruit),	×	—	—	—	—	—
,, (dry seed) .	×	—	—	—	—	—
Scilla verna *Huds.*, ...	×	—	—	—	—	—
Allium Babingtonii *Borr.*, . .	—	12	—	—	—	—
vineale *L.* (bulbils), . .	×	—	—	—	—	—
carinatum *L.* (bulbils), .	—	—	1	—	—	—
ursinum *L.*	—	1	—	—	—	—
Colchicum autumnale *L.*, . .	×, ×	—	—	—	—	—
Tofieldia palustris *Huds.*,. .	—	—	2	—	—	—
Narthecium ossifragum *Huds.*, .	—	12	—	—	—	*
JUNCACEAE.						
Juncus acutus *L.*, ...	×	—	1½	—	—	—
effusus *L.*, ...	×	—	—	—	—	*
inflexus *L.*, ...	×	—	—	—	—	*
balticus *Willd.*, ...	×	—	—	—	—	—
capitatus *Weigel*, . .	×	—	—	—	—	—
articulatus *L.*, ...	×, ×	—	—	—	—	*
acutiflorus *Ehrh.*, . .	×	—	—	—	—	—
squarrosus *L.*, ...	×	—	—	—	—	*
Gerardi Lois., ...	×	—	—	—	—	—
bufonius *L.*, ...	×, ×	—	—	—	—	*
mutabilis *Lam.*, ...	×	—	—	—	—	—
Luzula sylvatica *Gaud.*, . .	×	—	—	—	—	—
multiflora *Lej.*	—	—	2½	—	—	—
ERIOCAULACEAE.						
Eriocaulon articulatum *Morong*,	×	—	—	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
CYPERACEAE.						
Schoenus nigricans *L.*,	—	—	2	—	—	—
Cladium Mariscus *R. Br.*,	—	—	—	—	15 +	1–6m.
Rhynchospora alba *Vahl.*,	—	12	—	—	—	—
fusca *Ait.*,	—	—	2½	—	—	—
Eleocharis multicaulis *Sm.*,	×	12	—	—	—	—
Scirpus sylvaticus *L.*,	×	—	—	—	—	*
lacustris *L.*,	×	—	—	—	—	*
Tabernaemontani *Gmel.*,	—	6	1½	—	—	—
caespitosus *L.*,	×	—	—	—	—	—
pauciflorus *Lightf.*,	—	10	—	—	—	—
setaceus *L.*,	—	12	—	—	—	*
filiformis *Savi*,	—	12	1	—	—	—
Holoschoenus *L.*,	×	—	—	—	—	*
Blysmus rufus *Link*,	—	—	—	1	—	1–6m.
Eriophorum polystachion *L.*,	—	—	1	—	—	*
Carex dioica *L.*,	—	—	—	—	15 +	—
pulicaris *L.*,	—	—	1	—	—	—
incurva *Lightf.*,	—	—	—	2	—	—
vulpina *L.*,	—	—	—	—	15 +	12 + m.
muricata *L.*,	×	—	2½	—	—	—
divulsa *Stokes*,	×	—	3	—	—	—
paniculata *L.*,	—	—	—	4	—	12 + m.
diandra *Schrank*,	—	—	—	—	14 +, 15 +	—
paradoxa *Willd.*,	—	—	3	—	15 +	—
canescens *L.*,	—	—	—	—	15 +	1–6 m.
elata *Allioni*,	—	—	—	—	2, 2½, 9	—
acuta *L.*,	—	—	—	—	15 +	6–12 m.
aquatilis *Wahl.*,	—	—	—	—	15 +	—
fusca *Allioni*,	—	—	5	—	—	—
pallescens *L.*,	—	—	3	—	—	—
panicea *L.*,	—	—	2	—	15 +	*
limosa *L.*,	—	—	—	—	4½	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Carex pendula *Huds.*,	—	—	3, 4½	—	—	—
pilulifera *L.*,	×	—	2½	—	—	—
flacca *Schreb.*,	—	—	—	2⅓, 3	—	—
flava *L.*,	—	—	—	—	15 +, 15 +	6–12 m.
extensa *Good.*,	—	—	—	1, 1½	—	—
Hornschuchiana *Hoppe*,	—	—	3½	2⅓	—	—
helodes *Link*,	—	—	—	1	—	—
sylvatica *Huds.*,	—	10	—	—	—	—
Pseudo-cyperus *L.*,	—	—	—	—	9, 12	6–12 m.
vesicaria *L.*,	—	—	—	1½	1½	—
rostrata *Stokes*,	—	—	—	—	5	1–4 w.
riparia *Curt.*,	—	—	—	—	15 +, 15 +	—
GRAMINEAE.						
Panicum Crus-galli *L.*,	—	—	3	—	—	—
Setaria viridis *Beauv.*,	—	—	—	1⅔	—	—
glauca *Beauv.*,	—	—	—	2⅓	—	—
Phalaris canariensis *L.*,	×	—	—	—	—	—
arundinacea *L.*,	—	—	6	—	—	—
Anthoxanthum odoratum *L.*,	—	—	2, 2	—	—	—
Phleum pratense *L.*,	—	—	2	—	—	—
arenarium *L.*,	×	—	—	—	—	—
Alopecurus pratensis *L.*,	—	—	1, 3	—	—	—
Sesleria coerulea *Ard.*,	—	—	1	—	—	—
Nardus stricta *L.*,	—	—	1	—	—	—
Milium effusum *L.*,	—	—	2, 2	—	—	—
Apera Spica-venti *Beauv.*,	—	—	2	—	—	—
Agrostis alba *L.*,	—	—	2½, 6	—	—	—
tenuis *Sibth.*,	—	—	3	—	—	—
Polypogon monspeliensis *Desf.*,	—	—	1	—	—	—
Holcus lanatus *L.*,	—	—	4	—	—	—
Aira caespitosa *Beauv.*,	—	—	3, 3	—	—	—
flexuosa *L.*,	—	—	2, 3½	—	—	—

Table of Seed-buoyancy—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Aira caryophyllea *L.*,	—	3	1	—	—	—
praecox *L.*,	—	—	1	—	—	—
Trisetum flavescens *Beauv.*,	—	—	3, 4½	—	—	—
Avena pubescens *L.*,	—	—	2	—	—	—
pratensis *Huds.*,	—	—	2	—	—	—
Arrhenatherum elatius *M. & K.*,	—	—	3½	—	—	—
Sieglingia decumbens *Bernh.*,	—	—	1	—	—	—
Koeleria cristata *Pers.*,	—	—	3	—	—	—
Melica uniflora *Retz.*,	—	—	2	—	—	—
Molinia coerulea *Moench*,	—	—	3	—	—	—
Poa annua *L.*,	—	—	1	—	—	—
nemoralis *L.*,	—	—	3, 3	—	—	—
trivialis *L.*,	—	6	2	—	—	—
compressa *L.*,	—	—	1½	—	—	—
Glyceria aquatica *Wahlb.*,	—	—	5	—	—	*
fluitans *Br.*,	—	—	2½	—	—	—
plicata *Fr.*,	—	—	1	—	—	—
Schlerochloa festuciformis *R. & B.*,	—	—	2	—	—	—
distans *Bab.*,	—	—	2	—	—	—
Borreri *Bab.*,	—	—	2, 3	—	—	—
rigida *Link*,	—	—	1½	—	—	—
loliacea *Woods*,	—	—	—	3½	—	—
Briza media *L.*,	—	—	3	—	—	—
Catabrosa aquatica *Beauv.*,	—	—	2	—	—	—
Cynosurus cristatus *L.*,	—	—	1, 1	—	—	—
Dactylis glomerata *L.*,	—	—	3, 3	—	—	—
Festuca fasciculata *Forsk.*,	—	—	—	3½	—	—
Myuros *L.*,	×	—	1	—	—	—
bromoides *L.*,	—	—	1	—	—	—
ambigua *Le Gall.*,	—	—	1½	—	—	—
ovina *L.*,	—	—	1½, 2, 2½, 3	—	—	—
gigantea *Vill.*,	—	6	—	—	—	—
elatior *L.*,	—	—	2, 2, 3	—	—	—

TABLE OF SEED-BUOYANCY—*continued.*

Name.	Seeds all sunk in less than					Guppy's results. * = 0–7 days.
	One Minute.	Hours (1 to 24).	Days (1 to 7).	Weeks (1 to 4).	Months.	
Festuca pratensis *Huds.*,	—	—	3	1	—	—
Bromus erectus *Huds.*,	—	—	2	—	—	—
ramosus *Huds.*,	—	—	1½	—	—	—
sterilis *L.*,	—	6	—	—	—	—
secalinus *Bab.*,	—	6	—	—	—	—
mollis *Parl.*,	—	—	1	—	—	—
Brachypodium sylvaticum *R. & S.*,	—	—	2, 2½	—	—	—
Hordeum murinum *L.*,	—	—	1½, 2	—	—	—
Lolium perenne *L.*,	—	—	2, 3	—	—	—
temulentum *L.*,	—	—	1	—	—	—

ANALYSIS OF THE TABLE.

In order to obtain totals for this list, we select (still working for maximum efficiency), the highest figure given for each species whenever more than one figure is given—whether this refers to seed, fresh fruit, or dry fruit, or to Guppy's table. We then obtain the following result:—

	Total.	Percentage.
Sunk within a minute,	348	44·3
,, ,, one day,	99	12·6
,, ,, one week,	237	30·2
,, ,, one month,	40	5·1
,, ,, six months,	19	2·4
,, ,, twelve months,	12	1·5
Floated longer than twelve or fifteen months,	31	3·8
	786	99·9

It will be seen that of the 786 species experimented on, about 44 per cent. have seeds which sink at once in water; about 57 per cent. sink within twenty-four hours; about 87 per cent. within a week; this last figure closely approximates to Guppy's estimate of 90 per cent. as representing the proportion of the whole British flora which have seeds which sink within the same period (*op. cit.*, pp. 25, 535), but is arrived at from fuller material, and more directly.

For the sake of completeness, I now add the results obtained by Guppy upon species with which I did not experiment; a few further comments on the whole of the evidence available will then be useful.

ADDITIONAL BUOYANCY RESULTS FROM GUPPY'S LIST.*

Sank at once or in under 1 week, no mark. Floated for between 1 and 4 weeks, 1–4 w. Floated for between 1 and 6 months, 1–6 m.; 6–12 months, 6–12 m.: 12 months, 12+ m.

Thalictrum flavum *L.*
Ranunculus hederaceus *L.*
 acris *L.*
Castalia alba *Greene.*
Roemeria hybrida *DC.*
Radicula sylvestris *Druce.*
Armoracia amphibia *Peterm.*
Cardamine pratensis *L.*
Alyssum maritimum *L.*
Viola tricolor *L.*
Moenchia erecta *Gaertn.*
Myosoton aquaticum *Moench.*
Stellaria Holostea *L.*
Oxalis corniculata *L.*
Impatiens parviflora *DC.*
 biflora *Walter.* 6–12 m.
Acer campestre *L.*
Lathyrus maritimus *Big.* 1–6 m.
Fragaria vesca *L.*
Saxifraga granulata *L.*
Chrysosplenium alterniflorum *L.*
Myriophyllum spicatum *L.*
 alterniflorum *DC.*
Cicuta virosa *L.* 1-6 m.
Apium graveolens *L.*
 nodiflorum *Reich.*
 inundatum *Reich.*
Sium latifolium *L.* 1–6 m.
 erectum *Huds.* 1–6 m.
Oenanthe crocata *L.* 6–12 m.
Peucedanum palustre *Moench.* 1–6 m.
Hedera Helix *L.*
Chrysanthemum Leucanthemum *L.*
Matricaria Chamomilla *L.*
Tussilago Farfara *L.*
Petasites ovatus *Hill.*
Senecio palustris *Hook.*
Carduus palustris *L.*
 arvensis *Robs.*
Tragopogon porrifolius *L.*
Crepis foetida *L.*
Hottonia palustris *L.*
Lysimachia thyrsiflora *L.* 1–4 w.
Pinguicula lusitanica *L.*
Menyanthes trifoliata *L.* 1–6 m.
Nymphoides peltatum *R. & B.* 1–4 w.
Convolvulus sepium *L.* 12+ m.
 Soldanella *L.* 12+ m.
Cuscuta europaea *L.*
Myosotis versicolor *Sm.*
Borago officinalis *L.*
Linaria vulgaris *Mill.*
Veronica Anagallis-aquatica *L.*
 Beccabunga *L.*
Nepeta Cataria *L.*
Clinopodium Calamintha *Kuntze.*
Scutellaria galericulata *L.* 12 m.
Ballota nigra *L.*
Lamium purpureum *L.*
 album *L.*
 Galeobdolon *Crantz.*
Ajuga reptans *L.*
Salicornia europaea *L.*
Suaeda fruticosa *Forsk.*
Beta maritima *L.*
Rumex aquaticus *L.* 1–4 w.

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* I omit *Calla palustris*, which cannot claim rank even as an alien in the British flora.

Rumex Hydrolapathum *Huds.* 12+ m.
Polygonum maritimum *L.*
amphibium *L.*
Euphorbia amygdaloides *L.*
Ceratophyllum demersum *L.*
Urtica dioica *L.*
Alnus glutinosa *Gaertn.* 12+ m.
Corylus Avellana *L.* 1–4 w.
Quercus Robur *L.* 1–4 w.
Sparganium simplex *Huds.* 6–12 m.
Lemna minor *L.* 1–6 m.
gibba *L.*
Naias marina *L.*
Zannichellia palustris *L.*
Ruppia maritima *L.*
Potamogeton natans *L.* 12+ m.
lucens *L.* 6–12 m.
perfoliatus *L.* 1–6 m.
crispus *L.*
densus *L.*
obtusifolius *M. & K.*
Butomus umbellatus *L.*
Alisma ranunculoides *L.*
natans *L.*
Damasonium Alisma *Mill.*
Scheuchzeria palustris *L.* 1–6 m.
Hydrocharis Morsus-ranae *L.*
Iris foetidissima *L.*
Tamus communis *L.*
Fritillaria Meleagris *L.* 1–6 m.
Endymion non-scriptum *Garcke.*
Juncus maritimus *Lam.*
Luzula campestris *DC.*
Eleocharis palustris *R. & S.*
Scirpus fluitans *L.*
maritimus *L.* 1–4 w.
Eriophorum alpinum *L.*
vaginatum *L.*
Carex leporina *L.* 1–6 m.
echinata *Murr.* 12+ m.
remota *L.* 12+ m.
hirta *L.*
distans *L.*
acutiformis *Ehrh.* 12+ m.
Leersia oryzoides *Sw.*
Alopecurus geniculatus *L.*
Melica montana *Huds.*
Phragmites communis *Trin.* 1–4 w ?

ANALYSIS OF THE ABOVE LIST.

An analysis of this list shows :—

Sink at once or within a week,	83
Float for 1 to 4 weeks,	7
,, 1 to 6 months,	11
,, 6 to 12 months,	4
,, over 12 months,	9
Total	114

Taking the same periods from my own list, the corresponding numbers there are 684, 40, 19, 12, 31. If we add these to the figures just given, we obtain the following result for 900 British plants :—

	Total.	Percentage.
Sink at once or in a week,	767	85·2
Float for 1 to 4 weeks,	47	5·2
,, 1 to 6 months,	30	3·3
,, 6 to 12 months,	16	1·9
,, over 12 months,	40	4·4
Total	900	100·0

The drop from 87 per cent. to 85 per cent. in the final total, as compared with the total in my own experiments, is no doubt due to a fact which Guppy is careful to allow for in his own calculations; namely, that an undue proportion of plants having a high seed-buoyancy is included in his tables; which brings us back to near the 90 per cent. long ago estimated by Darwin.

As regards the balance, which possess the power of floating for periods varying from one week to over fifteen months, an examination of the combined lists fully bears out Guppy's conclusion that the buoyant-seeded plants in our flora are in the main inhabitants of either river-side or sea-shore. If we sub-divide these 133 buoyant species into groups, we may set down twenty-five of them as aquatics, forty-two as marsh-plants, five as bog-plants, seventeen as maritime, while the buoyancy of seven others is due to their fleshy fruits having been dried before testing; the balance (33 species) is made up mostly of plants of mesophile habitat. We may show this analysis as follows in the percentages of the total for each buoyancy-group:—

	BUOYANCY.			
	1–4 weeks.	1–6 months.	6–12 months.	Over 12 months.
Water-plants, ...	8·5	25·9	18·8	27·5
Marsh-plants, ...	25·5	14·8	50·0	45·0
Bog-plants, ...	2·1	11·1	—	2·5
Maritime plants, ...	10·6	29·6	12·5	5·0
Dried fleshy fruits, . . .	12·8	3·7	—	—
Balance (mostly mesophile), .	40·4	14·8	18·8	20·0
	99·9	99·9	100·1	100·0

It will be seen that aquatics and marsh-plants claim over half the total, maritime plants one-seventh. Bog-plants are but slightly represented. Dried fleshy fruits (a rare but quite possible dispersal-condition) supply a small contribution to the buoyant list, mainly for the shorter periods The balance of the buoyant seeds is made up of plants differing widely as regards both habit and habitat.

CAUSES AND DEGREES OF BUOYANCY.

Seeds which possess floating power owe their buoyancy to air which is contained within the outer coverings of the seed (or fruit), and the duration of buoyancy is determined by the permeability of the outer coverings. Sometimes the air is contained in spongy tissue in the peripheral wrappings, or in the albumen; sometimes its presence is due to the incomplete filling of the fruit by the seed, or of the seed by the albumen, or by the embryo. Guppy has some interesting notes on these points (*loc. cit.*, pp. 115, 116).

Although seeds vary considerably as regards the rate at which they sink when heavier than water, or the period during which they retain buoyancy, none are eventually very much heavier than water. And although it is extremely improbable that, when they have sunk, they could be conveyed by currents or other means across arms of the sea or lakes of any depth, they might still be transported for long distances along the beds of rivers, and similarly by currents over shallow sea-bottoms. Professor Oliver tells me that at Blakeney the greater part of the seed-drift takes place along the bottom, off-shore, the seeds being eventually cast up in quantities.

Buoyancy as displayed in the Leading Natural Orders.

If we study the general table from the point of view of the buoyancy of related plant-groups, we find that floating seeds are characteristic of certain orders, while in others absence of buoyancy is almost universal; in others, again, great variability in this respect exists. For instance, the seeds of Papaveraceae, Cruciferae, Caryophyllaceae, Geraniaceae, Leguminosae, Crassulaceae, Saxifragaceae, Primulaceae, Gentianaceae, Scrophulariaceae, and Juncaceae in almost all cases sink at once; it is to be noted that in almost all these orders it is the seed itself which is the unit of dispersal. On the other hand, Ranunculaceae, Compositae, Orobanchaceae, Orchidaceae, Cyperaceae, Gramineae are mostly possessed of buoyancy; in most of these the unit of dispersal is either a one-seeded indehiscent fruit containing or retaining air, or a seed with a loose test in which air is entangled. Among the orders which display wide variability as regards buoyancy are Rosaceae, Umbelliferae, Rubiaceae, Ericaceae, Boraginaceae, Labiatae, Polygonaceae.

We shall now look more closely into this point, and at the same time add notes on certain species which claim attention. The results given will be taken from my own table as given above, reference being made to Guppy's more generalized results where they supply additional evidence.

Ranunculaceae.

Species tested, 26. Almost all possess some floating power—½ to 5 days. *R. parviflorus*, *Trollius*, *Eranthis*, *Aquilegia*, and *Delphinium* alone sink at once. Guppy found *R. sceleratus* and *R. repens* to float for 6–12 months;

my own samples sank in a few days. Guppy notes that these two species vary as regards buoyancy.

Papaveraceae.

Species tested, 8. All sink at once.

Cruciferae.

Species tested, 49. The *seeds* of all sink at once, save for those of *Matthiola incana*, *Radicula Nasturtium-aquaticum*, *N. palustre*, and *Barbarea lyrata*, of which *N. palustre* alone floated for over a week. The dried *fruit* of *Crambe*, *Raphanus Raphanistrum*, and *R. maritimus* possesses considerable buoyancy, though their seeds have none.

Violaceae.

Eight species of *Viola* were tested; all floated for a few hours or days.

Caroyphyllaceae.

Species tested, 41. All sank at once, except *Honkenya*, the seeds of which floated (in fresh water) for 2 days. As regards this plant Guppy (*l. c.*, p. 541) gives a period of 10 days (and "a week or two" after a year's drying) for these seeds in fresh water, but found that, in salt water, 75 per cent. were still afloat after a year's immersion. The plant supplies an interesting exception to the rule that seeds which sink in fresh water sink also after a slightly longer period in salt water.

Hypericaceae.

Species tested, 11. Floating power slight, varying from 0 (the usual condition) to 1 day.

Geraniaceae.

Species tested, 15. All sink at once.

Leguminosae.

Species tested, 49. The *seeds* of all sink at once, except those of *Anthyllis Vulneraria* (1 day), and *Onobrychis viciaefolia* (2½ days). But the *fruit* of several Medicks and of *Trifolium arvense* floated for a few days, and these form the usual dispersal-unit of the species in question.

Rosaceae.

Species tested, 37. The fruits vary greatly in character, and are very variable in floating power, even within the same genus. The only species with apparently indefinite floating power are *Comarum palustre* (the only marsh-plant of the series) and *Potentilla Anserina*; *Spiraea Ulmaria* and *Agrimonia Eupatoria* follow with 1½ and 1 month. The behaviour of the fleshy-fruited genera (*Prunus, Rubus, Rosa, Crataegus, Pyrus*) will be referred to in a subsequent section.

Onagraceae.

Species tested, 8. Most Epilobiums float for up to 1 day.

Crassulaceae.

Of 7 Sedums, *S. Telephium* alone had any floating power (up to 2 days). *Cotyledon Umbilicus* floated for a few hours.

Saxifragaceae.

Species tested, 8. Of 6 Saxifrages, *S. stellaris* alone can float (1 day). *Parnassia palustris* floats for several weeks.

Umbelliferae.

Species tested, 34. Very variable. The species with apparently indefinite floating power are *Hydrocotyle vulgaris, Oenanthe aquatica, Angelica sylvestris.* Two maritime plants, *Haloscias scoticum* and *Crithmum maritimum,* follow with 2½ and 5 months respectively. Guppy adds *Sium latifolium, S. erectum, Peucedanum palustre* (all 1–6 months) and *Oenanthe crocata* (6–12 months). The 16 species which sink at once or within a day are all mesophile or xerophile (excluding halophile).

Rubiaceae.

Species tested, 9. Variable. The only marsh-plant among them, *Galium palustre,* far exceeds the others in buoyancy.

Valerianaceae.

The hollow chambers in the fruits of Valerianella do not much assist buoyancy, the maxima for four species being 2½, 2½, 5, and 14 days.

Compositae.

Species tested, 73. While only a few (15) sink at once, the only ones which float for over a week are *Artemisia maritima, Bidens cernua, B. tripartita, Carduus pratensis, Helminthia echioides,* the last being the only one which appeared to possess indefinite floating power. As regards the two species of *Bidens,* Guppy gives them each a period of 6–12 months; but my *cernua* sank within 3 weeks, and *tripartita* within 5 days. All through the Compositae, periods of ½ to 5 days prevail. The pappus-bearing species as a group show no greater nor less buoyancy than those whose fruits are devoid of appendages. In this connexion Moriz Kronfeld,[1] experimenting with *Taraxacum* and *Crepis foetida,* found that the buoyancy of the fruits was

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[1] Über einige Verbreitungsmittel der Compositenfrüchte. Sitz. k. Akad der Wissensch., Wien, Math.-Nat. Klasse, xci, Abth. i, pp. 414–428. 1885.

greatly diminished by separating the pappus from the achene, the achenes sinking soon, while the complete fruit remained afloat. This is an effect of the entanglement of air-bubbles in the pappus, as the following experiment showed:—

Twenty fruits of *Taraxacum* were divided above the achene, and the achenes and pappuses, as well as twenty more entire fruits, were immersed in water, care being taken to expel from the pappus the bubble of air which frequently lodges at the point whence the pappus-hairs radiate. At the end of twenty-four hours—

Of 20 fruits, ...	17 had sunk.
Of 20 achenes, ...	15 ,,
Of 20 pappuses, ...	20 ,,

The three fruits which still floated were then divided above the achene, whereupon their three pappuses sank at once. So that the total result of 24 hours' immersion was the sinking of 40 pappuses and 32 achenes. This shows that, when free of air-bubbles, the pappus hinders instead of assisting the floating of the Dandelion fruit. *Carduus lanceolatus*, *Crepis biennis*, and *Sonchus arvensis*, tested similarly, all gave a slightly higher buoyancy for the achene than for the bubble-free pappus. But in nature, an air-bubble frequently lodges at the base of the shuttlecock of bristles, and maintains its position tenaciously; so that in this way the pappus may cause the fruit to float until the bubble is expelled by rough treatment, or is dissolved in the water; and it may thus materially aid the floating power of the fruit.

Ericaceae.

Species tested, 10. Buoyancy low. *Erica vagans* (3½ days) and *Pyrola minor* (2½ days) alone float for over a day. The dried fruits of *Arctostaphylos Uva-ursi* float for a day or so, but those of *Arbutus* sink at once, as do its seeds and fresh fruit.

Primulaceae.

Species tested, 9. *Lysimachia vulgaris*, the only marsh-plant among them, alone possesses any buoyancy (one day in my samples, 1–4 weeks in Guppy's). Guppy adds *L. thyrsiflora*, with a 1–4 weeks' buoyancy.

Boraginaceae.

Species tested, 10. Buoyancy variable and low, 4½ days being the maximum (in *Myosotis caespitosa*).

Orbanchaceae.

The extremely light seeds of the Broom-rapes do not help them to float. Six species tested sank in between 1 hour and 4 days.

Scrophulariaceae.

Species tested, 35. Of these 24 sank at once, and 7 more within an hour. There remain only *Pedicularis palustris,* which floats for over a month, and *Rhinanthus Crista-Galli,* a sample of which sank within a fortnight. Guppy says the latter is variable in buoyancy, and gives it a period of over 6 months. The large number which have no buoyancy include several marsh-plants.

Labiatae.

Species tested, 29. Variable, but only three float for over a week. These three varied much in buoyancy:—*Mentha pubescens,* 6½ days (mihi) to over 6 months (Guppy); *Lycopus europaeus,* 2½ days to over 15 months; *Stachys palustris,* 1–6 months (Guppy) to over 15 months (mihi). The remaining 26 species in my list include only one marsh-plant, *Scutellaria minor.*

Polygonaceae.

Species tested, 18. Polygonums vary much in buoyancy, and Docks still more.

Euphorbiaceae.

Species tested, 12. Variable, the most buoyant species being the maritime Euphorbias.

Amentaceae.

Only 6 species tested. Very variable.

Typhaceae.

Typhas float for some days, Sparganiums for many months.

Naiadaceae.

Six Pondweeds tested by me showed a very uniform buoyancy of 1 to 2½ days, but one of these, and three out of six other species tested by Guppy, floated for many months. *Naias, Ruppia, Zannichellia,* and *Triglochin* have all a short period of flotation.

Orchidaceae.

Species tested, 9. Very variable, buoyancy ranging from 0 in *Neotinea intacta* to over 15 months in *Epipactis longifolia.*

Liliaceae.

Species tested, 12. Buoyancy very low. The bulbils of Alliums sink at once, as do the fleshy fruits of *Convallaria* and *Polygonatum.*

Juncaceae.

Species tested, 13. Eleven species of *Juncus* all sink at once, excepting a second sample of *J. acutus,* which floated 1½ day.

Cyperaceae.

Species tested, 44. It is in the genus *Carex* that a large proportion of the most buoyant "seeds" are found. The inflated seed-vessel retains its air for indefinite periods, and often keeps the seed afloat till the envelope decays, if germination does not take place in the meantime. Of 29 Carices tested, 10 were still afloat after 15 months' immersion, and 5 more floated for from 1 to 12 months. Only one species (*C. sylvatica*) had a maximum buoyancy-period of less than 1 day. The other genera of Cyperaceae (15 species) are much less buoyant; 4 of the species tested sank at once, 4 more within a day, 5 more within a week, leaving only *Blysmus* (1 month (mihi)), 1–6 months (Guppy), and *Cladium* (over 15 months (mihi)), 1–6 months (Guppy).

Gramineae.

The great majority (52 out of 62 species tested) sank with great uniformity in between 1 and 7 days. Five sank in less than a day, and the rest (5 species) in under a month.

HIGH BUOYANCY IN MARITIME AND MARSH PLANTS.

Guppy demonstrated from his series of experiments the interesting fact that almost all the species known to possess considerable seed-buoyancy had their habitat by the sea or on river-sides, and he discusses this question fully (*l.c.*, pp. 24–39). The same fact comes out prominently in the table on p. 49, in which the plants whose seeds are buoyant are analysed according to their habitats. The remarks above on buoyancy in the leading Natural Orders, point also to the same conclusion. In many genera of which the species occupy a variety of habitats, the fact comes out noticeably that the marsh-dwellers or shore-haunters have a higher seed-buoyancy than those species which inhabit other situations. But this will be seen to be by no means an invariable rule. The following comments on certain genera bear upon this point:—

Ranunculus.—Excepting *R. parviflorus*, which sinks at once, the *lowest* buoyancy is found in some aquatic species and in *R. Lingua*, while those of higher buoyancy include *R. Auricomus*, *R. bulbosus*, and *R. arvensis*. However, *R. Flammula* has the highest buoyancy of all.

Lathyrus.—The seeds of *L. maritimus* float for months (Sernander, Norman, Guppy, &c.). Those of five other species sink at once.

Potentilla.—*P. Anserina*, also *Comarum palustre*, which is often placed in *Potentilla*, were still afloat after 15 months' immersion. The seeds of six other species all sank within 12 hours.

Galium.—The only marsh species, *G. palustre*, floats for months. Five other species sink within a day.

Artemisia.—*A. maritima* floats for over a week. *A. Absinthium* and *A. vulgaris* sink at once.

Senecio.—*S. aquaticus* and apparently *S. palustris* display a buoyancy not above the low average of the genus.

Stachys.—*S. palustris* floats for many months. The other three British species sink within a day.

Rumex.—*R. conglomeratus*, *R. sanguineus*, *R. Hydrolapathum*, and *R. crispus* are far more buoyant than *R. pulcher*, *R. obtusifolius*, *R. aquaticus*, *R. Acetosa*, *R. Acetosella.*

Polygonum.—*P. Roberti* floats for over a month; *P. Hydropiper* for a week; 9 other species, including *P. maritimum* and *P. minus*, for a few days only or not at all.

Euphorbia.—*E. Paralias* floats for a month or two (Guppy; only a few days in my specimens); eight other species, including *E. portlandica*, for a few days or not at all.

Juncus.—Buoyancy nil, save for 1½ day in the case of *J. acutus.*

Scirpus.—Buoyancy very low, variable.

Carex.—Already commented on (p. 55).

See also the notes on Natural Orders above.

In *Vicia*, *Hypericum*, *Epilobium*, *Plantago* we find no noticeable higher buoyancy in the marsh or maritime species as compared with others.

It will be seen from these notes and those on Natural Orders, and from the Table, that, while high buoyancy is found mainly in marsh and maritime species, it is not the rule even in these; exceptions are numerous, and definite relationships do not exist between buoyancy and habitat, or buoyancy and any other character. The only general conclusion that can be drawn is that buoyancy is very seldom found in *seeds*; it occurs mainly in *indehiscent fruits.*

THE CASE OF FLESHY FRUITS.

As pointed out on a previous page, the natural unit of dispersal in the case of fleshy fruits, such as berries or drupes, may be whole fruit, in either a fresh or dried condition; or it may be the seed or stone freed from its fleshy envelope. Birds are the cause of the scattering of vast numbers of the seeds contained in berries and so on; these may subsequently get into streams, and aquatic dispersal may follow endozoic dispersal. It is therefore desirable that the buoyancy of such fruits, both fresh and dried, should be tested, in addition to that of the contained seeds. I find my results in this direction are not very complete. Guppy's results do not help much, as he tested but few fruits of this sort; and in those he recorded it is not quite certain what state he assumed to be the natural dispersal-condition of the species.

However, the following results are suggestive:—

BUOYANCY OF FLESHY FRUITS.

[Time is given in days. × = sinks at once.]

Name.	Fresh fruit.	Dry fruit.	Dry seed.	Guppy's results.
Actaea spicata, ...	68	—	—	—
Berberis vulgaris, . . .	4	1	×	0–7
Ilex Aquifolium, . . .	1½, 2	10	10	0–7
Euonymus europaeus, . .	×	60	2½	7–28
Rhamnus catharticus, . .	—	6	3½	—
Frangula, ...	19	10	½	—
Prunus spinosa, . . .	×	—	× (stone)	—
Padus, ...	—	—	½ (stone)	—
Rubus Idaeus, . . .	×	—	× (stone)	—
fruticosus, . . .	×	2	×, 1 (stone)	—
saxatilis, . . .	—	—	½ (stone)	—
Rosa spinosissima, . . .	42	—	×	—
mollis, ...	—	—	1½	—
tomentosa, . . .	×	14	×	—
canina, ...	×	—	½	—
arvensis, ...	$\frac{1}{24}$	42	×	0–7
Crataegus Oxyacantha, . .	14	21	×	7–28
Pyrus Aucuparia, . . .	×	14	×	—
Malus, ...	—	—	×	—
latifolia, ...	×	—	×	—
Cornus suecica, . . .	—	—	7	—
Viscum album, . . .	×	¼	×	0–7
Sambucus nigra, . . .	×	—	×	0–7
Viburnum Opulus, . .	×	—	×	—
Lantana, ...	—	2	2	—
Lonicera Periclymenum, . .	×	×	×	—
Arbutus Unedo, . . .	×	×	×	—
Arctostaphylos Uva-ursi, .	—	7, 10	—	—
Ligustrum vulgare, . .	×	28	×	0–7

BUOYANCY OF FLESHY FRUITS—*continued.*

Name.	Fresh fruit.	Dry fruit.	Dry seed.	Guppy's results.
Solanum nigrum, . . .	×	—	×	0–7
Dulcamara, . . .	×	7	×	0–7
Atropa Belladonna, . .	1, 1	—	×	—
Hippophae Rhamnoides, . .	—	—	×	—
Empetrum nigrum, . .	×	7	7	—
Taxus baccata, . . .	×	×	×	0–7
Juniperus communis, . .	—	21, 28	—	—
Arum maculatum, . . .	×	—	×	0–7
Convallaria majalis, . .	×	—	×	—
Polygonatum multiflorum, .	×	×	×	—

It will be seen that while the majority of these fleshy fruits sink at once, their buoyancy is usually increased, sometimes very largely, by drying. Taking the seventeen plants in which the buoyancy of both fresh and dried fruit was observed, we find that the result of drying is to increase the buoyancy from an average of 2·3 days to an average of 12·9 days. While the fresh fruit of only four of these seventeen plants was buoyant, the dried fruit of all but five of them floated for some time. In the case of *Berberis vulgaris* and of *Rhamnus Frangula*, the effect of drying was to *diminish* the buoyancy.

When dried fruits, such as rose-hips, are placed in fresh water, and air is not excluded, fermentation often sets in, and the fruit, either before or after sinking, becomes inflated with gases, and floats buoyantly until disintegration sets in ; what the result of this is on the vitality of the seeds was not tested.

The seeds of a large majority have no buoyancy, even when dried. All but twelve out of the thirty-six sank at once, and of these twelve only *Ilex Aquifolium*, *Cornus suecica*, and *Empetrum nigrum* floated for a week or ten days.

VARIABILITY OF BUOYANCY IN SEEDS OF THE SAME SPECIES.

In any batch of seed—even in a group of seeds taken from the same seed-vessel—a considerable variation in buoyancy exists. In the case of those that sink at once, the lightest seeds will take twice, or three times, or even four times as long to reach the bottom as the heaviest seeds will. Similarly, in those which float, the most buoyant seeds will sometimes float up to four times as long as the least buoyant. On the whole, to obtain the average buoyancy of the seeds given in my list, one might perhaps divide my figures by three. But while this variability exists among the individual seeds, it was found that if any batch of seed be divided, its sections behave very uniformly as regards maximum and minimum buoyancy, showing that the test employed is a satisfactory one.

At the same time, batches of seed collected in different places at different times occasionally displayed, when compared, a difference in their buoyancy far exceeding the difference observed within any one batch. While some of the discrepancies between Guppy's results and my own, as shown in the table, are possibly explicable by different conditions of experiment, the same cannot apply to different batches tested by myself under similar conditions.

The most striking cases of variability of buoyancy, taken from the table, are listed below :—

Ranunculus sceleratus—3½ days. (Guppy, 6–12 months.)
R. repens—3½ days. (Guppy, 6–12 months.)
Radicula palustris—3 hours, 12 days.
Raphanus Raphanistrum (dry fruit)—5 days, 4 months.
Comarum palustre—2 months, 15+ months. (Guppy, 12+ months.)
Haloscias scoticum—5½ days, 2½ months.
Bidens cernua—3 weeks. (Guppy, 6–12 months.)
Helminthia echioides—12 hours, 1 day, 15+ months. (Guppy, 0–7 days.)
Rhinanthus Crista-Galli—2 weeks. (Guppy, 6+ months.)
Lycopus europaeus—2½ days, 15+ months. (Guppy, 12+ months.)
Mentha pubescens—6½ days. (Guppy, 6+ months.)
Atriplex patula—sinks at once. (Guppy, 6+ months.)
Euphorbia Paralias—4 days. (Guppy, 1–6 months.)
Potamogeton polygonifolius—1½ day. (Guppy, 6–12 months.)
Sagittaria sagittifolia—1 week. (Guppy, 6+ months.)
Iris Pseud-acorus—3½ weeks. (Guppy, 12+ months.)
Sparganium erectum—1 week, 15+ months. (Guppy, 12+ months.)
Epipactis longifolia—1 month, 15+ months.
Blysmus rufus—1 week. (Guppy, 1–6 months.)
Carex paniculata—4 weeks. (Guppy, 12 + months.)
C. elata—2 months, 9 months.
C. panicea—2 days, 15+ months.
C. paradoxa—3 days, 15+ months.
C. vesicaria—10 days, 42 days.

I am not prepared to account for these cases of variability, nor to say how many of the discrepancies will be reduced or ruled out by further experiments on the species in question. Some of them may be due to immature or unsound seed (though trouble was taken to eliminate this source of error). But, as recognized by Guppy, considerable variability does exist in certain species. To determine its limits and its causes, the experiments on the variable species would have to be considerably extended. The fact that Guppy's buoyancy periods are usually greater than mine suggests that the difference may be due to his (presumably) using salt water, while my tests were mostly made in fresh water. But several of my batches, tested in salt water, gave results differing but slightly from the fresh-water results.

BUOYANCY OF FRUITING BRANCHES.

It is evident that, even if the seeds of a species sink at once, wide dispersal may still be effected if branches or crowns with fruit attached possess a considerable power of floating. This was pointed out long ago by Darwin. Accidents of one sort or another—storms, the subsidence of overhanging banks, the trampling of animals—occasionally precipitate plants or portions of them into rivers. The buoyancy of branches or fruiting crowns, both fresh and after thorough drying, was tested in the case of a few plants of different habit, with the result shown below. In the table (in which the numerals represent days) the buoyancy of the seeds, and of fresh and dry fruit where a succulent fruit occurred, is added for comparison wherever the information was available.

—	Branch.		Seed.[1]	Fruit.	
	Fresh.	Dry.	—	Fresh.	Dry.
Hypericum elatum,	4	4	1	—	—
Ilex Aquifolium,	14	4	10½	2	—
Sarothamnus scoparius,	1	1	0	—	—
Alchemilla alpina,	3	2½	2½	—	—
Dryas octopetala,	3	4	—	1	—
Rosa spinosissima,	1	4½	0	45	—
canina,	1	4	½	0	—
eglanteria,	12	14	5	—	—
Crataegus Oxyacantha,	5	6	0	14	21
Sedum Telephium,	7	6½	2	—	—
Saxifraga umbrosa,	40	3	0	—	—
Geum,	1	1	0	—	—
Viscum album,	0	0	0	0	¼
Artemisia maritima,	4	4	9	—	—
Achillea Millefolium,	3	4	1	—	—
Daboecia cantabrica,	2	4	$\frac{1}{24}$	—	—
Erica mediterranea,	13½	6½	$\frac{1}{24}$	—	—
vagans,	6	5½	3½	—	—
ciliaris,	5	4	—	—	—
Tetralix,	3	4	0	—	—
Mackaii,	3½	4	—	—	—
Calluna vulgaris,	5	4	0	—	—
Arbutus Unedo,	0	1	0	0	0
Limonium binervosum,	3	2	—	—	—
Ligustrum vulgare,	1	3	0	0	—
Solanum Dulcamara,	1	1½	0	0	—
Antirrhinum majus,	15	10	0	—	—
Euphorbia Peplus,	2	5½	0	—	—
Taxus baccata,	2	4	0	0	—

It will be seen that among these 29 plants, which include representatives of 15 different Natural Orders, and also plants of very different growth—herbs, shrubs, and trees—the effect of using branches

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[1] Or dry indehiscent fruit.

instead of seeds as the dispersal-unit is to increase materially the buoyancy; while the seeds of 14 of the 29 plants sink at once (probably more than 14, as information is not forthcoming with reference to four of the species), in the case of branches absence of buoyancy occurs in only 2 species when fresh, and 1 species when dry, and the average buoyancy of the branches, whether fresh or dried, is considerably higher than that of the seeds. So that this exceptional means of dispersal tends towards a wider distribution by water.

As regards a second point—the buoyancy of dried branches as compared with fresh ones—it is seen that the effect of drying is usually (in 14 cases) to increase to quite a slight extent their power of floating. In many (10) other cases drying actually diminishes buoyancy, sometimes to a great extent. Two remarkable instances of this kind are displayed by *Saxifraga umbrosa* and *Erica mediterranea*. In the case of *S. umbrosa* I tested several varieties of the species. While some sank in from 1½ to 6 days when fresh, two others—a native and a garden form respectively—remained afloat for periods of 25 and 40 days. The same specimens thoroughly dried sank in a few days. As regards *Erica mediterranea*, its buoyancy depends largely (as in the case of other heaths) on air imprisoned in the withered corollas. Probably under natural conditions of dispersal, where the branches were being tossed about in a river or in the sea, the air would be expelled from or dissolved out of the corollas more rapidly than under the comparatively tranquil conditions of an experimental tank.

Even leaving out of account these two exceptional species (whose inclusion would make the average buoyancy of the fresh branches considerably greater than that of the dried branches), we still find that the average buoyancy of the remainder is but very slightly increased by drying, the average time of floating of the fresh branches being 3·9 days, and of the dried branches 4·2 days. It will be noted that this result is much less favourable to the idea of increase of dispersal-efficiency by drying than that obtained by Darwin from a similar series of experiments, and quoted in the "Origin of Species," chapter xii. Darwin does not give a list of the plants he experimented upon; and in my own case the number of species tested is too small to permit of any generalization.

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BY

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[COMMUNICATED BY SIR WALTER N. HARTLEY, F.R.S.]

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IV.

ON A VIOLET COLOURING-MATTER AND ITS PRODUCTION BY A CERTAIN BACTERIUM.

By W. J. HARTLEY, B.A.

[COMMUNICATED BY SIR WALTER N. HARTLEY, F.R.S.]

[Read May 20; Published July 11, 1913.]

In the course of an examination of the waters used in certain Irish creameries a sample was examined in which a chromogenic bacterium of a bright violet colour was found. The sample was delivered at the Royal College of Science, Dublin, on April 17th, 1912, from a creamery in County Wexford. On the same day 0·1 and 1·0 c.c. were plated out in gelatine in the usual manner, and incubated in the dark at 20° C.; on April 23rd it was noticed that two colonies had a distinctly blue appearance, and sub-cultures were made from one of them.

The cultural characters were found to be variable. For instance, gelatine was sometimes liquefied in seven, sometimes in fourteen, and sometimes not in twenty-one days.

The thermal death-point after exposure to moist heat for one minute is apparently between 50° and 55° C. Sub-cultures from a culture previously heated to 50° C. produce little colour and liquefy rapidly; otherwise liquefaction rarely or never precedes colour-production.

Colour-production varies curiously. Agar colonies show colour in concentric rings. Agar streak-cultures usually produce colour on the margin, spreading inwards and intensifying till the tenth day. Sometimes colour is not produced for twenty-one days, and liquefaction may then occur.

Colour was not produced unless more water were present than was sufficient for the growth of the organism. The addition of sterile water to a well-grown, colourless, partly desiccated culture seventy-two days old, was followed by colour and liquefaction in forty-eight hours.

Experiments to find whether the colour is excreted or contained in the organism were inconclusive. With the exception of the statement of Macé,[1]

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[1] Macé, Traité de Bactériologie, 1897, pp. 849–853.

that *B. violaceus* produces spores, the cultural characteristics of this organism, owing to their variation, agree with his description of both *B. violaceus* and *B. ianthinus.*

In order to test the effect of media on colour-production, an inorganic food-basis solution was made up as follows:—

0·5 grm.	$NaCl$.
1·0 grm.	KH_2PO_4.
0·5 grm.	$MgSO_4$.
Trace of	$CaCl_2$.
0·003	$MgCO_3$.

Distilled water to 1 litre.

This solution was divided into five parts, to each of which was added 1·5 per cent. of agar-agar as follows:—

No. 1. Inorganic food-basis with agar.
,, 2. Same as No. 1, with two per cent. lactose.
,, 3. ,, ,, ,, two per cent. starch.
,, 4. ,, ,, ,, two per cent. urea.
,, 5. ,, ,, ,, two per cent. peptone.

Slope cultures of the bacillus were made on all these media and incubated at 20° C. Slight growth occurred on Nos. 1 and 4; better growth on Nos. 2 and 3; and good growth and colour were found on No. 5 (the peptone) in about five or six days.

Cultures were made in a 0·29 per cent. potassium nitrate solution free from nitrites; and after five days' incubation the medium was found to contain nitrites, showing the reduction of nitrates by the bacillus.

Preparation and Separation of the Pigment.

Slices of potato were sterilized in Petri dishes, and inoculated by quickly pouring over them an emulsion of bacteria. This emulsion was obtained by pouring about 10 c.c. of sterile water into a well-coloured culture of the bacillus on an agar slope. After four or five days' incubation at 20°, the potatoes appeared to be covered with a violet dew or "shagreenlike" growth; and on the eighth, ninth, or tenth day the growth appeared *more blue* and slimy, the slime being due in part to the partial decomposition of the potato. The growth was then lightly scraped off the potato with a spatula and placed in a "Sohxlet thimble." The colour was extracted by distilling absolute alcohol on to the growth until no more colour came over with the alcohol in the siphon tube. The solution was then placed in a boiling tube and allowed to stand for two or three days, and afterwards decanted or pipetted off, leaving about 10 c.c. of solution. This last part sometimes contained a deposit of dead bacteria and other matter which had siphoned over.

A part of the clear violet solution was now tested for starch, a possible impurity from the potato, with a negative result. The solution was then evaporated down to dryness, partly on a water-bath and partly *in vacuo* ; when dry it was a dark blue, almost black in mass, amorphous solid. It dissolved readily in cold alcohol, giving a violet solution, and in ether a purple solution, as also with chloroform. A volume of 1·5 c.c. was placed in each of the ten tubes, and tested with the following reagents :—

To No. 1 was added 0·5 c.c. of Normal KOH. The colour changed to blue, to green, and then to yellow.

To No. 2 was added 0·5 c.c. Na_2CO_3 Normal. The colour changed to a dark blue.

To No. 3 was added 0·1 c.c. " Concentrated " H_2SO_4. The colour changed to green.

To No. 4 was added 0·3 c.c. " Concentrated " H_2SO_4. The colour changed to yellow.

To No. 5 was added 0·2 c.c. HNO_3 " Concentrated." The colour changed to yellow.

To No. 6 were added 2 drops of strong Ammonia. The solution was immediately bleached ; the addition of acetic acid failed to restore any colour. With dilute Ammonia it turned green before bleaching.

To No. 7 was added a drop of bromine water. Colour instantly destroyed.

To No. 8 was added 0·5 c.c. H_2O_2. An opalescent blue, possibly due to partial precipitation of the colouring matter by water.

To No. 9 was added 0·5 c.c. $SnCl_2$. Gives a colourless solution on standing.

To No. 10 was added 0·5 c.c. ether. Gives a purple tinge to the alcoholic solution.

The pigment will act as an indicator to acids if sufficient alkali be present to turn the violet solution blue. The addition of acids turns it green, as, for example, weak hydrochloric acid. If an ethereal chloroform or alcohol solution be evaporated from a test-tube, the dissolved pigment will precipitate on the glass and show the same colour as the solution from which it precipitated. Some 20 c.c. of the alcoholic solution were poured through a column of precipitated chalk, in the hope that if two pigments were present they would separate, but this did not occur.

In order to test the dyeing properties of the colouring-matter, some 20 c.c. of the alcoholic solution (containing 0·02 grms. solid) were placed in a flask connected with a reflux condenser. Some pieces of linen, wool, silk, and cotton were washed with soap and water, and placed in running water over night, when small dry portions were added to the solution and boiled for two hours. On removal it was found that neither the wool, silk, nor cotton was changed in colour; but the linen when laid on white paper was faintly blue. This test with silk- and wool-fibres serves to distinguish the colour from that of a solution of aniline violet. The colour reactions with acids and alkalis are also different.

Some 5·0 c.c. of an alcoholic solution containing 0·005 grm. of solid were placed in an air-tight glass weighing-bottle. This was placed in a window for twenty-four days, during which time it was not exposed to more than twelve hours' direct sunlight, and at the end of the period the solution was almost colourless; only a faint trace of red remained.

The Absorption Spectrum of the Colouring-Matter.

A portion of the first alcoholic solution was evaporated partly on a water-bath and partly *in vacuo*, and repeatedly weighed till constant, the weight of the colouring-matter being 0·022 grm. A preliminary examination was made with a miscrospectroscope by Zeiss, conveniently fitted with a scale of wave-lengths.

A glass tube, 27 m.m. long by 10 mm. internal diameter, was cemented vertically to a thin glass microscope-slide, and in this the solution was placed. Thicknesses of solution greater than 10 mm. showed transmission of the blue, indigo, and a portion of the violet rays, or from a little beyond the solar line F to a point half way between G and H. There was total absorption of the red rays from A to beyond B; but a narrow band of bright red light with its centre about C was transmitted. Small thicknesses showed transmission of the bright red rays from λ 670 to λ 640, and absorption from about λ 640 to λ 490, the rays beyond F being transmitted.

A more precise examination of the same solution was made with one of Hilger's fixed deviation wave-length spectroscopes, illumination being by sunlight directed on to the slit by a heliostat.

No rays were transmitted through a thickness of 25 mm.; through smaller thicknesses, the measurements resembled those obtained with the Zeiss instrument and, stated generally, the rays less refrangible than λ 6700 were absorbed; the bright red rays from about λ 6670 to about λ 6240 were fully transmitted.

The rays from λ 6100 to near F (λ 4900) were absorbed; but a feeble transmission near the green $b^1\,b^2$ group to beyond F was seen, with a complete transmission from λ 4900 to λ 4000. The variations in the sunlight on the few occasions when it was available made it difficult to measure accurately; and therefore it was decided to rely on the photographs of the absorption-spectra, which were taken in the following manner:—

A weighed quantity of the dried pigment, 0·022 grm., was dissolved in 5 c.c. of "absolute" alcohol and successively diluted to 10, 15, 20, 40, 80, and 160 c.c. (see Curve). (The solid was *not* entirely dissolved by 5 c.c. of cold alcohol, but was completely dissolved by 10 c.c.)

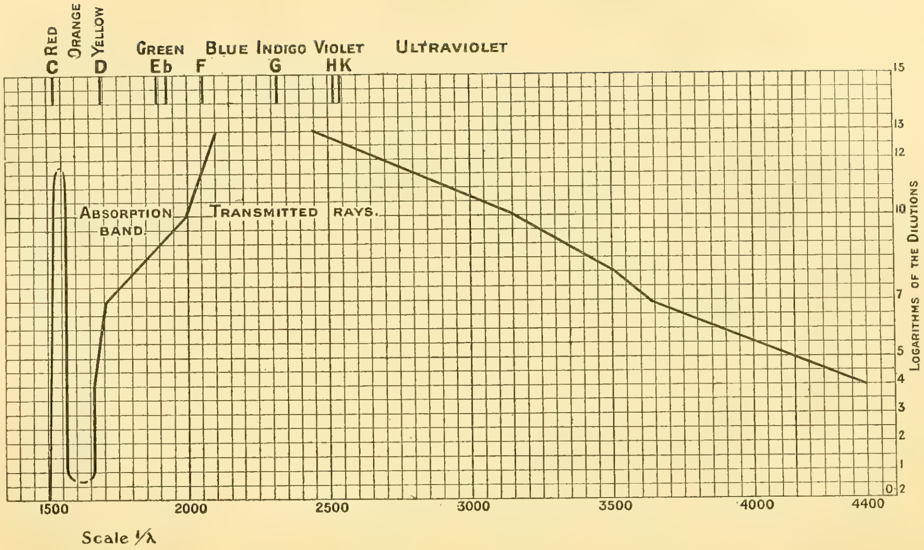
The spectrum of the alcohol was photographed through the same thickness (5 mm.). It transmitted all the rays to λ 2495 strongly, and feebly to λ 2196. The source of light was the continuous rays and metallic lines emitted by a condensed spark passing between electrodes, one of which was composed of an alloy of cadmium, 15 per cent. tin, 85 per cent.; the other cadmium, 15 per cent. lead, 85 per cent.

The cell was of glass with quartz ends, giving a layer of liquid 5 mm. thick. The instrument was a quartz spectrograph, photographing lines, from λ 7500 to λ 2145 in focus on one plate, and extending to a length of more than 100 mm.

The plates were Wratten and Wainwright's panchromatic spectrum plates.

The rays from the spark were condensed by a large quartz lens on the cell, which was placed so that a sharp image of the spark was focussed on the slit of the instrument, the exposure for each photograph being one minute.

[DIAGRAM AND TABLES.



Absorption Curve of the Colouring-Matter of *Bacillus violaceus*. 0·022 grm. of the solid dissolved in 5 c.c. of alcohol. Thickness of the layers of liquid 5 mm. Spectra at various dilutions from 5 to 160 c.c.

DILUTIONS OF SOLUTION.	5 c.c.*		10 c.c.		15 c.c.		20 c.c.	
DESCRIPTION OF SPECTRUM.	Frequencies.	Wave-lengths.	Frequencies.	Wave-lengths.	Frequencies.	Wave-lengths.	Frequencies.	Wave-lengths.
Spectrum begins at . . .	(2105)	(4750)	1515 to 1553	6600 to 6439	1515 to 1553	6600 to 6439	1515 to 1553	6600 to 6439
Band of absorption from . .	—	—	**1553** to **2000**	**6439** to **5000**	**1553** to **1709**	**6439** to **5850**	**1553** to **1696**	**6439** to **5893**
Rays transmitted ; feeble from .	2105 to 2439	4750 to 4100	2000 to 2105	5000 to 4750	1709 to 2083	5850 to 4800	1696 to 2000	5893 to 5000
Strong Spectrum from . .	—	—	2105 to 2765	4750 to 3616	2083 to 3001	4800 to 3332	2000 to 3325	5000 to 3007
Feeble Spectrum from . .	—	—	2765 to 3144	3616 to 3180	3001 to 3508	3332 to 2850	3325 to 3640	3007 to 2747
Total absorption beyond . .	(2439)	(4100)	3144	3180	3508	2850	3640	2747

* At 5 c.c., transmitted rays are very feeble.

DILUTIONS OF SOLUTION.	40 c.c.		80 c.c.		160 c.c.		alcohol only.	
DESCRIPTION OF SPECTRA.	Frequencies.	Wave-lengths.	Frequencies.	Wave-lengths.	Frequencies.	Wave-lengths.	Frequencies.	Wave-lengths.
Spectrum begins at . . .	1515 to 1553	6600 to 6439	1515 to 1553	6600 to 6439	1515 to 1666	6600 to 6000	1481	6750
Band of absorption from . .	**1553** to **1666**	**6439** to **6000**	**1553** to **1666**	**6439** to **6000**	—	—	—	—
Rays transmitted ; feeble from .	1666 to 1754	6000 to 5700	1666 to 1859	6000 to 5378	—	—	—	—
Strong Spectrum from . .	1754 to 3462	5700 to 2888	1853 to 3638	5378 to 2747	1666 to 4116	6000 to 2429	1515 to 4008	6600 to 2495
Feeble Spectrum from . .	3462 to 3523	2888 to 2838	3640 to 4415	2747 to 2265	4116 to 4405	2429 to 2270	4008 to 4553	2495 to 2196
Total Absorption beyond . .	3523	2838	4415	2265	4405	2270	4553	2196

The bleached solution in the 5 mm. cell transmitted the coloured rays, while showing a continuous absorption in the ultra-violet like that of the original colour in the same cell, but at a dilution of 15 c.c.; and in the 2 mm. cell, the bleached solution showed a continuous absorption in the ultra-violet resembling that of the original colour at a dilution of 40 c.c. through 5 mm. The measurements of the spectra are as follows:—

Alcoholic solution bleached by exposure to sunlight.
Concentration one part in 800 approximately.
0·005 grm. in 5 c.c. of absolute alcohol.

Thickness of cell, 5 mm. Coloured rays all transmitted.

Spectrum strong to 1/λ	3002	λ 3332
,, feeble to	3510	2850
Total absorption beyond	3510	2850

Thickness of cell, 3 mm. Coloured rays all transmitted.

Spectrum strong to 1/λ	3464	λ 2888
,, feeble	3523	2838
Total absorption beyond	3523	2838

The alcohol alone through 5 mm. transmits all rays to 1/λ 4555, or λ 2196.

There is no absorption-band in the ultra-violet.[1]

Inquiries into previous work upon these bacterial colouring-matters disclosed a paper, "Sur une nouvelle espèce de microbe chromogène Bacterium Rosaceum Metalloides," par G. F. Dowdeswell, M.A. ("Annales de Micrographie," vol. i, 1888–89), in which the examination of the colouring-matter was described by Professor W. N. Hartley. Unfortunately no definite weight of substance was taken, but the spectra of different thicknesses of solution were examined. It was decided to examine the violet colouring-matter in a similar manner and to compare the curve obtained with the curves of other similar colouring-matters from different sources.

A second paper, which was not obtainable till after all the experimental work was finished, was, "Die Bedeutung der Bakterienfarbstoffe für die Unterscheidung der Arten," von Dr. Paul Schneider (Karlsruhe, 1895).

Schneider describes the colour-reactions and spectra of some thirty different colouring-matters obtained from various organisms, among which are *Bacillus*

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[1] This distinguishes the colouring-matter from the violet and blue triphenylmethane dyes, the indophenols, and indigo, all of which have a chromogenic nucleus with an aromatic origin. Curves of these are shown in the Chem. Soc. Trans., li, 1887. See also Uhler and Wood's Atlas of Absorption-Spectra.

violaceus, and *Bacillus ianthinus*, the colouring-matters of which he says are identical. The reactions he obtained with this pigment proved to be similar to those I had obtained, except where the quantity of reagent is different. He used a Bunsen-Kirchhoff spectroscope with a photographed scale for examining the visible rays. The scale was entirely arbitrary and without reference to Fraunhofer's lines or to wave-lengths. The weight of substance dissolved is not stated, nor is the thickness of the layer of liquid recorded.

He states that the red rays are entirely transmitted, which is not the case according to either optical examination or the photographed spectra of the violet solutions I have described.

It is interesting to compare the absorption curve of this natural colouring-matter with that of Hoffmann's violet (Chem. Soc. Trans. li, 152–202, 1887).

0·416 grm. of Hoffmann's violet was dissolved in 100 c.c. of alcohol and diluted to 500 c.c., 2500 c.c., and 12,500 c.c., which dilutions were examined through 5, 4, 3, 2, 1 mm. Expressed in dilutions only, the same curve would be given by 0·208 grm. of solid, dissolved in 5 c.c. of alcohol, observed through 5 mm., and diluted to a concentration of 0·0208 grm., in 625 c.c., when absorption ceases. The spectrum of Hoffmann's violet, in the above-mentioned paper, is therefore strictly comparable with the spectrum of the bacterial colour violet, 0·022 grm. of which was dissolved in 5 c.c. of alcohol, and diluted to 160 c.c., when absorption ceases.

It is clear that Hoffmann's violet at similar dilutions shows four absorption-bands instead of one as does the natural colour, and that absorption continues to a greater dilution in the case of Hoffmann's violet, the colour intensity of which is nearly four times as great. Kopp[1] examined methyl violet (Geigy) and describes a sharp absorption-band overlying D, which withstood dilution to 1 grm. in 250 litres.

Further inquiries into research on plant and animal colouring-matters[2] showed that the colours of somewhat similar appearance have been more or less briefly examined:—

Lecoq de Boisbaudran extracted a violet colouring-matter from a bacterium grown on a starch-paste, which he does not describe.[3] The colour dissolved in alcohol gave an absorption-band about D, or from λ 600 to λ 563, with a maximum intensity at λ 581. Thick layers of the solution transmit the red rays fully only as far as λ 670, and faintly to 662, with a trace of light at 481.

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[1] Bull. Soc. Industr. de Mulhouse, xlviii, 946–950. 1878.

[2] Kayser's Handbuch der Spectroskopie, vol. iv.

[3] Comptes Rendus. xciv, 1882.

Moseley investigated janthinin, which gives three bands in dilute neutral solution, and one in an acid solution about D.[1] The bands were drawn, described, and measured.

Krukenburg also found a blue colour growing on moist fibrin.[2] It dissolved in alcohol with a violet-blue colour, and gave a band about D, which, he says, at once resembles and yet differs from that of certain aniline dyes.

An indigo-blue colouring-matter was obtained from water bacteria by Heinrich Claessen in 1890.[3]

Molisch, working with *Rhodobacterium capsulatum*, extracted from it by alcohol a green colouring-matter, "*bacteriochlorin*," and from the residual red-brown mass of bacteria he got a carmine-red colour "*bacteriopurpurin*" by extraction with carbon disulphide.[4] Bacteriochlorin has a band about D, from λ 615 to λ 565 which is typical of, and due to, the bacteriochlorin; the spectrum begins at λ 650 and ends at λ 525. The bacteriopurpurin which appears to give the colour to the organism has two absorption-bands, the first about λ 585–555, and the second from λ 540–515, while yet a third is supposed to exist. Molisch describes the separation of the two colouring matters from the growth on a microscopic scale by alcohol, and by carbon disulphide, and by alcohol and olive oil.

Similar experiments repeated with *Bacillus violaceus* failed to show any separation of colours whatever.

I should say that the violet colours extracted by Schneider from *Bacillus violaceus* and *Bacillus ianthinus* may be, as he says, identical; but, like Lecoq de Boisbaudran's colour, they differ from the colouring-matter which is the subject of this paper, in that they completely transmit the less refrangible red.

The colouring-matter janthinin is evidently different from either Schneider's or mine, for it is fluorescent and has three absorption-bands.

The measurements of the absorption-band given by bacteriochlorin and also the length of its transmitted spectrum show it to be different from the colouring-matter here described; while bacteriopurpurin has two, if not three, bands, the first of which resembles that of janthinin.

The colouring-matter of *Bacillus violaceus* differs from the dyes in the absence of absorption-bands in the ultra-violet, and from the plant and animal colouring-matters because of the continuous absorption of the rays

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[1] Quart. Journ. Micros. xvii, pp. 1–23. 1877.

[2] Physiologische Studien., 5 Abtheil., pp. 43–47. 1881.

[3] Centralblatt für Bakteriologie. 1890.

[4] Die Purpurbacterien. Molisch. Jena, 1907.

in the red less refrangible than λ 6600, and the transmission at nearly all thicknesses of the rays from λ 6600 to λ 6439.

There was brought to my notice recently a paper by Miss Wheldale on the production of Anthocyanin, in which she states hypothetical reactions for its production by oxidases acting on colourless chromogens present in plant-tissues in combination with glucosides.

The conditions under which colour was produced by *Bacillus violaceus* are not apparently at variance with the conditions presumably required by the hypothetical reactions.

H. E. and E. F. Armstrong,[1] in a paper on "The Origin of Osmotic Effects—III," describe Guignard's reaction for the detection of cyanophoric glucosides by means of sodium picrate paper in proximity to macerated tissue, which is incubated at 37°C. in an atmosphere of chloroform vapour. This test gave a positive result with my cultures.

As I have been unable to find sufficient record of either the chemical reactions or the absorption-spectra of Anthocyanin, I cannot say whether it is a substance resembling the violet colouring-matter of *B. violaceus*; but the latter appears to be formed in a similar manner, and I propose for purposes of comparison to investigate Anthocyanin as the subject of a further paper.

I hope with further opportunity to make a more complete investigation of the nature and biological significance of these bacterial colouring-matters.

Finally, I wish to offer my sincerest thanks to the Department of Agriculture and Technical Instruction for Ireland, for granting me a Research Scholarship at the Royal College of Science; and I also wish to thank Professor Morgan and Mr. Houston, for giving me every facility for my work in the Chemical and Bacteriological Laboratories.

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[1] Proceedings Roy. Soc., vol. lxxxii, series B. No. 559, p. 558.

THE

SCIENTIFIC PROCEEDINGS

OF THE

ROYAL DUBLIN SOCIETY.

Vol. XIV. (N.S.), No. 5. JULY, 1913.

THE EFFECT OF A LOW POTENTIAL CURRENT ON PHOTOGRAPHIC PLATES.

BY

REV. H. V. GILL, S.J., B.A.

(PLATES I, II.)

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[1] Proceedings Roy. Soc., vol. lxxxii, series B. No. 559, p. 558.

V.

THE EFFECT OF A LOW POTENTIAL ELECTRIC CURRENT ON PHOTOGRAPHIC PLATES.

By REV. H. V. GILL, S.J., B.A. (Cantab.), Belvedere College, Dublin.

(Plates I. and II.)

[Read May 20. Published July 17, 1913.]

In this paper I propose to give an account of some experiments made during the course of last year, which seem likely to help towards the solution of certain problems presented by the obscure phenomena connected with the blackening of a photographic plate. I do not at this stage propose to enter into a detailed examination or discussion of the results I have obtained, but will content myself with describing the conditions under which the experiments were made and the results obtained. These experiments were carried out during irregular intervals which occurred in the midst of other work, and do not pretend to be exhaustive, but it seems better to publish them such as they are, rather than postpone doing so indefinitely.

A considerable amount of experimental work has been done concerning the nature of the electric discharge, by causing sparks to pass over the sensitive surface of dry photographic plates. The question naturally arises whether the record obtained on development of the plate is to be attributed to some electric reaction, or simply to the luminosity of the discharge.

It seemed worth while making some experiments with currents which certainly were not luminous, to determine the part played by the purely electric elements of the discharge. Experiments were made in the first instance on dry photographic plates, the source of electricity being a 'water battery' consisting of 200 small Zn–Cu cells which could be used in multiples of 20. As the potential difference between Zn and Cu may be taken at 0·9 volt, the complete series gave a potential difference of about 180 volts. As tested by a gold-leaf electrometer, the voltage of the battery remained constant for many months. The strength of the current from such a battery is very minute, and no attempt was made to measure it. Exposures of from twelve to twenty-four hours were made with this battery in the way presently to be described. As a rule the whole series was employed, but definite results were obtained with a few cells in series. The feeble results obtained by this method were sufficiently definite to point to certain conclusions which seemed worth examining under circumstances which would produce more strongly marked reactions. The experiments were therefore repeated, making use of damp photographic plates and a series of small storage-cells. As the results obtained in this second series of experiments include, with increased distinctness, all those which had been observed in the original investigation, I have confined my description to them.

Method of making the experiments.—The plates were soaked in clean water for five or ten minutes, and were then pressed for a moment between sheets of clean blotting-paper to remove any superfluous water from front and back. The electrodes, which I shall call *anode* and *kathode,* were coins or other pieces of flat metal connected by wires to the terminals of a battery of storage-cells giving a potential difference of 400 volts. These coins were placed on the gelatine surface of the plate and kept in position by means of wooden clips. The 'exposure' occupied about ten minutes. The plates were then developed in the ordinary way. In this paper the figures represent the plates as they appeared on development, so that the blackened portions of the original photographic plates are black in the figures.

Effect produced at the kathode.—In the experiments on dry plates no effect was observed at the kathode—a fact which had its counterpart in the experiment now to be described. In general the effect produced at the kathode did not depend on the nature of the metal employed, and was less marked than that at the anode, but was present even when no blackening was produced at the anode. As will be seen from the figures, the kathode effect consists of a number of ray-like projections stretching out on the side facing the anode, being much less, or altogether absent, on the more distant side. This was accompanied by a slight blistering of the moist gelatine surface, as if gas had been liberated. The blistering was visible before development, but the change of colour did not appear until the plate had been developed (Plate I., fig. 1).[1]

General effect at anode.—The effect at the anode is much more marked than that at the kathode, though not always resulting in a blackening of the plate. This effect is characteristic of the nature of the metal used as electrode, and appears to consist of two reactions, both of which are not always observed. One of these reactions, which appears to be common to all metals, and which does not of itself result in a blackening of the plate on development, may be

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[1] All the figures are full size.

observed in the red light of the dark room during progress of the exposure. This reaction consists in a slight elevation or swelling of the gelatine surface of the plate, which is seen to spread out more or less uniformly around the anode. This reaction does not seem to give rise to a blackening of the plate, as will appear immediately The other anode effect is apparently connected closely with, or influenced by, that just referred to, but is not always present. This second reaction results in a blackening of the plate on development. The former of these reactions would appear to limit the distance to which the latter extends. Sometimes the blackening of the plate extends to the limits of the former, and sometimes not so far.

The rate at which these effects spread out gradually falls off, and at the same time the current decreases. In one experiment the current fell from an initial value of 4 to 8 milliamperes in ten minutes. The falling off of the current seems to be due to some polarization effect, and explains the fact that an exposure of ten minutes produces almost as marked a result as one of an hour's duration. Sometimes there is a metallic deposit at the point of contact of the anode.

It will be necessary to examine certain cases in detail. In some of these cases it will be sufficient to illustrate the anode effect, as that at the kathode is practically the same in every case.

Copper anode.—In the case of copper the two effects seem to be produced and the blackening of the plate extends from the metal to the limiting boundary of the other reaction. Plate I., fig. 2, shows the result obtained by using a piece of copper plating. It will be noticed that the kathode effect is much less marked, and is most evident at the corners. This effect seems to follow the direction of the lines of force. The result obtained by making use of copper coins is not so intense as when purer copper is employed.

Silver anode.—The result obtained when a silver coin is employed is characteristic. The space round the coin is divided into two clearly defined regions. Nearer the coin the plate is not blackened; in fact, sometimes, as in the case of Plate I., fig. 3, the developed plate is extraordinarily clear in this region, and far more transparent than in any other portion of the plate, even than in those parts which were not exposed to any luminous or electric influences. Outside this transparent ring is a region of intense blackening. This ring extends around the whole of the inner ring, but it is generally further on the side facing the kathode.

Iron anode.—The result obtained in this case is similar to that produced by silver (Plate I., fig. 4).

Nickel anode.—Produces a slightly different effect, though comparable to that obtained when iron is used.

Gold coin anode.—The result obtained in this way is very different from those already noticed. The effect produced around the coin is extremely regular (Plate I., fig. 5). In this case the two reactions described in the beginning of this paper are to be observed. There is the outside ring indicating the distance to which the swelling of the gelatine has extended. The space inside this ring does not blacken on development. Around the coin is the blackened region stretching out from the coin in ray-like prominences. The distance to which these rays extend is limited by the outer ring. The blackening effect seems to travel out at a slower rate than the other reaction, until it reaches the limiting ring, beyond which it does not proceed. This splash-like effect is characteristic of gold coins, and may be in some way connected with the copper which is in the alloy. The regularity of the stain produced by gold coins is also noticeable. Plate II., fig. 6, represents a plate on which were placed copper, gold, and silver coins as anodes; the kathode being a gold coin.

Platinum anode.—When platinum is employed, no blackening is produced on the development of the plate. Some platinum foil was folded round a coin and the exposure made in the usual way. There was no effect in addition to the slight swelling of the gelatine, which could be noticed even after the plate had been developed, but not as a black stain. It would seem from this result that the reaction resulting in the blackening of the plate is a complicated one necessitating the presence of the two reactions to which I have referred. A very instructive result was obtained which will be of value in helping to explain the phenomenon. Plate II., fig. 7, represents the effect produced by a copper coin covered with platinum foil, as already described; but in this instance there happened to be some small holes in the platinum where it was bent round the edge of the coin. As will be noticed in the figure, there is no blackening produced by the platinum as a whole, but at a few points black lines project out radially. These lines correspond to the holes in the platinum foil, and are evidently due to the copper of the coin around which the platinum was folded. It will be noticed that these lines terminate on the circumference of the outer circle already referred to, pointing to a connexion between the two reactions.

Bismuth anode.—Produces the result shown in Plate II., fig. 8.

Carbon anode.—A piece of electric-light carbon, ground smooth, gave the result shown in Plate II., fig. 9.

Cobalt and cadmium anodes gave rise to no darkening of the plate, though the other effect was produced.

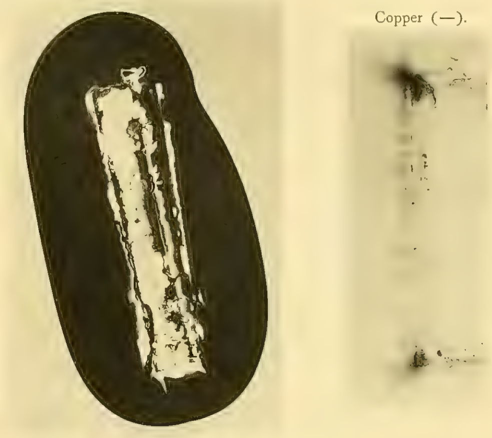
Zinc anode did not produce blackening of plate except at actual point of contact. The non-blackening effect was very marked, and it was noticed that a fogging of the plate did not affect this portion of the plate to the same extent as the remainder.

Some facts observed.—When the anode is placed near enough to the kathode to allow the region affected by the anode to reach that round the kathode, it is observed that at the junction of the two regions the blackening of the plate is sometimes more marked, but the anode effect seems to be arrested and to travel no further (Plate II., fig. 10).

Conclusion.—The results of these experiments suggest the possibility of their being connected with the reactions studied by Lodge, Whethem, and others in gelatinous and other solutions, and the results obtained are no doubt due to some action of metallic ions on the sensitive salts contained in the coating of the photographic plates.

While many suggestions might be made as to the nature of the reactions involved in these phenomena, I do not propose to discuss the action of the various substances employed in giving rise to a blackening of the plate. The question is one involving very obscure and little known reactions, and in the present state of our knowledge it is sufficient to put on record these results as a contribution to the accumulation of facts which have been ascertained concerning the blackening of photographic plates.

Fig. (1).



Copper (+) 10 mins. 400 volts.

Copper (—).

Fig. (2).

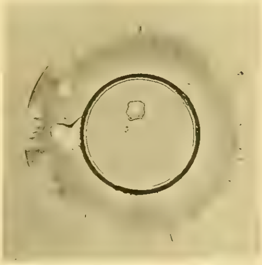
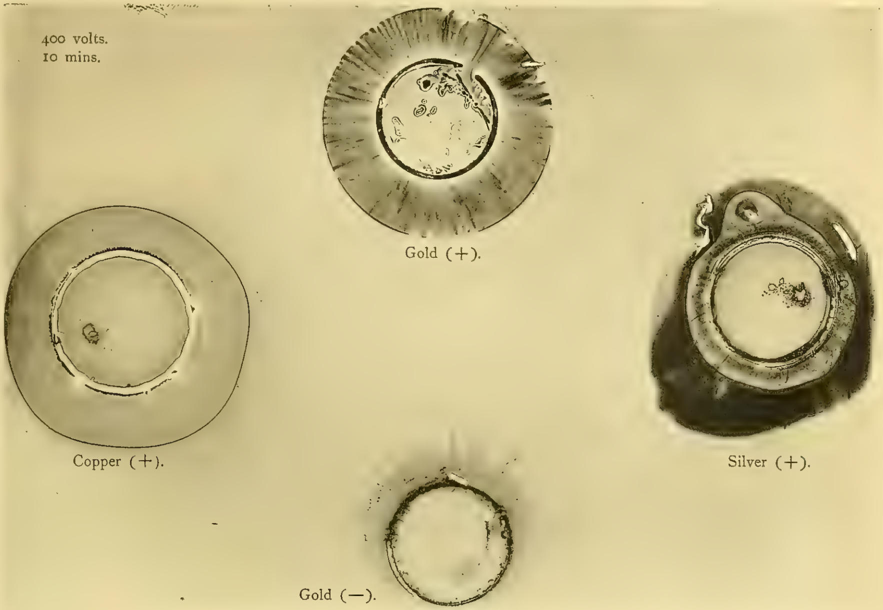


Fig. (5).

Fig. (3).

Fig. (4).



Gold (+).

Copper (+).

Silver (+).

Gold (—).

Fig. (6).

Fig. (7).

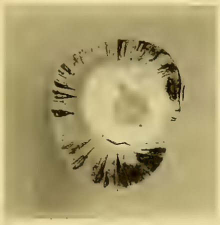


Fig. (9).

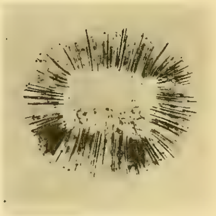


Fig. (8).

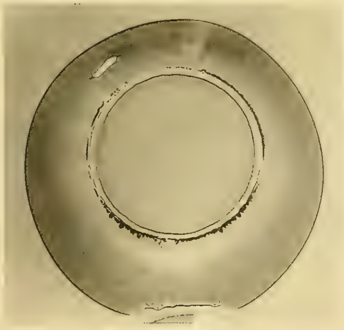


Fig. (10).

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Vol. XIV. (N.S.), No. 6. AUGUST, 1913.

THE MARITIME AND MARINE LICHENS OF HOWTH.

BY

MATILDA C. KNOWLES.

[COMMUNICATED BY R. LLOYD PRAEGER.]

(PLATES III.–IX and MAP.)

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VI.

THE MARITIME AND MARINE LICHENS OF HOWTH.

By MATILDA C. KNOWLES.

[COMMUNICATED BY R. LLOYD PRAEGER.]

(PLATES III–IX, AND MAP.)

[Read JUNE 24. Published AUGUST 16, 1913.]

TABLE OF CONTENTS.

I. INTRODUCTION.

HOWTH HEAD forms the northern boundary of Dublin Bay. It stands well out into the Irish Sea, an isolated area of high rocky land of about 4 square miles in extent, the highest point being about 560 feet above sea-level. A low isthmus of sand and gravel scarcely half a mile wide, and lying about 10 feet above ordinary high-water mark, connects it with the mainland. This isthmus is part of the raised beach that extends along the County Dublin coast from Clontarf northwards.

THE ROCKS (5) are entirely of the Palaeozoic series, being mainly Cambrian grits, shales, and quartzites, much contorted and interspersed here and there with bands of pure white quartz and numerous dykes of igneous rock, many of which may be seen on the sea-cliffs, and on the shore at low tide. Beds of Carboniferous limestone, resting unconformably on the Cambrian series, lie along the western side of the promontory, stretching from Sutton to Howth Harbour and Balscadden Bay, where they are exposed on the shore.

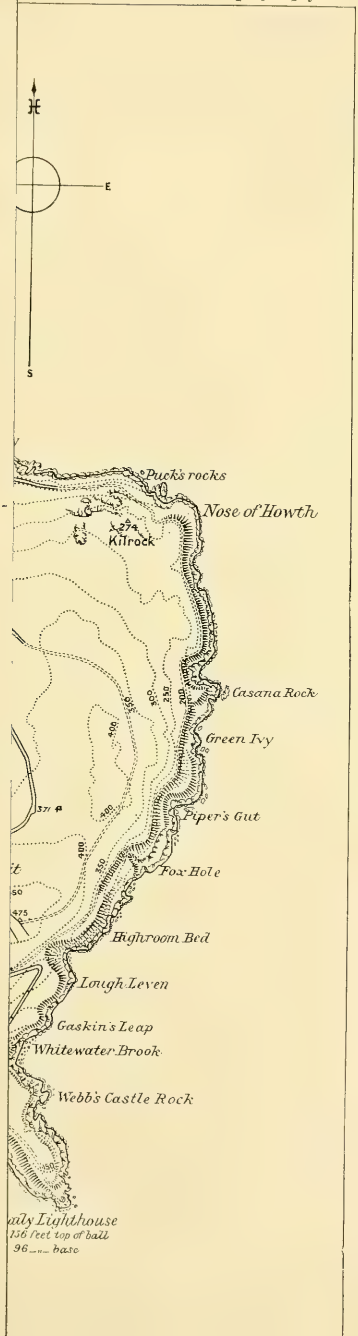
THE COAST-LINE is about 8 miles in circumference, and faces all points of the compass. Rising rather suddenly from about sea-level on the west, by far the greater part of it is composed of steep precipices, varying in height from 100 to 300 feet. These are broken up by shallow bays and narrow inlets, and here and there are rocky outliers, the most important of these last being the quartzite pinnacles known as the Needles, which lie between Drumleck Point and the Broad Strand. The upper part of the cliffs is covered by drift and Boulder-clay, in which marine shells and other calcareous matter are embedded, and which in many of the bays descend to the beach, and obscure the rocky surface.

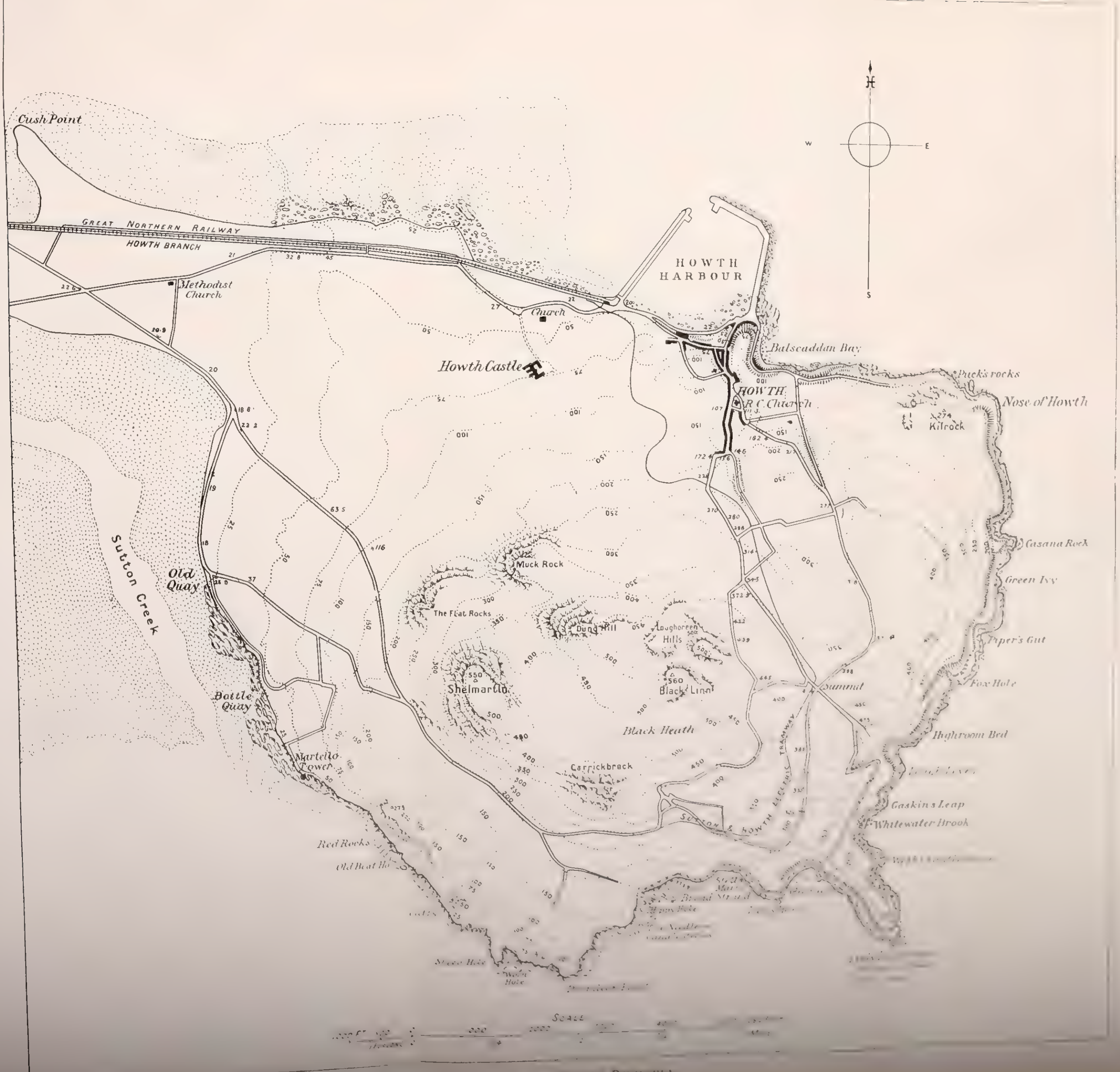
The south and south-west coasts are the most accessible. On the south-west the sea washes the base of the cliffs at ordinary high water, but as the tide retreats a low stretch of foreshore is laid bare, which enables one to explore almost the whole of this coast. Towards the Sutton end, and as far as Old Boat-house, the foreshore is for the most part composed of strewn boulders on a sandy bottom, but from Old Boat-house to Drumleck Point it is largely solid rock. The south shore has a narrow beach uncovered at high water, which varies with the undulations of the coastline, being low and sandy or pebbly in the shallow bays, and higher and composed of large blocks of rock on the more outstanding parts, so that even at full tide, except at Lion's Head, one can walk all along this shore from Hippy Hole to the Baily Lighthouse. The eastern coast is much more precipitous, the cliffs descending steeply into deep water. Only at one or two places, as at White Water Brook, the bay between High Room Bed and Lough Leven, and at Casana, where there are grassy slopes with accumulations of dislodged rock about high-water mark, is it possible to reach sea-level. Balscadden Bay on the north coast is easy of access; along the greater part of this coast there is a rough, rocky tract exposed at low water over which one can scramble to examine the vegetation on the rock-surfaces, and on the cliff-faces.

Tides rise and fall all round the coast. Spring rise is about 13 feet, but with southerly winds it may be as much as 15 feet, and with northerly winds low water may be a couple of feet below low water of ordinary tides. Neap-rise is 10 feet; neap-range, 8 feet. The highest tides are always about midday, which limits low-water collecting to the summer months.

Winds.—The prevalent winds are from the south-west and west, and are warm and moist. The force of these winds is greatly moderated by the highlands of County Wicklow, which lie in their track, and on which they deposit a good share of their moisture before reaching the north shores of Dublin Bay. In the spring months there are spells of north and east winds, which are cold and parching.

[*To face page* 80.





Map of the Howth Peninsula.

CLIMATE.—The climate of Howth is mild and equable. There is seldom any frost or snow. Rain falls on a comparatively great number of days throughout the year. No details have been published about the mean annual temperature or the mean annual rainfall; but Sir John Moore informs me that the climate of Howth is more maritime and rather drier than that of the city of Dublin. In his book on "The Climate of Ireland" (**18**) the mean annual temperature for the city of Dublin, calculated on a mean of forty years, is given as 49·5° F., and the mean annual rainfall as 28 inches.

Owing to the steep character of the northern and eastern coasts, the cliff-faces receive very little direct sunlight. The morning sun soon passes over to the south-west, leaving them cold and shady. This, no doubt, accounts for the prevalence of alpine species on these coasts. The less steep south and south-west coasts are bathed in the afternoon sunshine, and face the prevalent winds, which bring moisture. The lichens of these coasts therefore grow under much more favourable conditions. The Broad Strand is one of the sunniest and most sheltered spots on the whole coast of Ireland; and on its earth-covered cliffs several species of lichens occur that have so far been found only in the Channel Islands.

MOISTURE.—The main source of moisture is the rainfall, but fogs are frequent in late autumn and winter; and at these seasons the whole headland is frequently enveloped in mist. During the winter months the cliff-faces are often dripping wet from surface drainage, but in the summer this supply is largely cut off and the vegetation is sometimes subjected to long spells of drought. There are no streams of importance flowing into the sea; but here and there are small rills, and several springs and small waterfalls which overflow and spread over the cliffs and the rocks on the shore. The most important of these is White Water Brook; others occur at Red Rocks and Balscadden Bay. The shore rocks below Earlscliffe are in some places kept constantly damp by fresh water oozing from the base of the cliffs. At these places, and where fresh water flows, quite a different set of lichens and algae are to be found.

EXPOSURE.—No part of the coast can be described as exposed, though there are places that are relatively much more exposed than others. For the greater part of the year the seas are calm; and except during storms, which are of rare occurrence, the main part of the cliff-faces lies beyond the reach of the spray. Nowhere on the Howth coast are the cliff-faces subjected to the perpetual drenching that those on the west coast of Ireland experience; and, as a consequence, the area covered with marine lichens is much more restricted. Owing to the steep and indented nature of the Howth coast-line, sheltered and exposed conditions alternate with some rapidity.

The algae give, perhaps, the best indication of the amount of exposure to which these shores are subjected. All round the south and south-west coasts *Pelvetia cananiculatus*, *Fucus spiralis*, *Ascophyllum nodosum*, *Fucus vesiculosus*, and *Fucus serratus* are general and abundant (only from the steeper cliffs at Drumleck Point, from the sea-faces of the Needles, and from Lion's Head, was Pelvetia absent). In the shallow bays of Broad Strand and Glenaveena *F. spiralis*, var. *platycarpus*, replaces the species. *Porphyra umbilicalis* is also general, and usually forms a well-marked zone on the cliffs above Pelvetia; on the flat shores of Broad Strand it extends almost throughout the whole neap-range. It is most common in the Pelvetia belt, and just below it, but it occasionally grows in large patches above Pelvetia, and where fresh water flows is frequently associated with *Enteromorpha intestinalis.* These are algae of sheltered and semi-exposed coasts.

Except during easterly gales, which, as a rule, only occur in the spring, when the spray sometimes rises as high as the Cliff-walk, there is very little wave-action along the east coast, and the cliff-faces are seldom wet with sea-water for more than a few feet above high-water mark.

At Casana, one of the few accessible spots on this coast, the following algae were noted:—*Porphyra umbilicalis* with the narrow form var. *linearis* in two zones on the large boulders lying just above spring-tide level, *P. umbilicalis* being nearer the sea and extending down into a belt of Fucus, on which *Rhodymenia palmata* was growing epiphytically. Mr. A. D. Cotton considers that this Fucus comes near *F. vesiculosus*, var. *evesiculosus*, of exposed coasts, but that it indicates much less exposure than there is on the south shore of Clare Island. *Pelvetia cananiculatus* only occurred here and there as odd plants in the most sheltered spots amongst the boulders. *Ascophyllum nodosum* was not seen at any of the places examined on this coast, though it is general and abundant along the greater part of the north coast. On the limestone area at Balscadden Bay a narrow form of *Fucus vesiculosus* without bladders occurs above *Fucus serratus*, the last being exceedingly abundant. *Ascophyllum nodosum* is absent from the limestone, but occurs on the silicious rocks on the opposite side of the bay.

Previous work.—From a study of all the available literature it would seem that the lichens of the sea-shore have been the subject of very little investigation. Since the publication of Nylander's account of the Lichens of Pornic in 1861 (**20**), which was one of the first dealing with a maritime area, Weddell's paper on the Lichens of l'Ile d'Yeu (**30**) is the most important contribution that has been made on the subject. Weddell's paper deals chiefly with the systematic side of the question, though ecological factors are not entirely overlooked, as he gives copious notes containing much valuable and interesting information about the habitat and range of many of the species in his list. In the short introduction to the paper he divides the lichens of the rocky shores into three groups: (1) *Marine*—those growing on the rocks that are covered at every tide; (2) *Semi-marine*, those that, without requiring a complete immersion, benefit by the splashings they receive from the waves breaking at the base of the rocks, of which they occupy the sides; (3) *Maritime*—those that grow beyond reach of the waves, but come under the influence of the salt breeze. *Verrucaria maura* and *Lichina confinis* are mentioned as belonging to the semi-marine group, and *Lichina pygmaea* as an example of the marine group; but no further reference is made to this classification in the paper.

More recently, Sanstede has published several important papers on the lichens of the German North Sea islands (**24** and **25**). He also is mainly concerned with the systematic side of the subject, but there are some ecological notes, and references are made to the species that grew on rocks splashed by the salt water and to those preferring higher levels, and lists of species are given.

In his treatise on plant-ecology, Warming (**29**) refers to the zonal distribution of the vegetation of the sea-shore, and describes three belts of lichens as characteristic of the coasts of Denmark and Sweden, and of other northern countries. He says: "Lying lowest on the shore, where the rocks are very frequently wet, is *Verrucaria maura*, a very thin black scaly lichen divided into small pieces. . . . Higher up the rocks are reddish yellow with *Placodium murale*, which is accompanied by *Xanthoria parietina*. Above this follows a belt of *Ramalina scopulorum*; here the action of the salt water is reduced to almost nothing." There are scattered references to the lichen vegetation of the sea-coasts in other publications; but, so far, the ecological side of the subject has received very little attention from lichenologists. Practically all that has been written about the ecology of the lichens of the sea-coasts refers to the marine group and is to be found in the writings of marine algologists such as Börgesen (**2**), Jónsson (**9**), Joubin (**10**), Cotton (**3**), and others, who, in describing the various marine algal associations, incidentally give some account of the lichen vegetation found between tide-marks. By far the most complete account, and the only one that has been published for our own coasts, is that contained in A. D. Cotton's paper on the Marine Algae of the Clare Island Survey. Here we find a very full and interesting description of the Lichina communities of those coasts, and in his account of the "Hildenbrandtia-Verrucaria association" Mr. Cotton supplies a good deal of information about the distribution and habitat of *Verrucaria mucosa* and *V. maura*, and suggests that there may be other species in the association.

During the last three years I have paid some attention to the lichen vegetation of the Howth coasts; and in this paper I make an attempt to describe it in its ecological as well as in its systematic aspect. My account is necessarily very incomplete, partly because so little has been written on the subject that in an initial effort there is the fear that one may make mistakes, but mainly because the subject is an exceedingly difficult one. The fruticose and foliaceous lichens are comparatively easy to recognize in the field; but the majority of crustaceous species are so minute that it is not possible to identify them *in situ*; and, as every specimen has to be submitted to the final test of the microscope, much laborious collecting and examination, involving a great expenditure of time, is necessary to obtain even an approximate idea of their distribution.

AREA INVESTIGATED.—A broad pathway known as the "Cliff-walk" runs along the top of the cliffs on the east coast from the Nose to the Baily Lighthouse, and there is a narrow track at similar levels on the south and south-west coasts. These form the upper boundary of the area investigated, which extends thence down the shore as far as low spring-tide, the lowest level at which lichens were found growing.

A detailed examination of the whole coast was impossible, and my observations were made mainly at various spots on the more accessible south and south-west shores. Red Rocks, the Old Boat-house, Drumleck Point, the Needles, Broad Strand, and Lion's Head received special attention as affording different degrees of shelter and exposure; and large collections were made at these places from different levels, which were examined microscopically at home to verify notes made in the field. The same procedure was followed for the places examined on the east coast, viz.: White Water Brook, Gaskin's Leap, High Room Bed, and Casana. The northern coast was also examined in the same way.

Assistance.—In working out the large mass of material collected I frequently sent critical specimens to Miss Lorrain Smith for her opinion, and I wish here to record my indebtedness to her for the time and trouble she gave to them. It has been a great satisfaction to me to have her confirmation of my identifications. I am also indebted to her for suggestions about new species, and for checking the measurements of those described in this paper; for assistance in connexion with the Ramalina attachments, and for many other kindnesses received when I visited the British Museum, to compare some of the Howth lichens with Crombie's type specimens. To Mr. A. D. Cotton I owe thanks for notes about the algae; and I must also offer my sincerest thanks to Miss N. McArdle, for the care and accuracy with which she identified the various mosses mentioned in this paper, and for help in collecting material on many occasions.

II. Formations and Associations.

Lichens are found growing on many different kinds of sub-strata, but there are three on which they occur more abundantly than on any others, viz. rocks, bark of trees and wood, and the soil. Considering them from this point of view, lichenologists have arranged them into the three following groups:—

1. Saxicolous lichens—those growing on rock and stones.
2. Corticolous lichens—those found on bark of trees and on wood.
3. Terricolous lichens—those that grow on the soil.

According to the most recent conceptions of plant ecologists as to the meaning of the term 'formation,' these three groups might, perhaps, represent three formations. But as many of the details of the lichen-communities of the Howth coasts are not sufficiently worked out to enable one to arrive at an accurate estimate of their standing, I have thought it better, until some other area has been investigated, to abstain from employing the terms 'formation' and 'association,' and, for the purpose of describing the lichen-vegetation of these coasts, to make use of the three long-established groups above mentioned. All three groups are found on the Howth shores, the saxicolous being the most abundant. The Blackthorn thickets on the cliffs above the Broad Strand afford the only habitat for the corticolous forms. Those that grow on the ground, the terricolous, are largely represented, and have been studied mainly on the earth cliffs at Earlscliffe and at Glenaveena.

1. *The Lichens of the Rocky Coast* (*Saxicolous*).

i. *Silicious Rocks.*

The greater part of the coast is composed of silicious rocks; but, as has been stated, there is a small outcrop of limestone on the shore at Balscadden Bay. As several lichens not found on the silicious strata grow on this limestone area, its vegetation will be considered separately. The zonal distribution of the lichens on the sea-shore, to which Warming has drawn attention, and which has been referred to already at page 83, is very well seen along the greater part of the Howth shores. As one walks along the top of the headlands when the tide is low, a dark band, which seems like a stain on the rock-surface, can be distinctly traced on the cliff-faces and on the rocks of the seashore. At high spring-tide this dark band is almost hidden by the water, but it becomes wider as the tide falls; and at low spring-tide it is seen to end, on the cliffs and high rocks, in a well-defined line running parallel with the surface of the sea at a few feet below the level of ordinary high tide, contrasting in a striking manner with the paler band of the barnacle-covered rock below it. These two bands, the dark lichen band and the paler barnacle-covered area, are to be seen on all the cliffs round the headlands, the dark band rising higher on the parts which stand more out to sea; but they are most conspicuous on the steep cliffs rising out of the deep water on the eastern coast, on which, at low spring-tide, yet another dark band composed of algae (Fuci and Laminaria) can be distinguished below the barnacles. This dark lichen-band is wider on sloping surfaces and on flat shores, and is most intense in colour a little above and below high neap-tide level, shading off upwards and ending in a ragged uneven outline. On the rocks of the Broad Strand and other low shores, these dark lichen-growths stretch from the highest spring-tide mark to the lowest spring-tide mark, on the upper part of the shore forming a continuous sheet of vegetation which is most conspicuous above the ordinary high-tide mark, where it is unobscured by algae, but tailing off and becoming patchy as low-water mark is approached. The upper part of this dark lichen-band is occupied by the *Verrucaria maura* belt referred to by Warming. Below *Verrucaria maura* another belt occurs not mentioned by Warming—the belt of marine Verrucarias. Besides these there are two other belts or zones which form elements of the dark lichen-band, those of the shrubby Lichinas, *L. confinis* and *L. pygmaea*. The former of these grows along the upper, and the latter along the lower limits of the *Verrucaria maura* belt. Above the dark band the orange belt of *Placodium murorum*, *Physcia parietina*, &c., stands out conspicuously, and above the orange belt the grey-green growths of the Ramalinas occupy a wide area. These three colour-belts occur all round the coast, and can be well seen by standing on the shore just below high neap-tide level and looking towards the cliffs. On the beach at Stella Maris they occur with beautiful regularity. The Ramalina belt would correspond to Weddell's maritime group; the main part of the orange belt and the *Verrucaria maura* belt would be included in his semi-marine group; while those species growing below high neap-tide level would constitute his marine group. As, however, the arrangement in belts, which might perhaps be described as linear associations, seems to be the natural way in which the lichens of the sea-shore group themselves, I have decided to adopt it rather than Weddell's classification in describing the lichen-vegetation of the Howth coasts.

In many places the lichen-belts exhibit a further subdivision into zones which vary somewhat according to the lie of the shore and the amount of exposure, &c., but these variations will be dealt with when describing each belt in detail.

The belts will be taken up in the following order:—

(1) The Ramalina Belt.
(2) The Orange Belt.
(3) The Lichina Vegetation.
(4) The *Verrucaria maura* Belt.
(5) The Belt of Marine Verrucarias.

(1) The Ramalina Belt.

The Ramalina belt is a very wide one, stretching from just above the high-water mark of spring-tide to the tops of the highest cliffs; it even extends inland beyond our area, covering all the rocky knolls between Red Rocks and Drumleck Point on the south and south-west coasts, and many of the rocks facing the sea above the Cliff-walk on the east coast are also covered with a stunted growth of Ramalinas. The belt is well developed both in sheltered and in exposed situations, but the most extensive colonies and the strongest growths are always to be found on the windy sides of the hard, rough quartzite cliffs of the south and south-west coasts. The more easily disintegrated slates and shales seldom carry a good crop of Ramalinas. On the drier, more sheltered and shady eastern and northern coasts the Ramalina growths, though widespread, are usually sparser, and the tufts more stunted and less healthy-looking.

On the Howth coast the Ramalinas grow in two well-marked zones, those of the lower zone being usually very fertile, the plants consisting of straight, stiff, simple or slightly branched fronds of a pale grey-green or straw colour; while the Ramalinas of the upper zone are usually barren, have much-branched fronds of a darker colour than those of the lower zone, and of a somewhat glaucous appearance, the extreme tips of which are incurved. The paler colour of the lower Ramalinas is no doubt to some extent due to the fact that they grow on rocks and boulders along the verge of high-tide mark, or at a similar level on the cliffs, where they are frequently splashed by spray, and washed free from dust and other extraneous matter The upper Ramalinas growing on the cliffs beyond the ordinary spray-zone, or on the land sides of rocks and boulders of the beach, do not come under this cleansing influence, and their darker colour may be partly due to dust and particles of earth, &c., accumulating on the surface of the thallus. The glaucous appearance of the upper Ramalinas is, however, very distinct.

So far as one can judge in the field, the Ramalinas of the lower zone all belong to *R. scopulorum*. *R. scopulorum* is, however, morphologically similar to *R. cuspidata*, and can only be distinguished from it by treatment with hydrate of potash, when *R. scopulorum* gives the reaction medulla yellow, then red, while *R. cuspidata* gives no reaction. In order to see if both species were present on the Howth shores, and if possible to find out the conditions under which they grow, a large series of Ramalinas was collected at various parts of the south and south-west coasts from both zones, and treated with the potash solution.

The results showed that, among the lower Ramalinas, those that gave no reaction when treated with potash (that is, *Ramalina cuspidata* of the floras), grew chiefly between Sutton and Old Boat-house, inside the shelter of Dublin Bay, and were extremely scarce beyond Drumleck Point; while the Ramalinas in which the medulla turned first yellow and then red under the potash (*R. scopulorum*) were very scarce near Sutton, but became more abundant as the coast-line approached the open sea until, at Lion's Head and the Baily peninsula, the most outstanding parts of the coast, they were the only Ramalinas met with. The upper Ramalinas were less responsive to the potash treatment, but here and there tufts were met with at all levels on the cliffs at Red Rocks, at Drumleck Point, and at other places that changed colour. On the east coast also the majority of the specimens from the upper zone gave no reaction. These results tally with Olivier's account (**21**) of the habitat of *R. cuspidata*, which species he describes as growing further from the sea than *R. scopulorum*, and as sometimes extending inland for a considerable distance. Crombie (**4**), too, gives the habitat of *R. cuspidata* as "rocks and boulders in maritime districts, rarely on hills at a distance from the sea." There are, however, those specimens in which the reaction was indecisive, the cortex turning faint yellow, or a rich coppery colour, while the medulla remained unchanged, or showed only a very faint stain. How are these specimens to be classified? Under *R. cuspidata* or *R. scopulorum*? As already stated, this test with potash is the only means of distinguishing the two species; but since it is not associated with any differences in form or in microscopic structure, it seems to be a very unsatisfactory character on which to found a species. Weddell disregards it, and includes *R. cuspidata* under *R. scopulorum*. Harmand (**6**), in discussing the value of the reaction tests, considers that at least they have "une valeur documentaire, et qu'une flore descriptive ne peut les passer sous silence"; while Zopf (**32**) has found in *R. cuspidata* cuspidatic acid ($C_{16}H_{20}O_{10}$), an acid only met with in this species, and in *R. scopulorum* scopularic acid ($C_{19}H_{16}O_9$), soluble in potash, giving yellow and then red colours on solution, and only found in the latter species.

It would appear from the experiments made on the Howth Ramalinas that the change in colour produced by the potash is in some way due to the action of the salt water, as it seems to vary in intensity according as the Ramalinas are often or seldom wetted by the sea. The first parts to take the stain are the young shoots, the apothecia, and the pustulated areas. These are the most absorbent parts of the lichen. The older and firmer parts do not respond so readily, and sometimes do not change colour at all, or only after repeated applications of the potash, or after it has lain on the surface for five or ten minutes, or even longer. Altogether, I have found the reaction test so unsatisfactory that for the purpose of describing the lower Ramalina zone I have followed Weddell, and have grouped all these Ramalinas together under *R. scopulorum.*

The Lower Ramalina Zone (R. scopulorum).—*Ramalina scopulorum* is always found within reach of the spray, and covers with a tufty growth the tops and sea-faces of the large rocks that lie on the shore a little above high spring-tide level. Where the sea washes the base of the cliffs at high-water, as at Drumleck Point and other places, *Ramalina scopulorum* rises up the cliffs and forms a broad or narrow zone according to the slope, rising higher in more exposed places. In the lower part of the zone it occurs in irregular tufts here and there on the rocks, sometimes associated with *Lichina confinis*, *Placodium lobulatum*, and *Verrucaria maura*. Upwards it overlaps and gradually disappears amongst the upper Ramalinas. Plate IV, fig. 1, shows *Ramalina scopulorum* as it grows on the rocky flat at Old Boat-house. Here it covers an extensive area, sheltering an undergrowth of Physcias and crustaceous species, in places growing side by side with *Spergularia rupestris, Aster Tripolium, Statice occidentalis*, and other halophytes.

Where *Ramalina scopulorum* is much exposed to the wind, or where it grows on rocks that receive an unusual amount of splashing from the waves, the fronds are broader, bluntish at the ends, and have the surface much roughened by lumpy excrescences, which have been described by Crombie (4) and Leighton (12) as spermogones, but which Zopf (31) considers are due to galls. This form answers to the descriptions of *R. scopulorum* Ach. var. *incrassata* Nyl., and is more frequent in rather exposed situations, and on the rocks nearest to the sea. It is seldom fertile, but apothecia are occasionally met with on some of the fronds.

On dry rocks another form occurs in which the whole plant is smaller, the fronds more slender and unbranched, and the apothecia frequently terminal. This form grows on the shore at Lion's Head, at Worn Hole, and at several other places round the coast. It has the appearance of being a starved form, growing where there is less wind and moisture.

Other plants were noticed answering to various forms described by Olivier and Harmand, but most of these occurred only as single tufts here and there amongst the typical forms, and seemed to be accidental. The fronds of *R. scopulorum* sometimes showed a tendency to branch, which was more pronounced in the plants growing further from the open sea or on rocks where they were protected from the salt water.

Ramalina scopulorum is not so abundant on the east and north coasts. Easterly winds are dry and infrequent, consequently there is not so much moisture or spray on this part of the peninsula. The plants are small, with slender bristles which are sometimes thickly covered towards the ends with young apothecia and spermogones or galls, and resemble the form described as growing on dry sheltered rocks on the south and south-west coasts.

The lower Ramalinas grow in tufts from a corticated cushion-like base which is often of considerable area, and in old-established tufts may be more than ½ inch in thickness at the centre. From the lower surface numerous slender branching hyphæ penetrate in rhizoid-like strands between the irregularities of the rock, and wind themselves round the small projections, sometimes enclosing particles of the rock in their meshes. As the tissues of the fronds are continuous with those of the basal area, they are thus securely anchored to the substratum. The Ramalina tufts are always more numerous on rocks with rough surfaces. When they grow on smoother rocks, they are more frequent on those that are already covered with crustaceous species. Fresh growths seem usually to spring from the periphery of the attachment area, and young fronds are often to be seen springing up in considerable numbers to replace those that have been broken off by the wind and waves. In this way the Ramalina tufts may renew their growths almost indefinitely. It is quite common to see almost all the old growth shorn off at one side of the basal cushion and a crop of stiff young fronds springing up from another part.

Besides the young growths which arise from the basal cushions, the beginnings of new colonies—probably sporelings—were frequently met with. On one small piece of rock chipped off a flat boulder on the shore at Stella Maris, the surface of which was completely disguised by a covering of Buellias, 27 small colonies were counted on an area of about 2 square inches, and a great part of the boulder was dotted in the same way. The colonies were all in early stages of development, many of them were entirely crustaceous and not much larger than a pin-head, but others had already put forth small tufts of slender upright fronds. On a higher rock above this flat boulder were many fine colonies of mature fertile plants of *Ramalina scopulorum.*

The Upper Ramalina Zone.—The Ramalinas of the upper zone are much more abundant and seem to vary more in appearance than those of the *Ramalina scopulorum* zone. Three well-marked forms occur, which in places pass almost imperceptibly into each other. The prevailing form is shown in Plate IV, fig. 2, and for the present it may be called *Ramalina A.*

Ramalina A. grows above the ordinary spray-zone, and generally forms a sward-like growth on the weather sides of the lower cliffs, the plants growing both larger and closer together along the upper edges where the rock and earthy covering meet, or in the neighbourhood of the various tufts of Thrift or other flowering plants that occupy crevices in the cliff-faces. This is well seen in the photograph. In these situations there is a more constant supply of moisture either from surface drainage or from drippings from the leaves of the flowering-plants during rainy weather.

The thallus of *Ramalina A.* has a glaucous appearance and is much branched, though it sometimes appears to consist of simple fronds from the branching having taken place low down near the point of attachment. Many of the branches are dilated and fistular and have the upper surface covered with tuberculations, from which, as well as from the sides of the branches, numerous small abortive growths frequently spring. The lower surface of the thallus is usually of a paler colour, and a section shows the gonidia congregated in groups close to the upper surface, while very few are to be seen on the lower surface. Patches of young growths in all stages of development are of frequent occurrence on the barer parts of the rock. Plate VII, fig. 1, shows a sward of young growth of *Ramalina A.* as it grows on the granite boundary-stones near the Martello Tower, Sutton.

At higher levels the sward-like growths of *Ramalina A.* disappear, and are replaced by isolated tufts which are scattered irregularly on the rock-surface. At the same time the thallus becomes smaller and more amorphous, and the pustulations and disorganized areas more numerous. Gradually *Ramalina A.* passes into a very small amorphous form which may be called *Ramalina B.*, and which seems to be peculiar to the vertical or almost vertical western and south-western faces of the highest cliffs.

Ramalina B.—Some idea of the way in which this form grows on the steep rocks of the hill lying close to the sea at Red Rocks may be got from Plate V. This hill, which is composed of an exceedingly hard and close-grained quartzite, rises to about 210 feet above sea-level. It slopes gently from the crest in a south-east and easterly direction, but the west and south-west sides consist almost entirely of perpendicular rock-walls, the smooth surface being broken only here and there by an occasional ledge or crevice on which a few plants of Erica and Calluna have established themselves.

On these steep rock-walls *Ramalina B.* is the only lichen growth. On the western faces, indeed, it is the only visible vegetation. The individual tufts are very small and amorphous, with the fronds much lacerated, and are dotted all over the quartzites, in some places sparingly, in others grouped into dark masses. Some of the smallest tufts measured less than ¼ of an inch in diameter, and they seldom exceeded ½ an inch in height. Here and there small crustaceous spots were to be seen which appeared to be the starting-points of new tufts.

On the south-western or sea-faces of the cliffs the plants are somewhat larger, but everywhere they are closely adpressed to the rock-surface. At lower levels the fronds lengthen, the growth becomes closer, and gradually assumes the characters of *Ramalina A.*

The conditions under which *Ramalina B.* grows on these steep cliffs are very severe. The surface of the extremely hard and close-grained quartzite retains little moisture and affords scanty foothold. The plants are exposed to the full force of the prevalent winds. Except in violent south-westerly gales, they are well above the spray, so that the only moisture they receive is what is carried to them by the west and south-west winds in rainy weather, or what they absorb from the atmosphere during mists and fogs, which are sometimes frequent along the coast in autumn and winter. In spring and summer there are often long spells of dry, sunny weather during which *Ramalina B.* becomes very much burnt up and brittle, crumbling at the slightest touch. Warming says (29, p. 240) that crustaceous lichens are among the first colonizers. But on these steep rock-faces there is a complete absence of any of the crustaceous species. The problem, therefore, as to how *Ramalina B.* has obtained a foothold on these very hard precipitous rocks, which are too inhospitable even for crustaceous species, is an interesting and puzzling one.

On the south-eastern slopes the incline is gentle and the rocks are more sheltered. Here the Ramalina growths are sparser, and there is a good subvegetation of crustaceous lichens, *Buellia ryssolea* being one of the commonest species. Other Buellias, *Rhizocarpon geographicum*, *Lecanora glaucoma*, and *L. polytropa* also occur. On the flatter rocks where moisture lies longer, foliaceous species such as *Parmelia conspersa*, *P. fuliginosa*, *P. saxatilis*, and others form very large colonies, extending right up to the top of the hill. On the peaty soil bordering the rocky tracts, and on the bare patches here and there amongst the heather and low Gorse shrubs, several species of Cladonia and other earth-loving lichens abound.

The small amorphous *Ramalina B.* covers large areas at Drumleck Point and at other places on the south and south-west coasts. It is essentially a form of windy situations, and is characteristic of the highest and steepest cliff-faces, especially of those with a westerly or south-westerly aspect.

Ramalina C.—The third form in the upper zone may be called *Ramalina C.* It is found in shade and shelter. The thallus is rather softer in texture, much compressed, repeatedly branched in a more or less dichotomous fashion, and the tips of the branches are all elegantly incurved. In situations where the conditions are damper, the thallus of the older plants is densely cæspitose with the branches often interlaced. *Ramalina C.* is occasionally fertile. Sward-like growths of young plants are sometimes found in the neighbourhood of the fertile specimens. The individuals are of all sizes, and are of a paler colour. From the very earliest stages they show the forked branching and curled tips characteristic of the upper Ramalinas, the incurved tips of the fronds often resembling the terminal branches of a Ceramium shoot. Whether these young growths are sporelings or merely of vegetative origin I have not been able to determine. They are not confined to the vicinity of the fertile plants, but are frequently seen amongst the barren growths or springing from the attachment areas of the older plants.

Ramalina C. is found in all the sheltered bays between Drumleck Point and the Baily Lighthouse. It is most usual on the eastern sides of the cliffs; but at Glenaveena it frequently occurs on the sheltered and shaded land-faces of the shore-rocks, many of which carry good growths of *R. scopulorum* on their tops and on their sea-faces. On the east coast *Ramalina C.* is the general form, and is found chiefly on the low rocks that jut out from the grassy sward, the fronds being larger and sometimes fertile where the rocks are sheltered by the grass and bracken. In drier and less protected situations the plants are usually small and poorly developed.

The attachments of the upper Ramalinas vary somewhat in the different forms. In *Ramalina A.* and *Ramalina C.* there is often a continuous crustaceous substratum from which the fronds spring in tufts or singly. In *Ramalina B.* the individual plants are isolated or occur in small groups, and frequently consist of little more than the small crustaceous attachments. Some very fine colonies of *Ramalina A.* are to be seen on the boundary-stones which stand about 25 feet above sea-level on the edge of the grassy bank near the Martello Tower, Sutton. These pillars are made of granite—a rock foreign to the neighbourhood—and must have been completely bare of lichen growths when they were put up about 1811 during the scare about a Napoleonic invasion. Their surface is now almost entirely covered with a close sward of *Ramalina A.* So densely are the tufts growing on the surface that they seem to arise from a continuous crustaceous substratum. Along the periphery of the very few small bare spaces left, this crustaceous thallus can be seen encroaching on the uncovered rock, with here and there small masses of compact thalline granules just beginning to put forth the upright fronds. These granules are corticate on the upper surface and contain gonidia. From the lower surface slender hyphæ similar to those described on page 90 penetrate and ramify between the irregularities of the granite, thus anchoring the upright growths to the rock surface. On the coarser and more weathered quartzite rocks, where there is a sufficiently rough surface and a fair supply of moisture, a similar coalescence of the crustaceous attachments occurs, and the Ramalina growths have a sward-like nature; but on the smooth unweathered quartzites a sward of Ramalinas does not seem to be possible; the basal hyphæ are unable to penetrate even the surface of the rock; their hold is therefore very slight and the plants are easily dislodged.

The identification of these upper Ramalinas is a matter of some difficulty. If the reaction test is relied upon, the majority of them would fall under *Ramalina cuspidata*, subspecies *breviuscula*. No. 73 of Leighton's "Lichenes Britannici," from the top of Roseberry Crags, and No. 47 of Mudd's "Lichenes Britannicorum," which Crombie includes under this subspecies, are identical with many of the Ramalinas from the upper growths. *Ramalina A.* and some of the larger specimens of *Ramalina B.* resemble var. *crassa*. The young growths of *Ramalina A.* and of *Ramalina C.* answer exactly to Crombie's description of forma *gracilescens* of the same subspecies, and might be referred to this were it not for the fact that many of their fronds stained beautiful yellow and red colours when touched with the potash. If the reaction test is disregarded, then the fact that the upper Ramalinas are almost entirely barren would point to their being forms or varieties of *Ramalina scopulorum* growing beyond the natural habitat of this species. Before leaving the Ramalina belt I would like to refer to some interesting points mentioned by Sir J. D. Hooker in "Flora Antarctica" (7), about the Ramalinas of Fuegia and the Falkland Islands, which I have only come across since my account of the Howth Ramalinas was written. He states that seven varieties of *Ramalina scopulorum* are found in particular habitats along the coasts of those countries. Of these only one variety produces fruit. This variety, he says, is identical with the English *Ramalina scopulorum*, and inhabits rocks at a considerable elevation and at a distance from the sea, while the barren forms are all found nearer the sea. This is the exact reverse of what occurs along the Howth coasts. Sir Joseph Hooker, however, points out that the conditions of climate in the situations where the fertile Ramalina grows on the Falkland Islands, approach more nearly to those in which *Ramalina scopulorum* grows in England than do the situations nearer the sea which are moister in Fuegia and the Falkland Islands than they are in our own country.

Two other species are frequent, but not general, in the upper Ramalina zone, viz. *Ramalina Curnowii* and *R. subfarinacea*.

Ramalina Curnowii was only seen on the south-west coast, where it grew in tufts dotted irregularly here and there amongst the plants of *Ramalina A.* on the upper edge of the cliffs between Old Boat-house and Drumleck Point. Some of the plants gave the *R. scopulorum* reaction, and should perhaps be referred to var. *armorica* of that species; but they are identical in form, etc., with *Ramalina Curnowii.*

R. subfarinacea is confined to the coast near the neck of the peninsula and occurs both on the Sutton and Howth sides. At Sutton it grows on rocks cropping out from the soil at some little distance from the sea near Martello Tower associated with *Lecanora glaucoma*, *Rhizocarpon geographicum*, *Parmelia saxatilis*, and *Evernia prunastri*; this last species I have not encountered on a rocky substratum elsewhere on these coasts. On the Howth side *R. subfarinacea* was much more abundant. The rocks both above and below the Cliff-walk between Casana and Kilrock were studded with small tufts of this species growing amongst *Parmelia perlata*, *P. saxatilis*, *P. physodes*, and other foliaceous lichens. It also occurred but less abundantly, with *Lecanora glaucoma*, *Rhizocarpon geographicum*, and *L. polytropa*. *R. subfarinacea* seems to prefer shady and rather moist situations. The largest plants were gathered from the steep face of a rock which was shaded and sheltered by Bracken and tall grasses, and were associated with very luxuriant and sparingly fertile plants of *Ramalina C.*

Although the Ramalina vegetation has not been previously described in detail from any particular locality, it has been referred to by Warming (29) and Ostenfeld (22), and its position on the coast has been more or less defined. In his account of the "Coast-cliff plant-formation" of the Faeröes, Ostenfeld mentions among the lithophyta of that formation the "Grimmia-Weissia Association" and the "Ramalina Association" as forming two zones on the cliffs, the latter being nearer the sea. He also reproduces a photograph by F. Börgesen of the "Coast-cliff plant-formation at the Skanse, in the vicinity of Thorshavn," in which *Ramalina scopulorum* and Placodium sp. are shown as the dominant species. Warming's *Ramalina scopulorum* belt has already been referred to, page 83. It evidently corresponds with Ostenfeld's Ramalina Association.

The Subvegetation of the Ramalina Belt.—From top to bottom of the Ramalina belt, where the growth is not so dense as to exclude too much light and air, numerous foliaceous and crustaceous lichens often find shelter amongst the tufts of the Ramalina fronds. Some, such as several of the Parmelias, *Physcia aquila*, *Buellia canescens*, *Lecanora atra*, *L. glaucoma*, *Rhizocarpon geographicum*, and many others, push their way towards the shore, and others, such as *Physcia parietina*, *Placodium lobulatum*, *Lecanora* *prosechoides*, and *Lichina confinis*, whose natural habitat seems to be the rocks about high-water mark, extend their range upwards.

This subvegetation occurs in more or less distinct zones, in the following order, descending towards the sea:—

Parmelias.
Physcia aquila.
Physcia parietina.
Crustaceous species.

The Parmelias are abundant on Howth, and extend up to the very summit of the headland. They are usually found on the most weathered rocks, in situations where they are sure of a fair supply of moisture, and where there is shelter from severe winds. Along the upper edge of the cliffs they are often associated with the two mosses, *Grimmia maritima* and *Weissia rupestris.* Here they seem to have reached their ordinary seaward limit; but the shelter and protection afforded by the Ramalina growths enable them to penetrate to lower levels, and in moist shady places they may even reach the shore. Under these conditions they are frequently accompanied by the two mosses mentioned above.

The following are the species of Parmelia most often met with amongst the Ramalinas of the Howth coasts:—

Parmelia conspersa.
  Mougeotii.
  prolixa.
  fuliginosa.

Parmelia perlata.
  saxatilis.
  omphalodes.
  physodes.

The species in the first list are of common occurrence all round the headland, but are much more abundant along the south-west coast. Those in the second list, being more alpine in character, keep to higher levels on the sunny south-west coasts, but are the usual forms on the eastern and northern coasts, where they are sometimes seen growing on rocks almost at sea-level. *Parmelia prolixa* is the most general of all the species mentioned, and the one which penetrates furthest towards the sea. It does not often grow associated with any other species, but forms large stretches of pure brownish-green growths, fertile and vigorous, on the sloping surfaces of the rocks, mainly on those with an easterly incline and in sheltered and rather shady situations. It is also common on the bare flat tops of the low cliffs of the south-west coast, just above the shaggy growths of Ramalina which fringe their outlines, and is here often intermixed with tufts of the two mosses already mentioned. On sloping rocks *Parmelia prolixa* covers bare spaces amongst the Ramalinas, and may be seen frequently competing with them for room. Where the Parmelia has established itself only a few poor fronds of Ramalina are to be seen here and there sticking up through the close mat of its thallus. On the shady rocks on the shore at Glenaveena and at several other places *Parmelia prolixa* is very abundant, covering large areas on the landward faces of the rocks and on the top growing with *Physcia aquila* and *Lecanora parella*, and pushing its way in amongst the tufts of *Ramalina scopulorum.* On the moister and more shady faces of the rocks lying along the foot of the cliffs the isidiose form grows with the species. This form was also noted in several places on the eastern coast. *Parmelia prolixa* is partial to quartz rocks, but it also occurs on the shales; and, though it seems to prefer rather smooth and flat or slightly sloping rocks, it sometimes grows on steep shady surfaces.

Parmelia conspersa and *Parmelia fuliginosa* grow at higher levels than *P. prolixa.* On the south-west coast they are usually associated with *Ramalina B.* Amongst the Ramalinas of the lower cliffs they grow as isolated patches and are of so rare occurrence that they can hardly be described as forming part of the subvegetation at these levels.

The way in which these two species cover the bare rocky tracts of the western and higher slopes of the hill above Red Rocks has been referred to already on page 92, and may be taken as typical of the mode of growth all over the headland. *Parmelia conspersa* occupies large areas on the flat rocks that lie level with the soil and on the sides and bottoms of the shallow channels over which the surface-water runs.

Parmelia fuliginosa is more usual on the rather steeper surfaces, but the two species frequently grow together. The pale yellowish-green circular growths of *P. conspersa* are always fertile, and with the dark-brown velvety thallus of the less abundant *P. fuliginosa* make a beautiful piece of colour against the background of the reddish quartzites. *Parmelia Mougeotii* is occasionally associated with them near the top of the hill and somewhat resembles *P. conspersa*; but it is easily distinguished by the more finely divided thallus, the darker central area of which is covered with little pale yellow sorediate dots.

Parmelia omphalodes and *P. saxatilis* are very scarce inside the Ramalina area on the south-west coast, but are of general occurrence on the east and northern coasts, and are sometimes found a good way down the cliffs on bare places amongst the Ramalina tufts. *Parmelia perlata* and sub-species *ciliata* are also frequent on the east coast.

Parmelia physodes is abundant on the headland, growing on the rocks and on Heather stems and on the Blackthorn shrubs. The sorediate form *labrosa* is more general than the species; but both are to be seen on bare rocks amongst the sparser growths of the upper Ramalinas all round the coast. The species is most abundant on the north coast, but is not often encountered very far down the cliff-faces. It is usually associated with *Parmelia perlata* and *P. saxatilis*. *P. physodes* is not fertile on the Howth coasts.

The Physcias.—Three species, *Physcia aquila, Ph. parietina*, and *Ph. stellaris* subsp. *tenella*, are abundant.

Physcia aquila likes shady sheltered situations, and above all a north-east aspect. It is more frequently found on the shore-rocks than on the cliffs, and often covers wide expanded areas with its brown thallus. In favourable situations the growth is vigorous, and it may often enough be seen pushing its way down the shore, swamping the Ramalinas and clearing a space for its thallus on the rock. When growing amongst the Ramalinas the thallus of *Physcia aquila* forms small circular patches round the attachment areas of the fronds. These patches increase in size, and often overgrow the Ramalinas and exterminate them. Where the Ramalina growths are close and sward-like the Physcia thallus may often be seen growing on the surface of the fronds working up towards the light and air much in the way that Ivy climbs up the bark of trees, the Physcia thallus being so closely attached to the Ramalina fronds that it seems to be epiphytic on them. If *Physcia aquila* likes shelter and shade and a north-east aspect, *Physcia parietina* may be looked for amongst the Ramalinas on sunny rocks in rather exposed situations and especially on those facing south or south-west. In several places along the Howth shores *Physcia parietina* was noticed growing on the Ramalina fronds in a way similar to that already described for *Physcia aquila*, and also on dead Thrift stems and over the Thrift leaves in the same way. *Physcia tenella* is most frequently associated with *Physcia parietina*. Other species also occur in the Ramalina belt, but not in any abundance.

The Crustaceous Species.—The connexion between the thallus of the crustaceous lichens and the rocks on which they grow is so much more intimate than is the case with the foliaceous species, that they are enabled to thrive under more adverse conditions and to colonize steeper surfaces. The crustaceous subvegetation is therefore most often found on the steeper rocks where conditions of shelter and moisture are insufficient to support the foliose forms, and it is almost the only subvegetation met with in the lower reaches of the Ramalina growths.

A complete list of the crustaceous lichens found amongst the Ramalinas would include almost all those that have been found on the rocky Howth coasts, with the exception of the truly marine species.

The impossibility of recognizing the majority of these minute lichens in the field has made it a very difficult matter to obtain more than a scanty knowledge of their distribution. The following broad general facts may be stated about the crustaceous subvegetation as a whole :—

(1) Crustaceous lichens occur throughout the belt, but are most abundant in the lower part amongst the *Ramalina scopulorum* growths.

(2) Some species are peculiar in the upper part of the belt. Others are only found in the lower part of it.

(3) Some species are more abundant in shade and others in sunny aspects.

Of those that occur throughout the whole belt, *Buellia canescens*, *B. ryssolea*, *B. spuria*, *B. colludens*, *B. myriocarpa*, *Lecanora atra*, *L. subfusca* var. *campestris*, *L. ferruginea* var. *festiva*, *L. smaragdula*, *L. simplex* var. *strepsodina*, and *Opegrapha calcarea* are some of the more frequent species.

The Buellias, as a whole, are most often met with on dry, sunny rocks; but some species prefer shade and shelter.

Buellia canescens likes shade, and is often seen in very deep shade growing either round the attachments of Ramalinas in the centre of dense growths or covering the under-surface of slightly overhanging rocks, the tops of which are clothed with Ramalinas. In such very shady situations as these the thallus is always greenish in colour. This species was more frequently seen on the eastern and northern coasts; on the south and south-west coasts it grew most often on rocks with easterly or northerly aspects. On the Howth shores *Buellia canescens* is widespread, but always barren.

Buellia ryssolea is very common amongst the Ramalinas, and often covers large areas of rock outside the Ramalina growths with its grey map-like thallus. It likes sloping shady rocks; and on the Howth coasts it is usually to be seen on those with an easterly aspect. It avoids the pure quartz rock. In many places growths of this lichen where they encountered quartz veins stopped short on either side, leaving the quartz uncovered as a conspicuous white band in the middle.

Lecanora parella is a very scarce species. It only occurs here and there on the Broad Strand on the higher parts of the beach, and at one or two places along the south-west coast. It was not seen on the cliffs, and seems to be absent from the eastern and northern coasts. The growths were most usual on rocks with a westerly slope, and among the *Ramalina scopulorum* bristles, but were nowhere vigorous. The scarcity of this lichen along the Howth shores is very remarkable, but seems to be chiefly due to the absence of high rocks and boulders on the shore above the spray-zone. *Lecanora parella* is abundant along the west coast of Ireland, being one of the dominant lichens on Clare Island (**28**), and on the coasts of N. Mayo and N. Galway, where it covers the rocks and walls at a little distance from the sea with a continuous creamy growth.

Opegrapha calcarea.—This species is extremely abundant on the grits and coarse quartzites. It was not seen on the slates and shales. Among the Ramalinas it is often associated with *Lecanora atra* and *Buellia ryssolea*; but it prefers rather more shade than do these two species. *Opegrapha calcarea* is most frequent where the Ramalina growths are scanty. The form *heteromorpha* usually grows nearer the sea than the species, and often forms a wide band on steep cliffs below the Ramalinas. At Hippy Hole it grows in this way unassociated with any other species, the blackish fruits so close together as to visibly darken the surface of the rock. On these cliffs the Opegrapha growth is a couple of yards high in places, and recognizable from some distance off. Further west behind the Needles this lichen forms similar colonies, and is here associated with *Verrucaria murina* var. *pusilla*: *Lichina confinis* was also growing at the same level in weathered holes on the rock-surface. At the east end of Broad Strand the high boulders are entirely covered with *Opegrapha calcarea* on the overhanging shady sides facing away from the sea, *Ramalina scopulorum* being abundant on the tops of the rocks with *Opegrapha calcarea* and *Lecanora atra* as undergrowths. On the east coast *Opegrapha calcarea* is also abundant; but it generally grows below the Ramalinas. On the harbour wall it forms a narrow band above the Placodiums.

Lecanora atra usually grows nearer the sea than *L. parella*. It is one of the most abundant species met with among the Ramalinas, and is often found on rocks where it is wetted by sea-water. Its most usual habitat on the Howth coasts is on the shady side of sloping rocks, where the Ramalina tufts are sparse. Plate VI shows some colonies of *Lecanora atra* and *Buellia ryssolea* in the *Ramalina scopulorum* zone at Old Boat-house.

Buellia colludens, *B. stellulata*, *Lecanora smaragdula*, and *L. simplex* f. *strepsodina* are other species that are common as subvegetation in the Ramalina belt. They are more often met with on rather steep sunny cliffs; as a rule they avoid the quartzites, and are common on the shales and schists.

The species that seem to be more or less confined to the upper part of the Ramalina belt are *Lecanora glaucoma*, *L. polytropa* *Rhizocarpon geographicum*, *Lecidea rivulosa*, *L. contigua*, and *Pertusaria concreta* f. *Westringii*. These are mostly alpine forms. *Lecanora glaucoma*, *L. polytropa*, and *Rhizocarpon geographicum* are always associated on the south and south-west coasts; they seldom penetrate more than a little way into the Ramalinas, though at Red Rocks and at Lion's Head one or two patches were seen on the rocks of the shore; these, however, may have been on the rocks before they were dislodged from the cliffs above. On the east, and more especially on the northern coasts, *Rhizocarpon geographicum*, *Lecanora glaucoma*, and *Pertusaria* *concreta* f. *Westringii* form an almost continuous covering on the rocks both above and below the Cliff-walk, descending in some places nearly to sea-level, and are very common as undergrowth among the Ramalinas of these coasts. On the north coast *Lecidea rivulosa* and *L. contigua* are associated with them, and all grow together on Ramalina-covered rocks.

All the species mentioned above are invaders from higher levels, working their way towards the shore. Those species that on sheltered shores grow naturally below the Ramalinas, such as *Placodium murorum*, *P. lobulatum*, *Rhizocarpon alboatrum*, and *Lecanora prosechoides*, are often found in exposed areas among the Ramalinas.

Rhizocarpon alboatrum is very common on the Howth coasts, and sometimes covers considerable areas of rock unassociated with any other species. It is most general on the coarse quartzites; and when it occurs as a pure growth is found on rather steep rocks, in shelter, between the orange belt and the Ramalinas. Where the seas are rough, *Rh. alboatrum* rises amongst the Ramalina tufts, but on low sheltered shores it is very abundant in the upper part of the orange belt, where it is often associated with *Rinodina exigua*, *Buellia myriocarpa*, *Lecanora Hageni*, *L. umbrina*, and others.

The subvegetation of the eastern and northern coasts is largely composed of species that are common in alpine and upland regions. This is in a great measure due to the steepness of the rocks and also to the colder and drier conditions prevailing on these coasts. On the sunnier and moister south and south-west coast the subvegetation is almost entirely made up of lowland forms. On these coasts the rocks dip at rather low angles towards the east and south-east, and often present steep precipitous faces towards the west and south-west. The subvegetation is always more plentiful and there is a greater preponderance of foliaceous species on the gentler slopes where moisture lies longer and where there is shelter from the prevalent winds. The composition of the rock and the amount of weathering of the surface have also a great influence on the abundance and nature of the species. The complete absence of crustaceous lichens from the steep walls of hard quartzite at Red Rocks that are covered with growths of *Ramalina B.* has already been remarked upon. Where the cliffs are composed of softer rocks, as the shales and grits at Hippy Hole and near the Needles, the steep westerly sides support a thinner growth of Ramalinas, but are completely clothed with a covering of crustaceous species, such as Buellias, Lecanoras, and others.

(2) **The Orange Belt.**

Below the Ramalinas and between them and the sea several deep yellow or orange-coloured lichens form a belt of varying width all round the coast.

In summer the colour of these lichens is so brilliant that the belt is easily recognized from a considerable distance. It may be very distinctly traced on the cliffs near the Needles and on the rocks of the Broad Strand as one walks along the top of the cliffs from Drumleck Point towards Baily. On the east coast it may also be seen on the western and southern slopes of the lower cliffs. In winter or during long spells of wet weather it assumes a greenish-yellow colour and is not quite so conspicuous.

The most abundant species and those which give the characteristic orange colour to the belt are :—

Physcia parietina.
Placodium murorum.
    tegularis.
Placodium decipiens.
    lobulatum.

In addition to these bright-coloured lichens several others less noticeable on account of their grey or nondescript colour, also form important elements of the belt. The chief of these are :—

Lecanora prosechoides.
    umbrina.
    Hageni.
Rhizocarpon alboatrum.
Biatorina lenticularis.
Rinodina exigua *var.* demissa.
Opegrapha calcarea *f.* heteromorpha.

Of the species in the first list *Physcia parietina* is more yellowish in hue, and usually forms a distinct colour band above the Placodiums. The effigurate thallus of the rarer *Placodium murorum* is more frequently seen in the middle of the belt, while the more crustaceous *P. lobulatum* comes lowest on the shore. This arrangement is well seen on several parts of the Broad Strand. Here on the stones and low shore-rocks that lie just above the ordinary high-tide level, *Placodium lobulatum* grows abundantly, covering the rocks with a continuous sheet of brilliant colour. In some places it extends down the shore into the *Pelvetia cananiculatus* belt. At this level, however, the Placodium does not form a continuous sheet, but variegates with greenish-orange spots the black crust of *Verrucaria maura*. *Placodium murorum* and *Physcia parietina* are also occasionally found in the Pelvetia belt, but the normal position of these two species is at a higher level, where they usually form two consecutive zones in the order stated from below upwards. All three lichens are frequently submerged at high spring-tide.

On the sheltered sunny beach of the bay below Earlscliffe, the waves roll gently over the rocks with scarcely any splash except in the roughest weather. But a little further to the east higher and bolder rocks occur standing rather more out to sea. As one approaches these rocks the sequence of the three lichens is broken ; *Physcia parietina* has moved up under the Ramalinas for shelter, and a greenish grey crustaceous lichen (*Lecanora prosechoides*) has intruded itself between the Placodiums and the Ramalinas, covering the steeper sea-faces of the rocks, and especially those with an easterly or south-easterly aspect, with a close and continuous crust. As the shore curves back again into shelter to form the bay west of Lion's Head (Stella Maris), *Lecanora prosechoides* sinks out of sight amongst the Placodiums, and *Physcia parietina* emerges from under the Ramalinas to occupy its normal position in the upper part of the Orange Belt. The same variations may be traced on the cliffs. On sheltered and gently sloping faces *Physcia parietina* and the Placodiums form two more or less distinct parallel bands overlapping in the middle. But where the surface is steeper and where the seas are rougher and there is rather more dash of water, *Physcia parietina* and *Placodium murorum* seem to disappear, leaving *Placodium lobulatum* the sole representative of the orange lichens, while *Lecanora prosechoides* at once makes its appearance above it as a greyish band, varying in width from 3 to 6 feet according to slope and exposure. Where *Placodium lobulatum* and the Lecanora meet the rock-surface is variegated with spots of grey and orange. On the steep quartzites of the Needles and at other places round the coast, the band of *L. prosechoides* is also frequently interspersed with spots of a darker grey lichen which agrees with Weddell's *L. prosechoides* var. *aeruginosus*. Although *P. lobulatum* and *L. prosechoides* are frequently found growing together, *L. prosechoides* is more common on the shady steep eastern faces of the cliffs; while *P. lobulatum* likes sunshine and is more usual on the south and south-westerly slopes, on very sunny exposed rocks being always of a much browner colour than usual.

Physcia parietina, like most foliaceous lichens, requires a fair supply of moisture and some protection from severe winds. On sunny sheltered rocks it is of a brilliant colour and is always well fertile. On more exposed rocks and in shade it seldom produces apothecia. In deep shade the thallus is always of a greenish colour.

Some of the most extensive colonies of *Physcia parietina* found on the Howth coasts occur on the top of the cliffs at Drumleck Point. The cliffs here rise almost perpendicularly, and at about 50–80 feet above sea-level slope back at a low angle like the roof of a house. These rocky slopes pass upwards into a gravelly bank studded with Statice, Crithmum, and Plantains. The rock-surface near the edge of the cliff is covered with a mixed growth of *Placodium lobulatum* and *Lecanora prosechoides*, with here and there a few plants of *Spergularia rupestris* and *Cochlearia officinalis* in the cracks and fissures. Further from the edge where the surface is rougher and on the stones embedded in the bank above, *Physcia parietina* is a sheet of colour.

In the open the thallus is well developed, though it is seldom fertile, but where it covers the stones amongst the flowering plants which are close enough to give shelter without too much shade, it is usually well supplied with apothecia. On these rock-slopes, which face towards the west and south-west, *Physcia parietina* gets all the afternoon sun, and at the same time faces the prevalent winds, which bring a good supply of moisture in wet weather. These alternate bakings and soakings, combined with a fairly sheltered habitat, seem to be the conditions under which *Ph. parietina* thrives best. Along the eastern coasts *Ph. parietina* forms sheets similar to those described above, but it is less often fertile.

Placodium murorum is much less common on the Howth shores than either *P. lobulatum* or *Physcia parietina.* It is difficult to make out what are the conditions under which it thrives best. On sheltered shores it usually forms a narrow band below *Physcia parietina*, and in more exposed places, as on the tops of the cliffs at Drumleck, it is mixed up with the Physcia growths. It seems to be absent from or very scarce on the east coast, where it is usually replaced by *Placodium tegularis.*

Placodium tegularis is very frequent on the eastern and northern coasts, and occurs but seldom on the south and south-west shores. It grows very freely on the cliffs at Gaskin's Leap. The rocky slopes of these cliffs are very similar to those at Drumleck Point. The aspect also is mainly south-west, but the situation is more shaded by surrounding high rocks. The rocks are shales, and the surface is rough and much broken up, and seabirds frequently congregate on it. On the sunnier projections *Placodium tegularis* is of a deep orange colour and covered with apothecia; but in shady crevices and on the easterly sides of the projections it often covers quite large patches of the surface with greenish barren growths.

Placodium decipiens forms good colonies on the Needles and on similar high quartzite rocks. Like *P. tegularis,* it is only fertile on sunny slopes where the aspect is westerly. In shade, as, for instance, on the north-east faces of the Needles, it forms a thick close greenish-yellow covering of barren growths.

The species of the second list are frequent all round the coast. In shelter on the flat shores of the Broad Strand they are inconspicuous and grow chiefly amongst the orange lichens, seldom forming patches of any extent. On the cliffs and on steep rocks in more exposed situations, however, they often form pure colonies, sometimes of considerable area, above the orange lichens. The way in which *Lecanora prosechoides* grows on steep quartzite rocks where there is a moderate amount of wave-action, has been already described on page 103, the growths of *Opegrapha calcarea* f. *hetero-*

morpha on page 100, and those of *Rhizocarpon alboatrum*, page 101. All three seem to be abundant round the peninsula.

Epiphytes.—Leciographa parasitica was noted in one or two places on the Broad Strand growing on *Physcia parietina*, and in almost every specimen of *L. prosechoides* that was examined from all parts of the coast the apothecia were found to be infested with *Arthonia varians.*

(3) The Lichina Vegetation.

Though *Lichina confinis* belongs to the semi-marine lichens and *L. pygmaea* to the marine group, I have followed A. D. Cotton (**3**) in considering them together. Both species are widespread on the Howth coasts, *L. confinis* usually occurring along the inner fringe of high-tide mark between the orange lichens and *Verrucaria maura*, and slightly overlapping both these belts, *Lichina pygmaea* at a lower level below *Verrucaria maura* and usually defining the lower limits of this lichen, but in certain situations extending down as far as low neap-tide. The seaweed Pelvetia may be taken as forming the upper limit of *L. pygmaea* and the lowest level at which *L. confinis* grows on these shores. Both species are found in sheltered and in exposed situations, but *L. pygmaea* is absent from the most sheltered parts of the south coast, and neither species was seen on the exposed and outer face of the Needles.

Vigorous growths of the Lichina vegetation occur on the south-west coast between Red Rocks and the Needles. At Old Boat-house *Lichina confinis* covers a fairly wide area on the top of a rough rocky plateau, *L. pygmaea* forming irregular patches on the sea-faces of the same rocks. On steep rocks with an easterly aspect in this same locality *L. confinis* forms a narrow zone of about 4 inches wide between a broad band of *Lecanora prosechoides* and *Verrucaria maura*, whilst others facing west showed the following lichen and algal zones from top to bottom:—

Placodium lobulatum (spotted with a few tufts of *Lichina confinis*).
Verrucaria maura.
Pelvetia cananiculatus.
Lichina pygmœa and *Verrucaria striatula* f. *continua.*
Verrucaria mucosa.
Barnacles on which *Arthopyrenia foveolata* was abundant.
Fucus sp.

The three upper bands were narrow ones.

On the western and weather side of Drumleck Point *Lichina confinis* grows thinly on the top of the cliff amongst a poor growth of *Ramalina* *scopulorum* more than 50 feet above sea-level. Rounding the Point one finds on the eastern side the Lichinas forming two zones at much lower levels on the cliff-face, a narrow band of *L. confinis* running along the upper line of the *Verrucaria maura* belt; while below *V. maura*, *L. pygmaea* forms a marked band about 10 inches in depth. At the same level as *L. pygmaea* on this cliff-face, but nearer the land and more in shelter, *Catanella opuntia* grows in an equally wide band, which at a little distance looks like a continuation of the *L. pygmaea* zone. This alga, which is abundant round the Howth coasts as an undergrowth of *Pelvetia cananiculatus* and *Fucus spiralis*, forms in shade, where these species are absent, a band on the steeper cliffs below *V. maura.* On the Needles and on Drumleck Point Catanella often grows in close proximity to *L. pygmaea*; the latter, is however, always found more towards the open sea and is easily distinguished by the hard, crisp feel of the growth, Catanella being soft and yielding to the touch.

On the inner face of Needles *Lichina confinis* forms a band 4–5 inches wide above *V. maura* and between it and a band of *Placodium lobulatum* 3 feet wide. On the western face of the pinnacles both Lichinas occur in their relative positions; but from the outer and exposed faces both species are absent.

On the sloping cliffs behind the Needles the zonal distribution of the two species is very well seen, the zone of *L. pygmaea* being 3 feet wide and running along the cliffs for 30 to 40 yards. Below the cliffs on the rocky foreshore *L. pygmaea* also occurs and is distributed throughout the whole neap-range, the most vigorous patches being found near low water on the rather higher parts of the rocks which project up above a close mat of *Laurencia pinnatifida* and *Gigartina mamillosa.*

On the sheltered shore below Earlscliffe *L. pygmaea* is very scarce and the *Lichina confinis* growth is thin and occurs lower on the shore than usual, in some places being seen amongst the Pelvetias; but good growths of both species are found on the high rough rocks and boulders that lie about the middle of the Broad Strand, *L. pygmaea* growing most vigorously on the steep sea faces and western sides of the higher boulders where it meets the wind and is splashed by the waves, forming patches of considerable size around the attachments of Pelvetia and *Fucus spiralis*, *Lichina confinis* growing a little distance above Pelvetia, and in some cases rising up into the Ramalina belt. On the east coast *Lichina confinis* and *L. pygmaea* form two narrow bands above and below the *Verrucaria maura* belt respectively; in the few places examined *L. pygmaea* was most often seen about 4 feet below high-water mark of spring-tides.

From the above account it will be seen that *L. confinis* and *L. pygmaea* are both widespread along the Howth shores, *L. pygmaea* preferring rough surfaces and steep rocks which face the breeze and around which the sea breaks. Its range extends from the lowest limits of *Verrucaria maura* as far as low neap-tide and the growth is best developed in the upper part of its range among the Pelvetias and immediately below them. It is most common on the rough quartzites and grits, but it also occurs on the slates and sometimes on the pure quartz bands. *Lichina confinis* grows usually above *V. maura* in sheltered places; on flat shores its range is, however, frequently coextensive with that of *V. maura.*

Where *Lichina confinis* is found growing high on the cliffs beyond its natural habitat, in situations where it only receives occasional showers of spray and is mainly dependent on rain for moisture, the fronds are seldom fertile. The greatest height above sea-level at which it was noticed on the Howth coasts was that quoted from Drumleck Point, viz., 50 feet. On the west coast of Ireland, however, *L. confinis* often rises to very much greater heights. I have gathered it on the tops of the cliffs of High Island, off the coast of Galway, 200 feet above sea-level.

The zonal distribution of the Lichinas on the sea-shore has long been known to botanists. Sir William Hooker and Nylander were among the first to draw attention to it. Nylander, in his account of the Lichens of Pornic (**20**), describes *Lichina confinis, Verrucaria maura,* and *L. pygmaea* as forming three distinct zones, *L. confinis* along the upper part and *L. pygmaea* along the lower part of the beach, with *V. maura* in an intermediate position; and he says that all three species are wholly submerged by the incoming tide. Weddell describes a similar arrangement on the coast of l'Ile d'Yeu, but points out that during low tides *L. confinis* is often not submerged at all. On the Howth coasts this is also the case. A. D. Cotton's account of the Lichina communities in the Clare Island Survey area shows that the same zonal distribution exists on the west coast of Ireland. The Lichina vegetation of the Howth coasts is very similar. The growth on the low sheltered shores of Broad Strand resembles very closely that described by Mr. Cotton for the coasts of Clew Bay and Achill Sound.

(4) The Verrucaria maura Belt.

This belt normally occupies the part of the shore between high neap- and high spring-tide marks. It lies chiefly above *Pelvetia cananiculatus*, but where this seaweed is absent *Lichina pygmaea* frequently determines the lower boundary. On flat shores *V. maura* sometimes seems to occur throughout the whole range of Pelvetia; but, when this is so, examination always shows that *V. maura* is found only on the rather higher parts of the rocks, where it is submerged later than Pelvetia by the incoming tide. The belt is nowhere a wide one on the Howth coasts, and is always more restricted in area on steep shores in sheltered localities. On the steep cliffs it is often only a couple of feet wide; but it covers a greater extent on the low shore of Broad Strand, at Stella Maris, measuring more than a couple of yards in places. The limits are always well defined in sheltered localities, but where the coast is much broken, and there is spray, *V. maura* grows at higher levels, and the outlines of the belt are very irregular, the growth in the upper part being scattered, and rising on the rocks as far as the spray reaches. On Drumleck Point, and at various other places on the south-west coast, *V. maura* is found in spots and streaks among the Ramalinas. But nowhere, even in the most exposed parts of the coast, does *V. maura* cover the cliff-faces with a continuous crust more than 8–10 feet wide.

The belt is most conspicuous and best developed between Red Rocks and Baily on the south-west and south coasts. Along the Sutton Creek from Old Quay to Red Rocks the shores are very flat, with a few low rocks lying above ordinary high tide-mark. The bottom of the sea is sandy, and the constant scour of the sand-charged water over the rocks prevents the growth of crustaceous lichens. *V. maura* is here found only in small patches in hollows and cracks about high spring-tide level. At the Martello Tower, however, where the rocks stand higher, a great part of the surface is closely covered with a good growth of the lichen. Between these high rocks and Red Rocks the foreshore is composed of strewn rocks and boulders on a sandy bottom, and all crustaceous lichen growths are conspicuously absent from the rocks between tide-marks. Along the Broad Strand *V. maura* grows abundantly. In some places the rocks are so uneven that it is difficult to see the limits of the belt, but, on the boulders lying above Pelvetia at Stella Maris, where the shore sweeps round towards Lion's Head, these are beautifully defined. On the steeper east coast the belt is narrow, and lies mainly between the two Lichinas, which form two well-marked bands, *L. confinis* above and *L. pygmaea* below it on the rocks.

Verrucaria maura is the dominant lichen in the belt, but others, *V. memnonia*, *V. prominula*, and *V. aquatilis*, contribute to its formation, and are characteristic of certain situations. *V. scotina* also occurs occasionally. Besides these a number of other lichens, invaders from the belts above and below, are often found. *Arthopyrenia halodytes*, *A. leptotera*, and *A. halizoa* are some of the most frequent from the lower belt, but they are not able to endure the same amount of exposure to sunlight and air as *V. maura*, and do not grow in the open at these levels, but always occur in shade, or in cracks and crevices, where they find conditions most nearly analogous to those of their natural habitat. The invaders from the orange belt are, as a rule, only associated with *V. maura* along the upper limits; but on the flat shores of Broad Strand, and in several other sheltered localities, *Placodium murorum* and *Lecanora prosechoides* were seen in places spotting the dark crusts of *V. maura* throughout its whole range. *Verrucaria maura* normally forms extensive stretches of pure growth about the middle of its range, and seems to be best developed in rather exposed and sunny situations. It grows on both flat and sloping rocks, on smooth and broken surfaces, on the quartzites and on the shales, covering them with a close, dark, scaly crust. It avoids the pure quartz bands, and when growing on the coarse-grained quartzites and grits the thallus is always thin and scattered, being often indicated only by a faint brownish stain. The whole *V. maura* belt is submerged only during the highest spring-tides; but even during these periods the upper part of the belt is covered only for a very short time each day, and in calm weather in summer, during neap-tides, the main part of the belt is often left exposed for a long time. Where *V. maura* grows under drier conditions than usual, the thallus is much more finely areolated and scabrid. Weddell has named this form var. *aractina*. On the dry sunny shores of Broad Strand var. *aractina* forms a narrow zone along the upper part of the belt, but it was not noticed on the cliffs. Towards the lower limits of its range, where it is more often wetted by the sea, and in shade, *V. maura* gradually passes into the var. *memnonia*, which is characterized by having the thallus more gelatinous, and almost devoid of areolations.

The natural habitat of *V. memnonia* is the lower part of the *V. maura* belt; but on shady cliffs it seems sometimes almost to replace *V. maura*. It is most commonly seen on the cliffs, where it may be distinctly traced as a narrow zone immediately below *V. maura*, from which it is easily distinguished by its more shiny thallus. On the shady side of Drumleck Point, at Worn Hole, and at various other parts of the coast, *V. memnonia* forms good colonies on the cliff-faces 3 to 4 feet above ordinary high-tide level; and on the sloping cliffs behind the Needles, and at Casana on the east coast, it is often found under the narrow fronds of *Porphyra linearis*. *V. memnonia* is usually fertile on the Howth coasts, except when growing in deep shade, or at lower levels than usual.

On the south side of Balscadden Bay *V. memnonia*, *V. prominula*, and *Arthopyrenia halodytes* are associated, the reddish-brown spots of *A. halodytes*, some of them nearly an inch in diameter, conspicuously variegating the dark thallus of the Verrucarias. *A. halodytes* is found also amongst the *V. maura* growths in cracks and fissures where spray lies, and on the sides and bottoms of small rock-pools above ordinary high tide. On the top of the rocky plateau at Old Boat-house, and at other places, there are numerous small shallow rock-pools of this nature, which are often dry in summer, but in winter are filled by spray and rain. On the sides of the deeper pools, *A. halodytes* is sometimes seen as a narrow band below *V. maura*, and is here often associated with the crustaceous red alga *Hildenbrandtia prototypus*, and the bottoms of the shallower hollows are also frequently coated with a crust of these two species. These situations are similar to those which Weddell describes as the usual habitat of *A. halodytes* on the coast of France.

At various places round the coast fresh water flows over the rocks. At Red Rocks and White Water Brook small rills enter the sea, at other places small springs occur on the top of the cliffs which overflow and moisten the surface or drop on the shore below and spread over the rocks when the tide is out. Two or three of these small waterfalls occur on the north coast between Balscadden Bay and the Nose. At other parts surface drainage, which is considerable in winter, runs down the grooves and cracks on the cliff-faces, and on the Broad Strand large tracts of rock on the beach are kept constantly damp by fresh water oozing from the base of the cliffs. These areas are often easily recognized by the presence of the green seaweed *Enteromorpha intestinalis.* In situations such as the above-mentioned the continuity of the *V. maura* belt is broken and another dark encrusting vegetation appears, which is composed mainly of colonies of *V. aquatilis.* On the wet cliff-faces these growths are seen as dark streaks, and extend right up to the top in many places. Where the supply of fresh water is scanty, *V. aquatilis* does not descend below the *V. maura* belt; but where the flow is constant and fairly plentiful it covers much larger areas and is found as far down the shore as the influence of the fresh water lasts. On the south shore of Balscadden Bay *V. aquatilis* forms black patches here and there on the moist cliff-faces growing in the upper part with the moss *Weissia verticillata*, and lower with *Enteromorpha intestinalis*, which begins a few feet above high spring-tide mark, and accompanies *V. aquatilis* throughout the Pelvetia zone down the shore, well into the *Fucus spiralis* belt.

On the damp shaly rocks lying above high-water mark on the Broad Strand a similar, but much sparser, growth of *V. aquatilis* occurs. *Arthopyrenia halodytes* is here associated with *V. aquatilis* on some of the rocks, and on others it covers the surface with a warm brown stain of pure growth. *A. leptotera* is also frequent. This last species does not, however, extend up the beach as far as *A. halodytes*, but is confined to crevices and to the moister parts of the rock-surface.

On the Harbour walls, where there is a more or less constant supply of fresh water from surface drainage, *V. aquatilis* forms a thin interrupted band just above a copious growth of Enteromorpha. In several places *V. prominula* var. *viridans* was found associated, and occasional small patches of *V. maura* occurred here and there. The thalli of the Verrucarias were, however, so obscured by a copious growth of Gloeocapsa and other unicellular algae that the species were not easily recognized.

The *Verrucaria maura* belt forms a transition zone between the maritime and the truly marine lichens, and constitutes Weddell's semi-marine group. It has been referred to by various lichenologists; Nylander describes it as forming a definite zone on the shores of Pornic limited by the two Lichinas; Weddell defines its habitat on the coast of Brittany as rocks that are submerged or wet by the waves at high tide. Warming records it as being widespread along Arctic and northern seas, and says that on Swedish and Danish coasts it occurs on rocks that are "very frequently wet," and he quotes Beck von Mannegetta's (**15**) account of the "often pitch-black crusts" of *V. maura* that colonize the wet rocks along the Adriatic. For the British coasts, A. D. Cotton (**3**, p. 20) describes it as forming, on the exposed coasts of the Clare Island Survey Area, "a band a short distance above the Pelvetia zone"; and Miss Lorrain Smith (**28**, p. 3) describes "the rocks bordering the sea," and the "great cliffs" of the north-west coast of Clare Island as "black with an unbroken growth of *Verrucaria maura*."

On the exposed coasts of the West of Ireland *Verrucaria maura* often grows at great heights above sea-level. On the small uninhabited High Island off the coast of County Galway, where the seas are severe and the island in winter storms is swept from end to end by the spray, *V. maura* and *Lichina confinis* form a zone along the top of the cliffs in places more than 200 feet above the ocean.

(5) **The Belt of Marine Verrucarias.**

This belt comprises all the marine encrusting lichens, those that are submerged by the incoming tide for a longer or shorter period each day. It lies mainly inside neap range, but on low flat shores, where the tides rise and fall gently, straggling colonies are found as far down as the lowest spring-tide level. The lichens of this belt form a more or less continuous covering on the rocks of the upper part of the beach, but the growth becomes meagre and spotty nearer low water. The dominant species are *Verrucaria microspora*, *V. striatula*, and *V. mucosa*; and *Arthopyrenia halodytes*, which has already been mentioned in connexion with the *Verrucaria maura* belt, is also abundant in the upper part of this belt. Besides these, three other species, *Arthopyrenia halizoa*, *A. leptotera*, and *A. marina*, are sometimes present, but are nowhere plentiful; *Arthopyrenia foveolata* and *A. litoralis* are common on barnacles, but as the two last-mentioned species do not seem to grow on the silicious rocks, they are not included as elements of the belt as developed on these coasts; their distribution is given in connexion with the marine lichens of the limestone area.

The composition of the belt varies chiefly with the nature of the rock-surface. On smooth rocks *Verrucaria mucosa* is the prevailing species, while *V. microspora* seems to be more abundant on the rough quartzites and grits. Both species are very common round the coasts, and have a wide vertical range, extending on the south coast from the Pelvetia zone down to the lowest ebb-tide mark; but the main part of their growth lies in the *Fucus spiralis* belt.

Verrucaria microspora seems to be better able to accommodate itself to a variety of conditions than *V. mucosa*, as it grows on both rough and smooth surfaces, and on hard and soft rocks. Its vertical distribution also seems to be slightly greater; it is more often found at Pelvetia level, and also is the more usual of the two species on the rocks inside the Laminaria belt. When growing on the soft shales, the thallus of *V. microspora* is almost evanescent, and the perithecia are somewhat larger than usual. Along its upper and lower limits it also varies. In its lowest limits *V. microspora* is exposed to the air for only a very short time each day during spring-tides, and can receive only very little fresh water, while at neap-tide it is completely submerged for days at a time. The spores of specimens from low spring-tide level were mainly abortive; but one or two fertile spores were found, which were slightly longer and narrower than normal, but otherwise characteristic. Considering that both *V. microspora* and *V. mucosa* are able to live submerged for long periods, it is rather curious that neither species was seen in any of the small rock-pools in the upper part of the shore. Amongst the Pelvetias, *V. microspora* var. *mucosula*, in which the perithecia are smaller and more numerous and the spores rounder than in the species, occurs. It is frequent on the Broad Strand and at several other places on the south coast. This variety seems always to grow at a higher level than *V. microspora*, and is often associated with *Arthopyrenia halodytes* and with *Verrucaria striatula*, f. *continua*, which is very common on the Howth shores. *V. striatula* f. *continua* is very abundant along the south-west coast between Red Rocks and the Old Boat-house, and often forms a narrow zone below Pelvetia on the steep rocks. It is also found on the cliffs behind the Needles at the same level, and on the rocks of Broad Strand. The species, in which the thallus consists of small, scattered ridges, seems to be characteristic of steep rock-faces, in rather exposed parts, usually growing with *Lichina pygmaea* and *V. mucosa*. It is nowhere plentiful, and appears chiefly to grow only on pure quartz rock and on the close-grained quartzites. On Broad Strand a specimen that seems to be *V. striatula* was collected from the volcanic dyke at low spring-tide level, but the spores were abortive. Both the species and the form were noted on the coasts of Galway at Cleggan, growing in the same way as on the Howth coasts—the species on steep shady rock-faces, in rather exposed places, with *Lichina pygmaea* and *V. mucosa*, and the form on both sloping and steep rocks, at the same level on the shore, but more in shelter.

Some of the most extensive colonies of *Verrucaria mucosa* seen on the Howth coasts cover the boulders of the beach at Stella Maris. This beach below high neap-tide level is composed of rather small smooth quartzite boulders, pretty much of a size. A belt of Pelvetia, about a yard wide, runs along the upper part of neap-range, and immediately below this *Fucus spiralis* forms a band of thin growth among the boulders of nearly double this width. The remainder of the foreshore is covered mainly by a wide bed of *Ascophyllum nodosum*, which stretches down almost to low-water. In the *Fucus spiralis* belt *V. mucosa* is very conspicuous as a continuous dark-green, almost black band of greasy appearance, stretching along the shore, clearly separated from the dull black band of *V. maura*, which covers the larger boulders above it. Amongst the Ascophyllum plants every piece of bare rocky surface is covered with the same dark greasy-looking stain. On the upper part of the beach *V. mucosa* completely covers both the tops and the sides of the boulders, but nearer low-water it is mainly found on the upper surfaces, the sides being covered with the reddish crusts of *Hildenbrandtia prototypus*. The growth of *V. mucosa* on this beach is almost pure, and somewhat resembles that described by Mr. Cotton as covering the boulders at Portlea, Clare Island. *V. mucosa*, however, reaches higher levels on the more sheltered Howth shores, and has a wider vertical distribution. When growing in shade the thallus of *V. mucosa* is of a bright green, and contrasts with the darker colour of the lichen when found on flat rocks exposed to the sun. In a small narrow cave in the cliffs behind the Needles, accessible at low-water, the transition from the ordinary dark colour to the bright-green shade-form is easily traced. Near the mouth of the cave *V. mucosa* forms a dark band 4 feet wide on the sides, which gradually passes into a band of brighter green as the light decreases. In the furthest recesses (the cave is only about 15–20 feet deep) *V. mucosa* is entirely replaced by the red alga *Hildenbrandtia prototypus*, and a velvety growth of *Rhodochorton Rothii* covers boulders on the floor. On the north coast the sides of several shallow caves exposed at low tide are covered in a similar way with a beautiful emerald-green growth of *V. mucosa*.

The partiality of *V. mucosa* for smooth surfaces is plainly seen on the Broad Strand. On the smooth boulders at Stella Maris it covers a wide area with an almost pure growth; but below Earlscliffe, where the foreshore is mainly bed-rock of various textures and of different heights and degrees of smoothness, *V. mucosa* forms an interrupted growth, and occurs chiefly on the harder rocks and smoother surfaces. *V. microspora* is much more abundant on this part of the strand. Similarly on the cliffs, *V. mucosa* seems to be altogether absent from the grits. In this connexion it is worth noting that I recently explored a small tract of the coast of Antrim near Fair Head, where the rocks are coarse Carboniferous sandstone, without finding any trace of *V. mucosa*; while *V. microspora, Lichina pygmaea*, and *Arthopyrenia halodytes* were abundant.

The details as to the habitat and distribution of *V. mucosa* in other countries are exceedingly meagre. Weddell, who searched the shores of l'Ile d'Yeu in vain for it, says it has been observed on the coast of north Europe, but that the only actual localities from which it is known in France are in the neighbourhood of Cherbourg, where it grows on stones in the beds of small rivers or streams flowing into the sea, in places where the current is most rapid. This habitat is rather similar to that in which *V. aquatilis* grows on the Howth coasts, but, though carefully looked for in places where fresh water flows, *V. mucosa* was nowhere met with on these coasts.

Arthopyrenia halodytes has such a wide vertical range, and grows in such a variety of situations, that it is very difficult to say what is the natural habitat of this species on the Howth shores. In so far as it enters into the composition of the *V. maura* belt, its distribution has been gone into already and is very similar to that given by Weddell for the coasts of l'Ile d'Yeu, where it is chiefly associated with *V. maura* and *L. confinis.* On the Howth coasts *A. halodytes* has a much wider vertical range, but it seems to be most abundant just above and below high neap-tide level. In the lower Verrucaria belt it is usually associated with *Verrucaria microspora* and *V. striatula* f. *continua.* On sunny sheltered shores both *A. halodytes* and *V. microspora* are common amongst the Pelvetia growths, and on Broad Strand extend from this level down as far as low neap-tide. *A. halodytes*, is however, much less abundant at low levels than *V. microspora.* On steep rocks, at several places between Red Rocks and Old Boat-house, *A. halodytes* sometimes forms a narrow warm-brown band on the rough quartzites between *V. maura* and Pelvetia, but it also grows with *V. microspora* and *V. striatula,* f. *continua,* in the Pelvetia belt and at lower levels on these same rocks. On the more exposed coasts *A. halodytes* is less commonly seen in the lower Verrucaria belt. *A. halodytes* var. *tenuiscula* is found at various places on the south coast. It grows chiefly on the hard smooth quartzites, sometimes appearing as small light-coloured patches amongst the *V. mucosa* growths.

The chief requirement of *A. halodytes* seems to be a moist situation; and on the Howth coasts it appears to be rather indifferent as to whether the moisture is salt or fresh water.

Algologists have usually grouped *V. maura* and the marine lichens with Hildenbrandtia, and have treated them as one association. Börgesen (2, p. 711), in describing the "Hildenbrandtia formation" on exposed coasts of the Faeröes, says: "It covers the rocks with a dense mat of various colours to a considerable height, i.e. up to more than 2 feet above the level of the sea and down into the coralline formation. The uppermost part of it mostly consists of lichens which, according to the Rev. Deichmann Branth, belong to different species of Verrucaria." A. D. Cotton describes a similar vegetation as growing on the coasts of the Clare Island Survey district, from both exposed and sheltered shores, under the name of the "Hildenbrandtia-Verrucaria Association," and suggests including in the association all the littoral encrusting species that are soft and not calcareous. He mentions *Verrucaria maura*, *V. mucosa*, and *Hildenbrandtia prototypus* as being the characteristic species.

On the sheltered Howth coasts Hildenbrandtia has the same distribution as the lower Verrucarias, extending throughout neap-range, being most conspicuous and best developed nearer low-water mark, while the Verrucarias form a more continuous growth on the upper part of the shore. The red or yellowish-red crusts of Hildenbrandtia form such a striking contrast in colour to the green and blackish-green thalli of the Verrucarias that it is a comparatively easy matter to trace their distribution. They seem rather to form two overlapping belts, Hildenbrandtia ascending the beach and extending its range upwards under the protection of the larger algae or by occupying the bottoms of shallow pools or the cracks and crevices in the rocks; the Verrucarias, on the other hand, taking advantage of every bare spot to penetrate further towards the sea, in the lowest parts colonizing those projections that are first exposed to sun and air as the tide retreats. On the cliffs the true sequence of the two belts is more easily seen. In the small cave referred to, page 113, Hildenbrandtia forms a distinct zone on the walls below *Verrucaria mucosa*, just inside the entrance; and at various other places near the Needles Hildenbrandtia also mainly occurs below the Verrucarias, both growths rising higher in shade than in the more open situations. On the more exposed and broken parts of the coasts Hildenbrandtia may occasionally be seen in the *Verrucaria maura* belt; but where this is so, the two plants live under quite different conditions; moisture and shade seem to be necessities for the Hildenbrandtia, and at this level it is found only in the cracks and hollows and on the bottoms of shallow pools; whereas *Verrucaria maura* grows best on rocks fully exposed to sun and air and can endure drought for many successive days.

ii. *Calcareous Rocks.*

The Carboniferous limestones which overlap the Cambrian rocks at the western end of the peninsula stretch from Sutton to Balscadden Bay. On the Sutton side, though they reach the shore, they are almost entirely obscured by the Boulder-clay; but near the village of Howth on the north coast they are exposed in the east corner of the Harbour, and on the west of Balscadden Bay, where they form a long, low-lying stretch of foreshore with some higher strata about high-tide mark. Plate VIII, taken at about half-tide, shows the lie of the rocks. Behind them are the stratified drift-banks on which Balscadden Terrace is built. The waves wash the base of these banks during high spring-tides. The limestones are mainly rather soft and light-coloured; but here and there tracts of a dark brown limestone, harder in texture and dolomitic in character, are mixed with them.

The lichens so common on the Cambrian strata are almost absent from the limestones; where they occur it is only as thin, poorly developed patches. How far this is due to the nature of the rock or to other causes it is not easy to say without an examination of a larger area of limestone shore; but it seems probable that the composition of the rock has some effect on the lichen growths, as three species not found on the silicious rocks are the prevailing forms on the limestone.

There are no Ramalinas; but this is most likely due to the absence of high rocks and to the situation being close to habitations. The orange lichens are very poor, a few spots of *Placodium calopisma* and *Lecanora citrina* being the only representatives. Associated with them were scattered colonies of *Rhizocarpon alboatrum, Rinodina exigua* var. *demissa*, and *Lecanora galactina.*

Verrucaria maura, and all species with gelatinous thalli, with the exception of a few spots of *V. aquatilis,* which grew in the weathered hollows on the tops of the rocks above high-water mark, were entirely absent above half-tide.

The algal growths, too, seemed to begin at lower levels than on the Cambrian shores. They were, first, a narrow band of *Fucus vesiculosus.* Below this was a wide and very fine bed of *F. serratus,* which lower passed into an equally luxuriant growth of Laminarias (*L. saccharina* and *L. digitata*), *L. saccharina* rising higher on the shore in the troughs and hollows of the rocks. The tops of the rocks near low water being covered in many places with a close mat of *Laurencia pinnatifida* and *Gigartina mamillosa*, left very little unoccupied space for the lichens to grow on.

The surface of the apparently bare rocks lying along the top of the beach at about high neap-tide level, when examined with a lens, was seen to be thickly dotted with the little black fruits of Arthopyrenias. Two species—*Arthopyrenia litoralis* and *A. foveolata*—were abundant and were met with throughout the whole neap-range. There is literally hardly an inch of bare rock that they have not colonized; *A. foveolata* is most usual on rocks in the upper levels; nearer low water it grows indifferently on the rock and on barnacle shells; if anything, it is more abundant on the barnacles. *A. foveolata* is more general than *A. litoralis* in the upper part of the beach, its insculpt perithecia being better adapted to withstand the drier conditions. It is usual on flat surfaces, while *A. litoralis*, which has prominent perithecia, grows most often on the sides of the rock and in more shade; nearer low-water both species are to be found on the most exposed noses of the strata. Both species prefer a rather soft substratum, and are more frequently seen on the pale grey limestone; where they occur on the dolomites it is usually on the embedded fossils. On the south and south-west coasts *A. litoralis* and *A. foveolata* are also found, but only on barnacle shells never on the rocky substratum; *A. foveolata* is very abundant on these coasts; *A. litoralis* is very rare.

The Verrucarias, so common on the south and south-west shores, are very poorly represented on the limestone. *V. mucosa* and *V. microspora* were the only species met with. They are entirely absent from the upper part of neap-range, and only make their appearance about the middle of the *Fucus serratus* belt, occurring as thin patches here and there under the shade of the algae. They extend down the shore as far as low-water, and are most usual on the harder dolomites. *Verrucaria Lorrain-Smithii*, a new species, not seen on the silicious rocks, was associated with them near low-water. This last species is extremely inconspicuous and difficult to detect; the thallus is very thin, sometimes evanescent, and the perithecia so very minute that they can only be seen with a strong lens. The absence of gelatinous species from the upper part of the shore may be due to the fact that calcareous rocks dry up much more quickly and are more easily heated than silicious strata.

A few tufts of *Lichina confinis* were seen on the dolomites in the south-east corner of the Harbour; *V. microspora*, too, was present here, but the growth was very scanty.

2. *Terricolous Lichens.*

The earth-loving lichens do not seem to be very plentiful on the Howth coasts. In certain localities they cover fairly large areas, but the number of species is small. The Cladonias are much the most abundant. *Cladonia cervicornis* forms innumerable small tussocks on peaty soil amongst the heather and other vegetation on the upper part of the cliffs, and is frequent with *Cladonia coccifera* along the south-west and eastern coasts. *Cladonia sobolifera* is not so common. It occurs at lower levels on mossy soil or on mossy rocks. *Cladonia pyxidata* grows everywhere; on shady earth-banks with a well-developed foliaceous thallus which carries a plentiful crop of podetia, but on dry soil it consists of small scattered and ill-developed leaflets, and is always barren. On steep shady banks, leprarious growths of various Cladonias and wide bands of granular growths, lurid grey in colour, but sometimes mixed with sulphur-yellow patches, are common. These last are always barren, and seem to be mainly shade growths of *Lecidea granulosa*. They are very common along the south-west coast on steep cliffs, between the grassy sward and the rock, and on earthy ledges. Fertile growths of this Lecidea are also common on peaty soil in various localities. At Old Boat-house they are associated with *Cladonia cervicornis*, *Biatorina synothea*, and subsp. *subnigrata*, with *Bilimbia aromatica* and *B. melaena*. On the banks south-east of Earlscliffe, similar fertile growths of *Lecidea granulosa* and *Bilimbia melaena* were found.

The most important colonies of earth-loving lichens were seen on the earth-banks on the south-west coast. The upper part of the cliffs along this coast is to a great extent covered with Boulder-clay and drift, which in some places reach the shore, and almost obscure the rocky surface. These earth-banks are most accessible below Earlscliffe and at Glenaveena, where there is a maze of paths leading from top to bottom that makes it an easy matter to explore the slopes. At Earlscliffe the banks vary from 100–120 feet in height. The vegetation in the upper part consists of coarse grasses and strong-growing plants such as Bracken, Brambles, Thistles, Burdocks, Nettles, and refuse from the Earlscliffe garden. In spring groves of yellow cabbage plants, Wild Mignonette, and Opium Poppies make a beautiful sight. Thickets of Blackthorn, through which the White Bryony scrambles, also occur at various levels. The only bare soil in these upper reaches lies round the rabbit-holes, with which the ground is riddled, and it is too disturbed for lichens or any vegetation to take hold on it. Under the Blackthorns the shade is too deep, but on the humus at the edge of the thickets various crustaceous species were found. *Lecanora subfusca* var. *campestris*, and *Buellia myriocarpa*, which thickly covers the soil and the dead twigs, were the most common. Even the grass-leaves and other vegetable matter embedded in the soil were black with the fruits of this last species.

On the lower slopes the surface is rather steeper, and the vegetation less rank, and of a more halophilous character. Scurvy-grass, Sea Spurry, Sea Lavender, Thrift, Plantains, and Sea Campion are abundant. Bare patches of ground, sometimes of considerable area, occur here and there by the paths, and amongst the flowering plants. The soil seems to be derived chiefly from the disintegration of very friable and finely laminated shales, masses of which crop out above the surface in many places. It is a stiff, almost white clay, very powdery in dry weather, and very sticky and close in wet weather. These apparent bare patches are covered with a kind of broken skin formed of crustaceous lichens, grey, green, yellow, black, and brown, all mingled together in some places, but in others forming large colonies of pure growths. Dotted here and there amongst them is the small moss *Phascum muticum*, occupying the little hollows in the surface, and in some places being almost completely buried in the soil.

On this white clayey soil *Acarospora benedarensis*, a new species, is one of the most abundant lichens, and is always found in the driest and sunniest situations. The thallus consists of scattered squamules of all sizes, which sometimes seem to coalesce and form a fissured but more or less continuous growth. It is a very efficient earth-binder. The under-surface of the thallus has apparently no defining layer, and the hyphal filaments penetrate the fine powdery soil, ramifying and branching in all directions, and enclosing particles of soil in their tissues. *Lecanora epixantha* is frequently associated with it, but is of less vigorous growth, and is seldom fertile when growing on the bare soil. *Lecanora umbrina* is also frequently associated, and forms good colonies. At various places the soil is full of little chips of shale of all sizes and shapes and in all stages of disintegration. These chips and the intervening spaces are thickly covered with the neat little apothecia of various Buellias, of which *B. myriocarpa*, *B. saxatilis*, and *B. Schaereri* are the most frequent species. Most of the chips of rock are lying loose on the ground, and might easily be stirred by the wind, but the fact that their upper surfaces are covered with these lichens bears strong testimony as to the sheltered nature of the habitat.

On the outskirts of most of the bare areas a sort of transition vegetation made up of mosses and lichens grows between the crustaceous vegetation and the flowering plants. These three zones may be distinctly seen in Plate VII, fig. 2. The most usual mosses are *Pottia Heimii*, *Weissia rupestris*, and *Bryum argenteum* var. *lanatum*, and mixed with them are the fruticose and foliose lichens *Cladonia sobolifera*, *C. pyxidata*, and *Physcia speciosa*, also several crustaceous species that seem to prefer these situations to all others, the most abundant being *Lecanora leucospeirea*, which is fertile only in rather shady spots, *Lecanora holophaea*, and *Bacidia muscorum*. Others are also met with but not so often. *Biatorina contristans* is very rare and only grows in the damper and shadier places; while *Bacidia umbrina*, though occasionally found amongst the mosses, is more usual on bare ground associated with *Lecanora epixantha* and *Acarospora benedarensis.*

At various places along the south-west coast, where the soil is hard and caked, *Lecidea coarctata* var. *glebulosa* frequently forms patches of considerable size by the paths.

Quite a different set of lichens grow on the slopes at Glenaveena. The soil on these banks is darker than at Earlscliffe, and contains marine shells (glacial fossils) and a good deal of other calcareous matter. Lime-loving species both of flowering plants and lichens are common, especially on the lower slopes. Bracken and short coarse grass clothe the upper reaches; but lower *Geranium sanguineum*, *Viola hirta*, *Carlina vulgaris*, *Chlora perfoliata*, and *Poterium Sanguisorba* are common. The three mosses *Weissia rupestris*, *Brachythecium plumosum* and *Pottia Heimii* are also abundant. Amongst these mossy growths and on bare ground *Dermatocarpon hepaticum* is very widespread. Its squamules, which are in all stages of development, in the young stages, with their upturned edges, look very like the apothecia of some other lichen growth; associated with it are *Bilimbia aromatica* and *Biatorina coeruleonigricans*, the latter exceedingly abundant, the white pruinose thallus and apothecia being very conspicuous. Here and there on some of the mossy tufts *Lecanora luteoalba* is sometimes seen; and *L. aurantiaca* var. *erythrella* is occasional on the bare soil. Numerous patches of dark Collemas are frequent amongst the short grasses by the path, *C. pulposum* and *C. cristata* being the most general species, but the former was seldom fertile. In damp shady places near the foot of the cliffs *Cladonia pungens* and *C. furcata* form large patches, and *Cladonia deformis* is also occasionally seen. The Peltigeras are very rare all round the Howth coasts, though fairly common inland. The only species noted were *P. rufescens* on shady grassy banks at White Water Brook and one or two plants of *P. canina* along the east coast.

At the base of the earthy cliffs multitudes of small stones and chips of rock form a sort of talus, and occur either embedded in the soil or more or less covered by the soil washed down from above. On the exposed parts of these stones numerous species may always be looked for, *Buellia myriocarpa*, *B. verruculosa*, *B. colludens*, *B. stellulata*, *B. confervoides*, *Rinodina exigua*, *Lecanora Hageni*, *L. crenulata*, *L. subfusca* var. *campestris*, *L. vitellina*, *L. caesiorufa*, *Verrucaria nigrescens*, *Porina chlorotica*, &c. *Lecanora simplex* f. *strepsodina*, *Acarosporas maragdula*, and *A. fuscata* are also frequent on the schistose rocks, generally occurring along the lines of the fissures or outlining any unevennesses of the surface.

3. *Corticolous Lichens.*

There are no trees in our area, and consequently corticolous species are very scarce. The Blackthorn scrub on the cliffs overlooking Broad Strand supplies almost the only habitat for these lichens. The main part of the collecting was done below Earlscliffe. The scrub here is composed almost entirely of Blackthorns; a Whitethorn occurs here and there, but no different species were noticed on these. Gorse and Bramble stems and an Elder bush were also searched, but they yielded no lichens. One species, *Bilimbia Naegeli*, was collected on heather-stems on the south-west coast; but with this exception all the others are from the Blackthorns. As the scrub is extremely dense and the light very subdued in the interior, most of the lichens grew on the outer fringe of the thickets or on the twigs and branches on the top surface. The lichen-growths were nowhere abundant or vigorous, but they showed a tendency to occur in strata, the Ramalinas and Evernia being more usual on the scrub of the upper part of the bank where there was more breeze, while the Physcias and crustaceous species were more common on the thickets in the lower reaches. *Evernia prunastri* and three species of Ramalina—*R. fastigiata*, *R. farinacea*, and *R. intermedia*—were about equally abundant, but none of them was really plentiful; they mostly occurred as tufts on the smaller twigs and branches in the upper thickets. Several Parmelias—*P. sulcata*, *P. exasperata*, *P. laevigata*—covered the upper surfaces of the larger branches. The Parmelias seem to require a certain amount of shade and protection from wind; but they were not found in the interior of the thicket where the light was dim. *Physcia lychnea* was very general on the Blackthorns nearer the shore, and seemed to flourish best in the forks of the small branches, its patches of greenish-yellow fronds being usually fertile. In deep shade it was much greener in colour, but took on a yellowish hue, and seemed to be more generally fertile where the light was stronger. *Physcia aipolia*, and *P. tribacia* were occasionally found in the upper stretches of scrub and with them *P. pulverulenta*, *P. tenella*, and *P. pityrea*, the last-mentioned species being barren and much rarer than any of the others. Several crustaceous lichens were also collected; *Buellia myriocarpa* was comparatively plentiful both on living and on dead wood, *Lecanora varia*, *L. angulosa*, *L. chlarona*, *Rinodina sophodes*, *Lecidea parasema*, *L. elaeochroma* were general, often growing mixed up together on the same branch. *Arthopyrenia epidermidis*, *Acrocordia gemmata*, *Graphina inusta*, *Pyrenula nitida*, and *Lecanora symmictera* also occurred, but were scarce.

On the smooth shining bark of the Blackthorns lichens are not really plentiful; where found they were for the most part growing in the forks of the branches or where the surface was broken and somewhat roughened and cracked.

III. Systematic List.

The arrangement and nomenclature of the "Monograph of British Lichens," vols. I and II, are followed in this list. In the case of the Lecanorei the sections into which Crombie divided that large genus are indicated; and, as these are generally accepted as of generic rank, some of the species are referred to under them as generic names in the text: for instance, *Lecanora* (*Placodium*) *murorum* of the list is *Placodium murorum* of the text.

The list contains altogether 181 species, 13 subspecies, and 25 varieties. Of these, 3 species are new to science; 25 species, 4 subspecies, and 9 varieties are new to Ireland; and, including these, there are altogether 108 species new to district L2 (Dublin and Wicklow) of Mr. Adams' biological sub-division of Ireland.

The species new to Ireland are marked with an asterisk, and those new to Co. Dublin with a dagger.

List of Species.

Family **COLLEMACEI.**

Tribe **Lichinei.**

Lichina pygmaea Ag.—On rocks between neap-tide marks; general round the coast, mainly in the upper part of neap-range.

L. confinis Ag.—On rocks; general round the coast in the upper part of the *Verrucaria maura* belt.

Tribe **Collemei.**

Collema pulposum Ach.—On soil, chiefly calcareous—Earlscliffe, Glenaveena, White Water Brook, &c.; frequent.

†C. cristatum Hoffm.—On mossy rocks and on soil, Glenaveena; on walls, Sutton; rare.

Family **LICHENACEI.**

Tribe **Sphaerophorei.**

Sphaerophorus coralloides Pers.—On mossy rocks chiefly on the east and north coasts; abundant on the headland, but not common in our area.

Tribe **Ramalinei.**

Ramalina farinacea Ach.—On Blackthorns, Earlscliffe.

†subsp. **intermedia** Nyl.—On Blackthorns, Earlscliffe, associated with the species and more abundant.

R. fastigiata Ach.—On Blackthorns, Earlscliffe.

R. scopulorum Ach.—Abundant on rocks all round the coast; occasional specimens were noted growing on soil in crevices of rocks at Bottle Quay and on the root-stalks of Thrift plants, Old Boat-house.

†var. **incrassata** Nyl.—On exposed rocks, frequent.

*var. **armorica** Nyl.—Only noted on shady cliffs near the Needles; rare.

†**R. subfarinacea** Nyl.—On low rocks near the Martello Tower, Sutton; abundant between Casana and the Nose.

†**R. cuspidata** Nyl.—(See page 88.)

Ramalina A. (*R. cuspidata* Nyl. subsp. *breviuscula* Nyl. ?) (see page 94).

Ramalina B. (*R. cuspidata* Nyl. var. *crassa* Del. ?) (see page 94).

Ramalina C. (*R. cuspidata* Nyl. subsp. *breviuscula* Nyl. forma *gracilescens* Cromb. ?) (see page 94).

†**R. Curnowii** Cromb.—Frequent in breezy situations along the upper edge of the low cliffs; on the west coast only.

Tribe **Usneei.**

†**Usnea hirta** Hoffm.—Here and there associated with Parmelias along the upper edge of the cliffs; rare in our area.

Tribe **Parmeliei.**

Evernia prunastri Ach.—On Blackthorns, Earlscliffe, and on rocks on the west coast; frequent.

f. **sorediata** Ach.—Blackthorns, Earlscliffe; rare.

Parmelia perlata Ach.—On Blackthorns, Earlscliffe, and on shady rocks at various places round the coast; rare in our area.

subsp. **ciliata** Nyl.—Mossy rocks, chiefly on the east coast.

P. laevigata Ach.—On Blackthorns, rare; and rocks, frequent.

†**P. revoluta** Nyl.—On mossy rocks, east coast; rare.

P. saxatilis Ach.—On rocks, Drumleck and Glenaveena; common on the east coast.

f. **furfuracea** Schaer.—With the species, Glenaveena; rare.

†**P. sulcata** Tayl.—On Blackthorns, Earlscliffe; rare.

P. omphalodes Ach.—Rocks, rare on west coast, common on the east coast in the Ramalina belt.

P. caperata Ach.—On rocks chiefly in shade, Worn Hole, Drumleck and Casana; not common in our area.

P. conspersa Ach.—Abundant all round the headland, descending to sea-level on the east and north coasts.

†**P. Mougeotii** Schaer.—On Red Rocks and occasionally on rocks by the Cliff-walk on the east coast; rare in our area.

†**P. exasperata** Nyl.—On Blackthorns, Earlscliffe; rare.

P. prolixa Nyl.—General round the coast.

†subsp. **Delisei** Nyl. var. **isidiascens** Nyl.—On shady rocks with the species, Glenaveena.

†**P. fuliginosa** Nyl.—Frequent all round the coast.

P. physodes Ach.—Occasionally found along the upper edge of the cliffs on heather stems and on rocks; though abundant on the peninsula, it is not often found inside our area.

f. **labrosa** Ach.—Drumleck Point, and at several places on the east coast.

Tribe **Peltigerei**.

Peltigera canina Hoffm.—Grassy slopes, Casana; very scarce in our area.

P. rufescens Hoffm.—White Water Brook, on rocks in shady situations; rare in our area.

Tribe **Physciei**.

Physcia parietina De Not.—General and abundant on rocks all round the coast. This species was seen growing on the root-stalks of Thrift at Old Boat-house, and over the stems and leaves of the same plant at Glenaveena.

f. **virescens** Nyl.—On rocks in shady situations, Gaskin's Leap, and at several other places on east coast; frequent and usually barren.

*var. **aureola** Nyl.—On rocks with the species, Lion's Head.

†var. **ectanea** Nyl.—Abundant on dry sunny rocks near the Needles.

Ph. lychnea Nyl.—Abundant on Blackthorns between Hippy Hole and Lion's Head; fertile except in deep shade.

†**Ph. leucomela** Mich.—On the ground, associated with *Cladonia cervicornis* and various mosses, Earlscliffe; rare.

Ph. pulverulenta Nyl.—On Blackthorns, Earlscliffe; rare.

Ph. aquila Nyl.—On rocks, chiefly in shade, in the Ramalina belt; very common.

Ph. stellaris Nyl.

†var. **leptalea** Nyl.—On rocks, associated with *Ph. parietina*; frequent.

†var. **subobscura** Nyl.—On shady rocks, Old Boat-house, with *Ph. parietina*; rare.

subsp. **tenella** Nyl.—On rocks just above high-water mark, Bottle Quay and Broad Strand; common.

†**Ph. aipolia** Nyl.—On the sea-wall, Sutton; frequent.

***Ph. tribacia** Nyl.—On Blackthorns; rare.

Ph. erosa Leight.—On rocks at the base of the cliffs, above high-water mark, Broad Strand; frequent.

†**Ph. lithotea** Nyl.—In depressions of rocks about high-water mark, Bottle Quay, associated with *Ph. tenella* and *Lecanora epixantha*; frequent.

Tribe **Lecano-Lecideei.**

Sub-tribe **Pannariei.**

†**Pannularia nigra** Nyl. subsp. **psotina** Cromb.—Sea-wall, Sutton; rare.

Sub-tribe **Lecanorei.**

Lecanora (Squamaria) crassa Ach.—In crevices of rock on the shore between Lough Leven and High Room Bed; rare.

Lecanora (Placodium) murorum Ach.—Rocks about high spring-tide level; chiefly in sheltered situations.

* subsp. **decipiens** Nyl.—On the Needles; abundant.

*subsp. **tegularis** Nyl.—Occasional on the south and south-west coasts, but more frequent on the east coast, where it seems largely to replace the species. It is very plentiful on the cliffs at Gaskin's Leap. *L. tegularis* differs from *L. murorum* chiefly in its concave apothecia and in the smaller size of the spores. Crombie describes the margin of the apothecium of *L. tegularis* as entire. In the Howth specimens it is crenulate or sub-crenulate, but as the spores agree with Crombie's measurements (being $10–11\mu \times 3–4\mu$), and as Nylander, in his original description of *L. tegularis*, says nothing about the character of the margin of the apothecium, I have placed the Howth specimens under this subspecies.

†**L. (P.) callopisma** Ach.—Seen only on the limestone at Balscadden Bay, and on the Harbour wall; rare.

†subsp. **sympagea** Nyl.—With the species; rare.

†**L. (P.) lobulata** Somm.—Abundant all round the coast on rocks that are washed by the waves at high spring-tides.

***L. (P.) miniatula** Nyl.—On quartzite rocks above high-tide mark on the Sutton shore. This plant comes very near *L. murorum* subsp. *tegularis* Nyl., differing from it chiefly in the deep tawny vermilion colour of the thallus; in the Howth specimens of *L. tegularis* the crenulate margin of the apothecium is a further difference. These differences hardly seem to be specific, but, as Crombie has separated the two plants, I follow him in the matter.

†**Lecanora (Candelaria) vitellina** Ach.—Frequent on rocks, especially on the east coast, the growths usually occurring as yellow lines in the fissures and along the unevennesses of the rock-surfaces.

†**L. (C.) epixantha** Nyl.—On rocks and on the ground. General on the south and south-west coasts; rare on the east coast. On a rocky substratum this species is always fertile, but, when growing on the ground, the thallus is thinner, more dispersed, and usually barren. In the Howth specimens the spores are simple and often slightly curved, with the cell-contents more or less concentrated at the ends, indicating their polaribilocular character. No truly polaribilocular spores, however, were seen in the specimens examined. The measurements $16–19\mu \times 5–7\mu$ agree with Crombie's. *L. epixantha* is sometimes associated with *L. vitellina*, and is not distinguishable from it in the field. Under the microscope, however, they are easily separated by the 8-spored ascus of *L. epixantha*. At Bottle Quay *L. epixantha* grows on low rocks about high-water mark. In specimens collected from this situation the thallus was completely obscured by a dark algal growth (Gloeocapsa sp.?), which gave the lichen much the aspect of a Biatorina, with minute, pale yellow apothecia. The only previous Irish record is from Giant's Stairs, Co. Cork.

Lecanora (Caloplaca) citrina Ach.—Abundant on sea-walls and rocks near the villages of Howth and Sutton, and occasionally at other places round the coast.

***L. (C.) incrustans** Ach.—On crumbling rocks, Broad Strand, associated with *L. Hageni*; rare.

L. (C.) aurantiaca Nyl.—On an old wooden post, Earlscliffe; rare.

†subsp. **erythrella** Nyl.—Rocks and earthbanks, Glenaveena; rare.

†**L. (C.) ferruginea** Nyl.—On rocks; frequent.

†var. **festiva** Nyl.—On rocks in the Ramalina belt; common.

†**L. (C.) caesiorufa** Nyl.—On schistose rocks, and on stones at the base of the earthbanks, Earlscliffe, Glenaveena, and White Water Brook; frequent.

†**L. (C.) Turneriana** Nyl.—White Water Brook; rare.

***L. (C.) albolutescens** Nyl.—On calcareous soil, Glenaveena, amongst tufts of *Pottia Heimii* associated with *Dermatocarpon hepaticum* and

Biatorina coeruleonigricans. Crombie gives the habitat of this species as "granitic rocks in upland tracts." The habitat of the Howth plant is very different; but the specimens, although growing on the ground and near the sea, agree in all particulars with Crombie's description of *L. albolutescens*, and are identical in appearance with the specimens of this species in his herbarium in the British Museum. The only British records are from N. England.

†**L. (C.) pyracea** Nyl.—On stones and schistose rocks at the foot of earth-banks, Broad Strand, and at White Water Brook associated with *L. exigua* and *Physcia tenella*; rare.

†**L. (C.) vitellinula** Nyl.—Limestone rocks, Balscadden Bay; rare.

†**Lecanora holophaea** Nyl.—In crevices of rocks, and on the upturned edges of strata at various places on the south and south-west coasts, and at High Room Bed on the east coast; rare. This species is frequently associated with *Ph. parietina*, *L. lobulata*, and *Rhizocarpon alboatrum* on these coasts.

***L. leucospeirea** Nyl.—This species was seen only on the earth-banks between Sutton and the Baily Lighthouse. It is abundant, but often infertile. At Earlscliffe it is associated with *L. epixantha*, *Acarospora benedarensis*, and the small moss *Phascum muticum*. As a rule, in sunny situations, the squamules are small and scattered, but in shade they form conspicuous, pale, greenish-white patches, are better developed, and more usually fertile. *L. leucospeirea* is distinguished from *L. holophaea* chiefly by the whiter, and more scattered thallus, and by the smaller spores, which, in the Howth specimens, measure $10–12\mu \times 3\mu$. The only previous records are from the Island of Jersey, where it is said to be very rare.

†**L. Ralfsii** Cromb.—On the Needles, associated with *L. prosechoides*; very scarce.

†**L. spodomela** Nyl.—On coarse quartzites at the base of the earth-banks, Earlscliffe, and on the cliffs behind the Needles. Crombie describes *L. spodomela* as having much the aspect of *L. sophodes*. The Howth specimens might easily be mistaken for that species without a microscopic examination. The spores are slightly narrower than Crombie's measurements, being $10–16\mu \times 5–6\mu$, and are very rarely three-septate.

Lecanora (Rinodina) sophodes Ach., subsp. **laevigata** Nyl.—Frequent on rocks, Broad Strand, and abundant on pebbles embedded in the cement walls of an old boat-house on the shore; also on sea-wall at Sutton.

L. (R.) exigua Nyl.—Frequent on rocks about high-water mark.

f. **demissa** Stiz.—On rocks above high-water mark at Broad Strand and Sutton. *L. exigua* is frequently associated with *L. Hageni*. *L. exigua*

f. *demissa* is most often found with *L. Hageni* f. *calcigena*, and they usually occur at a lower level on the shore, being associated on Broad Strand with *V. maura* in some places.

***Lecanora (R.) subexigua** Nyl.—On limestone rocks above high-water mark, Balscadden, and on quartzites on the Broad Strand, associated with *Buellia myriocarpa* and *L. exigua*; rare. The specimens in Crombie's herbarium in the British Museum have a rather more continuous thallus and slightly smaller apothecia, but the entire thick wall of the apothecium of the Howth specimens and the measurements of the spores, which are consistently $12–15\mu \times 6\mu$, agree with Crombie's description of the species. Previously known in the British Isles only from S.W. England.

†**L. (R.) confragosa** Nyl.—Occasionally on hard quartzites at high levels, Red Rocks; more frequent on the east coast, but seldom growing inside our area. The Howth specimens agree well with Curnow's plant from the Lizard collected in 1873, which was erroneously recorded as subsp. *crassescens*, but placed by Crombie under *L. confragosa*.

†**L. (R.) milvina** Ach.—On schistose rocks at base of the earth-banks, Broad Strand, associated with *Acarospora benedarensis*; rare.

†**Lecanora galactina** Ach.—On walls about Sutton, Balscadden, and Howth Harbour, associated with *L. citrina*; very abundant.

L. subfusca Nyl.—On old wooden palings, Earlscliffe; rare.

var. **campestris** Nyl.—Frequent on stones and rocks lying at the base of the earth-banks, Broad Strand.

†**L. rugosa** Nyl., subsp. **chlarona** Nyl.—On Blackthorns, Earlscliffe; rare.

†**L. atrynea** Nyl.—On rocks amongst Ramalina growths at Sheep Hole; rare.

L. coilocarpa Nyl.—On rocks, Sheep Hole; rare.

†**L. albella** Ach.—On Blackthorns, Earlscliffe; rare.

†**L. angulosa** Ach.—On Blackthorns, Earlscliffe; rare.

L. glaucoma Ach.—Very widespread on the Howth Peninsula and common inside our area, especially along the east coast. It is usually associated with *Rhizocarpon geographicum*, and is frequently infested by *Leciographa glaucomaria*.

f. **decussata** Cromb.—In several places on the east coast.

f. **complanata** Leight.—On slate rocks, Kilrock.

* var. **inflexa** Johns.—With the species, but in more shady situations, White Water Brook.

†**L. umbrina** Nyl.—On rocks about high-water mark, Broad Strand, and at other places on the south coast, also on stones lying level with the soil, Earlscliffe; frequent.

†L. **crenulata** Nyl.—Here and there on rough quartzites.

†L. **Hageni** Ach.—Frequent and general round the coast on stones lying level with the soil, and on rocks at the base of the earth-banks, sometimes also on the ground associated with *Buellia myriocarpa* and *L. epixantha.*

f. **calcigena** Nyl.—On rocks on the shore, Broad Strand; frequent. This form seems to grow nearer the sea than the species.

†L. **prosechoides** Nyl.—Abundant on rocks splashed by the sea at high tide. The thallus varies a good deal in the Howth plant, being smooth, continuous, and much expanded on steep rock-faces, but more dispersed and unequal on low-lying flat rocks, where it is more often associated with other lichens. The apothecia also vary a good deal in size and colour. In a note about the species Crombie remarks that it is sometimes the host of *Arthonia varians.* On the Howth coast *A. varians* is almost invariably present on the apothecia.. Hardly a specimen was gathered on which the parasite was not detected. Sandstede (**24**), in his account of the lichens of the German islands in the North Sea, mentions a three-septate parasite as occurring on *L. prosechoides*, which is very probably *A. varians*, but he does not give it a name.

* var. **aeruginosa** Wedd.—On quartz rocks near Needles, associated with the species.

* var. **melacarpoides** Nyl.—With *Rhizocarpon alboatrum* on the top of the sea-wall, Sutton.

*L. **prosechoidiza** Nyl.—At various places on the south-west coast in situations similar to those in which *L. prosechoides* grows, and on calcareous rocks at Balscadden. *L. prosechoidiza* seems to be closely related to *L. prosechoides*, and may be only a state of that plant. The main characters which distinguish it from *L. prosechoides* are the greyer thallus, the conglutinate paraphyses, and the different reaction with iodine. It is much less common than *L. prosechoides.*

*L. **conferta** Nyl.—On quartzites, Sutton shore, and on the Harbour wall, Howth; rare.

L. **sulphurea** Ach.—Only seen on steep, rather shady rocks at the west end of Broad Strand.

†L. **epanora** Ach.—On slate rocks; rare.

L. **varia** Ach.—On Blackthorns, Earlscliffe, and on dead twigs lying on the ground; common.

†L. **symmictera** Nyl.—On Blackthorns, Earlscliffe; rare.

†L. **polytropa** Schaer. All round the coast; frequent.

L. **atra** Ach.—General all round the coast in the Ramalina belt, but nowhere abundant.

Lecanora (Lecania) erysibe Nyl.—Here and there along the Broad Strand on rocks about high-water mark.

***L. (L.) spodophaeiza** Nyl.—Bottle Quay. On rocks about high-water mark, associated with *Placodium murorum* and *Verrucaria maura.* Crombie describes the spores of this species as simple, or often spuriously one-septate. In the Howth specimens the spores seem to be almost constantly one-septate. The white fimbriate circumference and the granular nature of the thallus and the larger spores separate it from *L. prosechoides*, which grows in the same habitat. The only previous records are from the Island of Jersey.

***L. (L.) actaea** Nyl.—Only seen on the quartzites at Red Rocks; rare. The specimens from this locality agree very well with Larbalastier's from St. Aubin's Fort, Jersey, in the British Museum Herbarium. The only previous records are from the Channel Islands.

***LECANIA ATRYNIOIDES** Nov. Sp.[1]—Thallus greyish, rimoso-diffract, thin, effuse; hypothallus dark; apothecia dark reddish-brown, crowded and often angulose $\frac{1}{2}$–1mm in diameter, margined when young, becoming convex with age; margin thin, whitish, entire or slightly sub-crenulate and sub-persistent; paraphyses sub-discrete, jointed, clavate and blackish-brown at the apices; hypothecium colourless; spores 8 in the ascus colourless, ellipsoid, 1-septate, and occasionally constricted at the septum, sometimes slightly curved, $10-14\mu \times 4-6\mu$; hymenial gelatine, deep blue, then violet with iodine. Spermogones with slender arcuate spermatia, which are borne on the tips of simple sterigmata.

Habitat.—Schistose rocks about high spring tide-level, Red Rocks and Broad Strand. This lichen has a general resemblance to *Lecanora atrynea*, and grows in similar situations. In some of its characteristics it agrees with *Lecaniella arenaria* (Anzi) Jatta, "Flora Italica," p. 400. I have not been able to see a specimen of this lichen, but as the Howth plant differs from Jatta's description in having larger and sub-persistently margined apothecia, more discrete paraphyses, and broader spores, I have, after some hesitation, described it as a new species.

Lecanora (Ochrolechia) tartarea Ach.—Shady rocks, White Water Brook; rare.

L. (O.) parella Ach.—Chiefly on the south and south-west coasts; widespread but nowhere abundant.

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[1] Thallus cinerascens, tenuis rimosus vel evanescens, irregulariter effusus; apothecia aggregata prominula $\frac{1}{2}$–1 mm. diam., planiuscula dein convexa; margine thallino pallido, tenui, sub-persistente; paraphysibus subdiscretis, incondite, articulosis, clavis, fuscis ad apices; sporae octonae, ellipticae, uniseptatae, quandoque incurvae, $10-14\,\mu \times 4-6\,\mu$. Ad saxa siliceo-arinarea circum locos aestuum maximorum. Sutton.

L. (O.) pallescens Nyl.—On Blackthorns, Earlscliffe; rare.

Lecanora (Aspicilia) calcarea Somm.—On the limestone; rare.

†**Lecanora (Acarospora) fuscata** Nyl.—On grits and shales in sunny situations; frequent.

†**L. (A.) rufescens** Nyl.—On grits and quartzites, Broad Strand; frequent.

†**L. (A.) smaragdula** Nyl.—Very common on dry rocks in sunny situations all round the coast, associated with *Buellia spp.* and *L. simplex*, f. *strepsodina.*

†**L. (A.) Heppii** Nyl.—On grits, Hippy Hole, associated with *Rhizocarpon petraeum*; rare.

L. (A.) pruinosa Nyl.—On walls, Sutton; rare.

†**L. (A.) simplex** Nyl., f. **strepsodina** Ach.—On loose stones at the base of earth-banks, and on schistose rocks all round the coast; very common.

***ACAROSPORA BENEDARENSIS**[1] Nov. Sp.[2]—Thallus dark brown, thick, glebulose-squamulose; squamules discrete or sometimes confluent, pale beneath, 1–7 mm. wide and about ½–1 mm. thick, K– CaCl–; apothecia at first completely immersed, innate, concave, concolorous with the thallus, 1 or more in each squamule, reddish-brown when moist, margin thick, entire, persistent; paraphyses slender, conglutinate, sparingly branched, yellow-brown at the apices; hypothecium sordid grumous; ascus clavate, 80–100μ × 15–20 μ, myriospored; spores, 3–4·5μ × 1–2·5μ; spermogones numerous; spermatia 2–5μ × 1–1·5μ.

When treated with iodine the first part of the apothecium to take the stain is the hypothecium, which at once turns deep indigo-blue, passing off into sordid green later; the hymenial gelatine gradually takes on a rich clear wine-red colour.

Habitat—On dry clayey soil and on disintegrating fine shales in sheltered sunny situations on the earth-banks at Broad Strand.

Sub-tribe **PERTUSARIEI.**

Pertusaria dealbata Nyl. f. **corallina** Cromb.—Broad Strand; rare.

P. ceuthocarpa Turn. and Borr.—East coast; rare and infertile.

P. concreta Nyl. On schistose rocks chiefly on the east coast, associated with *Lecanora glaucoma* and *Rhizocarpon geographicum*; not common in our area.

f. **Westringii** Nyl.—On the cliffs near the Needles; rare.

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[1] Thallus effusus glebuloso-squamulosus, squamuli rimoso-discreti vel confluentes, castaneo-nigri, subtus pallidiores, 1–7 mm. lati, circa ½ ad 1 mm. alti; apothecia primo immersa, semper urceolata, disco plano, margine crasso, prominente, paraphysibus gracilibus, parce ramosis, sursum flavo-brunis, conglutinatis; hypothecio pallido-griseo, grumoso; ascus clavatus, 80–100μ longus, 15–20μ latus; sporis minutissimis 3–4·5μ longis, 1–2·5μ latis; hypothecium iodo violascens, dein sordido-olivaceum; hymenium pulchre vino-rubescens. Ad terras argillaceas. Earlscliffe.

[2] From Ben Edar, the Celtic name for Howth.

Tribe CLADONIEI.

Cladonia pyxidata Fr.—On peaty ground between the rock and the grass along the top of the low cliffs and on the earth-banks, Broad Strand; frequent.

C. cervicornis Schaer.—On the ground, abundant all round the coast, chiefly in earthy crevices among the rocks, and on stony places between the grass and the rock. At Old Boat-house it grows on peaty ground almost within reach of the waves amongst rocks covered with Ramalinas and Physcias.

†**C. sobolifera** Nyl.—Commonly associated with the previous species on the slopes at Earlscliffe and Glenaveena; the thallus occurs in all stages of development, in some places forming large patches amongst the mosses *Phascum muticum* and *Pottia Heimii.*

C. degenerans Flörke.—In shady places amongst fallen rocks at the base of the cliffs; rare.

†**C. pungens** Flörke.—In damp shady places on the cliffs, Glenaveena; rare.

†**C. squamosa** Hoffm.—Associated with mosses in the crevices of rocks at the base of the cliffs, Glenaveena and Broad Strand; frequent.

†**Coccifera** Schaer.—On peaty soil, in the crevices of rocks and on bare places among heather along the top of the cliffs; common.

Sub-tribe LECIDEEI.

†**Lecidea coarctata** Nyl.—Rocks on the banks above Broad Strand; rare.

†var. **glebulosa** Crombie.—On the ground, Earlscliffe and Glenaveena; more frequent than the species.

†**L. arridens** Nyl.—Only seen on coarse quartzites near the top of the earth-banks, Broad Strand.

†**L. granulosa** Schaer.—On peaty soil, Old Boat-house, and at other places on the south and south-west coasts, associated with *Cladonia cervicornis*, *Bilimbia aromatica*, and *B. melaena.* The plant is often barren, forming large glaucous-grey patches on the earth between the rock and the grass in shady situations; common.

†**L. protrusa** Fr.—On rocks and stones at base of cliffs, Broad Strand and White Water Brook; frequent.

L. parasema Ach.—On Blackthorns; common.

†var. **elaeochroma** Ach.—With the species, but less common.

L. contigua Fr.—Plentiful on east and north coasts.

†var. **flavicunda** Nyl.—North coast; rare.

*L. **sorediza** Nyl.—Only seen on the cliffs at White Water Brook.

†L. **albocoerulescens** Ach.—On shady rocks, west end of Broad Strand; abundant.

†L. **confluens** Ach.—On rocks, north coast; frequent.

L. **rivulosa** Ach.—On rocks here and there on south and south-west coasts; frequent. On the east and north coasts it grows with *Lecanora glaucoma* and *Rhizocarpon geographicum.*

†L. **griseoatra** Schaer.—On the cliffs, Hippy Hole.

*L. **imponens** Leight.—On *Lecanora polytropa*, east coast. The only other localities in which this species has been found are near Fishguard in Wales.

Biatorina coeruleonigricans A. L. Sm.—On calcareous soil, Glenaveena; very common on the lower part of the earth-banks with *Dermatocarpon hepaticum, Bilimbia aromatica,* &c.

*B. **synothea** Koerb.—On soil, Old Boat-house, associated with *Cladonia cervicornis,* and in similar situations at Earlscliffe. This species has not hitherto been noted as growing on the ground, and the Howth specimens are interesting on this account. The spores are typical, measuring $10–12\mu \times 3–4\mu$, and the epithecium gives the characteristic violet colour when treated with K. The spermogones are numerous, measuring $4–5\mu \times 2\mu$. The thallus of the Howth specimens is usually greenish.

* subsp. **subnigrata** A. L. Sm.—On schistose rocks near the Needles; scarce. Distinguished from the species chiefly by the stouter spores.

†B. **lenticularis** Koerb.—Plentiful all round the coast. On the Broad Strand it grows with *Placodium lobulatum* on rocks where it is wetted by the sea-water at high tide.

f. **nigricans** Arnold.—Occasionally with the species.

†B. **chalybeia** Mudd.—On quartzite rocks near the Needles, associated with *Buellia canescens*; frequent.

† subsp. **chloroscotina** A. L. Sm.—On slates and schists, in shade, Broad Strand, and at High Room Bed; rare.

*B. **contristans** A. L. Sm.—The specimens which I have referred to this species were gathered at Earlscliffe, where they were growing on the ground, in a dark shady situation, amongst mosses, and associated with *Bacidia umbrina.* The apothecia are frequently conglomerate; but the size of the spores and the other characters agree with the description in the "Monograph of British Lichens." The only previous record in the British Isles is from the summit of Ben Lawers, so that the Howth locality, being near sea-level, is an interesting extension of the range of this species.

Bilimbia aromatica Jatta.—Frequent on sea-walls about Sutton and Howth; in crevices of rocks at various places round the coast, and on calcareous soil at Glenaveena.

B. melaena Arnold.—On peaty soil, Old Boat-house and Lion's Head; rare.

Bacidia muscorum Mudd.—Here and there amongst tufts of the moss *Pottia Heimii*, on the earth-banks and at Glenaveena; rare.

†**B. umbrina** Branth. & Rostr.—On the earth-banks, Earlscliffe, associated with *Biatorina contristans* and *Lecanora epixantha*; frequent.

Buellia canescens De Not.—In shady places, all round the coast; common.

†**B. spuria** Koerb.—On dry sunny cliffs near the Needles, and on stones at the base of the cliffs, Broad Strand; rare.

†**B. myriocarpa** Mudd.—Common all round the coast, very plentiful on the rocks lying at the foot of the cliffs, Broad Strand. At Earlscliffe it is equally abundant on rock, bark, and soil, and covers the surface of dead twigs and stems of grasses lying on the ground (see page 118). The form *leprosa* is most usual on the soil, and forms *areolata*, *ecrustacea*, and *opegraphina* were all noted on rocks on the Broad Strand.

***B. Schaereri** De Not.—On the ground and on chips of rock lying on the surface of the earth-banks, Broad Strand, associated with the above, which it closely resembles, but from which it is distinguished by the smaller and more distinctly margined apothecia, the concrete paraphyses, and by the smaller and paler-coloured spores, which in the Howth specimens measure consistently $8–10\mu \times 4\mu$; common.

†**B. aethalea** Th. Fr.—On schistose rocks on the shore, Broad Strand; rare.

†**B. verruculosa** Mudd.—Frequent round the coast on dry sunny rocks, but nowhere plentiful.

†**B. saxatilis** Koerb.—On small stones, Earlscliffe, and on schistose rocks near the Needles; scarce.

***B. ryssolea** A. L. Sm.—A very common species, especially on the south and south-west coasts, on rocks in shady places, often covering many square yards of the surface with a close grey map-like growth, very similar in appearance to that of *Lecidea rivulosa*, but rather paler in colour. The Howth specimens vary a little from the description in the "Monograph of British Lichens," the spores being slightly constricted at the septum. The only previous record for this species is from south-west Wales, where it grows on the Caradoc sandstones.

†**B. stellulata** Mudd.—On rocks and stones; common.

***B. impressula** A. L. Sm.—On slaty rocks on the east coast; rare. I have not been able to see specimens of this species for comparison, but the Howth plant agrees so well with the description of *B. impressula* in vol. II of the "Monograph of British Lichens," that I have little hesitation in referring it to this species. The apothecia are small, circumscribed, and often three or more together. The spores measure 12–14μ × 8–9 μ, and are slightly constricted at the septum, which is rather thick. The hypothecium is pale, brownish-yellow, the paraphyses are indistinct, and the hymenial gelatine turns bluish with iodine.

The only previous records are from hilly districts in Wales.

†**B. atrata** Mudd.—East coast; frequent.

†**B. colludens** Tuckerm.—On rocks all round the coast; common.

†**B. confervoides** Krempelh.—On slaty rocks in dry situations, and often associated with *L. simplex* f. *strepsodina*; common.

B. atroalba Th. Fr.—Only noted on dry rocks near the Needles, but it is probably frequent.

†**Leciographa parasitica** Massal.—On the thallus of *Physcia parietina*, about high-water mark, Broad Strand. The spores of the Howth specimens are very occasionally four-septate; but in all other respects the plant agrees with the descriptions of this species.

***L. glaucomaria** A. L. Sm.—On the thallus of *Lecanora glaucoma* at White Water Brook, and at other places on the east coast; rare.

Rhizocarpon alboatrum Th. Fr.—A very common species on rocks about high-water mark, associated with *Placodium lobulatum* and *Lecanora prosechoides* (see page 101).

†var. **venustum** A. L. Sm.—Forms pure colonies on quartzite rocks splashed by the sea at high tide, near the Martello Tower, Sutton, and at various other places on the south-west coast.

†var. **epipolia** A. L. Sm.—Frequent on rocks and stones at the base of the earth-banks, Broad Strand; also on the limestones at Balscadden Bay.

Rh. geographicum DC.—On rocks from sea-level to the top of the highest cliffs on the east coast; less general at low levels on the south and south-west coasts. It is usually associated with *Lecanora glaucoma*, and is frequently infested with *Muellerella polyspora* Hepp.

Rh. petraeum Massal.—Occasionally on schistose and quartzite rocks and stones on the shore above high-water mark, Broad Strand; frequent on the east coast.

†var. **excentricum** A. L. Sm.—On limestone, Balscadden; rare.

†**Rh. confervoides** DC.—On quartzites, frequent round the coast.

Tribe GRAPHIDEI.

†**Arthonia punctiformis** Ach.—On Blackthorns, Earlscliffe; rare. The only previous Irish records are from Co. Galway.

†**A. paralia** Nyl.—Here and there on the Harbour wall associated with *Placodium murorum*; rare. The only previous record is Cleggan, Co. Galway.

A. varians Nyl.—Abundant all round the coast on the apothecia of *Lecanora prosechoides*. The only previous Irish record is from Lambay Island, Co. Dublin.

†**A. subvarians** Nyl.—Frequent on *Lecanora galactina*. The only previous Irish record is Castlebar, Co. Mayo.

Opegrapha atra Pers.—On Blackthorns, Earlscliffe.

O. saxicola Ach.—Broad Strand, associated with *Buellia stellulata*; frequent.

†var. **Decandollei** Stiz. f. **clarescens** A. L. Sm.—On rocks above Casana, and on the Nose of Howth; rare.

†**O. calcarea** Turn.—On grits and quartzites in the Ramalina belt all round the coast (see page 100).

f. **heteromorpha** A. L. Sm.—General and usually found nearer the sea than the species.

†**O. confluens** Stiz.—Only noted on the east coast; rare.

†**Graphina Ruiziana** Muell.-Arg.—On Blackthorns and on dead wood, Earlscliffe; rare. This species is usual on the surface of the wood where branches have been snapped off at right angles. The thallus is whitish, and the apothecia occur in prominent broken lines radiating from pith to bark. The spores of the Howth specimens are slightly smaller than the measurements in vol. II of the "Monograph of British Lichens," being $24–35\mu \times 9–15\mu$.

Tribe PYRENOCARPEI.

†**Dermatocarpon hepaticum** Th. Fr.—Very abundant on the earth-cliffs at Glenaveena, associated with *Biatorina coeruleonigricans*, &c. (see page 120).

Verrucaria maura Wahlenb.—On rocks above high neap-tide level; very common.

*var. **aractina** Wahlenb.—In sunny situations and at higher levels than the species; Broad Strand.

†var. **memnonia** Koerb.—In shade at a lower level than the species common round the coast.

V. mucosa Wahlenb.—On rocks inside neap-range; common. This species is recorded from stones in the beds of streams on the French coast.

Weddell, who has examined some of these specimens, says they correspond almost exactly with those in the exsiccata of Th. Fries; but he noticed a difference in the form and size of the spores of the French specimens, the majority of which were broadly elliptical or almost globose, measuring 6–8μ × 6–7μ. These correspond exactly with the measurements of the spores of *V. aquatilis*, which is common where fresh water flows on the Howth shores; *V. mucosa*, on the contrary, is never met with in these situations, but is confined to rocks between tide-marks. The spores of *V. mucosa* from Howth measure 7–10μ × 4μ, or occasionally 7–10μ × 5μ.

†**V. microspora** Nyl.—On rocks inside neap-tide marks associated with *V. mucosa*; common. The spores of the Howth specimens vary somewhat in size and shape. The type, in which the spores are elliptical and measure 9–10μ × 4–5μ, is most abundant below the Pelvetia zone; towards low water the spores are frequently longer, often measuring 14μ × 5μ.

*var. **mucosula** Wedd.—In the upper part of neap-range; frequent. The thallus of this variety is thinner, the perithecia are smaller and more numerous, and the spores are shorter and broader than in the type. Sandstede (**24**) records it from similar levels on the German islands of the North Sea.

†**V. striatula** Wahlenb.—On steep shady quartzites in the upper part of neap-range, on the more exposed parts of the coast, associated with *Lichina pygmaea*, *V. microspora*, and *V. mucosa.*

The main characteristics of *V. striatula* lie in—

(1) the thallus, which consists of small scattered or dendritically arranged ridges;

(2) the flat perithecia with large shallow apical depressions, which in the Howth plant are usually surrounded by a shiny margin;

(3) the small obtuse-ended spores, which measure 6–9μ × 4μ.

A study of this lichen as it grows on the Howth coasts shows that while there are specimens that answer to the above description there are others which differ only in having a continuous thallus, with small ridges and dots scattered irregularly on the surface, but sometimes radiating towards the circumference. This state of the lichen, which I call forma *continua*,[1] to distinguish it from the species, is much more abundant, and is usually found on a greater variety of rock and in more sheltered situations.

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[1] f. *continua.* Thallus continuus, niger vel nigro-virescens, thallina dorsa parva incondite dispersa.

V. striatula and *V. microspora* are often associated, and the two species are sometimes confused. *V. microspora,* however, may be easily distinguished by the hemispherical perithecia, which are not depressed at the top, but open by a small pore, and by the slightly larger elliptical spores, which measure 9–10μ × 4–5μ.

***V. scotina** Wedd.—Specimens which may be referred to this species were collected in the upper part of the *V. maura* belt at Drumleck Point, Old Boat-house, and on Ireland's Eye. The main characteristics and those which distinguish *V. scotina* from *V. maura*, with which it is associated at these places, seem to be the large prominent conical or hemispherical perithecia and the entire or sub-entire perithecial wall. The spores of the two species are very similar. In the specimens referred to *V. scotina* from Drumleck Point the thallus is almost evanescent. In those from Ireland's Eye and Old Boat-house the thallus is thick, black, somewhat cracked and areolate.

†**V. aquatilis** Mudd.—Plentiful at several places round the coast where fresh water flows and on the Harbour wall. The spores are sometimes slightly longer than the measurements given in the "Monograph of British Lichens," being 8–10μ × 5–7μ.

V. nigrescens Pers.—On rocks and stones lying above high-water mark, Broad Strand, associated with *Buellia colludens* and *Lecanora simplex* f. *strepsodina*; on the rocky cliffs at White Water Brook and on rocks at Martello Tower, Sutton; common.

†**V. murina** Leight.—On rocks, White Water Brook; rare.

* var. **pusilla** Arn.—Only seen on cliffs at Hippy Hole, where it was abundant with *Opegrapha calcarea.*

†**V. prominula** Nyl.—At Worn Hole and on shady rocks on the south side of Balscadden Bay, associated with *Arthopyrenia halodytes* and *V. memnonia.*

†var. **viridans** Nyl.—Occasionally found with the species.

V. rupestris Schrad.—On rocks and stones lying above high-water mark, Broad Strand; frequent.

* **VERRUCARIA LORRAIN-SMITHII** Nov. Sp.[1]—Thallus blackish-green, gelatinous, thin, continuous, or sometimes almost evanescent; perithecia excessively minute, about 120–180 μ in diam., only visible with a powerful lens, scattered, hemispherical, shining, opening by a very minute

---

[1] Thallus nigrovirescens, gelatinosus, tenuissimus interdum evanescens; perithecia dispersa, minutissima, aterrima, lucida, hemisphaerica, dimidiata, circa 120–180μ diam.; apice ostiolo minutissimo, 15–20 μ diam.; paraphysibus obsoletis; ascus ellipticus, 20–25 μ × 10–12 μ; sporis 8-nis, semper simplicibus, oblongo-elongatis, angustioribus, rectis vel leviter curvatis 15–18 μ × 2–4 μ. Ad saxa calcaria in locis inter aestus minimos et maximos nascens.

pore which measures 15–20 μ in diam., perithecial wall dimidiate; ascus elliptical, 20–25 μ × 10–12 μ; spores eight in the ascus, simple, rod-like, oblong-elongate or slightly curved, 15–18 μ × 2–4 μ, regularly or irregularly grouped in the ascus.

Habitat.—Limestone rock inside neap-range at Balscadden Bay, more abundant near low-water mark. It is associated with *V. microspora*, *Arthopyrenia litoralis* and *A. foveolata.*

In Sandstede's paper on the lichens of the German North Sea islands (**25**, p. 16) Bouley de Lesdain has described a new species—*Verrucaria Sandstedei*—which in the effuse thallus and the size of the ascus is almost identical with *Verrucaria Lorrain-Smithii.* The two species, however, are separated by the character and size of the perithecia, which in *V. Sandstedei* are immersed, sometimes confluent, and measure 1·5–2mm., whilst in *V. Lorrain-Smithii* they are prominent and excessively minute, measuring only about 175 μ in diameter. The spores, too, of *V. Sandstedei* are both longer and broader than those of *V. Lorrain-Smithii*, the former measuring 16–21 μ × 3–5·5 (rarius 4) μ, and the latter 15–18 μ × 2–4 μ.

Thelidium cataractarum Mudd.—On shady cliffs with a north aspect, Balscadden. The Howth plant differs in some points from Mudd's specimens in the British Museum. The thallus is lighter in colour and more farinaceous-tartareous, and the asci are more elongate. The spores also, though agreeing in colour, size and septation, are very distinctively guttulate—a feature not noticed in those of Mudd's specimens. These differences are probably due to the maritime habitat, and do not seem to me to be sufficient to justify the creation of a new species.

Acrocordia gemmata Koerb.—On Blackthorns, Earlscliffe; rare.

†**Arthopyrenia epidermidis** Mudd.—On Blackthorns, Earlscliffe; rare.

†**A. punctiformis** Arn.—On Blackthorns, Earlscliffe; rare.

†**A. litoralis** A. L. Sm.—Found only on limestone rocks inside neap-range, Balscadden Bay, and on barnacles, Old Boat-house.

The size of the perithecia in the Howth specimens varies a good deal, some of the largest measuring 0·4–0·5 mm. in diameter; the asci are cylindrical, and also variable in size, measuring 80–150 μ × 15 μ,; the spore measurements are 15–22 μ × 5–7 μ, and are larger than those given in the "Monograph of British Lichens."

†**A. foveolata** A. L. Sm.—On barnacle shells all round the coast, and on limestone rocks between tide-marks, Balscadden Bay, associated with *A. litoralis*; very abundant.

†**A. leptotera** A. L. Sm.—On Broad Strand and Ireland's Eye, on rocks

associated with *Lichina pygmaea* and *Verrucaria striatula*. *A. leptotera* also occurs both on the Broad Strand and at Balscadden Bay associated with *Verrucaria aquatilis* on rocks over which fresh water frequently flows. This species is distinguished from the other marine Arthopyrenias by the dark gelatinous thallus; the very minute perithecia, which are thickly scattered on the surface; by the dimidiate perithecial wall and by the narrow spores, which, in the Howth specimens, are of an almost uniform width throughout, and measure 15–17μ × 5 μ.

***A. halodytes** Oliv.—Frequent all round the coast on the silicious rocks Not seen on the limestones.

* var. **tenuiscula** Wedd.—With the species on hard smooth quartzites, Lion's Head.

***A. halizoa** A. L. Sm.—Limestone rocks, Balscadden.

***A. marina** A. L. Sm.—East end of Broad Strand with *A. leptotera*. The narrow three-septate spores of this species are very distinct, and easily separate it from the other marine Arthopyrenias. In the Howth specimens the thallus is less developed than that of *A. leptotera*, with which it is sometimes associated.

†**Porina chlorotica** Wainio.—On the Nose of Howth with *V. maura*; frequent on moist shady quartzites on the east coast; occasional on the Broad Strand.

Pyrenula nitida Ach.—On Blackthorns, Earlscliffe; rare.

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EXPLANATION OF PLATE IX.

1. Young growths of *Ramalina scopulorum.*

2. Young growths of the same from the upper Ramalina zone.

3–6. *Lecania atrynioides* nov. sp.

  4. Longitudinal section through one of the apothecia.

  5. Ascus and paraphyses.

  6. Spores.

7–10. *Acarospora benedarensis* nov. sp.

  8. Longitudinal section through an apothecium.

  9. Ascus and paraphyses.

  10. Spores.

11–13. *Verrucaria Lorrain-Smithii* nov. sp.

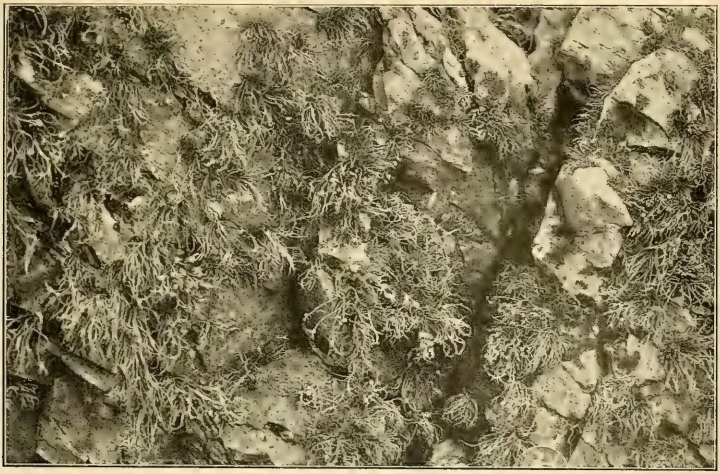
  11. A perithecium showing the very minute ostiole.

  12. Asci with spores variously grouped.

  13. Five spores.

R. Welch, *Photo.*

VIEW SHOWING THE NATURE OF THE BEACH AT GLENAVEENA, AND THE SLOPE OF THE CLIFFS.

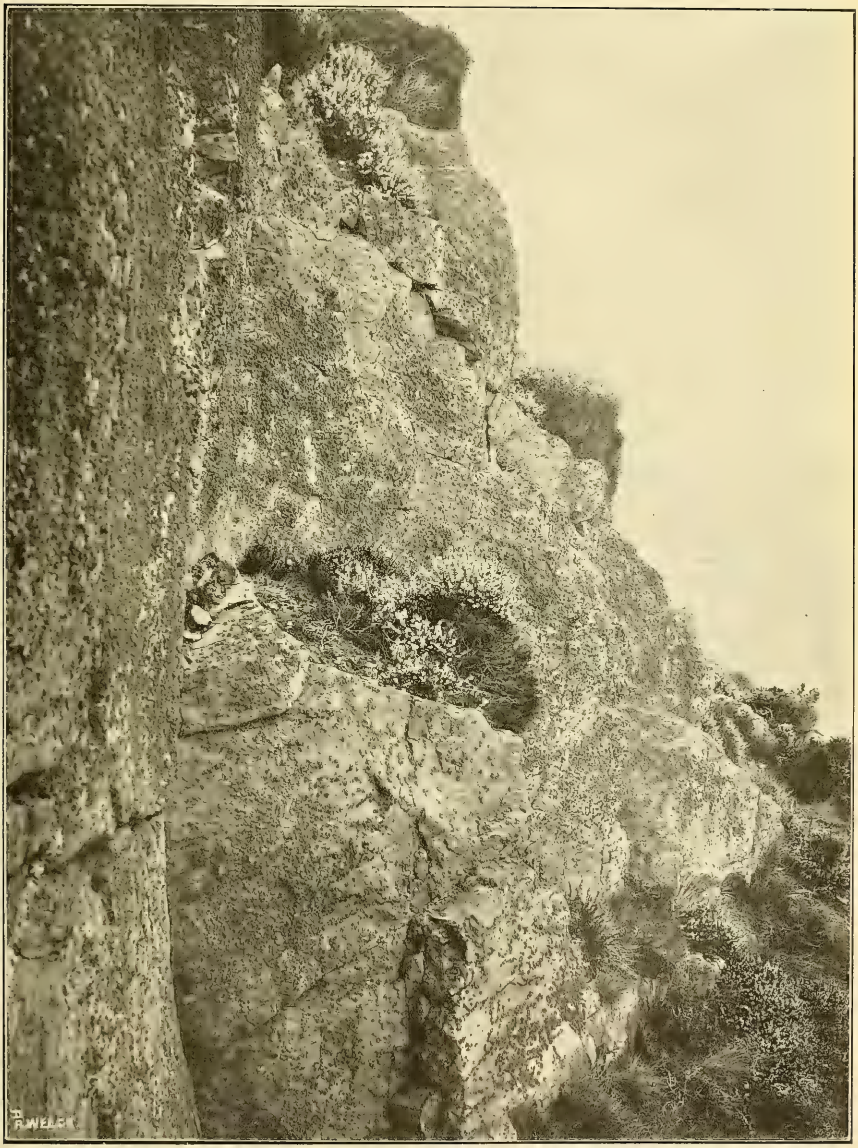


R. Welch, *Photo.*

FIG. 1.—*Ramalina scopulorum.*
RAMALINA BELT. LOWER FERTILE ZONE.

R. Welch, *Photo.*

FIG. 2.—*Ramalina A.*
RAMALINA BELT. UPPER BARREN ZONE.

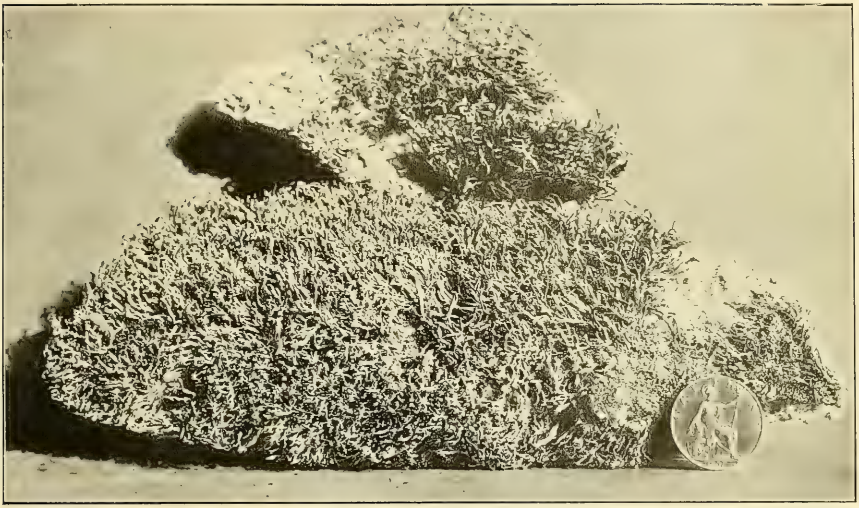


R. Welch, *Photo.*

Ramalina B.—On the quartzite cliffs at Red Rocks. [Note the absence of crustaceous lichens.]

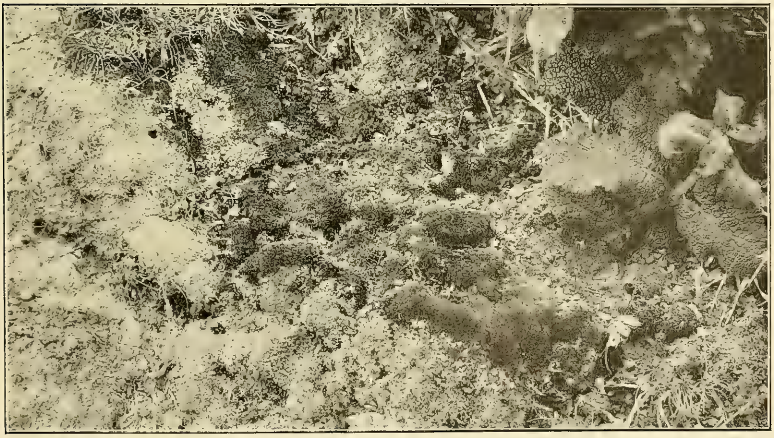
R. Welch, *Photo.*

Lecanora atra (GREY PATCHES), AND *Buellia ryssolea* (DARK PATCHES), IN THE *Ramalina scopulorum* BELT.



R. Welch, *Photo.*

FIG. 1.—SWARD OF YOUNG RAMALINAS FROM THE BOUNDARY STONES NEAR THE MARTELLO TOWER, SUTTON.

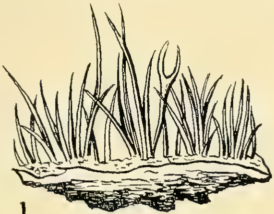


R. Welch, *Photo.*

FIG. 2.—SHOWING THE ZONATION OF THE VEGETATION ON THE OUTSKIRTS OF THE BARE AREAS, EARLSCLIFFE.

R. Welch, *Photo.*

LIMESTONE FORESHORE, BALSCADDEN BAY, THE HABITAT OF *Verrucaria Lorrain-Smithii.*



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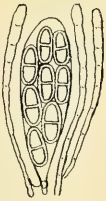
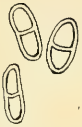
3 ×5



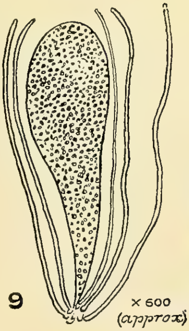
5 ×600 (*approx*)



6 × 900 (*approx*)

4 × 70 (*approx*)

10 × 600 (*approx.*)



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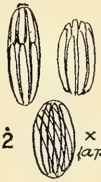

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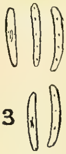

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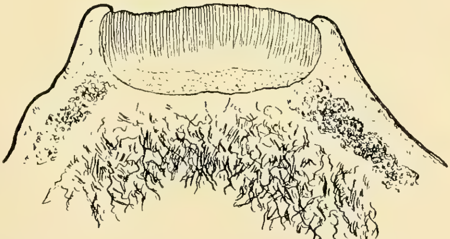

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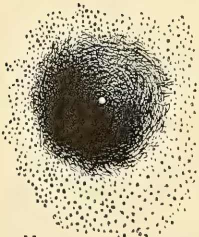

11 × 120 (*approx*)

Eileen E. Barnes, del.

M'Farlane & Erskine.

THE

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Vol. XIV. (N.S.), No. 7. AUGUST, 1913.

OXYDASES AND THEIR INHIBITORS IN PLANT TISSUES.

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EXPLANATION OF PLATE IX.

1. Young growths of *Ramalina scopulorum.*

2. Young growths of the same from the upper Ramalina zone.

3–6. *Lecania atrynioides* nov. sp.

    4. Longitudinal section through one of the apothecia.

    5. Ascus and paraphyses.

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    11. A perithecium showing the very minute ostiole.

    12. Asci with spores variously grouped.

    13. Five spores.

VII.

OXYDASES AND THEIR INHIBITORS IN PLANT TISSUES.

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[Read June 24. Published August 20, 1913.]

In the course of some work with Professor H. H. Dixon (9) on osmotic pressures in plants, the brown colour of the sap as expressed from many tissues was very noticeable. We subsequently found that this effect was well known to be due to the action of oxydases, as had been shown by Wheldale (28), Kastle (14), and others. In some cases, however, the sap was light in colour, and no oxydases could be detected. It has been pointed out by Aso (2) that tannin hinders the action of oxydase on the usual reagents employed for the detection of the enzyme, and Hunger (12) states that sugars behave in a similar way. We were able to account for a number of cases of undarkened sap by proving the presence of tannin. Some, however, remained over, and in view of the great importance now attaching to the action of respiratory enzymes, owing to the work of Palladin and his collaborators (18, 19, 20), to their production of organic acids, as shown by Weevers (27), to their rôle in plant pathology, as worked out by Bunzel (5, 6, 7), Woods (29), Shaw (25), and Suzuki (26), in the study of technology, as pointed out by Bailey (3), in the ripening of fruits and seeds, owing to the work of Appleman (1), Eckerson (11), and others, and in the study of genetics, by Keeble and Armstrong (15, 16, 17), it seemed advisable to make an attempt to explain why the oxydase could not be detected in certain saps.

The possibility of the presence of reducing agents at once presents itself, as such have been found by many workers, notably by Czapek (8), and by Schreiner and Sullivan in roots (23, 24).

Distribution of Oxydases in Certain Tissues.

The direct guaiacum oxydase reaction was given by the sap of the following leaves:—*Hedera Helix*, *Syringa vulgaris*, *Magnolia acuminata*, *Catalpa bignonioides*, *Fraxinus oxyphylla*, *F. excelsior*, *Helianthus multiflorus*;

ERRATUM.

W. R. G. Atkins.—Oxydases and their Inhibitors in Plant Tissues. Scient. Proc. Vol. XIV., No. 7, August, 1913.

Page 145, line 16 from bottom, for *Fraximus* read *Fraxinus*.

these all afforded dark-coloured sap. The saps which were undarkened could be divided into several classes as follows:—

I. Those which contained oxydase, but were deficient in organic peroxide, and accordingly only gave the guaiacum blue after the addition of hydrogen peroxide, viz., the indirect reaction. This division includes—

Wistaria sinensis, leaves.
„ „ petioles.
Chamaerops humilis, leaves.
Cordyline australis, leaves.
Equisetum Telmateia, vegetative shoot.
„ „ rhizome.
Pteris aquilina, very young leaves.
Eucalyptus globulus, petiole.

Of the above, the leaf-saps were pale green, the Eucalyptus petiole light red, and the Equisetum rhizome very light brown.

On the border-line between those affording saps of dark and those affording saps of light colour stands *Sambucus niger*, which gave a light-brown sap and direct guaiacum reaction when tested soon after pressing; but after standing in diffuse light for two hours it only gave the indirect reaction. Addition of the fresh extract, boiled for two minutes, failed to bring about the direct reaction; accordingly it appears that the organic peroxide is thermolabile. *Fraximus excelsior* also was found to give the direct action at one time, the indirect at another. These facts are in accord with the conclusions of Keeble and Armstrong (15), who regard the presence of the natural peroxide as variable.

II. Plant tissues yielding a light sap, in which the presence of tannin prevented the visible action of the oxydase: for example:—

Equisetum Telmateia, spike or cone.
Eucalyptus globulus, leaf.
Rosa rugosa, white petal.

III. Tissues in which the absence of dark sap and of the oxydase reaction in any form could not be ascribed to the inhibiting action of tannin, as in the following:—

Iris germanica, leaf.
Pteris aquilina, leaf.
Aspidium Filix-mas, leaf.

Being primarily concerned with cryoscopic determinations of osmotic pressures and with electrical conductivity measurements (10 A), these observations were laid aside till February, 1912, when the study was resumed.

The distribution of oxydase in microscopic sections of the tissues of a large number of plants was studied by means of the guaiacum reaction. On becoming acquainted with the researches of Keeble and Armstrong (15, 16, 17), other reagents, such as *a*-naphthol, benzidine, and *p*-phenylen diamin, were also employed. Alcoholic solutions of these penetrate tissues more readily than does guaiacum, and so have obvious advantages. It would be tedious to enumerate the tissues which afforded the direct or indirect reaction, but a few cases seem worthy of mention.

The very general presence of abundant oxydase in the phellogen layer of stems is remarkable, and suggests that the enzyme may have an important function in the formation of cork. In this connexion it may be remarked that while cork cells afford no oxydase reaction, they readily adsorb guaiacum on their walls. Thus a section which has been treated with this reagent, when further treated with potassium ferricyanide to detect tannin, immediately develops an intense blue in the cork, while the general blue colour of the cells containing oxydase is only slightly intensified.

This behaviour of cork is in marked contrast to that of sclerenchymatous tissue, the walls of which are very generally reactive with oxydase reagents, even when none of the tissue of the cortex or bundle is so, as in the *Iris germanica* leaf. In *Catalpa bignonioides* the cells of the cortex sheathing the sclerenchymatous strands of the stem gave a marked oxydase reaction with *a*-naphthol. Thus the evidence of the distribution of oxydase lends support to the view that the enzyme is concerned in the production of the hardened cell-walls.[1]

In carrying out some of these reactions on stems of *Rosa rugosa*, it was seen that while many cells contain tannin, as shown by the potassium ferrocyanide and ammonia reaction, the cells of the epidermis, colourless otherwise, afford a bright green colour with these reagents. The epidermal hairs also appear green; but I do not at present know to what this colour is due.

Petals of *Rosa rugosa*, white, were found to contain tannin, as did also the stamens. These did not darken perceptibly at the torn edges, nor did they give an oxydase reaction with guaiacum or *a*-naphthol. On the other hand, red petals of the same plant bleached when placed in an alcoholic solution of *a*-naphthol. A pink colour is produced on adding hydrogen peroxide; but this soon fades. The addition of water at this stage restores the bright-red colour, the veins becoming especially prominent. The purple colour given by the oxidation of *a*-naphthol appears only at the cut edges of

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[1] I find that Keeble and Armstrong (15) make a similar suggestion.

the petal. The explanation of this behaviour can be given in terms of Keeble and Armstrong's (15) theory, the decolourization being due to the operation of a reducing agent as dehydration proceeds, the oxydase being thrown out of action by the strong alcohol; the restoration of colour on diluting is thus due to the renewal of oxydase activity. The red in the veins is to be accounted for by the diffusion of the natural chromogen into them, thus bringing about contact between the bundle peroxydase and the chromogen. From the fact that the veins are red, not purple, it appears that the natural chromogen is more readily oxidized than is α-naphthol, though their intense colour is probably due to a combination of the two colours, for purple becomes visible along the torn edges of the veins. The natural chromogen is apparently oxidized by both the epidermal and the bundle oxydase of Keeble and Armstrong (17). Alcoholic benzidine decolourizes the red petal, and renders it slightly brownish, more markedly so in the veins, and this is brought about without the addition of hydrogen peroxide. *P*-phenylen diamin in alcoholic solution gives a brown, changing to dark green, the distribution being the same in the red petals as is the colour given by benzidine. With white petals, however, though no colour is given with α-naphthol and hydrogen peroxide, a very faint darkening appears in the veins of petals treated with benzidine and hydrogen peroxide, while *p*-phenylen diamin blackens the veins, and causes a slight general discolouration when followed by peroxide.

Another instance in which the inhibitory action of tannin is clearly brought out is the young and mature leaves of *Vitis Veitchii.* The sap of the young red leaves shows the direct action with guaiacum, whereas the more mature green leaves, which contain tannin, give no reaction. Young and old, however, both give the epidermal oxydase reaction with alcoholic benzidine, though they give no reaction for the bundle oxydase with α-naphthol.

With *Hedera Helix*, however, young and old leaves give the direct action with guaiacum when bruised, the epidermal reaction with alcoholic benzidine, and the indirect bundle oxydase reaction with α-naphthol and hydrogen peroxide.

The separation of tannin and oxydase had been effected by Aso (2), as the latter is precipitated by strong alcohol. Attempts were made to remove the tannin from the fertile stem of *Equisetum Telmateia* by means of gelatine, but this was not effective, or at least the enzyme could not be detected in the filtrate. The employment of potassium ferricyanide and ammonia, or of basic lead acetate, is ruled out, for these substances give a blue colour with guaiacum.

When leaves of *Cochlearia armoracia* are treated with alcholic α-naphthol and hydrogen peroxide, the presence of Keeble's "bundle peroxidase" is revealed in all the veins. But the surfaces of the leaves appear curiously mottled with purple. This colour is seen to be confined to the three, four, or five epidermal cells immediately abutting on the guard-cells of the stomata, the guard-cells themselves being unaffected.

Again, when leaves of *Tradescantia virginica* and *Wistaria sinensis* are placed in alcoholic benzidine, it is seen that the guard-cells and the four cells abutting on them become brown to a more marked degree than do the other epidermal cells. Thus they seem to be considerably richer in oxydase. If now a dilute solution of methylene blue be added to the leaf, after rinsing off the benzidine, the epidermal cells become blue,[1] with the exception of the guard-cells and the four adjacent cells, which remain brown, the nuclei being especially darkened.

This peculiar distribution of the enzyme may be related to the mechanism of the movement of the stomata. The question is being worked out further.

The distribution of oxydase and reducing agent in Iris germanica.

The localization of oxydase in *Iris germanica* is particularly interesting. As far back as 1820 Planche (21) had noticed that the fresh roots (? rhizome) of this plant gave the direct action with guaiacum. Sections across the root of Iris give the indirect action with guaiacum and hydrogen peroxide. Though the whole cortex gives the colour, it is most intense in the outermost and innermost layers, the walls of the endodermis being deeply stained.[2] The walls of the smaller vessels of the wood and of the central lignified tissue also give the reaction, though none is given by the bast or the large vessels of the wood. The whole root, when pounded up, turns guaiacum blue, so there can be no inhibitor present in the tissues which do not give the reaction, or more probably such a body is only present in small quantity. Sections through the rhizome show the indirect oxydase reaction throughout, especially in the thick-walled woody vessels of the bundles. Addition of potassium ferricyanide to this section brings about the appearance of a deep blue in the corky tissue, as already noted.

In the leaf-sections the only oxydase reaction is, as before, the indirect one with guaiacum, and that is limited to the wood walls and sclerenchyma of the bundles.[3] Further up the leaf it is entirely absent. Sap from the

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[1] I am indebted to Miss K. Hadden for this observation.

[2] Cf. the previous suggestion as to the function of oxydase in cell-walls.

[3] Under certain conditions the oxydase reaction may be widespread.

leaf as a whole fails to give any oxydase reaction, with the exception of that from the extreme tip which affords the indirect reaction with guaiacum. This irregular distribution leads one to suspect the presence of some reducing agent, which prevents the detection of the oxydase.

The purple flowers yield an intensely coloured sap when pressed. The brown markings at the bases of the perianth leaves are due to the optical combination of yellow plastids with a purple cell-sap. The purple pigment turns red with an acid, and this red is stable. With alkalis, however, a green fading to pale straw is produced. Neutralization does not restore the purple, nor does acid now turn the sap red. Thus alkalis quickly destroy the pigment. A very faint trace of ammonia vapour alters the purple to a beautiful shade of blue, but this fades through green to straw in a day. This coloured extract was employed as a reagent later on.

Analyses of leaf-sap of Iris germanica.[1]

In view of the above-mentioned peculiarities of *Iris germanica*, it seemed desirable to get an idea of the chemistry of its cells. It is well known that starch is not stored in the leaves of Iris, except in the guard-cells of the stomata, in which it is by no means invariably present, and in the white bases. In the rhizome and cortex of the root it is, however, plentiful.

The sap as pressed is of a very light-brown colour, and is strongly acid, malic acid being present. The absence of tannin is shown by the fact that the addition of ferric chloride to a portion of the sap, decolourized with animal charcoal, gave only the faintest darkening. Phenyl glucosazone was obtained in abundance from the sap, thus proving the presence of glucose in the usual manner, and on cooling the reaction-mixture a brownish sediment slowly settled down; this was supposed to be phenyl maltosazone, but it was not possible at the time to settle the question.

It has been shown by Hunger (12) that substances present in the cocoa-nut milk from unripe nuts inhibit the guaiacum oxydase reaction; these substances he believes to be sugars. I have found an intense direct guaiacum reaction to be given by the sap of *Hedera Helix*, the analysis of which by the polarimeter showed a large amount of sugar to be present, the rotation amounting to 1·40° in a 20 cm. tube. A plentiful crop of phenyl glucosazone crystals was obtained also. Accordingly, I am inclined to think the inhibitor in the cocoa-nut is not sugar. However, it seemed advisable to take into account the actual concentration of the sugars in Iris sap.

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[1] Most of the quantitative analytical work was done in conjunction with Professor H. H. Dixon in connexion with another research.

Fresh leaves were pressed at various times during February and March, 1912, and determinations of reducing power, rotatory angle, osmotic pressure, mean molecular weight, and electrical conductivity were made. It may here be remarked that though these values are recorded as being roughly comparative, they are neither absolute nor even strictly comparative values. For it has been shown by Dixon and Atkins (10) that the true concentration of the cell-sap is only obtained after the protoplasm is rendered permeable by immersion of the tissues in liquid-air before pressing.

Under Δ, P, M and $C \times 10^5$ in the following table are recorded the freezing-points determined by the thermo-electric method of cryoscopy (Dixon and Atkins (9)), the osmotic pressures calculated from them, the mean molecular weights obtained from the freezing-point and solid residue determinations, and the electrical conductivities:—

No.	Date.	Sample.	Δ	P.	M.	$C \times 10^5$
440	Feb. 17	Leaves, including white bases, . .	0·627	7·54	119	523
442	Feb. 17	Half-leaves, A, ...	0·540	6·49	135	554
443	Feb. 17	Half leaves, B, of 442, dried at 110° and made up to original weight with water,	0·704	8·47	182	783
448	Mar. 2	Green parts of leaves only, . .	0·592	7·12	120	453
449	Mar. 2	Bases of leaves used in 448, . .	0·558	6·71	115	468
454	Mar. 16	Green parts of leaves, . . .	0·530	6·38	133	414

It is clear that the low values of the mean molecular weights exclude the presence in quantity of the hexoses or higher sugars. With the exception of No. 443, the extracted dried leaves, the values found lie fairly close together.

It will be noticed that the electrical conductivities increase towards the close of the spring. This is probably an apparent effect, due to the greater number of young leaves employed.

The sugar analyses showed that the amounts of sugar present were not very great. In one case (No. 443), 0·48 grm. of dextrose and levulose was found per 100 c.c. of sap. with 0·65 grm. of cane-sugar. Yet this sap gave the strong reducing action and inhibited Hedera oxydase. In another case (No. 448) 3·32 grm. of dextrose were present, 1·90 grm. of levulose, and a small quantity of cane-sugar. Owing to the absence of starch in the leaves, these calculations were made on the assumption that no maltose was present. In making the analyses, the sap colloids were removed by basic-lead acetate and excess of lead by sulphuretted hydrogen. The latter was boiled off, and the sugars estimated by Fehling's solution and the polarimeter as described by Brown and Morris (4). Calculation shows that at the outside no more than half of the osmotic pressure is due to the sugars in Iris leaf sap. From these analyses it will be seen that the sugar concentrations are not such as to interfere with the reaction of the oxydase if present.

The presence of a strongly reducing substance other than the sugars is shown by the rapid decolourization of potassium permanganate solution by the juice of Iris, even in the cold. This effect is apparently not altered by boiling the juice. A small quantity of the leaf-juice quickly decolourizes the purple sap of the petals, and treatment with hydrogen peroxide does not restore the colour. Hydrogen peroxide alone slowly destroys the purple pigment. That this action of the Iris leaf-sap is a reducing one is made further clear by the fact that not only does it prevent the production of guaiacum blue when added to leaf-sap of *Hedera Helix*, but it also destroys the blue when it has been already produced by Hedera sap. When, however, Hedera sap is mixed with a little Iris sap and guaiacum, it is found that, after a certain quantity has been added, the further addition of hydrogen peroxide causes the blue colour to appear. Thus the Hedera sap no longer affords the direct reaction, as its supply of organic peroxide has been used up by the reducing agent of Iris. The reducing agent is not destroyed by boiling.

Dialysis of the sap in the presence of toluene was found to remove the reducing agent, for on testing the sap it was found to give the indirect oxydase reaction with guaiacum after dialysis for several days. The next day it was found to give the direct reaction, so apparently some of the organic peroxide remained behind. The sap employed for the dialysis had been filtered through an ordinary filter paper. This dialyzed sap also gave the catalase reaction. As dialysis proceeded, the effect of the sap upon the purple colouring matter of the petals was tried daily. The leaf-sap which gave the indirect oxydase reaction was found to have altogether lost the power of decolourizing the petal extract. Decolourization was, however, effected by the oxydase of *Hedera Helix* leaf.[1] So it appears that either reduction or further oxidation converts the purple pigment into a colourless substance. That the pigment is not merely extracted by alcohol is shown by the alcohol in contact with a petal becoming first purple and then colourless. Though neutral hydrogen peroxide slowly decolourizes

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[1] The possibility is not excluded that the oxydase obtains oxygen for oxidation at the expense of the pigment.

the purple extract, probably by oxidation, it must be remembered that this reagent can have a reducing action in the presence of an easily reducible substance, such, for instance, as silver hydroxide. Assuming it to be an oxidation, it was thought that the reducing action of the leaf-sap might restore the original purple, but this was not found to happen.

Precipitation of the enzymes by pouring the leaf-sap of Iris into strong alcohol also serves to separate the reducing substance which is found in the filtrate. The precipitate now gives the indirect guaiacum reaction, the colour being destroyed by addition of the filtrate.

Reducing agents in other plants.

In the preliminary experiments it was noticed that whereas very young leaves of *Pteris aquilina* gave the indirect guaiacum reaction, the mature leaves gave no reaction for oxydase or for tannin. *Polypodium aureum* leaves gave the indirect reaction, while *Aspidium Filix-mas* gave none, and even behaved like Iris leaf-sap, but not to such a marked extent. The pressed leaves afforded a light-green sap; this when dialyzed for several days gave the indirect guaiacum reaction, but further dialysis failed to bring about a direct reaction. It must be distinctly understood that these reducing agents are active in aqueous solution, and so are different from the reducing agents of Keeble, Armstrong, and Jones (16) to which the decolourization of flower anthocyan pigments is due, for these only become active as dehydration proceeds.

The pigments of the flower of Iris.

The flowers of Iris are roughly divided into two classes, the purple and the yellow. Of these the yellow owe their colour to a plastid pigment, while the purple is due to an anthocyan pigment dissolved in the cell vacuoles.

Six varieties will be considered here :—

1. Purple perianth leaves, with yellow hairs, and white streaked with brown markings at base, the latter being due to the presence of yellow plastids and purple sap in the same cells; the purple is very noticeable in the epidermal papillæ. This is *Iris germanica.*
2. Same as 1, but the purple is paler all over, *Iris pallida.*
3. Same as 1, but the purple is very intense, and contrasts sharply with large white patches, about equal in area to the purple region, *Iris pallida,* var. *victorine.*
4. Deep yellow, with red-brown markings at base, Iris sp.? Canadian.

5. Pale creamy yellow, red-brown markings much the same as in 4, *Iris laevigata.*
6. White with yellow at base, and extending up the central region, *Iris aureus.*

Perianth leaves of the above types were treated with alcoholic benzidine or *a*-naphthol, followed by hydrogen peroxide, as advocated by Keeble and Armstrong. In no case was the natural pigment, destroyed by immersion in alcohol, restored again by removal of the leaf to water. In this respect Iris[1] differs from many flowers studied by Keeble and Armstrong, and from *Rosa rugosa*, as noticed earlier in this paper. Possibly this is due to the lack of a sufficient supply of the chromogen, though absence of the "epidermal oxydase," detected by benzidine, or presence of the characteristic reducing substance of Iris, seems to be a more probable cause, as will be shown. It may here be noted that while *a*-naphthol is turned to a lilac colour by bundle peroxide, benzidine is turned first blue, then brown, by both bundle and epidermal oxydase.

After treatment with the above reagents it was seen that, in type 1, the purple flower, the bundle peroxidase was absent, except for traces in the tips of the veins, whereas in type 2, the pale purple, it was well marked. On the other hand, in the purple type the benzidine reaction was very pronounced in the veins, and distinct in the other cells, while in the pale purple the brown in the veins and other cells was not so pronounced. The production of the purple is accordingly seen to be due to the action of the epidermal peroxidase on the chromogen. In type 3 there was only the faintest reaction with benzidine in the veins, while with *a*-naphthol there was absolutely none. Since, however, the patches of the purple colour were originally present, it seems that this apparent absence of oxydase in any form is rather due to the presence of the reducing substance of Iris in the white patches, and its diffusion through all the tissues on the death of the protoplasm.

On examining the yellow flowers it is seen that they all possess the epidermal peroxidase, the pale yellow, type 5, having the greatest quantity, and the white and yellow, type 6, the least. Accordingly, the absence of purple is due to absence of chromogen, which only exists in the portions at the base, which appear red-brown. The bundle peroxidase, too, is plentiful in types 5 and 6, whereas in 4, the deep yellow, it is limited to the uppermost extremities of the veins. This is possibly due to the presence of the reducing substance in their lower portions.

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[1] Other species of Iris at present under observation recover in part their original pigment. The inhibitor in the petals may also be destroyed by hydrogen cyanide.

SUMMARY.

1. The presence of oxydase in abundance in the phellogen ring and in sclerenchymatous walls seems to point to this enzyme as being concerned in the production of cork and sclerenchyma.

2. Keeble and Armstrong's bundle peroxydase may extend to the epidermis, and has been detected in the three, four, or five cells immediately abutting on the guard-cells of *Cochlearia armoracia.* Epidermal oxydase is frequently most active in guard-cells and those immediately in contact with them, as in *Wistaria sinensis* and *Tradescantia virginica.*

3. Plants which yield a brown sap always give the direct oxydase reaction, while those which yield light-coloured saps either give the indirect action or contain tannin or some reducing agent.

4. The peculiar distribution of oxydase in *Iris germanica* has been shown to be only apparent, as the presence of a strong reducing agent prevents its detection in certain tissues. It is possible to remove the reducing agent by prolonged dialysis, and the presence of oxydase may then be demonstrated. The reducing substance is not a tannin or a sugar. The mature leaves of *Aspidium Filix-mas* also contain a reducing agent which masks the presence of oxydase. After dialysis of the leaf-sap, the indirect guaiacum reaction may be brought about. A more rapid method for separating the reducing substance and enzyme is that employed by Aso for tannin. The precipitation of the enzyme may be effected by pouring the sap into absolute alcohol. The precipitate when redissolved in water gives the indirect guaiacum reaction very intensely. This colour is destroyed by addition of the filtrate. Thus fresh evidence has been obtained in favour of the view that oxydases are universally present in plant tissues, though their immediate detection may be hindered by the presence of reducing agents.

5. The colours of the perianth leaves of six varieties of Iris have been shown to be due to the presence or absence of a yellow plastid pigment and an anthocyan pigment, which is formed by the action of the epidermal peroxydase on a chromogen. This production of pigment may be inhibited by the presence of the reducing substance of Iris leaf-sap. The latter is distinct from the decolourizing reducing substance of Keeble and Armstrong, as it is active in aqueous solution as opposed to strong alcohol.

I am indebted to Professor H. H. Dixon for the benefit of his advice and continued interest throughout the work.

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OXYDASES AND THEIR INHIBITORS IN PLANT TISSUES.

Part II.—The Flowers and Leaves of Iris.

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VIII.

OXYDASES AND THEIR INHIBITORS IN PLANT TISSUES.

Part II. THE FLOWERS AND LEAVES OF IRIS.

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[Read November 25, 1913. Published January 16, 1914.]

In the first paper by the writer under the above general title (1), it was shown that, in the leaf of *Iris germanica*, there exists a substance which prevents the detection of oxydases by the direct application of guaiacum solution and hydrogen peroxide. This inhibitor is effective both in leaf-sections and in sap from leaves pounded in a mortar. Furthermore a blue solution of oxidized guaiacum is immediately decolorized by the reducing action of this substance. Dialysis of the sap pressed from the leaves results in the disappearance of the reducing agent, leaving behind the oxydase or peroxydase. Active preparations of the enzymes may also be obtained by precipitating them with strong alcohol, as the reducing agent remains in solution.

The objects of the present research are (*a*) to determine qualitatively the effect of light and darkness upon the oxydases and reducing substances of Iris leaves; and (*b*) to study the relationship of oxydases and reducing agents to the production of colour in the flower of Iris, by means of the reagents, found suitable by Keeble and Armstrong (4, 5, 6) in their fascinating work on other types of flowers.

The Oxydase Reagents.

To detect oxydases in cut sections of Iris sp. a very dilute aqueous alcoholic solution of guaiacum resin was employed. This was followed by hydrogen peroxide also dilute and neutral; with both sections and perianth leaves dilute solutions of benzidine and α-naphthol in aqueous alcohol were used, with subsequent addition of the peroxide. The reaction is blue with guaiacum, blue to brown with benzidine, and a lilac purple with α-naphthol. The use of the two latter reagents in this connection has been advocated by Clarke (2), and by Keeble and Armstrong (4), and has been found of great value.[1] It appears that the results obtained may be interpreted in two different ways: (1) that the coloration of the reagents is due to specific oxydases, for certain tissues are found to react with one and not with another; (2) that one oxydase is present, but that in certain tissues reducing agents are located, that is to say substances more readily oxidized than the artificial or natural chromogen. Such reducing agents would rank as apparent inhibitors of oxydase action; but possibly substances which totally inhibit oxydase action are present. From the fact that, as shown by Keeble and Armstrong (5), for *Primula sinensis* and other plants, hydrogen cyanide is able to remove an inhibitor, after which all three reagents act intensely, it seems almost certain that the one oxydase interpretation is correct; and that the different natural and artificial chromogens are susceptible to its action to different extents on account of the interference of the inhibitor. These points will be more evident when the reactions with various flowers are considered in detail.

(*a*) OXYDASES IN *IRIS GERMANICA* LEAF.

As is well known, the sword-shaped leaf of *I. germanica* is peculiar in that it has been folded in two along its length, so that its morphological upper surface constitutes the inner side of the fold. This upper surface is devoid of stomata and is pressed against the under—namely, the outer—surface of the next adjacent leaf. The bases of the leaves are whitish. About half-way from the base the upper surface disappears altogether, so that the two sides of the leaf-blade are entirely bounded by what is morphologically the under-epidermis. The leaf is mainly composed of compact parenchyma traversed by parallel vascular bundles. In the latter the wood is toward the upper (inner) surface, and below it is the bast and a very noticeable triangular mass of sclerenchyma. There is a somewhat indefinite bundle-sheath round the whole.

Transverse sections of the green lower portion of the leaf, growing under normal conditions, when treated with guaiacum and peroxide frequently develop a blue colour in the wood and always in the sclerenchyma, but the bast, epidermis, and mesophyll are usually unaffected. Even when some colour is shown by the last two tissues, application of sap from the whole leaf pounded up destroys it. Treatment of sections with hydrogen cyanide serves to remove an inhibitor, so that when tested a blue colour appears all over. The upper portion of the leaf contains no inhibitor, for it gives

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[1] The problem of deciding between a slight positive result and a negative one with the reagents employed is always difficult, particularly in the epidermis and especially so with benzidine, as the solution may have a faint brown tinge even when quite fresh.

the blue reaction. Sometimes only the extreme tip is free; at other times the inhibitor is limited to the lower two-thirds of the leaf.

Treatment of similar sections with benzidine results in the appearance of brown in the bast, bundle sheath, and epidermis, also in the walls of the wood, though the sclerenchyma remains unaffected. Indeed it is remarkable how the cell-walls take on a brown colour, especially the thickenings of the guard-cells and epidermis generally. The endodermis of the root is another good instance of this staining of the walls. It should be noted that the mesophyll remains uncoloured, and that the epidermis is frequently inactive, owing to the presence of inhibitors as shown by hydrogen cyanide treatment.

The α-naphthol reagent behaves somewhat in the same way, giving purple with the epidermis, guard-cells, bast, wood, but not as a rule with the mesophyll, and never with the sclerenchyma. After cyanide treatment the mesophyll reacts positively; but the sclerenchyma is still colourless. It seems that the different behaviour with the various reagents may best be explained as due to the varying stability of their oxidation products to reducing substances. If the latter are aldehydes, the action of the cyanide may be readily attributed to a cyanhydrin formation. The coloration of sclerenchyma by guaiacum is probably due to the inclusion of traces of oxydase insufficient to act on the other reagents and to the complete absence of the inhibitor.[1]

Effect of Light and Darkness upon Oxydase in Leaves.

To test the effect of light and darkness two plants were brought from the gardens on July 18th; one was set in a dish in the laboratory dark press, while the other was put in the greenhouse. Both were kept well watered, but it is evident that their external conditions differed in many other respects as well as in the presence or absence of light; for the temperature, humidity, and circulation of air must have been quite dissimilar. Sections were examined at intervals over two months. These were obtained from the greenhouse control, green leaves in the dark, nearly colourless leaves in the dark, and young leaves about nine inches long which had grown from the rhizome during the period of total darkness. Contrary to what might be expected, the oxydase reactions were on the whole but little altered by growth in the dark; this shows that the inhibitor is certainly not a direct product of photosynthesis. It is true that at times the mesophyll of leaves from the dark press gave stronger reactions than those from the greenhouse, but at

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[1] Leaf sections which had been boiled showed none of these reactions with the oxydase reagents. The cuticle however, appeared green owing to the action of the dissolved chlorophyll upon it.

others the intensities and distribution of the oxydase reactions appeared to be identical. On the whole, it may be said that leaves from the dark contain the inhibitor in the lower half of the leaf, while those in the light have a more widespread distribution. It is, however, by no means certain that this is not due to individual variations or to the weakened condition of the plants in the dark.

(*b*) THE FLOWERS OF IRIS.

A number of species and forms were obtained during June and July, 1913, from the Botanic Gardens of Trinity College, Dublin. Care was taken to verify the garden names as far as possible. For this purpose Lynch's Handbook (9) and Dykes' Monograph (3) were consulted, as well as the specimens in the Trinity College Herbarium. I am, moreover, much indebted to Mr. Wild, of the Botanic Gardens, for assistance in identification.

Adopting the usual nomenclature, the outer drooping perianth leaves are termed the "falls," the usually erect inner three being known as "standards." Of these the lower part is the "haft" and the upper the "blade." Owing to the fact that the falls, which are opposite the stamens, are usually of a greater diversity of colour than the standards, the former have been employed in the majority of the tests.

Members of three large groups have been utilized, namely, the Xiphion, the Pogoniris, and the Apogon groups. The last is very large, and includes such different forms as the yellow flag Iris *I. pseudacorus*, the large Japanese *I. Kaempferi*, and the small-flowered *I. sibirica*. The members of this group seem to be less closely connected with each other than are those of others, and crosses are often sterile. The Pogoniris group contains those flowers which have a beard of multicellular hairs on the hafts of the falls, such as *I. germanica* and the sweet-scented *I. pallida*. The Xiphion group is usually well represented by the many garden forms of Spanish Iris, *I. Xiphium*, and of English Iris, *I. xiphioides*.

In Table I are recorded the results of the application of the various reagents to *I. xiphioides*. The first column is for the restoration of the natural colours by immersion of the leaf in water; these had previously been destroyed by soaking in strong spirit till colourless. Both processes were at air-temperature, and one day was allowed for the restoration, but several observations were made during that period. The other columns show the character of the reactions with benzidine and α-naphthol, hydrogen peroxide being added in each case to the extent of five drops of a neutral four-volume solution to each petri dish containing the specimens. It should be mentioned that exposure to the reagents without the addition of hydrogen peroxide only resulted in a slight browning of the epidermis in one instance (*I. Xiphium*, var. *Battandieri*, blanche superbe) after an exposure of forty minutes, whereas ten minutes after the addition a well-marked reaction had appeared in the veins and epidermis of a number of varieties.

An attempt at a colorimetric quantitative estimation is indicated by the number of plus signs. The chemistry of these changes is dealt with by Keeble, Armstrong, and Jones (8).

Table II shows the result of the application of the above two artificial chromogens after treatment of the perianth leaves with 0·2 per cent. hydrogen cyanide, and subsequent thorough washing. For this purpose leaves from the flower employed in the experiment of Table I were made use of in each case, the two treatments being carried on concurrently.

For convenience of reference the specimens are numbered consecutively through the various tables.

TABLE I.

Xiphion Group.

No.	*I. xiphioides* (Ehrb.)	Restoration of colour	Benzidine		α-Naphthol	
			Veins	Epidermis	Veins	Epidermis
1	Pale purple, falls, . . .	—	+ +	+	+ +	+
2	Pale red purple, mottled with more intense patches, falls and standards,	+ slight	+ +	+ +	+	+ traces
3	Dark purple, falls and standards, .	+ in patches	+ +	+ +	+	—
4	White with blue specks, yellow central patch, falls,	—	+ +	+ +	+ +	+ +
5	White, pale blue patches, falls. [Pale blue standards.]	—	+	—	+	—
6	Blue, yellow central patch surrounded by white, falls. [Blue standards.]	+ slight	....	....	+	—
7	Purple spots on pale blue, yellow central patch surrounded by white, falls. [Standard more intense purple.]	+ slight	+	—	+	—
8	Deep purple spots on purple, yellow central patch surrounded by white, falls. [Deep purple standards.]	+	+	+ partly	+ slight	—
9	Deep purple red, yellow in central patch surrounded by white. [Purple red standard.]	+	+	+ partly	+ slight	—

TABLE II.

No.	After HCN treatment	Benzidine		α-Naphthol	
		Veins	Epidermis	Veins	Epidermis
4	....	+++	+++	+++	+++
5	....	+++	+++	+++	+++
6	....	+++	+++	++	+
7	....	+++	+++	++	+
8	....	....	....	++	—
9	....	+++	+++	++	—

Discussion of Results of Tables I and II.

With regard to the restoration of the original anthocyan pigment (the yellow plastid pigment is never restored), the chief factor seems to be the intensity of colour in the untreated leaf. For the oxydase which was sufficient to produce the natural pigment is still there. Yet in some cases the diffusion of inhibitor from adjacent cells on the death of the protoplasm undoubtedly checks the re-formation of colour. The mechanism of this reaction has been fully discussed by Keeble and Armstrong (4), and their explanation is quoted in a former paper by the writer (1).

In connection with the oxydase distribution, it is apparent that α-naphthol is more susceptible to inhibitors than is benzidine. This is probably because its oxidation products, readily formed, part with oxygen just as readily. The same holds good for guaiacum. Experiments upon the destruction of the oxidation products of these artificial chromogens with *Iris germanica* leaf-sap bear out this view. Inspection of the table shows that no yellow is present in Nos. 1, 2, 3, and 5. Of these the first three show well-marked oxydase reactions and natural anthocyan pigments; whilst the last probably owes its white and pale-blue to the presence of an inhibitor, for here oxydase appears to be absent in the epidermis. No. 4 brings about a marked reaction with both reagents, so its white is due to absence of chromogen, except in a few spots. Accordingly this white is probably a Mendelian recessive. Evidently there is no appreciable amount of inhibitor present, although the yellow patch at the junction of haft and blade is usually productive of such a substance. Examination of Nos. 6, 7, 8, 9 shows that though the natural pigments are well developed, yet the oxydase reactions are not at all pronounced. Each yellow patch is seen to be surrounded by white in the natural state. Thus it appears that cells in the region of those containing yellow plastid pigments produce an inhibitor of colour-formation. Accordingly yellow is surrounded by white. This interpretation seems to be the correct one rather than the alternative of absence of chromogen; for the addition of a chromogen does not give any very decided reaction. Indeed it frequently appears that conditions suitable for the production of the natural pigment are suitable for the benzidine reaction, but not for the α-naphthol. Where no chromogen is present the epidermis usually gives an intense reaction with the latter reagent. The causes of the differences in the shades of colour—blue, purple, red, etc.—have been recently dealt with by Keeble, Armstrong, and Jones (8).

Table II shows the influence of treatment with hydrogen cyanide as described in removing the inhibitor. All the benzidine reactions are now very dark, while those with α-naphthol are much intensified. Here again it is noticeable that the presence of deep purple chromogen seems to inhibit the latter reaction in the epidermis; and this inhibitor is not removed by the cyanide, or it may be that twenty-four hours was not sufficient for its complete removal, or even that this body was regenerated by enzyme action while the last traces of the cyanide were being washed out. However, if it is correct that the white in No. 5 is due to the presence of the general inhibitor, and since the conditions of cyanide treatment have sufficed for its complete removal, it does appear as if the presence of the natural chromogen acted as an inhibitor on the production of the α-naphthol reaction.

Table III. contains the results afforded by the varieties of *I. Xiphium.*

TABLE III.

Xiphion Group (*continued*).

No.	*I. Xiphium*, Linn.	Restoration of colour	Benzidine		α-Naphthol	
			Veins	Epidermis	Veins	Epidermis
10	Var. *chrysolora*, Hort.; deep yellow in centre, sulphur yellow around, falls,	....	+ +	+ +	+ + +	+ + +
11	Var. *Battandieri*, Foster; form "blanche superbe," yellow in the centre, pure white around, falls,	....	+ +	+ +	+ +	+ +
12	Var. *lusitanica*, Ker; form "Thunderbolt," yellow in centre, bronzed purple around, falls,	+ partly	+ + +	+ + +	+ + bases + tips	— —
13	Var. ?; yellow in centre, a little light blue around, falls,	....	+ +	+ +	+ + +	+ + +
14	Var. ?; yellow in centre, intense blue around, falls,	+ partly	+ + +	+ + +	+ + +	+ + +

Discussion of Results of Table III.

Each of the above five varieties of *I. Xiphium* showed a sharply defined inhibition-area, which remained colourless when treated with the usual reagents. This area, on the haft of the falls, coincided with the distribution of the plastid yellow. Prolonged treatment with the reagents in part obliterated this white area, apparently because the inhibitor slowly diffused away.

As before, restoration of colour took place in the deeply coloured forms. This group is apparently characterized by the absence of inhibitor, except over the sharply defined area referred to above. A similar inhibition has been noticed by Keeble and Armstrong (4) over the yellow eye of *Primula sinensis.* The strong reaction with benzidine of the "Thunderbolt" and deep-blue (No. 14) forms is noteworthy, as it brings out the relation between oxydase action and intensity of natural pigmentation when undisturbed by inhibitors.

The absence of a-naphthol reaction in "Thunderbolt" epidermis agrees with what was previously noted. With the blue epidermis of No. 14 the reagent reacts strongly; here it seems as if a blue chromogen does not inhibit the reaction, while a purple does.

The yellow in "Thunderbolt," producing the bronzed shade, is probably a sap soluble yellow; for, on treatment with strong spirit for some hours, the falls were almost indistinguishable from those of the deep-blue No. 14, only the yellow on the haft remaining, as it had not all been dissolved out of the plastids; furthermore, the inhibition area coincided with the plastid distribution in this group. Direct observation must settle this point.

"Blanche superbe," having neither a sap-pigment nor inhibitor, would probably behave as a Mendelian recessive if crossed with an anthocyan-containing flower. The same applies to "chrysolora." In No. 13 the absence of chromogen is most likely the deciding factor, for there is a pronounced oxydase reaction with both reagents.

Table IV contains the results obtained with the Pogoniris group.

Table IV.

Pogoniris Group.

No.	—	Benzidine		α-Naphthol	
		Veins	Epidermis	Veins	Epidermis
15	*I. germanica*, Linn. ; dark purple, falls, .	+ + +	+ +	+ trace in periphery	—
16	*I. hybrida*, Retz ; var. "Victorine," violet blue, mottled with white, falls,	+	+	—	—
17	*I. pallida*, Lam. ; pale lilac, falls, . .	+	+	+ trace	—
18	*I. pallida*, var. ; pale lilac, falls, . .	+ +	+	+ +	—
19	*I. pallida*, var. "Queen of May," rosy lilac, falls,	+	+ trace	—	—
20	*I. variegata*, Linn. ; var., tawny pale yellow, falls. [Standards yellow.]	+	+	+ trace centrally	—
21	*I. variegata*, var. "Gracchus," crimson with white reticulations, falls. [Standards yellow.]	+	+	+ trace centrally	—

Discussion of Results of Table IV.

In this group the benzidine reaction is rather of a brown-yellow colour than of the usual dark brown. It will be noted that the α-naphthol reaction is invariably absent from the epidermis, and present only in traces in the veins, with one exception. The benzidine reaction is only really well developed in *I. germanica*. The garden "Victorine," which is given as a variety of *I. hybrida*, of doubtful origin, evidently owes its white mottlings to an inhibitor ; this diffuses and checks the oxydase reaction. In this specimen the colour is only partly restored for the same reason. The pale hues of *I. pallida* forms are probably due to the presence of inhibitors; but no material was available to test this point by the cyanide method.

Table V records the behaviour of the Apogon group.

Table V.

Apogon Group.

No.	—	Benzidine		*a*-Naphthol	
		Veins	Epidermis	Veins	Epidermis
22	*I. sibirica*, Linn.; white with blue veins and brown markings on haft, falls,	+ + +	+ +	+ trace	—
	and standards, ...	+ +	+	+ trace	—
23	*I. sibirica*, white with brown markings on haft, falls. Standards white,	+ +	+ slight	+ +	+ slight
24	*I. orientalis*, Thunb. non Miller; blue, with white streaks, and brown and red markings on haft, falls and standards,	+ + +	+	+ +	+ slight
		+ +	+	+ +	+ slight
25	*I. aurea*, Lindl.; bright yellow, falls, . .	+ +	+	+ +	—
26	*I. ochroleuca*, Linn. = *I. orientalis*, Miller: orange yellow in centre, pure white elsewhere, falls.	+ + +	+ +	+ +	+ +
	Standards ditto, ...	+ + +	+ + +	+ + +	+ + +
27	*I. tenuifolia*, Hort.[1]; white with blue veins, falls and standards. Shape of both falls and standards similar to *I. ochroleuca*.	+ + +	+ + +	+ +	+
28	*I. pseudacorus*, Linn.; yellow with brown markings on haft, falls,	+ +	+ slight	—	—
29	*I. pseudacorus*, Linn.; yellow without brown markings on falls,	+ +	+ slight	—	—
30	*I. sp.*; deep yellow with red brown markings on haft of falls,	+ + +	+ + +	+ + +	+ + +
31	*I. sp.*; pale sulphur yellow with brown markings on falls,	+ + +	+ + +	+ + +	+ + +
32	*I. Kaempferi*, Siebold; yellow centre, surrounded by white, red purple round edges widely, falls,	+ +	+ +	+ +	+ +

Discussion of Results of Table V.

In contrast to the preceding one this rather heterogeneous group invariably gives a well-marked benzidine reaction in the veins, and, on the whole, the epidermal reaction is more or less definite. With *a*-naphthol there is less uniformity. Considering first the two varieties of *I. sibirica* and the related *I. orientalis*, it is seen that the limiting factor in the blue-veined *I. sibirica* is the chromogen, for the benzidine reaction is given by veins and epidermis. The deep pigmentation of the veins, however, seems to inhibit the *a*-naphthol

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[1] It certainly is not *I. tenuifolia*, Pall., as seen by comparison with an herbarium specimen named by Karelin and Kiriloff.

reaction, for in the white variety the α-naphthol reaction is well marked in them. In *I. orientalis*, on the other hand, the epidermis gives a slight α-naphthol reaction; but in the veins it is well defined.

In *I. ochroleuca*, *I. tenuifolia* (?), and the deep and pale yellow varieties Nos. 30 and 31, anthocyan chromogens are absent, except where brown markings are on the hafts in the two latter cases, for the pronounced oxydase reactions of both veins and epidermis lead to this conclusion, there being no inhibitors present. In *I. Kaempferi* the reactions are on the whole well marked, though there is evidently an inhibition patch, white, extending beyond the yellow plastid area. In the two varieties of *I. pseudacorus* the distribution is quite different, for there is no trace of reaction with α-naphthol and hardly any with benzidine in the veins. Treatment with hydrogen cyanide, however, removes an inhibitor, so that just as intense a reaction is given as with the two other yellow varieties Nos. 30 and 31. Probably the yellows with inhibitor would behave as dominants if crossed with purple varieties, whereas those without inhibitor would very likely be recessives, the resulting hybrid having both yellow and purple superposed or mingled. The carrying out of breeding experiments with Iris varieties, on lines similar to the work of Keeble, Armstrong, and Jones on Primulas, would be of great interest.

The effect of the age of the flower upon its oxydase reactions was not investigated, for no more material was available owing to the lateness of the season. It is remarkable that the direct action was only observed in one case in traces; in all the others the addition of peroxide of hydrogen was necessary. All the flowers, however, were gathered in bright summer weather, so it is possible that the natural peroxides would have been found in unopened buds or at night, for Keeble and Armstrong have shown that the supply of these substances is augmented by keeping Primula flowers in the dark for a day. These questions, and others, such as deciding in which cases, if any, the yellow plastid pigment was replaced by a yellow sap pigment, await further investigation next summer.

Examination of a number of herbarium specimens of Iris shows that the flowers of some turn a dark brown in drying, while others remain of a light colour. Owing to the influence of the gums used, it is not possible to be dogmatic; but it seems almost certain that those which become brown, such as *I. germanica*, *I. florentina*, *I. sambucina*, *I. pyrenaica*, *I. acutiloba*, &c., are the ones with plentiful oxydase supply; whereas others which preserve their natural light shades, such as *I. pseudacorus*, *I. aequiloba*, *I. squalens*, *I. chinensis*, (epidermis), &c., contain inhibitors. It has been shown in this paper that *I. pseudacorus* does contain an inhibitor.

SUMMARY.

1. Prolonged darkness has no decided effect upon the distribution of the indirect oxydase (peroxidase) reactions or of the inhibitor in the leaf of *Iris germanica.*

2. The distribution of oxydase and inhibitor, in the flowers of a number of varieties of Iris, has been examined and correlated with the natural colouring of the flowers. Among these it is probable that both dominant and recessive whites and yellows will be found to occur, judging from the chemical reactions, an inhibitor being present in the dominants. Related varieties have very similar oxydase contents. On the whole, the behaviour of Iris flowers follows closely that of other species investigated by Keeble, Armstrong, and Jones.

I wish to record my indebtedness to Prof. H. H. Dixon for his continued interest in this work and his helpful criticism.

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GINKGOPHYLLUM KILTORKENSE sp. nov.

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(PLATES X–XII.)

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IX.

GINKGOPHYLLUM KILTORKENSE sp. nov.

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(Plates X–XII.)

[Read November 25, 1913. Published February 27, 1914.]

The discovery in the year 1896 by Hirasé of the occurrence (1) of ciliated antherozöids in *Ginkgo biloba*,[1] the Maiden-hair tree or *Salisburia adiantifolia*, justified more than ever the separation of this genus from the rest of the Coniferæ with their aërial mode of fertilization by non-motile male nuclei, and the institution, for its inclusion, of the group of Ginkgophyta or Ginkgoaceæ. It added interest also to the attempt to trace backwards in time through the rocks the first signs of the emergence of the group in the flora of the world.

To Heer (2) belongs the honour of proving that the representatives of Ginkgo show the characteristic foliage and reproductive organs.

The group reached its zenith in the mid-Jurassic period in such a form as Baiera. Some twenty species, found in nearly all parts of the world, even to 79° N., are on record for this epoch.

Though the presence of Ginkgoaceæ throughout the Tertiary and Mesozoic rocks is generally admitted, the case is different on descending to the Palæozoic rocks. There is not on record under any generic name anything more complete than leaves or foliage shoots suggestive of a

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[1] The most recent arboricultural account of *Ginkgo biloba* is given in the "Trees of Great Britain and Ireland" by Elwes and Henry, vol. i, pp. 52–62, pls. 21–23, 1906. I have it on the authority of my colleague, Professor Henry, that, statements to the contrary notwithstanding, there is no reliable evidence that Ginkgo is indigenous in any part of China. It owes its survival in this case to the commendable habit of the people of China and Japan of planting it in groves round their temples from time immemorial. J. S. Gardner (Monograph of the British Eocene Flora, J. Starkie Gardner and C. von Ettingshausen, Palæont. Soc., 1886, p. 100) describes the Ginkgo he found in the Tertiary basalt at Ardtun in the Isle of Mull as specifically identical with *Ginkgo biloba*, though in the Palæontographical Society's Memoir he describes the specimens as *G. adiantoides*. J. S. Gardner's naming is, he states, in accordance with custom, which requires a different specific name for an Eocene form even though identical with a living species.

Ginkgo affinity. There is all over the world a sharp demarcation in the flora and fauna between the Permian epoch, the youngest of the Palæozoic period, and the Triassic, the oldest of the Mesozoic beds. Groups of animals and plants flourishing in the Permian disappear at its termination abruptly as if cut off, to be replaced in the Triassic by other groups usually more highly organized. Does the evidence tend to show that the conditions induced by the Permian "catastrophe" favoured the first uprising and luxuriance in the Mesozoic of the Ginkgoaceæ? Their points of agreement with the Cordaïtaceæ, a group of Gymnosperms confined to the Palæozoic, have suggested that they arose from the Cordaïtaceæ, most abundant in the Upper Carboniferous, and that they replaced this group in part in the Mesozoic.

Of all the types of foliage hitherto described from the Palæozoic the one from the Permian rocks of Lodève, named by Saporta (3), *Ginkgophyllum Grasseti*, seems to come nearest to Ginkgo itself. In it the leaves are obscurely arranged distichously, and show a long petiole, 2 mm. wide, but with a decurrent base of attachment. The lamina is elongated, cuneiform, and several times bifurcate. Each terminal bilobed portion is truncate, toothed. The veins are shown dichotomously divided, though they are not described in detail. No reproductive organs are known. *G. Grasseti* comes very near Baiera, to which it is united by Heer, but its decurrent leaf-bases separate it from that genus. Whittleseya is another genus, the affinities of which are highly suggestive of Ginkgo. It has been described as the earliest member of the group, and is well represented by several species in the Carboniferous strata of Pennsylvania, &c.

Further, in the "Fossil Flora of Great Britain," Lindley and Hutton describe and figure a fossil from the Carboniferous strata under the name of *Noeggerathia flabellata* (4) which became subsequently the type of the genus Psygmophyllum created by Schimper (5), to include forms characterized by wedge- or fan-shaped leaves, traversed by dichotomously divided veins, passing as a few strands into a leafstalk formed by the gradual tapering of the lamina; the shape and venation of the leaf being the chief generic characters.

The earliest recorded specimen of this type is a leaf assigned to the genus by Nathorst (6) under the name of *Psygmophyllum Williamsoni*, sp. nov. from the Upper Devonian beds of Spitzbergen. *P. Williamsoni* is regarded by Nathorst as the most important of the remains discovered in the Devonian beds of Spitzbergen. As drawn, it is strikingly like the figure in "Fossil Flora" (plate xxix) of *N. flabellata*. In both, the obcuneiform or obtriangular simple leaf (or leaflet?) shows a broad apical or distal margin which, as far as traceable, was indented or lobed. The base tapers, so that its shape here is roughly that of the inverted acuminate apex of a foliage-leaf. In neither case is the forked venation so clearly observable and described as could be wished.

Seward (7) describes a portion of a leaf from the Permo-Carboniferous rocks of Kashmir under the name of *Psygmophyllum Hollandi*, which is highly suggestive of a form intermediate between *Ginkgophyllum kiltorkense* and a more recent Ginkgo. Its venation is unfortunately not fully preserved.

Archaeopteris archaetypus Schmalh. possesses Psygmophyllum-like pinnules, the largest being 6 cm. long and of the same width.

Nathorst states that the lateral veins in his Psygmophyllum appear to arise by repeated dichotomy or forking of the veins at the base of the leaf and not, as he says is the case in *Ginkgo biloba*, from two marginal bundles. This distinction as applied to Ginkgo, if found reliable, would constitute an important feature of distinction. It is one ascribed to Ginkgo by Renault, and has been made use of in the identification of fossil leaves. It is, I think, not maintainable. It is an error of description rather than of observation, due to the way of considering the venation. The leaf-scar of Ginkgo, horizontally oval, shows clearly the two scars or cicatrices indicative of the broken ends of the double leaf-trace. The two bundles running through the long leaf-stalk receive all the vascular bundles of their own halves of the actually or potentially bilobed fan-shaped leaf-blade. The leaf-blade is supplied throughout with dichotomously divided veins. The "marginal" vein is really the vein formed by the fusions, at intervals along the lateral edge of the lamina, of the forked veins, as they traverse the lamina from its expanded apex towards the leaf-base. At the point of constriction of the lamina to form the petiole, the vein on either side having received the fusions of the forking-veins of its half of the lamina, passes with its fellow as two main veins through the petiole, each being independent of the other, and belonging to its own half of the leaf, as the double leaf-trace, into the stem. There is dichotomous venation throughout the lamina of Ginkgo; and a "marginal" vein, from which forking veins are given off on its inner side towards the body of the lamina, does not exist.

The true origin of the so-called marginal vein, by the fusion of the forking veins in the body of the lamina is well brought out in the figure of the leaf-venation of *G. biloba* in the "Morphology of Gymnosperms," by Coulter and Chamberlain (8), (fig. 212, p. 187). Unfortunately this figure errs in one important respect. The veins of the lamina are shown fusing together to form a single vein in the petiole, though the "double leaf-trace" is recognized in the text as a Ginkgo character.

The Ginkgo type of leaf is unique among seed-bearing plants of to-day, and does not make its appearance in any Dicotyledon or Monocotyledon. Its venation is different from that of any recent ferns, even from suggestive forms like Actiniopteris (9) and Aneimia. Hence its venation is of considerable taxonomic value as a guide to palæobotanists in tracing the genus through time.

Interesting as is the deposit of fossil plants in the Upper Devonian beds of Kiltorcan, no signs of Gymnosperms have hitherto been observed there. It is a surprising fact that, whether due to absence of the fossil, or to insufficiency of exploration, there are no fossil Gymnosperms recorded for Ireland, except a few from the basaltic Miocene deposits of Co. Antrim, and a Sternbergia or Artisia, the pith-cast of the stem of Cordaites, from the Yellow Sandstone and Coal-Measure deposits of Cultra, Co. Down. When recently examining in detail a large quantity of material quarried for me this year, partly through the aid of the Royal Dublin Society, I was surprised to find in faint outline on one slab an impression which closer examination and magnification satisfied me was different from any type of fossil I had yet seen in these rocks.

A general idea of this discovery is obtainable from the illustrations, (Pl. X, figs. 1 and 2).

The leaf (or leaflet) is 7 cm. long, 5 cm. wide, is in general outline fan-shaped, and gradually tapers to form a ribbon-like petiole, 25 mm. long, 2–5 mm. wide. It is thus in general form Psygmophyllum-like. The impression in the rock is incomplete on one side (the left as looked at). Enough of it is, however, preserved to show that the leaf-blade was symmetrically and deeply divided into two lobes, and each of these again into two, themselves sub-divided, ribbon-like segments. The whole lamina is more or less palmately lobed, giving eight lobes in all. Its venation is clearly dichotomous. The petiole is traversed by four veins in its upper part, and, judging from the formation of these by the fusions of the veins from the lamina, these four, though lost in the impression of the basal part of the petiole, fuse in pairs, and form the double leaf-trace, so characteristic of Ginkgo and primitive Gymnosperms. It would be going too far to assert that this Ginkgophyllum possesses the double-leaf trace. The venation of *Ginkgophyllum kiltorkense* agrees, as far as traceable, with that of *G. biloba*. We have thus, persisting from the Devonian epoch to the present day, a Ginkgo type of megaphyllous leaf as interesting as is the microphyllous Equisetum within its range.

There is another feature in the venation of the Ginkgoaceæ to which I wish to direct special attention, as it seems to me to have a distinct bearing on the degree or depth of segmentation or lobation to which a lamina may go. The region in the lamina where the lobes meet is ordinary mesophyll tissue, free from veins. Further, the veins of one lobe do not unite with those of the adjoining one for some distance below the point of separation of the lobes. Thus the segmentation might be carried deeper and deeper until the point of junction of the veins, well towards the base of the leaf, is reached, giving a leaf-blade comparable to that of Baiera, with its numerous digitate segments, or conversely, the mesophyll might be imagined as filling up the free space between the segments, gradually obliterating them, until the lamina would assume the condition of the more or less bilobed leaf of Ginkgo itself. In pursuit of this idea I took a leaf of Ginkgo, and cut through the mesophyll between the forking-veins from the broad apex downwards, until the points of fusion or bifurcation of the veins were reached. The result, showing six main artificial lobes, is illustrated in Pl. XI, fig. 1. Its suggestion of the fossil, *Ginkgo digitata*, or of Baiera (10), or of the Kiltorcan specimen, is sufficiently obvious. The text-books give illustrations of abnormal leaves of Ginkgo deeply, occasionally palmately lobed. More interesting is the fact that one cotyledon of the Ginkgo seedling (when not both) is deeply bilobed, as are normally the first leaves of the seedling. A plant's embryonic leaves reveal the ancestral characters of its group; and those in Ginkgo show a closer connection with Ginkgophyllum than is evident from the examination of its adult leaves. "Pro-Ginkgo," in becoming "Eu-Ginkgo," in the course of time, has filled in the mesophyll, and has thus obliterated the lobes more or less completely. Its vascular bundles have become more numerous, too, by more frequent forking.

The disappearance of lobation is in keeping with the general conclusion of fossil evidence—viz., that the earlier types (Baiera) show more deeply lobed leaves, and that, as later Mesozoic passes into Tertiary, the normal wedge-shaped, simply lobed, or unlobed, long-stalked leaf, characteristic of the extant Ginkgo, to the final exclusion of the segmented type, predominates.

Still, with the warning furnished by the errors of the past in identifying as ferns many of the fossil plants of the Carboniferous epoch, now known to be Pteridosperms, by relying mainly on conclusions drawn from their foliage and its venation, it may seem rash to conclude from the leaf alone that the Kiltorcan specimen is undoubtedly a Ginkgophyte.

My own impression is that it is a true Ginkgo ancestor; and in consequence I prefer to use for it the generic name Ginkgophyllum, Saporta, rather than the name Psygmophyllum, Schimper, which, while less suggestive of prejudging than Saporta's name, is also less indicative and is better applied to the less segmented form of leaves, such as *Psygmophyllum Williamsoni* may have been. Indeed as I first read Saporta's account of *Ginkgophyllum Grasseti*, the type of his genus, I was much struck by its general agreement with the Irish specimen, which I propose accordingly to name *Ginkgophyllum kiltorkense.*

Ginkgophyllum kiltorkense and *Psygmophyllum Williamsoni* (if we regard the latter, as Nathorst is inclined to do, as a Ginkgophyte) are then the earliest known leaves of the Ginkgoaceæ, and are of interest as evidence of the occurrence of this group of Gymnosperms in the Devonian epoch, i.e., at as early a period as that of the Cordaïtaceæ. In the slab which yields this leaf of *Ginkgophyllum kiltorkense* there are other fossil impressions deserving notice. One of these is indicated at (*a*) in Pl. X, figs. 1 and 2, and magnified in Pl. XI, fig. 5. It appears to be a small detached piece of flattened stem folded on itself, 30 mm. long and 4 mm. wide. It is mainly interesting because it shows over its surface oval erect scars arranged in ascending spirals. The scars are 1·75 to 2 mm. long and 1 mm. wide. In each one can see a central straight dark line running the greater part of the length of the scar. The line ceases at a little distance from the more pointed end of the scar and leaves a clear space in it, though occasionally one may see signs of a dot-like speck in this space. The scars are, I take it, leaf-scars; and so far as arrangement and distance from one another (2 mm.) are concerned remind one of a Bothrodendron-like stem. The scars are not like those of this genus and are new to me. The general external surface of the stem is finely striate longitudinally (Pl. XII, fig. 1).

It is worthy of note that the leaf-scar is the same in length—2 mm.—as the width of the petiole of the associated leaf. On another slab is a fragment of stem bearing a small wedge-shaped leaf which suggests itself as the young leaf of *Ginkgophyllum kiltorkense* (Pl. XI, fig. 2, A). This leaf is inserted in a flattened vertically elongated base, i.e. as one would expect it to be if the stem and leaf-scars described, together with the leaf of *G. kiltorkense*, belong to the same adult plant. The figures which Fontaine (11) gives of the foliage shoots of Baiera (Baieropsis), e.g. *B. adiantifolia* and *B. foliosa*, show wedge-shaped leaves with vertically elongated bases of insertion, i.e. lobed, fan-shaped leaves of upright habit, so that stem and leaves look like a pinnate leaf.

Baiera foliosa Font. is a Ginkgophyte which occurs in the Mesozoic rocks of Europe and America. As quite recently redescribed by Berry (12), the plant possesses dwarf shoots or spurs like those of Ginkgo and stalked lobed leaves with the usual Baiera characters, arranged in a low spiral. The leaf-bosses are rounded, sub-rhomboidal, 2 mm. apart.

Another instructive fossil now known as a Ginkgophyte under the name Trichopitys was described by Renault as *Dicranophyllum gallicum* (13). In it the leaf-scars are close together without any free intervening stem-surface, and are Lepidodendron-like, as shown in Renault's figure. In this species the leaves are filamentous and forked. There is, so far as the descriptions go, nothing in either of the two illustrative cases to suggest that the Kiltorcan stem is not Ginkgoaceous. There is, of course, the possibility that the stem described is part of the same kind of plant as that giving the forked filaments seen in Pl. X, fig. 2, i.e. that we have at Kiltorcan a Ginkgophyllum and a Trichopitys. The finest piece of stem, apparently a deeper-seated portion of the same kind of stem as that shown in Pl. XI, fig. 5, is illustrated in Pl. XI, fig. 4.

The leaf-scars appear as leaf-gaps; and the linear scar within the leaf-scar seen in Pl. XII, fig. 1, is missing. The space between the leaf-scars is occupied by a well-developed system of lines or ridges forming a narrow network vertically elongated. This sculpturing suggests the cortical reticulate framework of fibres of a Lyginodendron (14). The whole stem, 5 cm. long and 1 cm. wide, showing three gaps with intervening space in a width of 5 mm., looks like a stem combining in itself characters found in part in Lyginodendron and in part in Bothrodendron. The impression of this stem is roughly comparable with the appearance of the skeleton of a fern-stem of erect habit. Potonié (15) mentions as possibly Gymnospermous certain stems from the middle Devonian of the Lenne Slates, &c. Though these are Araucarioxylon-like, they are considered mostly Pteridospermous. He writes similarly of certain Dicranophyllum (i.e. Trichopitys)—like remains described under various names from the Devonian beds of Central Europe, Australia, and Canada.

In the course of examination of the Kiltorcan material I have searched for the possible presence of seeds—a search which became more hopeful when the leaf of *Ginkgophyllum kiltorkense* revealed itself. Stalked bodies of the same size and shape as the Heterangium-like seeds of the Carboniferous Sandstone of Scotland described by Gordon, have been found; but until I am more satisfied by the examination of additional material, I prefer to postpone further reference to them, beyond mentioning that they appear to be Devonian seeds of, as yet, undetermined identity. Until *G. kiltorkense* comes to light[1] in a more complete form, I must resist the inclination to discuss its bearings on the inter-relationship of the various groups of Gymnosperms (7).

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[1] I have got a second specimen of its foliage from Kiltorcan since this paper went to press.

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EXPLANATION OF PLATES.

(The figures in Plates X and XI are from photographs of the specimens taken by Mr. T. Price.)

PLATE X.

Fig.

1. Foliage of *Ginkgophyllum kiltorkense* obscured by other plant impressions. ($\frac{1}{1}$).
2. Central part of fig. 1 magnified to show the venation. (See Pl. XII, fig. 3.)

PLATE XI.

Fig.

1. Artificially lobed leaf of *Ginkgo biloba*, as described in text.
2. A fragment showing apparently a young leaf of *G. kiltorkense* at A.

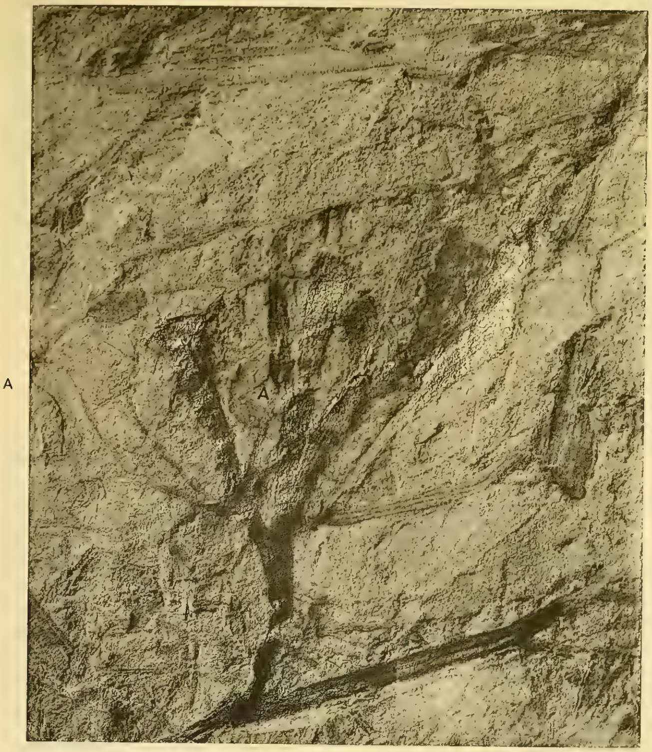
3 & 6. Seed-like bodies.

4. Stem impression with leaf-scars. (See text, p. 175.)
5. Enlarged illustration of the specimen marked A in Pl. X.

PLATE XII.

Fig.

1. Drawing of fragment of stem seen in Pl. X, fig. 1, A.
2. Drawing of a portion of the stem impression seen in Pl. XI, fig. 4.
3. Venation in leaf of *Ginkgophyllum kiltorkense*.
4. Stem (?) fragment in rock slab. Cf. Saporta's specimen of *G. Grasseti*
5. Seed-like body as photographed (Pl. XI, fig. 3).
6. Seed-like body as photographed (Pl. XI, fig. 6).



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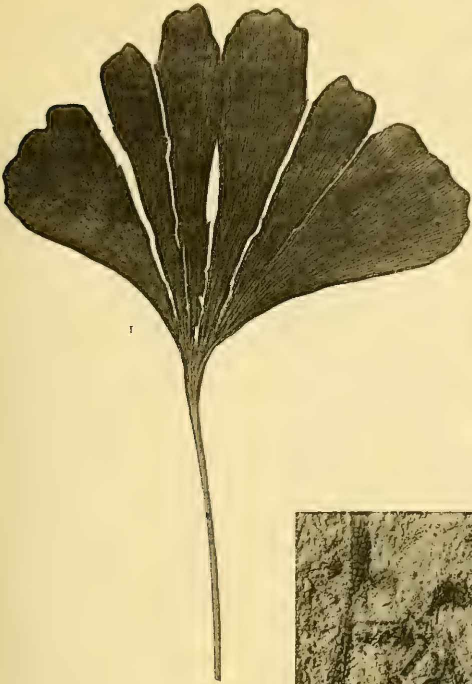

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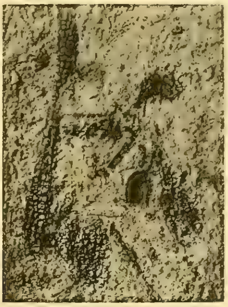

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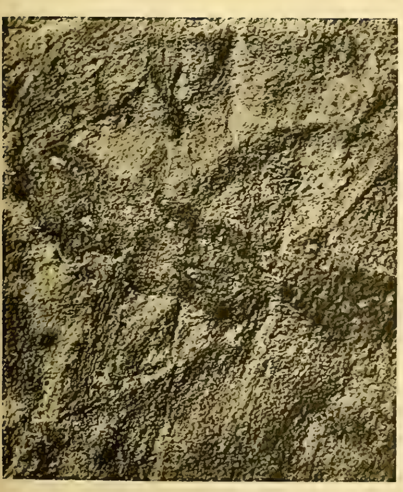

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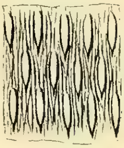

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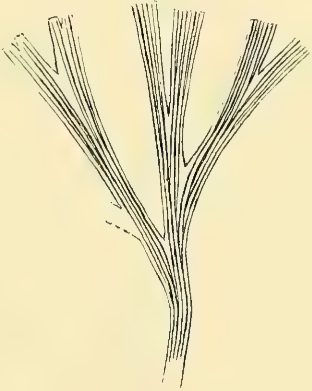

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M'Farlane & Erskine.

Ginkgophyllum kiltorkense, sp. nov.

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FURTHER OBSERVATIONS ON *PHYTOPHTHORA ERYTHROSEPTICA* PETHYB., AND ON THE DISEASE PRODUCED BY IT IN THE POTATO PLANT.

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FOR IRELAND.

(PLATE XIII.)

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X.

FURTHER OBSERVATIONS ON *PHYTOPHTHORA ERYTHROSEPTICA* PETHYB., AND ON THE DISEASE PRODUCED BY IT IN THE POTATO PLANT.

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Economic Botanist to the Department of Agriculture and Technical Instruction for Ireland.

(Plate XIII.)

[Read December 16, 1913. Published January 31, 1914.]

In a communication[1] brought before the Society early in the present year and published in March last, a description of a new form of rot in the potato tuber, caused by a new species of Phytophthora, was given.

Apart from the nature of the rot and the very considerable losses occasioned by it in certain parts of the country, the organism causing it (to which the name *Phytophthora erythroseptica* was given) is of peculiar interest, inasmuch as its sexual organs are developed in a manner which, at the time of publication of the paper referred to, was wholly novel and unprecedented.

Shortly stated, the course of events is as follows:—The oogonia and antheridia are borne on separate hyphae; the oogonial incept, at a very early stage, penetrates into the interior of the previously developed antheridium at or near its base, grows up through the latter, breaks out at its summit, and expands rapidly to form that portion of the oogonium in which shortly afterwards first an oosphere, and then a thick-walled oospore, develops. Whether fertilization actually occurs is not yet known; but, if it does, it probably takes place before the oogonial incept emerges from the antheridium and before the formation of the oosphere.

Cultural studies of several other members of the genus Phytophthora, which are described in the paper referred to, and, as regards *P. infestans* in

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[1] Pethybridge, G. H.: "On the Rotting of Potato Tubers by a new species of Phytophthora, having a method of sexual reproduction hitherto undescribed." Sci. Proc. Roy. Dublin Soc., vol. xiii (N.S.). No. 35, March, 1913.

particular, in a further paper[1] published coincidently with it, revealed the fact that, judging from the final state of affairs, the course of development of the sexual organs in *P. infestans* de Bary and in *P. Phaseoli* Thaxt. is similar to the peculiar one discovered for *P. erythroseptica.* It was further surmised that in all probability the development of these organs in *P. omnivora* var. *Arecae* Coleman follows an identical course.

On the other hand, it was found that the development of these organs in certain other members of the genus, such as *P. Cactorum* Schroet., *P. Fagi* Hart., and *P. Syringae* Kleb., was of the ordinary type in which an outgrowth from the antheridium penetrates the oogonium laterally, and thus effects the fertilization of the oosphere.

It was therefore suggested that the generic name Phytophthora should be reserved for those species (viz. *erythroseptica infestans*, *Phaseoli*, and probably *omnivora* var. *Arecae*) having the newly discovered mode of development of their sexual organs, and that those species which follow the *omnivora* or *Cactorum* type should be assembled in a new genus—Nozemia.

Shortly after the publication of the papers referred to further proof of the existence of such a novel mode of development of the sexual organs was adduced in the case of two other members of the genus occurring in India by Dastur[2] and by Butler and Kulkarni.[3] Dastur describes the development as it occurs in a new species (*P. parasitica*) which he had discovered as the cause of a disease in the Castor Oil plant; while Butler and Kulkarni deal with the Colocasia disease caused by *P. Colocasiae* (a species originally described by Raciborski, to whom the sexual organs were unknown), and show that the mode of development of the sexual organs here is similar to that which occurs in *P. parasitica.*

In all essential points the course of development of these organs in *P. parasitica* and *P. Colocasiae* is similar to that already described as occurring in *P. erythroseptica.*[4] Of the three species, *P. parasitica* appears to stand alone

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[1] Pethybridge, G. H., and P. A. Murphy: "On Pure Cultures of *Phytophthora infestans* de Bary, and the Development of Oospores." Sci. Proc. Roy. Dublin Soc., vol. xiii (N.S), No. 36, March, 1913.

[2] Dastur, J. F.: "On Phytophthora parasitica nov. spec. A new disease of the Castor Oil Plant." Mem. Dept. Agric., India. Bot. Ser., vol. v, No. 4, May, 1913.

[3] Butler, E. J., and G. S. Kulkarni: "Studies in Peronosporaceae. Colocasia Blight caused by Phytophthora Colocasiae Rac." Mem. Dep. Agric., India. Bot. Ser., vol. v, No. 5, May, 1913.

[4] Massee, in his recently published "Mildews, Rusts, and Smuts," London, 1913, in referring, on p. 206, to the apparent uniqueness amongst fungi of the mode of development of the oospore in *P. erythroseptica*, makes use, for some not very intelligible reason, of the expression, "Long may it remain so." Although the fact that I showed that both *P. Phaseoli* and *P. infestans* conform to the *erythroseptica* type in this respect, failed to have the effect of strangling this curious aspiration at its birth, perhaps the published observations on *P. parasitica* and *P. Colocasiae* here alluded to will be adequate to provide the *coup de grâce* for it while still in its infancy.

in respect of one detail, namely that, according to Dastur, in this species the oogonial incept (" origin " of Dastur) *may* arise on the same hypha as the antheridium, and indeed may arise from the base of the antheridium itself as an ingrowth. Butler, however, states that in *P. Colocasiae* " so far as could be determined the oogonium arises always from a different hypha from that which bears the antheridium. Sometimes, however, the stalks of both organs swell up, and a tangled mass results, which prevents their origin being ascertained." The same holds good more or less for *P. erythroseptica*; and antheridia and oogonia have so far never been seen other than on separate hyphae.

Although the development of the sexual organs follows essentially the same course in the three species mentioned there are in certain details of these organs, as well as in other characters, considerable morphological differences between them, and the three species are by no means to be regarded as identical.

In the paper on *P. erythroseptica* already published some important points in the character and behaviour of the fungus were not fully described, as they had at that time been but partially elucidated.

These points relate chiefly to:—

(1) The micro-chemical reactions of the walls and the mode of germination of the conidia (zoosporangia).

(2) The micro-chemical reactions of the walls of the sexual organs and the germination of the oospores.

(3) The occurrence of the fungus in parts of the potato plant other than the tubers (in which it had been found merely in its vegetative condition), and the occurrence in such parts of its sexual and asexual reproductive organs which hitherto had only been observed when it was grown as a saprophyte in pure culture on artificial media.

It is the object of the present paper to furnish information on these matters obtained from further researches carried out during the past season.

I desire to express to Mr. H. A. Lafferty, A.R.C.SC.I., a Research Scholar of the Royal College of Science for Ireland, who has been working in my laboratories for the past twelve months, my indebtedness for much assistance rendered in various ways (including the preparation of the drawings for text figs. 1 and 2) during the progress of the work the results of which form the basis of the present paper.

I. MICRO-CHEMICAL REACTIONS OF THE WALLS AND GERMINATION OF THE CONIDIA (ZOOSPORANGIA).

Production of Conidia.—It was stated in the former paper that, with possible exceptions in one or two isolated instances, the conidia of this fungus could only be obtained under water. It is just possible that in nature they may be produced in a saturated atmosphere, in contact with a moist substratum such as on dead underground tissues; but they are certainly not produced on aerial conidiophores, as is the case, for instance, in *P. infestans.* Essentially they are of an aquatic type, and are not subject to aerial dissemination. They have not even been seen to become detached from the hyphae bearing them, and they germinate while still attached to the latter.

They are easily obtained in considerable quantities when a small piece of medium, such as oat extract agar, on which the fungus is growing is submerged in water (preferably previously sterilized) in a small flask. They can also be obtained easily if small pieces of an affected tuber (in which the disease has not proceeded so far that the killed tissues are already invaded by saprophytes) are removed aseptically and placed in previously sterilized water. Bog water or the watery extract of a boggy soil has been found excellent for the purpose.[1] In the course of a few days at room-temperature mycelium (bearing both conidia and sexual organs) can be seen emanating from the pieces of media or tissue introduced.

The mode of origin of the conidia, as well as their general shape, was also described in the former paper. They do not possess an apical papilla, as is the case in *P. infestans* and some other species; but the apex is more or less blunt or even somewhat flattened. The contents when ripe are finely granulated; and usually a large oil-drop—sometimes two or more—occupies a position in the centre. At the base, or rather at the end of the hypha bearing the conidium, there is a somewhat large plug of cellulose, which effectually shuts off the contents of the hypha from those of the conidium.

Micro-Chemical Reactions of the Cell-walls.—The walls of the hyphae

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[1] Incidentally, it may be remarked that this latter method of procedure forms a very satisfactory method of diagnosing "Pink Rot" (the name suggested for the disease caused by this fungus in potato-tubers) with certainty. The affected tuber should be well washed, and, if in a particularly dirty condition, may be disinfected externally by being placed for a short time in mercuric chloride or formaldehyde solutions. It should, after drying, be cut open with a sterile knife, and small portions of the diseased tissue should be removed as carefully and quickly as possible from a region not near the skin and not far from the still healthy tissue, and dropped into sterile water in a small flask, closed with cotton-wool. In a few days not only conidia, but also the characteristic sexual organs of *P. erythroseptica*, will be produced.

bearing the conidia as well as those of the hyphae, making up the main mass of mycelium, are composed apparently of cellulose, seeing that they stain a purplish-violet colour when treated with chlor-zinc-iodide, or with sulphuric acid and iodine in potassium iodide.[1]

When conidia are placed in either chlor-zinc-iodide solution or in sulphuric acid and iodine in potassium iodide, their walls become stained a purplish-violet colour (indicating the presence of cellulose), with the exception of the thicker and more hyaline apical portions, which do not become stained at all by these two reagents. No traces of any yellow colouration of the walls or portions of them can be observed.

When placed in concentrated sulphuric acid, the conidia swell up and burst; and the contents, with the exception of the oil-drops, quickly disappear. The walls, excepting the apical portions, are rapidly dissolved; but very careful observation shows that they are rarely or never completely destroyed, although the remains of them become so exceedingly thin as to be easily overlooked. The apical portions of the conidial walls are considerably more resistant to the action of the strong acid, although they also become to some slight extent dissolved.

When placed without previous treatment in a solution of ammoniacal copper hydrate, the conidia usually burst, and the walls slowly dissolve. Even after standing for a fortnight, however, traces of the walls can still be discerned, and the apical portions are scarcely, if at all, affected by this reagent.

It would, therefore, appear that the conidial wall is composed largely of cellulose, but that its apical portion consists of some substance other than cellulose, which possibly forms an *extremely thin* outer layer over the remaining portions of the wall.

Germination of the Conidia. In the former paper it was stated that conidia might give rise to hyphae, and also (in a foot-note) that typical zoospores had been observed[2]; and the production on germination of germ-tubes direct, as well as of zoospores, has since been abundantly confirmed many times.

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[1] Dastur states that he found these two reagents not very reliable for cellulose reactions in the case of *P. parasitica*, and he placed more reliance on the action of calcium-chloride-iodide and phosphoric acid and iodine, as recommended by Mangin. The chlor-zinc-iodide used for *P. erythroseptica* was an old solution (almost certainly over thirteen years old), and concentrated sulphuric acid diluted with an equal volume of water was used along with iodine in potassium iodide, both reagents giving satisfactory results. These reagents, as well as the ammoniacal copper hydrate solutions (prepared from metallic copper and ammonium hydrate, as well as from copper sulphate, sodium hydrate, and ammonia), were tested, and found to work satisfactorily with cotton-wool and pure Swedish filter-paper.

[2] With a fatal facility for error, Massee (*loc. cit.*) makes the wholly erroneous statement, "As to whether the conidia produce zoospores or a germ-tube on germination is not known, . . ."

When conidia are transferred from flasks of bog-soil water to hanging drops of tap-water, or even when they develop in droplets of condensed water in cover-glass film-cultures, it frequently occurs that they burst at some point in their periphery, often, but not always, at the apex, and through the opening the disorganized contents more or less slowly ooze out. This condition is illustrated in Plate XIII, fig. 4. No satisfactory explanation for this behaviour can be given at present. It, however, seems to occur chiefly when the conidia are unripe, and may, perhaps be due to osmotic phenomena induced by a change in concentration in the surrounding medium.

The germ-tubes produced direct from the conidia do not differ essentially from ordinary hyphae. They may arise at the apex or, perhaps more frequently, a little to one side of it, but they may also arise at almost any other point on the periphery of the conidium. Two or three such germ-tubes may sometimes be seen arising from different points on one conidium; and very frequently a germ-tube gives rise to two or three branches almost immediately after it leaves the conidium. A conidium which has developed a germ-tube from near its apex is illustrated in Plate XIII, fig. 6.

The conditions which decide as to whether conidia shall produce germ-tubes or zoospores are not yet known. As was explained, however, in the former paper, considerable difficulty was at first experienced in securing the formation of zoospores. Further observations seem to show that attempts to stimulate their production by artificial means are useless, so long as the conidia are not fully ripe, and that when they are ripe such stimulation is not necessary.

The minimum time in which zoospores have been observed to be produced on germination is a period of seven days elapsing since the medium or tissue containing the fungus mycelium was placed in water to induce the formation of conidia; but in some cases this period extended over twelve days. When the necessary time has elapsed, zoospore formation can be observed with ease by removing mycelium bearing conidia from the bog-soil water in which they have developed to hanging-drops of tap-water at room-temperature. Zoospore production commences as a rule within about an hour after the preparation of these drops.

Previous to the liberation of the zoospores, the contents of the conidium (zoosporangium) change from a finely to a coarsely granular condition; the large oil-drop, if present, disappears as such, becoming broken up into a large number of smaller ones. Soon the segregation of the protoplasm into zoospore units begins to take place within the sporangium; and these units can be seen clearly, although their total number cannot usually be determined with certainty in any given case until they are liberated. At this stage the contents are slightly contracted from the walls, more particularly at the apex. Figs. 5 and 7, on Plate XIII, show zoosporangia in this condition, although the amount of contraction shown in fig. 5 is somewhat exaggerated owing to fixation with iodine and subsequent mounting in dilute glycerine.

The apical, thicker, and more hyaline portion of the sporangium-wall now begins to bulge rapidly outwards, becoming extremely thin as it stretches, and quickly assumes a more or less spherical form, sometimes almost equal in size to the sporangium itself. Practically simultaneously with this bulging of the wall, the mass of zoospores passes out, filling this bladder-like swelling, the extremely attenuated wall of which at once dissolves. The zoospores then disentangle themselves from one another, and swim actively away.

At times, however, the liberation of the zoospores is not so rapid, possibly because the place of exit is relatively narrower. The apical portion of the wall swells up, and becomes dissolved; and through the opening, the zoospores squeeze out more leisurely, separating from one another, as a rule, immediately on reaching the exterior. Not infrequently some of the zoospores remain behind in the sporangium, and ultimately produce germ-tubes *in situ*. Cases have also been observed in which the zoospores seem reluctant to leave the sporangium. One or two of them may get out, do not swim far away, and soon produce germ-tubes. The majority of them remain behind, more or less rounded off within the sporangium. A case of this kind is illustrated in fig. 8, Plate XIII.

The number of zoospores in each sporangium varies considerably, and is dependent on its size; numbers varying from eight to twenty-three have been observed as they leave the sporangium.

The shape of the zoospores is on the whole oval, and more or less bluntly pointed at one or both ends; but during the period of motility their shape is constantly changing. Sometimes zoospores possess more or less of a tail, which may be rather short and blunt, or nearly as long as the chief portion of the zoospore itself. In the latter case it often resembles a fine thread, with small portions of protoplasm attached to it at intervals.

Apart from these "tails," which are not of very frequent occurrence, each zoospore possesses two cilia, one being about twice and the other about three times as long as the zoospore. There is also in each zoospore a vacuole and a bright spot. In addition to this, when in active motion a darkish longitudinal line or groove appears to be present; but its nature, owing to the rapidity of motion, could not be determined with accuracy, and it disappears as soon as the movements begin to slow down and the zoospores come to rest. The cilia are not easily seen when the zoospore is at its height of activity; indeed under these conditions it is extremely rare to see the one directed forward in the line of progression, possibly on account of its rapid movements. The one directed backwards, however, is more easily seen, and appears to act as a kind of rudder.

Attempts to fix the zoospores satisfactorily when in a motile condition with the vapours of osmic acid, formalin, and chloroform, as well as with iodine solution, failed. These substances cause the zoospore to become shattered, leaving a mass of small granules in a hyaline matrix of irregularly rounded shape, larger than the living zoospore, without vacuole or bright spot, but with the cilia still present. The same thing occurs when a beam of sunlight is caused to fall on the zoospores by means of the sub-stage mirror of the microscope, just as they are leaving the sporangium.

The maximum and minimum periods of motility have not been determined; but in many cases, after swimming about for from twenty minutes to half an hour, the motion becomes more sluggish. The zoospore itself generally remains almost stationary, becomes nearly spherical in shape, and slowly lashes its two cilia. Soon all movement ceases, and a completely stationary spherical body results. For a few minutes longer the cilia can still be seen, after which they gradually fade from view, apparently becoming dissolved, and not withdrawn into the interior. It is at this stage that it can most clearly be seen that the two cilia have a common point of origin, or nearly so. As the cilia fade away the outline of the spore becomes more and more clearly defined, owing to the formation of a definite wall.

In this condition the spores have a diameter varying from 8μ to 14μ, the average being about 11μ. After a further period of an hour or two, each spore sends out a delicate germ-tube, into which the contents pass, leaving the spore empty and sometimes cut off from the germ-tube by a transverse wall, formed at some little distance from the spore. Germinated zoospores are illustrated in fig. 8, Plate XIII.

II. MICRO-CHEMICAL REACTIONS OF THE WALLS OF THE SEXUAL ORGANS AND GERMINATION OF THE OOSPORES.

For a detailed account of the peculiar method of development of the sexual organs reference should be made to the paper already quoted.

When the oospores are ripe the hyphae bearing the oogonium and the antheridium respectively, as well as the antheridium itself, are practically devoid of contents. The funnel-shaped base of the oogonium is clearly seen within the antheridium, and is closed by a thick hyaline plug.

The ripe oospore with its thick wall may or may not completely fill the spherical part of the oogonium. The wall itself not infrequently shows, in optical section, a series of radial striations, which, in surface view, present the appearance of pits of narrow diameter. The contents consist of a large central oil-drop (sometimes two or more smaller ones), staining almost black with osmic acid, surrounded by a peripheral mass of very finely granular protoplasm, in which is a rather large, refractive, oval body. This body also becomes fairly deeply stained with osmic acid, more so than the protoplasm in which it is embedded, but less so than the central oil-drop. Figs. 1 and 2, Plate XIII, show ripe oospores both in a living condition and after fixation with osmic acid vapour.

The ripe oospore has never been seen (except after artificial treatment) free from the oogonium and its permanently attached antheridium either in pure cultures or in material gathered in the field. It is, however, of course possible that during the winter the remains of the antheridium and oogonium may become disintegrated in the soil, thus setting the oospore free.

Micro-Chemical Reactions of the Walls of the Sexual Organs.—When the ripe sexual organs are treated with chlor-zinc-iodide solution, the empty hyphae, the antheridial wall, and that of the lower part of the oogonial funnel, including the hyaline plug, immediately become coloured purplish-violet, which later deepens somewhat to a claret colour. The oogonial wall with its included oospore becomes of a faint bluish tinge distinct from that of the antheridium.

With sulphuric acid and iodine in potassium iodide the same colour is produced in the hyphae, antheridial wall, base of funnel and plug, but practically no change takes place, at least for a considerable time, in the oogonium proper and its contained oospore.

If, however, gentle pressure be brought to bear on the cover-glass so as to burst the oogonium, the following changes occur rapidly with both of the reagents named, viz., the hyphae, antheridial wall, lower part of oogonial funnel and plug stain as before; the oogonial wall is of somewhat the same tinge, but not quite so deep, and around its periphery a very fine yellowish line can be made out; the thick wall of the oospore becomes a distinct blue colour with no tinge of red in it, quite distinct from the tint of the antheridium, &c.; while, if it is itself squashed, it is also seen (particularly clearly at the points of cleavage) to be surrounded by a very fine yellow line.

The walls of both the oogonium and the oospore are therefore seen to be each of a double nature. This fact is clearly demonstrated by the action of concentrated sulphuric acid on them. When this reagent is applied, the oospore, including its wall, swells up, the contents are destroyed (except the oil-drops), the inner and thicker part of its wall becomes dissolved, whilst the inner portion of the oogonial wall does likewise. Finally there are left, one within the other, two thin films, one being the outer part of the oospore wall, and the other that of the oogonial wall. The upper part of the funnel-shaped portion of the oogonial wall within the antheridium, and above the hyaline plug, also resists the action of concentrated sulphuric acid.

The outer part of the oogonial wall seems to differ slightly from that of the oospore wall in that the former is impermeable or only *very* slowly permeable to dilute sulphuric acid. It is for this reason that when the intact sexual organs are treated with sulphuric acid and iodine in potassium iodide the oogonial and the oospore walls do not become coloured. On the other hand, the outer part of the oogonial wall is penetrated at once—to some extent at least—by chlor-zinc-iodide solution; but the oospore wall stains more quickly and more deeply blue with this reagent when the oospore is previously artificially liberated from the oogonium, showing that the oogonial wall does offer some resistance to this reagent.

When the ripe sexual organs are placed, without previous treatment, in a freshly prepared solution of ammoniacal copper hydrate, the hyphae, antheridial wall, lower portion of oogonial funnel with its hyaline plug, together with the inner portion of the spherical part of the oogonial wall, become dissolved in the course of a few days, leaving the intact oospore surrounded by the slender outer part of the oogonial wall, which, as stated above, resists the action of concentrated sulphuric acid, and is coloured yellow with the iodine reagents mentioned.

Oospores liberated from their oogonia artificially, when allowed to remain in this reagent, show no signs whatever of the solution of their walls, even after a period of twenty-one days; neither does solution of the oospore walls occur with this reagent when the oospore is burst, and the reagent therefore in a position to attack its walls from the inside as well as the outside.

Germination of the Oospores.—It has not been found possible to cause germination to take place in only comparatively recently matured oospores; and the material on which the following description is based was derived from nine months' old cultures on oat extract agar slants. Examination of such slants reveals the occasional presence of already germinated oospores, so that nine months must be regarded as more than the minimum period of rest required. The stages in germination to be described were observed in hanging-drop preparations in sterilized bog-water at room-temperature. They require a period of two or three days for their completion.

The early stages in germination consist of a passage in the reverse order through the later phases exhibited during the ripening of the oospore. The structure of the ripe oospore was described on pp. 186 and 187. When germination is beginning, the large central oil-drop loses its identity, becoming broken up into smaller ones. The bright oval body disappears, the finely granular peripheral protoplasmic layer becomes coarsely granular, and the oospore becomes completely filled with what resembles a uniform but somewhat coarsely granular emulsion.

At this stage the thick wall of the oospore commences to be dissolved from within outwards; the rate of solution is not uniform all round; and hence the inner margin of this wall has now a more or less irregularly corroded appearance. (See fig. 3, Plate XIII.) Finally the oospore wall is reduced to the thickness of the outer layer alone, and is thus very slender.

The dissolution of the greater part of the thick oospore wall in the manner described strongly suggests that its function is not merely that of protection during the resting period, but that it also acts as a reserve-supply of food material; and it may be observed that since the blue colour produced in it by the iodine reagents previously mentioned is quite different from that produced in the antheridial and other walls, it seems fair to conclude that it consists of some special form of cellulose, which, however, is not directly soluble in ammoniacal copper hydrate solution.[1]

A germ-tube arises from the now thin-walled oospore, penetrates the oogonium wall, and so reaches the exterior, when it resembles an ordinary hypha. After growing in length to a greater or less extent, and frequently producing branches, conidia (zoosporangia) are produced in some cases, in others the germ-tube and its branches become ordinary hyphae; and in a few days a considerable and complex mass of mycelium results.

When the oospore completely fills the spherical portion of the oogonium, the point of penetration of the oogonial wall by the germ-tube coincides with the point of origin of the germ-tube itself from the oöspore. (See text fig. 1, p. 190.) When, however, the oospore does not fill the oogonium, its germ-tube may meander about to quite a considerable extent in the space

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[1] It is, of course, well known that, in some groups of the higher plants, cellulose is found deposited as a reserve material in thickened cell-walls; but, so far as I am aware, it has not previously been found stored in this manner amongst the fungi. The dissolution of this wall in the manner described was followed in all cases where the stages of germination of oospores were actually kept under observation from start to finish. One case was found, however, in an old culture where an oospore had already produced a germ-tube of considerable size, while the somewhat thin oospore wall, although having an irregularly waved inner margin, was not reduced in thickness so completely as to leave only its outer resistant larger. It would therefore appear that the *total* solution of the inner cellulose wall of the oospore need not necessarily precede the development of the germ-tube.

between the oospore and oogonial walls before it finds its exit through the latter, which may be a considerable distance from the point of origin of the germ-tube. A comparatively simple case of this kind is illustrated in text fig. 2, below.

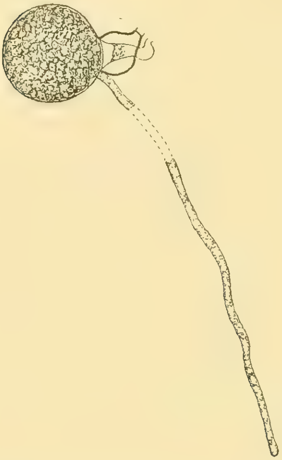

Fig. 1.

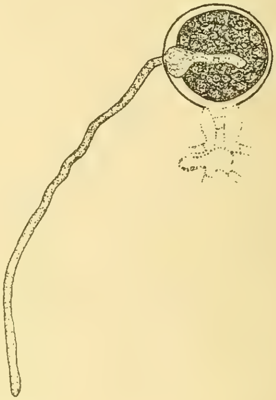

Fig. 2.

Fig. 1. Sexual organs containing an oospore, the stages of germination of which were watched in a hanging drop. At the outset the spore, which practically filled the spherical part of the oogonium, was provided with the normal thick wall and contents similar to those shown in Plate XIII, fig. 1. At the time of the production of the germ-tube, when the figure was drawn, this wall had become so thin as to be practically invisible. The germ-tube emerges through the oogonium wall at a point very slightly below the plane in which the drawing was made. × 510.

Fig. 2. Another set of sexual organs in which the stages of germination were followed in a hanging drop. Here the originally thick-walled oospore did not fill the oogonium. The germ-tube originates on the surface of the now thin-walled oospore, somewhat to the right of the centre, and penetrates the oogonium wall after traversing some distance in the space between the oospore and oogonium walls. Previous to penetration it becomes considerably swollen. × 510.

III. PARTS OF THE PLANT ATTACKED BY THE FUNGUS.

Up to the time of publication of the previous paper *P. erythroseptica* had been found only in the tubers, and in them merely in a vegetative condition. Prolonged and exhaustive search for conidia or oospores in affected tubers, after they had been removed from the soil, failed to reveal them, even when the tubers were so badly diseased as to be almost or quite rotten. The presence of the fungus and its reproductive organs was also sought for, although in a much less thorough manner, owing to limitations of time, in parts of the plant other than the tubers, but with negative results up to that time.

From the available evidence, however, it seemed quite clear that the primary source of infection must be the soil; and it was natural to suppose that, by some means or other, the soil became charged from an unknown source with the resting spores of the fungus. That the disease is contracted from soil is clear from two experiments carried out during 1913.

Two small plots were made at Clifden in bog-land which, within the memory of living man, had not previously been tilled. One of them was contaminated by adding to it a mixture of bruised and cut tubers affected with Pink Rot and soil from a plot which had produced plants bearing such tubers in 1912. The other was not thus treated, and served as a control plot. In each plot ten healthy tubers were planted. In the control plot two of these failed to produce plants, but in the contaminated plot seven behaved in this way. Unavoidable circumstances prevented investigations being made at the time to ascertain the cause of these failures.

Every plant in each plot was subjected to close observation, and towards the end of the season was dug and examined in detail with the aid of the microscope.

In the eight plants from the uncontaminated control plot, with the exception of a slight attack of the ordinary blight due to *P. infestans*, there were no signs of disease, and no traces of *P. erythroseptica* were found in any parts of them.

In the three plants from the contaminated plot, on the other hand, characteristic symptoms of disease (different from those due to ordinary blight, &c.) were observed in the plants before they were dug. The same symptoms were observed in many other cases in plants growing on land which had been cropped with potatoes for several successive seasons, and they will be described presently. After digging, *P. erythroseptica* was found to be present, and bearing its sexual organs in the partially decayed underground portions of the stalks of all three of these plants, while one of them bore an affected tuber.

The second experiment was carried out in pots. Eight large pots were filled with contaminated bog-soil, to which a quantity of bruised and cut affected tubers was further added. Two of these pots, with their contained soil, were sterilized by suitable treatment in the autoclave. In addition, two further pots were filled with non-contaminated peaty soil alone. A healthy tuber was planted in each of the ten pots. In the pots containing non-contaminated soil, and in those containing contaminated but subsequently sterilized soil, four plants grew which remained healthy; and on microscopic examination, they showed no signs of the presence of *P. erythroseptica*. In the six pots of non-sterilized contaminated soil, however, only two plants grew. One of these two plants was found to be quite healthy, but the other one, on microscopic examination, showed the presence of *P. erythroseptica*, bearing its sexual organs, in the underground portion of its stalk as well as in one of the rhizomes. Eighteen tubers were produced on this plant, seventeen of which were healthy, while one was almost entirely rotted away, leaving practically nothing but its skin. This tuber was attached to the above-mentioned rhizome; and lining the *inside* of its skin the sexual organs of the fungus were found in abundance. Up to the present this is the only tuber which has ever been found in which the resting spores of the fungus have been discovered; but it is the only one which has ever been examined after the rot has been allowed to complete its course, while the tuber remained undisturbed in the soil. Further trials are being made to ascertain whether, if the internal portions of affected tubers be allowed to rot away completely underground, the inner surface of the skin is, as a general rule, the seat of the production of oospores.

These two experiments therefore show—

(*a*) that contaminated soil may give rise to diseased plants;

(*b*) that parts of the plant other than the tubers may become attacked, and that the resting spores of the fungus may be formed in them;

(*c*) that the resting spores of the fungus may be found also on the inside of the skin of affected tubers which complete the rotting process under ground,

From the facts given in (*b*) and (*c*) it is easy to understand how the soil becomes contaminated.

In other plots of ground at Clifden, which had been cropped with potatoes for five successive years, and which therefore had had every chance of becoming seriously contaminated, attacked plants were plentiful in 1913;

and the fungus has now been found in such plants in the underground portions of the stalks, in the rhizomes and in the roots, always bearing sexual organs in abundance, and sometimes a few conidia.

As stated above, affected plants show symptoms of the disease in their overground stalks and foliage, as well as in their underground portions. These symptoms only become apparent, however, somewhat late in the season, so far at any rate as present observations show. They are as follows:—

From about the middle of August onwards[1] the foliage of affected plants is characterized by its pale green or almost yellow colour; while the margins of the leaflets are often rolled upwards and inwards, being brown, dry, and crisp. Scattered over the leaflets are larger or smaller brown and dead areas of tissue, which are either isolated or more or less continuous with the brown marginal portions. Frequently all the stalks of a plant are similarly affected; but cases have been observed where some of the stalks were diseased, while others remained healthy.

Very often close to the ground-level there is a conspicuous crop of aerial tubers, showing that the portion of that stalk below ground is injured or destroyed. If the affected stalks be cut across with a sharp knife, the three principal vascular bundles are sometimes seem to be somewhat browned, rarely strongly so. On pulling up such stalks, the underground portions are found to be rotten. The epidermal and cortical tissues are decayed, usually without strong blackening, and frequently the wood is exposed. The pith is destroyed, and a cavity is formed lined with cellular *débris* in which the sexual organs are invariably found, sometimes in extraordinary abundance. This rotting of the tissues at the base of the stalks does not as a rule proceed up the stalk beyond about the ground-level. The mycelium of the fungus has also been found in the wood-vessels; and its presence there probably accounts for some of the symptoms noticeable in the foliage.

Some of the roots and the rhizomes may be partially decayed, and if so the fungus with its reproductive organs is found in these also. Some of the tubers, both amongst those at or above the surface of the soil, as well as the deeper lying ones, are also usually affected; but a few cases have been observed in which the tubers up to the time of digging were free from the fungus, although it was present in the stalks, and in some cases in the rhizomes bearing tubers not yet attacked.

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[1] Where such diseases as the blight and the Sclerotium disease are not so prevalent as they are at Clifden, it might be possible to recognize the symptoms on a cursory glance somewhat earlier. Further, it is possible that the fungus may, by attacking the young sprouts, be the means of causing "misses."

In two cases where healthy tubers were still attached to rhizomes, portions of which at some distance from the tubers were diseased, it was found that on burying them in the soil the disease progressed through the rhizomes, entered the tubers, and destroyed them.

In its fundamental aspect the disease may be described as being of the "wilt" type, and it is accompanied by a rotting of portions of some or all of the underground parts of the plant. In this latter aspect the disease differs from the Verticillium wilt, which has been a subject of study at Clifden for some years, and which will be described in detail, it is hoped, in a later paper. In general outward appearance it closely resembles the disease known as Black Stalk Rot,[1] due to *Bacillus melanogenes* P. & M. Without a careful microscopical examination, indeed, it is in many cases impossible to distinguish with certainty between these two diseases. It may, however, be stated that in the case of the present disease (for which perhaps the name "Pink-Rot Wilt" may be suggested) the browning of the vascular bundles of affected stalks is not usually such a clearly marked character, and there is, as a rule, less or none of the external blackening of the epidermal and cortical tissues of the stalks which constitutes a well-marked feature of Black Stalk Rot. Furthermore, the fact that Black Stalk Rot is one of the earliest of the potato diseases to show itself in the season (being evident often in the month of June) serves to some extent to distinguish it from the Pink-Rot Wilt.

The investigations made up to the present unmistakably point to the conclusion that there exists a disease of the potato-plant as a whole (not merely a disease of the tubers, although this is perhaps the most serious aspect of the disease from the economic standpoint) which is caused by *P. erythroseptica*, and which has hitherto escaped observation. From one point of view the disease may be looked upon as a new one; but in all probability it is one of old standing, only its recognition as specific disease being really new.

It will be observed that, in the foregoing account, it has been assumed that the connection between the symptoms described and the presence of the parasitic fungus is a causal one. As regards the attack on the tubers, however, it has been proved definitely, by means of controlled inoculation experiments with pure cultures, that the fungus in question is undoubtedly the cause of the particular form of rot which occurs; and it must be admitted that experiments of a similar nature are necessary with the

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[1] Pethybridge, G. H., and P. A. Murphy: "A Bacterial Disease of the Potato Plant in Ireland, and the Organism causing it."—Proc. Roy. Irish Acad., vol. xxix, Sect. B, No. 1, 1911.

whole plant to prove with absolute certainty that the same fungus is the actual cause of the symptoms of disease observable.

The circumstantial evidence is, however, strong. Certainly the symptoms described do not at all resemble those of the blight or of the Stalk (Sclerotium) Disease, two of the most prevalent diseases on the Clifden plots. It might be argued that the affected plants described were cases of Black Stalk Rot, supplemented by the subsequent saprophytic invasion of *P. erythroseptica.* This is decidedly improbable, for this fungus has been proved to be a virulent parasite of the potato, destroying both living tubers and stalks when inoculated into them. Further, had the plants first been attacked by Black Stalk Rot, it is practically certain that their diseased condition would have been observed many weeks earlier than was the case. Lastly, the Black Stalk Rot disease has been eliminated almost completely from the plots at Clifden, so that there would not have been a sufficient number of such plants present in the plots to account for the observed prevalence of the Pink-Rot Wilt.

The actual details as to how the primary infection of the plant from the soil occurs are not yet known; but it is probable that the germ-tubes of the zoospores, conidia, and oospores possess the power of penetrating the epidermis of some portion or portions of the underground parts of the plant. That these germ-tubes are incapable of penetrating the superficial tissue when it consists of corky periderm seems to be probable from the fact that unwounded tubers invaded other than directly through the rhizome are of the greatest possible rarity.[1] Further, since healthy tubers, when pitted in close contact with cut or damaged diseased ones, do not become diseased, it would appear that the mycelium of the fungus is incapable of penetrating through the skin of the tuber. This is rendered all the more probable from the fact that the fungus does not grow out through the skin of affected tubers so long as the latter remains intact and unbroken.

With regard to the occurrence of the disease it has been seen up to the present only in Ireland; but there is no reason to suppose that it is strictly limited to this country. Particularly in districts where potatoes are cultivated too frequently in the same ground, it is likely that careful search will reveal its presence. It is on land of such a character that the losses caused by it are most serious, although it has been met with to some extent in parts of the country where better methods of farming prevail.

On poor reclaimed bog-soil at Clifden, Co. Galway, which had borne

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[1] This has been observed in only two cases, and even in these the fungus might have gained an entry through minute wounds, too small to be seen easily, or which had, subsequent to infection, become more or less obliterated.

potatoes for five years in succession, over twelve per cent. of the total yield of tubers was destroyed by this disease. On similar land two years in potatoes the loss was nearly four per cent., whereas on fresh land of the same character the loss was only 0·03 per cent.

It is obvious, therefore, that it takes some years for land to become contaminated to an extent sufficient to cause very serious losses; and if a proper rotation be followed, such losses are practically negligible.

Since the soil must become contaminated from the resting-spores present in the decayed portions of the plants, it is clear that collecting and burning the potato haulms previous to digging is a practice strongly to be commended; for if this be done, there is less chance of other areas becoming contaminated through such *débris* as might reach it *via* the manure-heap or in some other way. Further, it would be prudent to destroy or bury deeply all affected tubers when lifted, seeing that they too may possibly harbour the resting-spores of the fungus.

SUMMARY.

The present paper contains further observations on *Phytophthora erythroseptica* Pethyb., which in a former one was shown to be the cause of a specific rot ("Pink Rot") of the potato tuber.

Attention is directed to the fact that the peculiar mode of development of the sexual organs described for this fungus, and shown to occur also in *P. infestans* de Bary, *P. Phaseoli* Thaxt., has also been observed independently in *P. parasitica* n. sp. and *P. Colocasiae* Racib. by Dastur and by Butler and Kulkarni respectively. This brings up the total number of species in the genus Phytophthora, as restricted by the present author, to five certain species with a possible sixth, viz., *P. omnivora* var. *Arecae* Coleman.

The microchemical reactions of the walls of the hyphae, conidia, and sexual organs show that these are largely but not entirely composed of cellulose. The walls of the oogonium and of the oospore each consist of two layers, the thin, outer portions being coloured yellow with iodine reagents, and not dissolved by ammoniacal copper hydrate or concentrated sulphuric acid. The inner portion of each consists of cellulose, but that of the oospore differs from that of the oogonium in turning blue with iodine reagents instead of purplish-violet, and in being insoluble in ammoniacal copper hydrate.

The thicker, more hyaline apical portion of the conidium wall is not stained by iodine reagents, and is insoluble in concentrated sulphuric acid and in

ammoniacal copper hydrate. It is probable that an extremely thin layer of this material covers the whole surface of the conidium.

The conidia (zoosporangia) may germinate either by producing germ-tubes direct or by the formation of zoospores. The details, which do not differ essentially from those observed in other species of Phytophthora, are described.

The oospores have been caused to germinate after a suitable period of rest. Previous to the production of a germ-tube by the oospore, which then penetrates to the exterior through the oogonial wall, the thick inner portion of the wall of the oospore dissolves and apparently serves as a store of reserve carbohydrate food material.

Whereas formerly the reproductive organs of the fungus had only been observed in artificial pure cultures of the fungus as a saprophyte, they have now been found in all of the underground parts of the plant, including in one case a tuber.

Plants in the roots, rhizomes, stems, and tubers of which the fungus has been found, exhibit symptoms of disease in their sub-aerial organs, and it is believed that these symptoms, which are of the "wilt" type, are due to the invasion of the plant by the parasitic fungus. Hence the name "Pink-Rot Wilt" is suggested for the disease.

The disease is evidently contracted from the soil, and is of serious consequence only in land which has borne a crop of potatoes for several successive seasons.

December, 1913.

[EXPLANATION OF PLATE.

EXPLANATION OF PLATE XIII.

The reproductions of the photo-micrographs in this plate are from the original untouched negatives.

Fig. 1. Ripened sexual organs of *Phytophthora erythroseptica* photographed from living material. The oogonium, with its funnel-shaped base (containing a cellulose plug) within the antheridium, contains in its spherical portion an oospore with a thick wall, granular peripheral protoplasm in which the oval body can be seen, and a central large oil-drop. × 500.

Fig. 2. Ripened sexual organs after exposure to the vapour of osmic acid. The central oil-drop is very deeply stained, the oval body less so, and the granular protoplasm still less so. × 500.

Fig. 3. Sexual organs viewed from above, showing an oospore in the early stages of germination. Owing to the irregular manner in which the thick wall of the spore becomes dissolved, its inner margin is wavy. The two short, thin, hypha-like structures in contact with the object are portions of the medium, not of the fungus. Living material. × 900.

Fig. 4. A conidium which, instead of producing either a germ-tube or a number of zoospores, has burst at its apex, and the contents are oozing out. × 260.

Fig. 5. A conidium (zoosporangium) fixed with iodine in potassium iodide solution, showing the segregation of its contents into zoospore units. The thicker, flatter, apical portion of the wall is clearly distinguishable; but the contraction of the contents away from the wall is somewhat greater than is the case in living material. × 365.

Fig. 6. A conidium germinating by the production of a single germ-tube. This tube makes its exit a little to one side of the apex of the conidium, the apical, thicker, more hyaline part of the conidial wall being seen to the right near the base of the germ-tube. Living material. × 527.

Fig. 7. Two conidia (zoosporangia), one of which has already discharged its zoospores and become split during manipulation. The other shows the segregation of the contents into zoospore units previous to their emission, as seen from the basal end of the zoosporangium. Material fixed in iodine in potassium iodide solution. × 370.

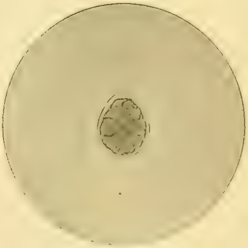
Fig. 8. A group of four zoosporangia. The uppermost one on the left contains undischarged zoospores; the one below it has emitted one zoospore, which has come to rest and developed a germ-tube. In the centre is a practically empty zoosporangium, while on the right is a partially emptied one, two of the three emitted zoospores having produced germ-tubes. Material fixed with osmic acid vapour. × 354.

1

2

3

4



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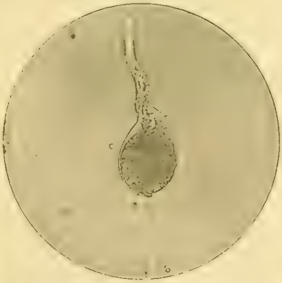

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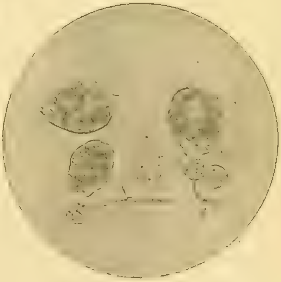

8

PHYTOPHTHORA ERYTHROSEPTICA Pethybridge.

THE
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OXYDASES AND THEIR INHIBITORS IN PLANT TISSUES. PART III: THE LOCALIZATION OF OXYDASES AND CATALASE IN SOME MARINE ALGÆ.

BY

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XI.

OXYDASES AND THEIR INHIBITORS IN PLANT TISSUES. PART III: THE LOCALIZATION OF OXYDASES AND CATALASE IN SOME MARINE ALGÆ.

By W. R. G. ATKINS, Sc.B., A.I.C.,
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[Read December 16, 1913. Published January 31, 1914.]

Notwithstanding the large amount of work which has been done in recent years in connection with the distribution of oxydases in plants, the problem of their localization in marine algæ has hitherto attracted little attention or none at all. In the present paper a short account is given of such work; and it is hoped that further details will soon be forthcoming.

For the detection of oxydases, very dilute solutions of guaiacum resin, benzidine, and α-naphthol in aqueous alcohol were employed; if no direct action appeared, a few drops of neutral four-volume hydrogen peroxide were added. It may be remarked that the above reagents for oxydases yield respectively a blue colour—a blue changing to brown, and a purple when oxidized. The addition of alcohol alone causes an evolution of gas-bubbles from algal tissue. Probably this may be accounted for by the forcing out of solution of dissolved air, because the same effect is produced by mixing alcohol and water. Whether this is a quantitatively sufficient explanation has not been tested. There remains the possibility that it is due in part to liberation of oxygen by reducing substances, which on the death of the protoplasm come into contact with easily reduced compounds. An example of this type is the action of hydrogen peroxide upon moist silver hydroxide.

$$2AgOH + H_2O_2 = 2Ag + 2H_2O + O_2.$$

Accordingly, to avoid this error in testing for catalase, hydrogen peroxide alone was added to the mashed algal tissue. Algæ which have been boiled fail to bring about the decomposition of the peroxide. Whether the browning of benzidine is in every case due to oxydase action will be considered later on.

Material.

The algæ tested were collected by the author in Cork Harbour towards the end of September and early in October, and kept in sea-water in the open air till their examination, which was carried out within two days of the time of gathering. The observations were subsequently further extended, and repeated in many cases, with specimens from Dublin Bay collected during November. Owing to the rapidity of bacterial action on these algæ, attention to the fresh condition of the material is essential.

To perform the tests, a portion of the uninjured thallus, or a section of it, was allowed to soak in the reagents α-naphthol and benzidine, whilst for the less permeable guaiacum either a section or a macerated thallus was employed.

The results obtained up to the present are recorded in the following tables:—

—	Catalase reaction	Oxydase reactions, indirect, with		
		Benzidine	Guaiacum	α-Naphthol
CHLOROPHYCEÆ.[1]				
Enteromorpha intestinalis, *Link.*,	+	+	—	—
Ulva Lactuca, *L.*,	+	+	—	—
Cladophora sp.,	....	+	—	—
PHÆOPHYCEÆ.				
I. PHÆOSPOREÆ.				
(*a*) *Ectocarpaceæ.*				
Chorda filum, *Stackh.*,	+	+	—	—
Desmarestia aculeata, *Lamx.*	+	+	—	—
(*b*) *Sphacelariaceæ.*				
Sphacelaria cirrhosa, *C. Ag.*,	+	+ +	—	—
(*c*) *Laminariaceæ.*				
Laminaria saccharina, *Lamx.*,	+	+	+ +	—
L. digitata, *Lamx.*,	+	+ +	+ +	—
II. CYCLOSPOREÆ.				
Fucaceæ.				
Fucus vesiculosus, *L.*,	+	+	—	—
F. serratus, *L.*,	+	+	—	—
F. platycarpus, *L.*,	+	+	—	—
Ascophyllum nodosum, *Le Jol.*,	+	+	—	—
Pelvetia canaliculata, *Dcne. & Thur.*,	+	+	—	—
Halidrys siliquosa, *Lyngb.*,	+	+	—	—

---

[1] Oltmann's classification has been followed throughout.

—	Catalase reaction	Oxydase reactions, indirect, with		
		Benzidine	Guaiacum	α-Naphthol
RHODOPHYCEÆ.				
I. BANGIALES.				
Porphyra laciniata, *J. Ag.*, ...	+	+	—	—
II. FLORIDEÆ.				
(1) *Gigartinales.*				
Chondrus crispus, *Stackh.*, ...	+	+	—	—
Cystoclonium purpurascens, *Kütz.*, . .	+ slight	+	+ slight	—
(2) *Rhodymeniales.*				
(*a*) *Rhodymeniaceæ.*				
Gracilaria confervoides, *Grev.*, .	+	+	+	—
G. multipartita, *Harv.*, ...	+		—	—
Rhodymenia palmata, *Grev.*, . .	+	+	—	—
Plocamium coccineum, *Lyngb.*, .	+	+	—	—
(*b*) *Delesseriaceæ.*				
Nitophyllum laceratum, *Grev.*, .	+	+	—	—
N. uncinatum, *J. Ag.*, . . .	+	+	—	—
Delesseria sinuosa, *Lamx.*, . .	+	+	—	—
D. sanguinea, *Lamx.*, ...	+	+ +	+	+ slight
Pteridium alatum, *J. Ag.*, . .	+	+ slight	—	—
(*c*) *Rhodomelaceæ.*				
Polysiphonia fastigiata, *Grev.*, .	+ +	+	+ slight	—
Heterosiphonia coccinea, *Falk.*, .	+ +	+	—	—
(3) *Cryptonemiales.*				
Furcellaria fastigiata,[1] *Lamx.* .	+	+	+ +	+ slight

Discussion of Tabulated Results.

Catalase is seen to be present in every specimen. But whereas in some cases the evolution of gas was slow, in others, such as Polysiphonia and Heterosiphonia, it was amazingly rapid. The highly viscous nature of the mashed-up algal tissue, in many instances, delays the movements of the bubbles.

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[1] Gives the direct guaiacum and benzidine reactions strongly, being the only alga that I have as yet found to behave thus. The dried specimen gave a decided but less intense reaction ten days later. In this, as in all other cases examined, no coloration of guaiacum was brought about by boiled material.

Inspection of the table makes it at once apparent that the oxydases are conspicuous by their absence, to judge from the reactions with *a*-naphthol and guaiacum. With the former reagent, in two cases only, a slight coloration was produced, though in both these the guaiacum blue was well marked. With the latter, seven algæ out of twenty-nine tested gave a positive result, and in two of these the coloration was only feeble. The behaviour of *Furcellaria fastigiata* is remarkable, for it alone gave the direct reactions with guaiacum and benzidine.

The benzidine reaction was always given, but only upon long standing in the majority of cases, so that it appears rather to be due to spontaneous oxidation than to enzyme action. This view is further borne out by the fact that boiled specimens also darkened when in benzidine solutions. Yet it seems to me that the depth of colour was not so great in the latter. Those algæ, however, which react with guaiacum also react rapidly with benzidine. Work on Iris (1, 2) has, however, shown that tissues which, owing to the presence of a reducing substance, fail to react with guaiacum or *a*-naphthol, in many cases produce a colour with benzidine. With the algæ there is a further source of difficulty in the natural pigmentation, which renders such reactions hard to observe. Microscopic sections do not in these cases afford much additional information, for, as in Iris, it is quite possible that an oxydase is active in the superficial, but not in the medullary tissue. An attempt was made to remove inhibitors by immersion of the thallus of *Ascophyllum nodosum* and of *Polysiphonia fastigiata* in 0·2 per cent. hydrogen cyanide with subsequent thorough washing. After such treatment, catalase, though strongly inhibited by the cyanide, as shown by Senter (8), was found to be active. This proves that the washing was thorough. Sections of Ascophyllum, however, gave no oxydase reactions; and Polysiphonia when macerated gave only a slight reaction with guaiacum, no more intense than before the treatment. Thus, in the large majority of algæ examined, there is no decisive evidence of the existence of oxydases; but, on the other hand, reducing agents are present (as will be shown later) of such a nature as to prevent the detection of the former by the usual methods.

In this connection the case of *Sphacelaria cirrhosa*, one of the filamentous Phæophyceæ, is of interest. Treatment with the benzidine reagent overnight produced no browning, but the addition of hydrogen peroxide brought about a rapid darkening, the colour being especially intense in the apical cells, by the subdivision of which the thallus increases in length. This clearly is a case of enzyme action, though no coloration was produced with *a*-naphthol, or even with guaiacum, when the thallus was pounded up and tested. The cell-walls, too, of this alga assumed a brown or delicate blue colour in the benzidine reagent. It was noticed that the mucilage connecting the cells of the diatoms Tabellaria and Pinnularia, epiphytic on Sphacellaria, also assumed a blue colour rapidly. Furthermore, slime pressed from the conceptacles of *Fucus serratus* gave this blue with benzidine and the peroxide. From their rapidity it appears that these reactions are due to oxydases; but the crucial test of boiling was not made. The natural pigment of the diatoms rendered it impossible to decide whether the benzidine reaction was produced inside the valves also. Recently Kylin (5) has obtained varieties of slime or mucilage from both brown and red algæ. All agree in remaining uncoloured by zinc chloro-iodide, and in giving the pentose reactions with phloroglucin and orcin, followed by hydrochloric acid in each case. It seems likely that oxydases are concerned in the production of these slimes, just as they probably function in the production of sclerenchyma, as pointed out in Part I of this series. From the fact that in many cases cellulose walls are coloured blue by benzidine, it appears that oxydases are intimately associated with many of the changes occurring in these so-called passive portions of the cell.

Some Reactions of Phæophycean Extracts.

Attempts were made to obtain active oxydase preparations from members of this class by precipitating dilute alcoholic aqueous extracts with alcohol, thus separating the enzyme from the soluble inhibitor, as was done in the case of *Iris germanica* leaf extract (1). Both *Pelvetia canaliculata* and *Ascophyllum nodosum* were tested in this way, the latter being allowed to soak for three days. The precipitate was washed well with spirit; but when water and the guaiacum reagent were added, no blue colour appeared, nor did subsequent addition of hydrogen peroxide produce an alteration. Perhaps the disintegration of the tissue was not sufficiently complete. Further attempts are being made in this direction.

The presence of an active reducing agent in these algæ is shown by the fact that a macerated *Fucus serratus* thallus yields a solution which almost decolorizes dilute aqueous methylene blue, changing it to a light greenish hue. The same almost colourless extract also destroys the guaiacum blue produced by *Hedera Helix*, leaving a light green colour only. The filtrate from the attempted enzyme extraction of Ascophyllum, which was of a golden brown colour, was also found to destroy the colour of methylene blue, for though producing but little effect when first added, a greenish colour appeared on warming. These solutions were faintly acid; this was noted because guaiacum blue is decolorized by alkalis.

Phæophycean Pigments.

With a view to obtaining colourless material on which to try the enzyme reactions, some experiments were made on the pigments derived from this group. A remarkable change from brown to green occurs when Fucus, Ascophyllum, or other brown algæ are boiled or steamed. This has long been known and has been examined in detail in recent years by Molisch (6, 7), and Tswett (9, 10). They, in common with previous investigators, regard the water-soluble brown pigment of the Phæophyceæ as due to post-mortem oxidations. It is easy to satisfy one's self that it is not due to an enzyme action, for Fucus, Ascophyllum, Pelvetia, and Halidrys acquire a dark brown colour when allowed to stand in air after having been boiled. This water-soluble brown pigment is termed "phycophæin." Molisch reserves the term "phæophyll" for a brown pigment occurring naturally, but not soluble in water. By reduction or other change taking place in its molecules a green pigment similar to chlorophyll is produced. This Molisch regards as the cause of the colour change which occurs, as I have myself observed, not only upon boiling, but when the protoplasm is made permeable by immersion in ethyl or amyl alcohol. That it is not due to an extraction of pigment is clear, for the alcohols remain untinged for a long time after the change is complete. Tswett, too, has carried out an elaborate investigation upon these pigments. He ascribes the natural colour to the mixture of chlorophyll a and γ, fucoxanthin, carotin, and fucoxanthophyll, and attributes the appearance of green to the solution or destruction of fucoxanthin which is red-brown in the solid state, but yellow in solution. There does not, however, seem to be evidence that this pigment exists in the plastids in the solid condition. I venture to suggest that this change on boiling may be explained at least equally well as follows: Water-soluble phycophæin is produced after death; so it seems reasonable to suppose that it is produced in the plastids of the superficial tissues during life. When the protoplasm is made permeable, by boiling or treatment with hormones such as the alcohols, reducing substances in the same or neighbouring cells interact with this phycophæin, converting it into a colourless body. Kylin (5) has this year brought forward evidence that phycophæin is nothing but oxidized fucosan. Fucosan he regards as a tannin-like substance, though not a typical tannin. Thus it it is quite possible that fucosan, like tannin, may be an inhibitor of oxydase action. Again, it may be that during life the oxidation of fucosan is a method by which these algæ store a supply of oxygen when it is abundant, as, for instance, during low water; this being given up if required when the supply is diminished by the returning tide.

As is well known, acids only slightly decrease the depth of coloration of phycophæin solutions; but I have observed that, making use of an extract of *Halidrys siliquosa*, the addition of a little zinc dust to the acid solution results in the rapid reduction of the pigment to a colourless substance, leaving only a pale yellowish solution. Treatment of the red-brown extract with slightly acid hydrogen peroxide results in the destruction of the pigment, with the production of a pale yellow, indistinguishable from that given by the action of nascent hydrogen in the zinc dust experiment already described. Here the peroxide is probably functioning as a reducing agent, as it does with silver hydroxide and in the well-known method for reducing a ferricyanide. In other recent papers Kylin (3, 4) maintains that Fucoideæ contain chlorophyll, carotin, xanthophyll, and phycoxanthin, but does not satisfactorily explain the death-change from brown to green. That this is a reduction, changing a brown "phæophyll" to a chlorophyll, is the view put forward by Molisch. That the change is a reduction appears to me to be very probable, but that the resulting body is chlorophyll seems to be in need of definite proof. That the coloured substance is phycophæin, shown by Kylin to be an oxidation product of fucosan, and only formed in small quantities during life, is in my opinion quite as reasonable a view as that of Molisch, though equally in need of direct proof.

Rhodophycean Pigments.

To satisfactorily examine the oxydase contents of the superficial cells, it is necessary to remove the pigments. My attempts in this line are as yet only of a preliminary nature; but it was observed that the red pigment in *Plocamium coccineum*, *Rhodymenia palmata*, and *Gracilaria multipartita* was decolorized by alkalis, the green having been dissolved out by alcohol. Addition of acids, sulphuric or acetic, restored the colour, but it was of a slightly more purple shade than the original pigment. It was further noticed that boiling for a minute sufficed to destroy the natural red colour, but that addition of an acid again restored it. This looks as if the acid, in this case, hydrolyzed a chromogen to produce an indicator-like body, whereas with alkalis the pigment may merely form a colourless salt.

Summary.

1. Catalase was found in all the species tested, being very active in some.

2. Out of a total of twenty-nine, one alga gave the direct oxydase reaction with guaiacum, whilst six gave the indirect—namely, on addition

of hydrogen peroxide. In two cases only was a colour produced with α-naphthol, and in these it was faint and indirect, but all reacted with benzidine indirectly. However, it is certain that the latter coloration was not always due to an oxydase, as it was also produced in boiled specimens.

3. The presence of water-soluble phycophæin in small quantity during life and its reduction to a colourless substance at death, is suggested as an explanation of the much-discussed colour-change occurring in brown algæ.

I have much pleasure in thanking Professor H. H. Dixon for the criticism and advice which he continually afforded me. I wish also to acknowledge my indebtedness to Professor K. Yendo, of the University of Sapporo, for his kindness in assisting me with the identification of the various algæ.

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NOTE ON THE SPREAD OF MORBID CHANGES THROUGH PLANTS FROM BRANCHES KILLED BY HEAT.

BY

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XII.

NOTE ON THE SPREAD OF MORBID CHANGES THROUGH PLANTS FROM BRANCHES KILLED BY HEAT.

By HENRY H. DIXON, Sc.D., F.R.S., Univ. Professor of Botany,
Trinity College, Dublin.

[Read December 16, 1913. Published February 3, 1914.]

Some years ago[1] I showed that, if a branch of a plant is killed by heat, and the rest of the plant is then supplied with water through this branch, some of the leaves on the uninjured branches may become injuriously affected, although there is no other interference with their direct supply from the roots. This result may be explained as due to the formation of poisonous bodies in the heated branch and their transference in the water-supply to the other branches.

The fact that the withering of the leaves in these cases is due to the contamination of the water-supply demonstrates the invalidity of the conclusion that the fading of leaves above a steamed region of a stem must be attributed to the cutting-off of the water-supply by interfering with the vital actions of the wood parenchyma and medullary ray cells in the stem.

Shortly after completing the experiments quoted above, I made additional experiments which seemed to me to bear out my previous conclusion in a still more striking manner. However, as the point seemed adequately established by former work,[2] the record of these experiments was put aside in favour of more pressing investigations.

Quite recently attention has been again directed to the subject by Ursprung.[3] He reiterates the earlier explanation, and states that he could observe no poisoning effects below the killed region such as were observed in my experiments. This negative observation in itself would, of course, mean nothing unless it were quite certain that a stream of water passed from the killed region to the healthy leaves, and so that there was an opportunity for the dissolved substances in the killed region to pass into the other parts of the plant. The partial restitution of turgescence observed by Ursprung, if wilting had not gone too far when the dead branches were supplied with water under

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[1] Roy. Dublin Soc. Proc., vol. x, 1905, p. 48.

[2] J. B. Overton: Transpiration and Sap-flow. Bot. Gaz., 1911, pp. 28 and 102. These papers contain a summary of the discussion, together with new and convincing experimental work.

[3] A. Ursprung: Zur Frage nach der Beteiligung lebender Zellen am Saftsteigen. Beihefte zum Botan. Centralblatt. Bd. xxviii (1912), Abt. I, p. 311.

pressure, may be explained as an effect one would expect due to the dilution of the poisons.

As further supporting the explanation which I have previously given of the observed wilting, the two following experiments carried out in the summer of 1911 may be of interest.

The object of these experiments was to see if it would be possible to more or less completely wash out the poisonous material from the killed region, and so prolong the life of the leaves above that region.

The subject of the first experiment was a pot-plant of *Prunus Cerasus* (fig. 1). The stem of this plant bifurcated at a level of about 40 cm. above the soil into two equal branches *B* and *C*. *B* produced two lateral branches *E* and *D* at 11 cms. and 34 cms. respectively above the bifurcation, while *C* had two smaller leafy branches about 15 cms. above the bifurcation, and terminated with a tuft of leafy branches above. The top of *B* above the base of *D* was removed, and, with suitable precautions to minimize the clogging of the surface, a rubber-tube was attached to it. The whole of *E*, except a few centimetres of its base, was cut away. The cut surface at the top of *B* was now supplied with distilled water under a head of 33 cms., and when the cut surface of *E*, by becoming moist, showed the arrival of the stream below, the intervening space of 23 cms. (shown dark in the figure) was lapped in cloth and sprayed with boiling-water. Meanwhile the leaves were protected from injury by being covered with damp cloths. The hot spraying lasted ten minutes. After it ceased the damp cloths were removed from all except the branch *D*. The transpiration of this branch was thus kept at a minimum during twenty-four hours, while the supply of distilled water was kept up to flush out the materials exuded into the water-ways of the heated region. During this time about 35 c.cs. were passed in at the top of *B*. After this the plant stood in a cool green-house under conditions favourable to transpiration.

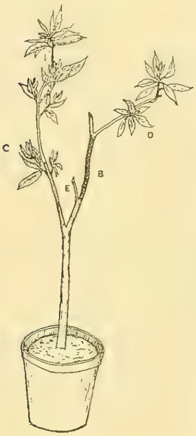

Fig. 1.

Plant of *Prunus Cerasus* used in Expt. 1. The dark region on the branch *B* indicates the part which was killed at the beginning of the experiment. The shaded region on branch *C* shows the part heated later.

Three days later it was observed that the lower leaves of *D* and a few of the leaves at the lower branches of *C* were slightly discoloured and curled at the edges—typical symptoms of the poisoning caused by heating the supply branch. Evidently heating part of *B* had affected not only the leaves above it, but also the effects had been transmitted backwards to *C*.

After seven days from the beginning of the experiment the leaves on *C* and *D*, which before had shown the slight changes just mentioned, had become quite withered and curled, and slight but similar changes were becoming apparent in a few of the upper leaves of *D*.

Fourteen days later—i.e., twenty-one from the beginning of the experiment—there were still four living leaves on *D*; but their veins were coloured red, and the edges of two were discoloured and brown. The remaining leaves on *D* were dry and crisp. On *C* most of the lower leaves had fallen, and those which were still attached were crisp and much discoloured. The remaining upper leaves were apparently still quite healthy.

The lower leaves on *C* were now removed; and the lower 23 cms. (shaded in the figure) of the branch were killed with hot water in the same manner as the portion of *B* had been, while the upper healthy leaves were protected by enveloping them in a damp cloth. Five days after this treatment all the leaves on *C* were stained and curled, their cells being evidently dead.

In this experiment we see that all the leaves above a length of stem of 23 cms. which has been killed by heat, show strongly marked morbid changes after five days if no attempt is made to wash out the killed portion; while in a similar case in which the heated region is flushed out with water, these changes are postponed for twenty-one days, and even then are not complete.

The experiment also demonstrates that leaves on an uninjured branch may be caused to wither by supplying them with water which has passed through a heated stem, although their normal supply is interfered with in no other way.

In the second experiment a pot-plant of *Cotoneaster frigida* was used (fig. 2). Distilled water was supplied at the cut end of the main stem; below this four lateral branches, *A*, *B*, *C*, and *D*, took origin, separated from one another by distances on the stem of 18 cms., 5 cms., and 22 cms. respectively from below upwards. From each of these, leafy secondary branches sprang. *D* supported only one secondary branch; and it was cut short by an old injury. *C* was lopped at the beginning of the experiment, and was left with only one secondary branch. *B* and *A* were not interfered with.

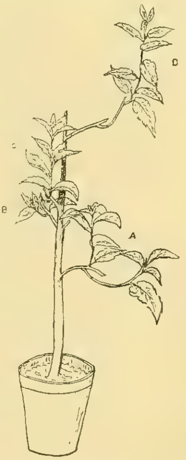

Fig. 2.

Plant of *Cotoneaster frigida* used in Experiment 2. The dark portion on the main stem indicates the region which was killed.

During twenty-four hours distilled water was supplied at the cut top of the main stem. Shortly after this supply had been started, the region of the stem, viz., 22 cms. between

C and *D*, was killed with hot water as in the previous experiment, while the leaves on all the branches were protected from injury. The heated region is shown dark in the figure.

Three days later blotchy discolourations appeared on the lower leaves of *D*, and less markedly on some of those of *A*, *B*, and *C*.

On the fourteenth day the four lowermost leaves of *D* were completely discoloured, and a small blotch had appeared on the fifth leaf from the base, while the seven leaves above were quite healthy. On *A* there were five leaves dead; on *B* two small ones and two large leaves blotched; on *C* there was one withered and crisp and one blotched.

At this stage 14 cms. at the base of *C* were heated, the leaves above being protected.

On the next day all the leaves of *C* were stained and beginning to curl. No further changes were noticed on *A* and *B*.

Six days after the heating of *C* all its leaves were dead, while still the uppermost leaf of *D* was unaffected, although its supply had now been drawn for twenty days through a piece of dead stem, 22 cms. in length.

The results of this experiment drive us to the conclusion that deleterious substances were washed from the stem below *D* and transferred into the lateral branches *A*, *B*, and *C*; for the changes in the leaves of *D* were no more noticeable than those in the leaves of the other branches, into which evidently the major part of the poisonous substances had been distributed. The changes observed in the leaves of *C* after it had been heated indicate the extent and rapidity of the injuries we might have expected in *D* had the heated region below its base not been washed out.

Both experiments seem to afford conclusive evidence that, with the killing of the cells of the stem by heat, changes are introduced into the sap which are largely responsible for the alterations in the leaves above. Hence the morbid changes there cannot validly be attributed to the cutting-off of the supply, but rather should be assigned to its contamination.

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BOTHRODENDRON KILTORKENSE, Haught. sp.: ITS STIGMARIA AND CONE.

BY

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PROFESSOR OF BOTANY IN THE ROYAL COLLEGE OF SCIENCE FOR IRELAND, DUBLIN.

(PLATES XIV–XVIII.)

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XIII.

BOTHRODENDRON KILTORKENSE, Haught. Sp. ITS STIGMARIA AND CONE.

By T. JOHNSON, D.Sc., F.L.S.,
Professor of Botany in the Royal College of Science for Ireland, Dublin.

(Plates XIV–XVIII.)

[Read December 16, 1913. Published February 27, 1914.]

In a paper on *Bothrodendron kiltorkense* I sought to show that Bothrodendron agrees with Lepidodendron and Sigillaria in the possession of a rhizomatous or rhizophorous organ (1) indistinguishable from a form of fossil commonly called *Stigmaria.* Recent excavations at Kiltorcan have supplied material which seems to leave no room for doubt that this is the case. The specimen figured (Plate XIV) is an impression, at one end of which are the typical Stigmarian appendages. Their characteristic scars are visible higher up on a wrinkled surface-marking which gradually changes to one like that seen in the ordinary aërial stem of *Bothrodendron kiltorkense.* The wrinkled surface, so well seen here, is to be attributed to shrinkage of the surface due to the collapse of the chambered cortical tissue of this fossilizing marsh-plant. At one point (Pl. XIV, a, a) there is a horizontal zigzag line marking off the more wrinkled lower region from the upper smoother surface. On this latter surface one can see clearly oblique rows or spirals of small rounded leaf-scars wide apart. The leaf-scars alternate with one another above and below, or are quincuncially placed (Plate XV, fig. 2). Thus in one continuous impression we have an aërial stem showing leaf-scars, with a sharp line of demarcation from the forking Stigmarian rhizome with its root appendages. The illustration is of interest in its bearing on a view that the Stigmarian appendages, which are also quincuncially arranged, may be modified leaves.

The horizontal streak represents, I take it, the ground- or mud-level of the marshy soil in which the Bothrodendron grew. It is worthy of note that the leaf-scar region shows that zonation to which I have called attention previously, as a possible sign of Calamarian affinity. The impression abundantly justifies the view that Bothrodendron passed at its base into a typical Stigmarian stump with appendages. I must refer readers interested in the question to the discussion on Stigmaria in my previous paper, but more particularly to the account of Stigmaria in Scott's "Studies in Fossil Botany" (2nd ed., 1908, vol. i, pp. 237–262).

D. White (2) describes a fossil tree-trunk from the Middle Devonian of Naples, New York,[1] which shows in its lower part Sigillaria characters, and in its upper regions Lepidodendron features. Bothrodendron traces are also observable. Though the expanded, truncate butt-end of the trunk does not show the Stigmarian "root," it does bear typical Stigmarian rootlets, or appendages, and shows on its much-wrinkled surface the regular Stigmarian scars. *Archæosigillaria primæva* (Rogers, sp.) is thus a remarkable primitive Lepidophyte, nearer the ancestral type than any of the three genera mentioned above, though *Protolepidodendron scharianum* Krejči (3) from the Devonian (?) of Bohemia is, White thinks, still more primitive.

About the middle of the last century *Stigmaria ficoides*, described as the commonest fossil of the English Coal-measures, was proved to be the underground "root" of Sigillaria and of Lepidodendron. The illustration here given shows that it must now be also accepted as the underground organ (rootstock) of Bothrodendron. There is no marked feature of distinction—morphological or structural—of the Stigmaria stage of one genus from that of either of the others; a sign in itself of the close affinity of the three.

Recent quarrying has also yielded interesting material of the cone of Bothrodendron. The illustration in Pl. XVI, fig. 2, shows that the cone was not a deciduous sessile one leaving a Ulodendroid scar, as restorations in other species of the genus have shown it, but that it was borne on a well-developed stalk which shows the ordinary leaf-scars continued to the very base of the cone. Its axis is short and thick, and bears numerous closely arranged sporophylls. These consist of a broadened fertile proximal portion lying horizontally at right angles to the axis of the cone, and an upturned filamentous or awl-shaped distal portion similar to the ordinary vegetative leaf (Cf. op. cit., fig. 1, pl. xxxvi). The dichotomous branching, giving the two stalked cones, is strictly comparable with the forking of the vegetative shoot. One specimen (Pl. XVI, fig. 1) in fact shows a forked shoot, one limb of which is vegetative, while the other ends in a cone.

The cone is heterosporous like that of Lepidostrobus. The lower

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[1] D. White: "A remarkable Fossil Tree-trunk from the Middle Devonic of New York." New York State Museum, Bulletin 107, Geological Papers, Albany, 1907.

sporophylls are female, and each bears a large sessile megasporangium containing numerous megaspores. The upper sporophylls are male, and their sporangia contain microspores. The cone is Lepidostrobus-like and not Selaginella-like, as is the *Bothrodendron* (*Bothrostrobus*) *mundum* cone described by Watson.

The cone of *B. kiltorkense* is a highly specialized structure, more differentiated than the strobilus of Lycopodium, and especially than that of *L. Selago*. The presence in the Devonian of such well-organized cones so clearly marked off from the sterile foliage shoot should give pause to the entertainment of the theory of the derivation of foliage leaves by sterilization of fertile sporophylls. In the case before us it seems simpler to regard the cone as arising by the development of the sporangium on the apical foliage leaf of the shoot, with the modification of form resulting from this additional function, than to accept the view that the cone—a circumscribed terminal region—is all that is left of the fertile sporophylls, the others having, by loss of their sporangia, become sterile foliage leaves.

In support of this latter view is the case of *Pinakodendron Ohmanni*, Weiss, as described by Kidston (4). In his well-illustrated account Kidston shows that the only difference between the sterile and fertile regions of this heterosporous Carboniferous Lepidophyte is the presence of four-spored megasporangia on the fertile leaves. The species shows the recent *Lycopodium Selago* type of 'strobilus.'

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2. WHITE, D.—"A remarkable Fossil Tree-trunk from the Middle Devonic of New York." New York Museum, Bulletin 107: Geological Papers, Albany, 1907.
3. KREJČI, J.—"Notiz über d. Reste von Landpflanzen in der böhmischen Silurformation." (Sitz.-Berichte d. königl. Böhm. Gesellsch. d. Wissensch. in Prag, Jahrgang, 1879, s. 203.)

   [The few lines of unillustrated description of this interesting primitive form seem inadequate.]
4. KIDSTON, R.—Les Végétaux Houillers recueillis dans le Hainault Belge. *Pinakodendron Ohmanni*, Weiss, pp. 166–172. Pl. xxiv, figs. 1–11, &c. (Mém. du Musée Roy. d'Hist. Nat. de Belgique, Bruxelles, t. iv, 1909.)

EXPLANATION OF PLATES.

(The Illustrations of Plates XV–XVI are from photographs by Mr. T. Price.)

PLATE XIV.

Root-stock of *Bothrodendron kiltorkense.* The letters A, A indicate the ground-level. Above this is the aërial stem, showing the distant leaf-scars. Below it, with traces of leaf-scars, the forking Stigmaria with appendages and scars. (From a water-colour drawing by Miss Barnes.) ($\frac{1}{1}$.)

PLATE XV.

Fig.
1. Root-stock of *B. kiltorkense.* (Cf. Pl. XIV.) ($\frac{3}{5}$.)
2. Illustration of the region around A, A in Pl. XIV to show the leaf-scars in oblique rows, and the wrinkled Stigmaria surface below. (Cf. Pl. XIV.) ($\frac{3}{2}$.)

PLATE XVI.

Fig.
1. A forking-shoot showing a sterile limb. The right limb is hidden in the rock, but ends in a cone to be seen on the other side of the slab.
2. Forked shoot bearing two cones. Note traces of distal ends of sporophylls. The counterpart of fig. 1 is on the underside of the slab, and gives fig. 2. Thus it is possible to reconstruct the actual terminal shoots of *B. kiltorkense.*

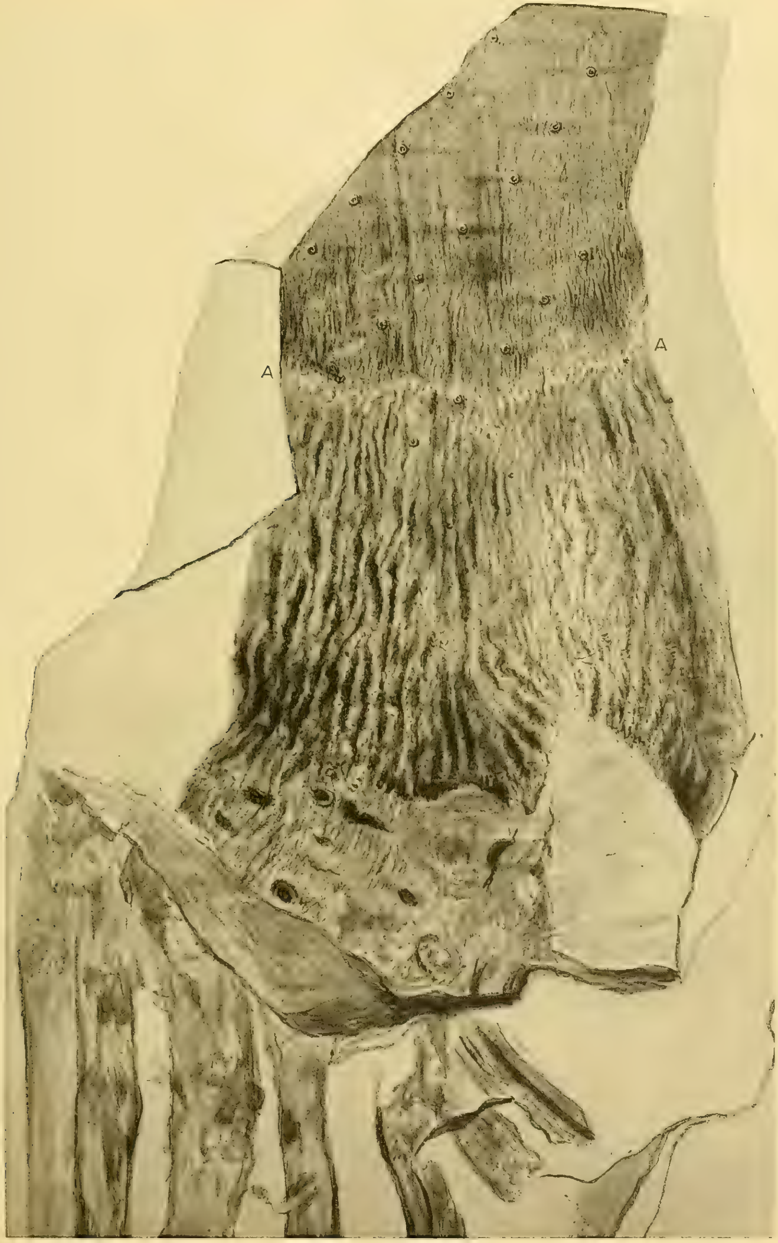
   Dichotomy is a pronounced feature. Sometimes the limbs of the terminal fork are sterile. (See my previous paper "On *Bothrodendron* (*Cyclostigma*) *kiltorkense*, Haughton, sp." in Scient. Proc. Roy. Dubl. Soc., vol. xiii, No. xxxiv, March, 1913.)

PLATE XVII.

Fig.
1 & 2 ($\frac{1}{1}$) Drawings of the sterile limb of the forking shoot photographed in Pl. XVI, fig. 1. They are the counterparts of one another. The crowded, confused arrangement of leaf-scars shows that the stem was flattened, causing the leaf-scars on both surfaces to appear in the impression.

PLATE XVIII.

Restoration of *Bothrodendron kiltorkense*, after Kidston's one of *B. punctatum*, L. & H.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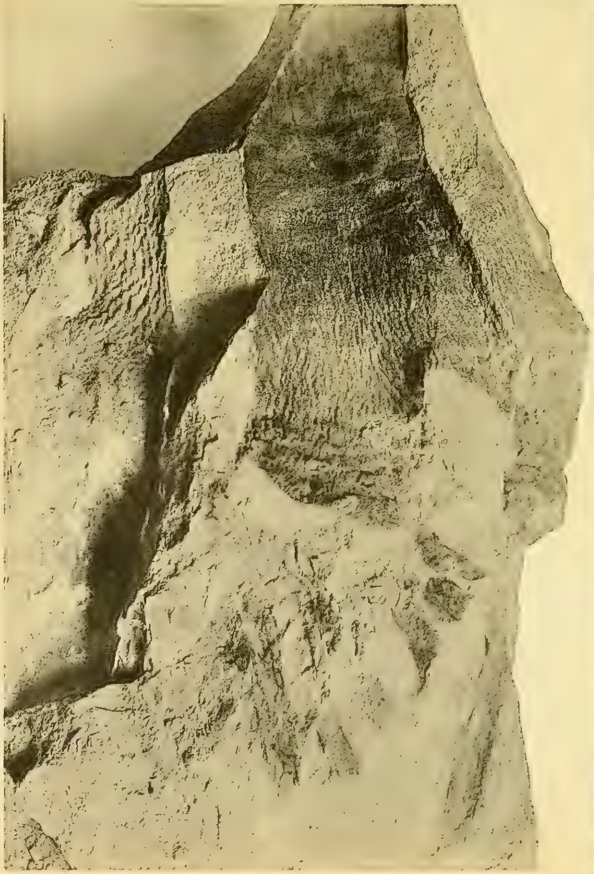

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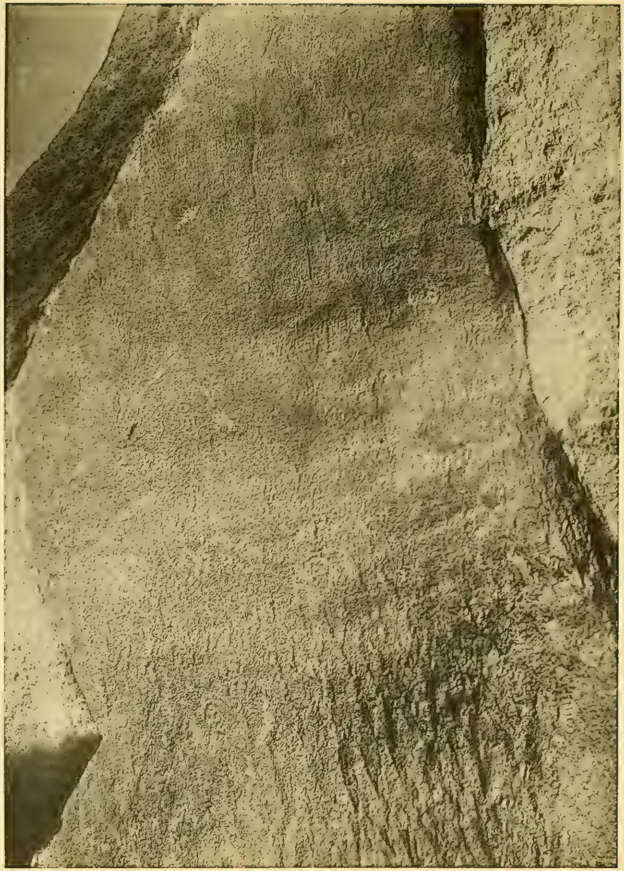

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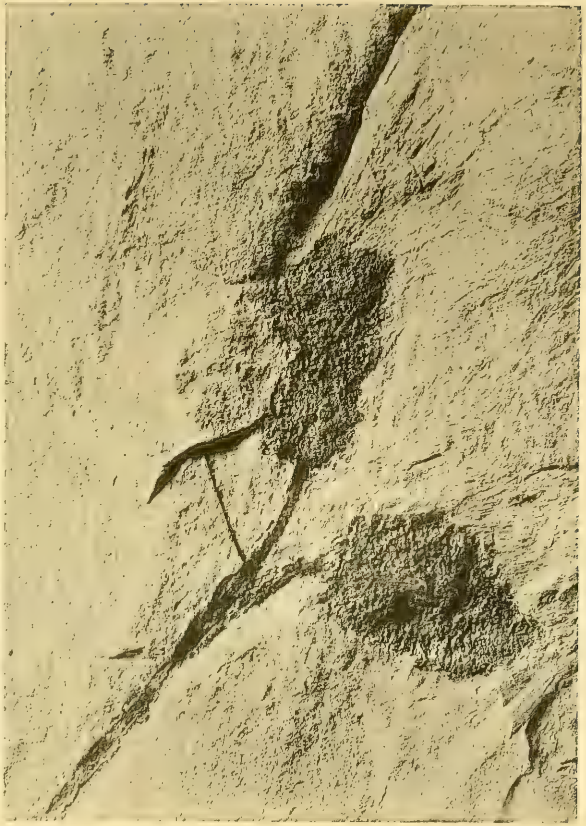

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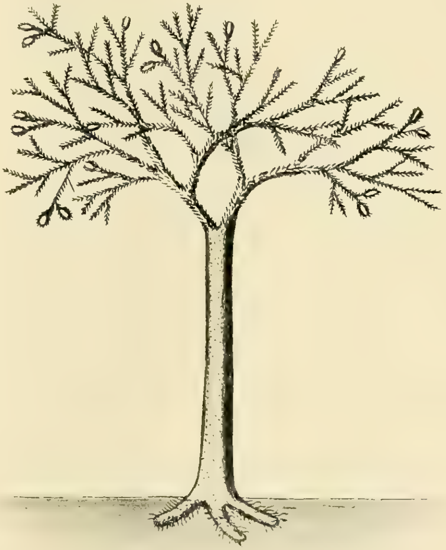
M'Farlane & Erskine.



M'Farlane & Erskine.

Restoration of *Bothrodendron kiltorkense*, after R. Kidston's one of *B. punctatum*, L. & H.

THE

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THE SUBSIDENCE OF TORSIONAL OSCILLATIONS IN NICKEL WIRES WHEN SUBJECTED TO THE INFLUENCE OF ALTERNATING MAGNETIC FIELDS.

BY

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XIV.

THE SUBSIDENCE OF TORSIONAL OSCILLATIONS IN NICKEL WIRES WHEN SUBJECTED TO THE INFLUENCE OF ALTERNATING MAGNETIC FIELDS.

By W. BROWN, B.Sc.,
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[Read January 27. Published February 20, 1914.]

About three years ago, one of us brought before this Society the results of some experiments on the subsidence of torsional oscillations in iron and nickel wires, when they were subjected to the influence of longitudinal magnetic fields, the magnetic fields being produced by continuous direct currents.[1] These results, among other things, showed a marked *increase* in the damping of the torsional oscillations in magnetic fields up to a certain value, depending on the longitudinal load on the wire, and for higher magnetic fields a remarkable *decrease* in the damping of the oscillations.

The present communication gives results obtained with nickel wires when they were subjected to the influence of alternating magnetic fields of different frequencies, and for comparison, each set of experiments, with the alternating magnetic fields, was preceded by an experiment with a direct longitudinal magnetic field of the *same* value, and also one in which the wire was under the influence of the vertical component of the Earth's magnetic field, about 0·45 c.g.s. units. The results of this latter have, however, only been recorded once in this paper, and that in the form of a curve in the figure (p. 221).

Drago[2] has shown that the rapidity of the damping of torsional oscillations in an *iron* wire diminishes under the influence of electrical oscillatory discharges and alternating currents. The wire he used was 20 cms. long and

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[1] Scient. Proc. Roy. Dub. Soc., vol. xiii (N.S.), No. 3, p. 32.

[2] Nuovo Cimento, serie vi, vol. iii.

0·03 cm. diameter, with a load of about 9×10^5 grammes per sq. cm., which is much larger than the loads employed in our experiments.

The method of our experiment was as follows:—A solenoid, 236 centimetres long, consisting of 7,707 turns of No. 18 double cotton covered copper wire arranged in four layers, and capable of producing an internal magnetic field of 41 c.g.s. units per ampere, was fixed vertically on a wall with the lower end about 30 centimetres from the surface of a slate slab. The nickel wire under test was suspended in the centre of the solenoid; the length of the wire was 226 centimetres, so that it was in a uniform magnetic field throughout its whole length. The wire was firmly fixed at the top end by means of a three-jaw centre clutch, which projected five centimetres into the solenoid. On the lower end of the wire there was fixed by the same means a cylindrical vibrator composed of lead and brass, with a small iron pin or spike projecting from its lower surface for the purpose of dipping into mercury.

A concave mirror was fixed on the stem of the vibrator in such a way that its reflecting surface was in the same plane as the centre of the wire. The torsional oscillations of the wire were observed by means of a spot of light on a semi-transparent millimetre scale placed at a distance of 167 centimetres from the mirror.

The maximum deflection of the light-spot which was used was at the division marked 300 on the scale, which corresponded to a torsion or twist of the lower end of the wire equal to an angle of about 5° 10′ on each side of the zero. The wire under test was set to oscillate round its own centre line—that is, without any pendulum-motion—until the light-spot was a little above the three-hundredth division; and, when it was just on this 300 mark, one started to count the oscillations and to read off the amplitude at every *fifth* vibration up till the *twentieth*, and then every *tenth* vibration until seventy vibrations in all had taken place.

For the purpose of setting the wire to oscillate properly, two independent direct-current circuits were employed, (1) a circuit including the wire under test, secondary battery, ammeter, reversing key, and a rheostat; (2) a circuit including the solenoid, secondary battery, reversing key, rheostat, and hot-wire milliammeter, which latter measured both the direct current and the alternating current round the solenoid.

By sending a given steady direct current round the solenoid, and a separate steady direct current through the wire, then, by means of the reversing key reversing the current through the wire, in unison with the oscillations of the wire or light-spot, the required amplitude of oscillation could be easily obtained; and when this amplitude was a little above the 300 mark on the scale, the direct currents were switched off both the wire and solenoid, and an equal alternating current immediately switched on to the solenoid only. The amplitudes of the oscillations were then read off on the scale until seventy vibrations had been observed.

It has been shown[1] that, in nickel wires, the greater the longitudinal load on the wire—up to a certain limit—the greater the twist of the free end, and that the magnetic field round the wire which gives this maximum twist increases with the load, the twist being due to the combined circular and longitudinal magnetization. It was also shown that the greatest damping of the torsional oscillations of the wire takes place under these conditions of load and longitudinal magnetic field. It seemed likely that the same conditions would hold with alternating magnetic fields; and at the beginning of the present investigation we proved experimentally that they do hold. Therefore the magnetic fields employed in the present research, whether continuous or alternating, were those corresponding to the given loads on the wire when the maximum twist was obtained. Thus, when the load on the wire was $0·5 \times 10^5$ grammes per sq. cm., the magnetic field round the wire was 13 c. g. s. units, and 17 c.g. s. units when the load was 10^5 grammes per sq. cm., and 20 c. g. s. units when the load was $1·5 \times 10^5$ grammes per sq. cm.

The nickel wire first tested was a No. 16 s. w. g. of diameter 0·1675 cm. in the physical state in which it was received from the manufacturer—that is, it was hard or of simple rigidity about 770×10^6 grammes per sq. cm. There were few tests made with the light load $0·5 \times 10^5$ grammes per sq. cm., because the torsional oscillations were so rapid that reliable readings of the amplitudes of the light-spot on the scale could not be made.

The load of 10^5 grammes per sq. cm. was therefore put on the wire, and observations made on the damping of the torsional oscillations, when magnetic fields of 17 c. g. s. units were round the wire—that is, a damping curve was first observed when this magnetic field was produced by a continuous, steady direct current round the solenoid, and observations again made when the magnetic field was produced by means of alternating currents of the same R. M. S. value as the direct current, and of frequencies 20, 30, 50, 100, and 140 per second.[2] The results are shown in Table I.

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[1] Scient. Proc. Roy. Dub. Soc., vol. xiii (N.S.), No. 3, pp. 31–37.

[2] Graphs of the alternating currents at the various frequencies were taken by means of the oscillograph. Those for frequencies 20 to 50 were very nearly sine curves; and the currents were obtained from a single-phase alternator direct-coupled to a direct current shunt motor. The currents at frequencies 100 and 140 were obtained from a small belt-driven inductor alternator; and their graphs, on analysis, showed the presence of the third harmonic of amplitude 2·3 as compared with 10·5, the amplitude of the fundamental on the same scale, the harmonic starting from zero in nearly phase-opposition to the fundamental.

Table I.

Number of Vibrations.	Amplitude of oscillations in scale divisions from an initial amplitude of 300, or 5° 10′ when subjected to an alternating magnetic field of 17 c.g.s. units, and frequencies :—					
	d. c.	20	30	50	100	140
0	300	300	300	300	300	300
5	294	285	285	285	290	290
10	288	271	271	272	280	280
15	282	257	259	259	270	270
20	276	244	246	248	260	261
30	265	221	224	226	243	244
40	254	199	203	206	227	228
50	245	179	185	188	212	214
60	235	160	167	171	198	200
70	226	143	151	155	184	187

If we plot these results in the form of curves, with the numbers in the first column as abscissæ, and the corresponding numbers in the other columns as ordinates, it will be seen that the damping curves obtained with the alternating magnetic fields all lie below the damping curve obtained with the direct longitudinal field, which is the reverse of what we obtain in the case of the soft nickel wire, as shown below.

It was observed during the experiments with the hard nickel wire that, after the alternating magnetic field had been round the wire, on attempting to start up the oscillations for the next experiment by means of direct currents through the wire and round the solenoid, the movements of the light-spot were more sluggish than formerly, or it took a longer time to get up to the maximum amplitude of 300 on the scale, which seemed as if the alternating magnetic field had made the wire for the time being partially *non-magnetic.*

This was very strongly marked when alternating magnetic fields of frequencies 100 and 140 per second were round the wire. For example, before subjecting the wire to these alternating magnetic fields, we sent an equivalent direct current round the solenoid, and a certain known direct current through the wire, and obtained a twist of the lower end of the wire corresponding to a steady deflection of the light-spot on the scale of forty-one *divisions* or *millimetres.*

We then switched off both these direct currents, and put on the solenoid an alternating magnetic field of frequency 140 per second, and of the same value as the direct magnetic field, for a period of *three* minutes; the alternating field was then switched off, and the vibrator and wire brought to rest. Direct currents of the same value as formerly were now put through the solenoid and wire, and the steady deflection obtained on the scale was only *one millimetre*, which showed that some decided change had taken place in the wire, which, for want of a better name, we have called *temporary magnetic fatigue.* That this effect is temporary is shown by the fact that it can be cured in several ways—(1) by putting an alternating magnetic field round the wire of lower frequency than that which caused the fatigue; then taking off the alternating field, and allowing the wire to rest for some time, it slowly recovers; in one case, however, we observed that it took seventeen hours to fully recover: (2) another way we found effective was to relieve the wire of its load, and send a direct current round the solenoid; this made the wire recover at once. The above was when the load on the wire was 10^5 grammes per sq. cm.

When the small load of $0{\cdot}5 \times 10^5$ grammes per sq. cm. was on the wire, and an alternating magnetic field of 13 c.g.s. units and frequency of 140 per second was round the wire for about three minutes, and then tested, the wire was found to be about half fatigued; and when the larger load of $1{\cdot}5 \times 10^5$ grammes per sq. cm. was on, and an alternating field of 20 c.g.s. units of frequency 140 per second for three minutes, again, on testing the wire, it was found to be about three-quarters fatigued.[1] This magnetic fatigue does not occur in soft nickel wires as shown below. We have, however, made arrangements for investigating further and more fully this property of hard nickel wires.

The wire was now taken down and made as soft as possible by the following means:—it was suspended in a vertical position and allowed to hang loosely under its own weight only, and raised to a bright cherry-red heat by means of a broad Bunsen burner by heating it from the top downwards three times in succession, and when cooled its simple rigidity was about 708×10^6 grammes per sq. cm. The wire was again put into position inside the solenoid, and the small load $0{\cdot}5 \times 10^5$ grammes per sq. cm. hung on the lower end; the damping of the torsional oscillations was observed as before, (1) when the wire was under the influence of the Earth's vertical force, (2) when a longitudinal magnetic field of 13 c.g.s. units was round it, and (3) when alternating magnetic fields of 13 c.g.s. units each and of frequencies 20, 30, 50, 100 per second were round the wire. The results obtained are shown in Table II.

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[1] This means that if the steady deflection is 40 divisions on the scale when the wire is fresh, the deflection when *half fatigued* is $(1 - \frac{1}{2})\,40 = 20$ divisions; similarly when the wire is *three-quarters fatigued* the deflection on the scale is $(1 - \frac{3}{4})\,40 = 10$ divisions; in the same way we get $(1 - 1)\,40 = 0$ for total fatigue.

TABLE II.

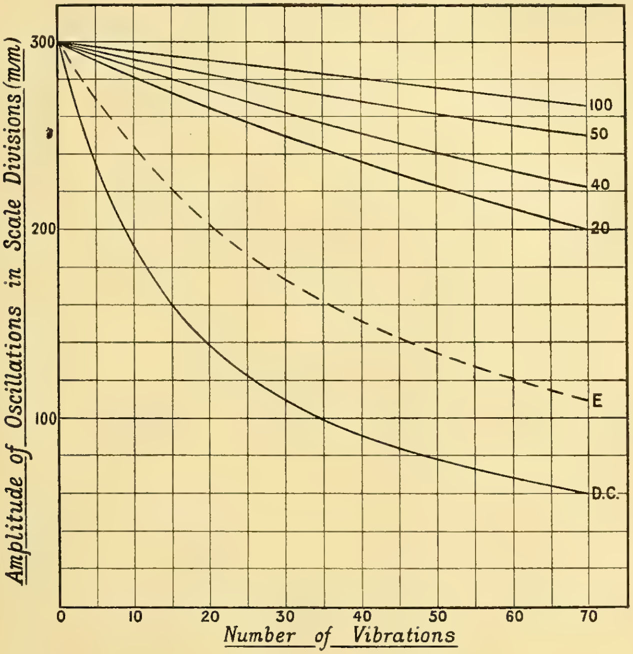
Number of Vibrations.	Amplitude of oscillations in scale divisions from an initial amplitude of 300 or 5° 10′ when subjected to an alternating magnetic field of 13 c.g.s. units, and frequencies :—				
	d. c.	20	30	50	100
0	300	300	300	300	300
5	235	289	289	291	294
10	191	279	279	282	288
15	160	269	270	274	282
20	137	259	261	267	277
30	106	241	243	252	267
40	87	224	227	239	258
50	72	208	212	228	249
60	62	194	198	217	242
70	54	181	186	208	235

The load on the wire was now increased to 10^5 grammes per sq. cm., and a similar set of experiments performed when a longitudinal magnetic field of 17 c.g.s. units was round the wire, as well as alternating magnetic fields of 17 c.g.s. units and of frequencies 20, 30, 40, 50, and 100 per second. The results are shown in Table III, and in the form of a curve in fig. 1.

TABLE III.

Number of Vibrations.	Amplitude of oscillations in scale divisions from an initial amplitude of 300 or 5° 10′, when subjected to an alternating magnetic field of 17 c.g.s. units, and frequencies :—					
	d. c.	20	30	40	50	100
0	300	300	300	300	300	300
5	235	291	293	294	296	298
10	192	282	286	287	292	295
15	162	274	279	281	288	293
20	139	266	272	275	284	290
30	110	251	259	263	276	285
40	91	237	247	252	269	280
50	78	224	235	242	262	275
60	68	212	223	232	256	270
70	59	201	213	222	250	265

The results in Table III are shown as curves in the figure (except those values in column four, which are left out so as not to crowd the figure) where the values in column *one* are taken as abscissæ, and the corresponding values in the other column as ordinates.



The curve marked D. C. is that obtained when a direct longitudinal magnetic field is round the wire; and the four top curves are those obtained when equivalent alternating magnetic fields of frequencies 20, 40, 50, and 100 per second are round the wire; whilst the dotted curve marked E is the one obtained when the Earth's vertical force, 0·45 c.g.s. unit, is on the wire, this latter being put in for comparison.

The effect of an alternating magnetic field in *decreasing* the damping of torsional oscillations in a soft nickel wire is very strikingly shown by these curves. The damping curve for a frequency of 100 per second, and an alternating magnetic field of 17 c.g.s. units, with a load of 10^5 grammes per sq. cm. on the wire, is almost identical with a damping curve obtained previously by one of us with a nickel wire of the same length, diameter, and softness, with a load of 1·5 × 10^5 grammes per sq. cm., but in *a continuous longitudinal magnetic field of 200 c.g.s. units.*

By comparing the values in Table I with those in Tables II and III, it will be seen that with the wire in the hard state (Table I), the damping curves due to the alternating magnetic fields at the different frequencies all lie *below* the curve obtained with the direct longitudinal field of equal value, whereas in the case of the soft wire (Tables II and III) the curves due to the alternating fields all lie *above* the curve obtained with the longitudinal field.

The physical state of the wire has a very marked effect on the rate of damping of the torsional oscillations, as shown in Table IV, where, for convenience of comparison, we have grouped the results for *hard* and *soft* wire respectively, the load in each case being 10^5 grammes per sq. cm., and magnetic field of 17 c.g.s. units, produced by a direct current and alternating currents of frequencies 20 and 100 per second.

TABLE IV.

Number of Vibrations.	Wire, Hard.			Wire, Soft.		
	d. c.	20	100	d. c.	20	100
0	300	300	300	300	300	300
5	294	285	290	235	291	298
10	288	271	280	192	282	295
15	282	257	270	162	274	293
20	276	244	260	139	266	290
30	265	221	243	110	251	285
40	254	199	227	91	237	280
50	245	179	212	78	224	275
60	235	160	198	68	212	270
70	226	143	184	59	201	265

The examination of the figures given in Table IV shows:—1. In a *hard* wire the damping due to an alternating magnetic field is *greater* than that due to an equivalent direct magnetic field; whilst, in the case of the *soft* wire, the damping due to the alternating field is *less* than that due to the

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[1] Scient. Proc. Roy. Dub. Soc., vol. xiii (N.S.), No. 3, p. 35.

equivalent direct field. 2. The damping due to a *direct field* is *much greater* in a *soft* nickel wire than in a *hard* one; whilst the damping due to an *alternating field* is *greater* in a *hard* nickel wire than in a *soft* one.

The same holds true when smaller or larger loads are on the wire; thus, with the load of $1\cdot5 \times 10^5$ grammes per sq. cm., and a magnetic field of 20 c.g.s. units round the wire, we obtained the following numbers, which are amplitudes of oscillations after seventy vibrations had taken place from the initial amplitude of 300 divisions on the scale :—

Hard Wire.	Soft Wire.	Frequency of Magnetic field.
300	300	d. c.
237	61	
300	300	50
199	248	
300	300	140
230	252	

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CHANGES PRODUCED IN THE SAP BY THE HEATING OF BRANCHES.

BY

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The same holds true when smaller or larger loads are on the wire; thus, with the load of $1{\cdot}5 \times 10^5$ grammes per sq. cm., and a magnetic field of 20 c.g.s. units round the wire, we obtained the following numbers, which are amplitudes of oscillations after seventy vibrations had taken place from the initial amplitude of 300 divisions on the scale :—

Hard Wire.	Soft Wire.	Frequency of Magnetic field.
300	300	d. c.
237	61	
300	300	50
199	248	
300	300	140
230	252	

XV.

CHANGES PRODUCED IN THE SAP BY THE HEATING OF BRANCHES.

By HENRY H. DIXON, Sc.D., F.R.S.,
University Professor of Botany, Trinity College, Dublin.

[Read January 27. Published March 10, 1914.]

A large accumulation of evidence shows that the withering and death of leaves supported by a branch which has been killed by heat are due chiefly to a change in the nature of the sap supplied to them, or, in other words, to the contamination of the sap-supply by substances emanating from the killed branch.[1]

A priori such a contamination seems inevitable. When the heat has killed a portion of the stem, the cells adjoining the water-tracts become permeable; and hence the dissolved substances in their vacuoles are set free into the upward current of sap in the tracheæ. The vacuoles contain acids carbohydrates, and salts, so that, even in the absence of corroborative observations, we would expect the sap to be enriched with these substances. Furthermore, very probably substances in the cells ordinarily not in solution would be brought into solution, and introduced into the sap by the higher temperature; possibly, too, some bodies might be precipitated from the rising sap by the higher temperature. Yet another change is to be anticipated. The heat will destroy any thermolabile substances in the sap and in the adjoining cells. Coagulation changes may also be expected.

It is not difficult to test these surmises experimentally; and indeed a colour-change in the sap issuing from heated stems has before now been recorded and commented upon.[2]

The sap extracted from various trees primarily for other experimental purposes incidentally provided material suitable for this investigation. The extraction was effected by means of a centrifuge. Short lengths of the branch to be investigated (9–10 cm. long × 2–2·5 cm. diam.) are placed in gilt buckets of a centrifuge, and the sap yielded after about five minutes'

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[1] J. B. Overton: "Transpiration and Sap-flow." Bot. Gaz., 1911, pp. 28 and 102.

[2] Henry H. Dixon: "Vitality and the Transmission of Water through the Stems of Plants." Proc. Roy. Dublin Soc., vol. xii, No. 3, 1909, p. 21, and Notes from the Bot. Sch., Trinity College, vol. ii, No. 1, p. 5.

rotation is collected. The quantities of sap obtained in this way are surprising. Whether in midsummer, autumn, or winter, I have found that four such pieces of the various woods used yielded about 3–5 c.c. In the same way sap was collected from pieces of steamed branches. These samples of sap could now be compared physically and chemically. In every case, as was anticipated, profound differences were found to exist between the characters of the saps drawn from the fresh and the steamed branches. Some of the results may be seen in the following table: under Δ the depression of the freezing-point, under C the conductivity is given. Also the reaction of the sap to litmus and the presence of oxydase are noted.

	Fresh.					Steamed.				
Name.	Δ	$C \times 10^4$	Acidity.	Colour.	Oxydase.	Δ	$C \times 10^4$	Acidity.	Colour.	Oxydase.
Fagus sylvatica,	0·083°	3	very faint	colourless	+	0·509°	19	marked	brown	0
Populus alba,	0·055°	2·7	very faint	colourless	+	0·231°	11	marked	pale brown	0
Ilex Aquifolium,	0·072°	6·4	very faint	pale grey	...	0·321°	28	marked	pale brown	0

The change in Δ brought about by steaming is due to the total increase of the dissolved substances, and indicates that the concentration of the sap has increased 4–6 times. The changes in conductivity (expressed as the reciprocals of the resistances measured in ohms) indicate the relative richness of the saps in electrolytes.[1] From the table it appears that the concentration of electrolytes has become 4–6 times greater by steaming. The development of strong acidity during the heating (observed in every case so far examined) shows that the increase of solutes is partly due to the introduction of acids into the sap.[2] In Fagus and Populus an oxydase was present[3] in the sap of the unsteamed stem, which coloured guaiacum tincture faintly blue. The blue was intensified by the addition of hydrogen peroxide. The oxydase was of course destroyed with heating. The oxydases were not looked for in the fresh sap of Ilex.

Qualitative tests on the sugars of the saps from the fresh and steamed branches indicated changes in these bodies also.

Where non-reducing sugars are present, they are of course hydrolysed during the steaming of the branch by the acid liberated, and appear after the heating as reducing sugars; examples of this were found in *Ilex Aquifolium,*

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[1] I am indebted to Mr. W. R. G. Atkins for the determination of these conductivities.

[2] The very faint acidity of the sap from the fresh branches may probably be ascribed to the sap set free from the injured cells at the ends of the pieces.

[3] It is possible that this oxydase was also derived from the cut cells.

Salix babylonica, and *Cotoneaster frigida*. In the last-mentioned, however, much larger quantities of reducing sugars were found present in the sap of the steamed branch than could have been formed by the inversion of the non-reducing sugars present in the sap of the fresh branch, so that we must assume that they were introduced into the sap from the neighbouring cells.

These tests are sufficient to justify the surmise that the physical and chemical nature of the sap is profoundly altered by steaming the branch through which it passes.

It is evident that the substances thus introduced into the sap must be swept along in the rising current till they reach the leaves, except for the material which is absorbed by the walls of the tracheæ, and by the cells adjoining the water tracts above the heated region. In the leaves those which are not in a form suitable for assimilation must accumulate; and if sufficient of the branch has been killed, the accumulation will ultimately—without any other poisonous action—plasmolyse the cells of the leaf.[1]

Reduction in the water-supply may be also brought about by the coagulation of colloids in the sap, and the consequent formation of plugs in the conducting tubes. This condition has been observed by several investigators.[2]

It seemed of interest to essay to find out if the sap in steamed branches contained any substance which acted as a protoplasmic poison, and not merely as a plasmolysing agent by simple accumulation. To test this point saps extracted from branches subjected to various treatments were applied to severed leaves of *Elodea canadensis*, and the effect on the cells of these leaves was microscopically controlled.

In the first place it was found that the cells remained normal, and protoplasmic streaming continued undiminished in the sap from fresh branches for at least five days, and probably much longer. This point was verified in the case of the sap of *Ilex Aquifolium*, *Prunus cerasus*, *Syringa vulgaris*, *Cotoneaster frigida*, and *Salix babylonica*. In contrast to the sap from the fresh branches, that from the steamed branches of all of these, with the exception of *Ilex Aquifolium*, produced lethal changes in the leaves of Elodea within two or three days. These changes consisted in a cessation of protoplasmic streaming, in the discoloration of the veins and margins, and in the contraction of the protoplasts of the cells all over the leaf, and their ultimate blackening. The contraction which occurs is not of the nature of plasmolysis; for more than a day is often required for its production, and it cannot be undone by the transference of the leaf into water.

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[1] It may be noted that Ursprung looked for plasmolysing effects in the root-hairs of *Impatiens Sultani* by a decoction of the same plant, but did not find any. Here, of course, concentration would not take place.

[2] J. B. Overton, loc. cit.

In each case a sample of sap centrifuged from the fresh branch was tested; other similar tests were made upon that centrifuged from a branch immediately after steaming, or centrifuged from a branch steamed a day or two previously, or with the liquid centrifuged from a branch which had a day or two previously been steamed and at once depleted of its sap by centrifuging, and refilled with distilled water. These two last tests were made in order to see if the poisonous materials are set free immediately into the sap on steaming, or whether they are produced as subsequent degradation-products of the cells.

In the table below, the sap obtained by centrifuging immediately after steaming is termed "steamed direct"; that which was centrifuged some days after steaming is called "steamed indirect"; while the liquid obtained from the steamed branches which had been emptied of their sap, and subsequently filled with water, is tabulated as "steamed indirect + water." The ciphers in the table indicate that no effect was observable on that day of the experiment under which the figure is placed, while a plus mark shows that an effect was observed. Query marks indicate that only some of the leaves tested were affected or that only slight protoplasmic contraction was observable. In each case three or four leaves were immersed in the liquid and used as tests.

TABLE.

Day of Experiment.	1st	2nd	3rd	4th	5th
Ilex Aquifolium, fresh,	0	0	0	0	0
steamed direct,	0	0	0	0	0
,, indirect,[1]	0	0	0	0	0
,, ,, + water,[1]	0	0	0	0	0
Prunus cerasus, fresh,	0	0	0	0	0
steamed direct,	0	+	+	+	+
,, indirect + water,	0	0	+	+	+
Cotoneaster frigida, fresh,	0	0	0	0	0
steamed indirect,	0	?	+	+	+
,, ,, + water,	?	+	+	+	+
Salix babylonica, fresh,	0	0	0	0	0
steamed indirect,	0	0	?	+	+
,, ,, + water,	0	0	0	+	+
Syringa vulgaris, fresh,	0	0	0	0	0
steamed direct,	0	+	+	+	+
,, indirect,	?	+	+	+	+
,, ,, + water,	?	+	+	+	+

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[1] When the sap had stood in contact with the dead cells for two or more days, discoloration of the veins and blackening of some of the cells without marked contraction occurred on the fifth day.

It may be noted that, in the case of *Cotoneaster frigida*, *Syringa vulgaris*, and *Ilex Aquifolium* (see foot-note on previous page), the liquid centrifuged from the steamed branch, after it was emptied of sap and filled with water, is more rapidly poisonous than the sap itself. In these cases probably a poison is formed in the cells after death, which is not sufficiently concentrated in the sap centrifuged immediately after steaming. The same explanation probably applies to the observation that the sap extracted from the Syringa branch immediately after steaming is not so quickly lethal as that drawn off a couple of days after death.

The slow generation of poisons indicated in these experiments probably affords an explanation of the fact that, even when steamed branches are washed out immediately after the heating, some of the leaves above perish from poisoning.[1]

The facts recorded in this note form additional evidence against the view that the leaves above a steamed branch perish because they are cut off from their water-supply by the death of the cells of the stem. They show that in every case profound changes are produced in the sap by the steaming, which will ultimately cause plasmolysis of the leaf-cells if these cells are not previously killed by poisonous substances produced in the heated region. The immediate or ultimate production of these poisons has been demonstrated in all the cases examined. As has been pointed out previously, the drying of the poisoned leaves is probably caused by the partial or complete plugging of the water-channels by colloids exuded from the heated cells, or coagulated in the sap.

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[1] Henry H. Dixon: "Spread of Morbid Changes through Plants from Branches killed by Heat." Proc. Roy. Dublin Soc., vol. xiv, No. 12, 1914, p. 205.

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ON THE TENSILE STRENGTH OF SAP.

BY

HENRY H. DIXON, Sc.D., F.R.S.,
UNIVERSITY PROFESSOR OF BOTANY, TRINITY COLLEGE, DUBLIN.

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XVI.

ON THE TENSILE STRENGTH OF SAP.

By HENRY H. DIXON. Sc.D., F.R.S.,
University Professor of Botany, Trinity College, Dublin.

[Read January 27. Published March 10, 1914.]

In 1893 Böhm (2) recorded an experiment in which a transpiring branch drew up a column of mercury above the contemporary barometric height. This experiment, in the light of our present knowledge, demonstrated clearly that the sap in the branch was in a state of tension.

Experiments made by Dr. Joly and the writer (5) in 1894, in which pieces cut from the wood of trees were enclosed in large sealed glass tubes without introducing weakness in the cohesion of the water, demonstrated the tensile strength of sap up to 7½ atmospheres. Further researches carried out by myself in 1903 (3), in which tension was applied by means of a column of mercury, showed that comparatively large pieces of living tissue, containing conducting tracts and their sap, together with parenchymatous cells, when introduced into water, did not spoil its cohesive properties. These tensile properties of sap were also demonstrated up to tensions of about 150 atm. in capillary tubes in later experiments (4).

It may be noticed that in none of these experiments was dissolved air intentionally excluded, but, rather, must have been present in the tensile liquid, owing to the procedure adopted in each experiment. In some, air-saturated water was in contact with the sap, which itself must have contained air.

A priori there appeared no reason to suspect that the tensile properties of the sap would be different from those of water. The passage of the sap through cell-walls must preclude the possibility of the entry of undissolved air, and the presence of traces of colloids in the sap could scarcely be supposed to introduce a weakness.[1]

In face of this evidence it is not a little surprising, then, to find that, basing his opinion apparently on four negative experiments, A. Ursprung (7)

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[1] In this connection it is worth recording that a soap film, made up with sap from the wood of Populus sp., even when sufficiently thin to show the 'black spot' was found to be wonderfully stable—apparently more stable than a similar soap film made up with distilled water. This demonstrates that a film of sap not more than 12 $\mu\mu$ thick can sustain at least twice the surface tension of the soap solution, and hence sets a minor limit to its cohesion of 42 atm. (cf. Proc. Roy. Dublin Soc., vol. xii (N. S.) No. 3, 1909, and Notes from the Bot. School, Trinity College, vol. ii, No. 1, p. 17).

supposes the sap of plants to have a negligible cohesion. Ursprung tested the sap (extracted from a bleeding plant) in an apparatus which is a modification of that described by Tait (6), and later by Askenasy (1). In it a porous evaporating surface draws up water, which in turn adheres to, and raises, a column of mercury. The sap, collected by a steel tube, was conducted by a rubber tube into a flask, and then drawn into the apparatus through a plug of cotton wool. For anyone who has experimented on the cohesion of liquids it will not be surprising that tension was not established in sap collected and introduced in this manner. One would expect by such a procedure that many insufficiently wetted particles of dust would be introduced, and that any considerable tension would be rendered impossible. Notwithstanding this, Ursprung actually did observe small tensions in three experiments, but these he rejects on what appear to me inadequate grounds. Two he sets aside because enough sap was not introduced; and the third is discarded because the sap was allowed to enter through the porous vessel into the tension-chamber. With regard to the first two, it would appear that the smallest amount of sap (if without cohesion) must destroy all tension; and it is hard to admit Ursprung's objection to the third, namely, that all the dissolved air was abstracted from the sap by passing through the porous substance, and hence the physical nature of the sap was changed.

I have recently found it easy to abstract sap in considerable quantities by centrifuging short lengths of decorticated stems; and in the following I have recorded some tests carried out on sap so obtained. I confess that, considering our present knowledge of the tensile properties of water, both containing and free from air, these experiments would have appeared to me superfluous, had it not been for the publication of Ursprung's results quoted above.

The method of experimentation was the same as that described in my previous paper (4); and it is to be noted that in the calculation of the tension full allowance is made for the distortion of the glass envelope during the contraction of the enclosed liquid, so that the method is not invalidated by Julius Meyer's objection, quoted by Ursprung (7).

In the first instance the sap centrifuged from pieces of branches of *Fagus sylvatica*, cut from about 70 feet above the level of the ground, was enclosed in the tension-tube. This sap, after collection, was boiled on three successive days for about one hour in order to secure the complete wetting of dust-particles fortuitously contained in it. After its last boiling it was exposed for twenty-four hours as a thin layer about 4 mm. deep to the air, but shielded from dust. In this way it must have become practically saturated with dissolved air. The capillary tube, into which it was now drawn by alternate heating and cooling, had been very carefully cleaned with a succession of chromic acid, caustic potash, and boiled water. After this cleaning, the tube was boiled in water for about an hour on three successive days, heating and cooling being effected in the same water. The tube was emptied before each boiling, and allowed to fill with the freshly boiled water. The object of this was to thoroughly wet the tube, and any dust-particles it contained, by bringing all undissolved air on their surfaces into solution. The tube after filling with the sap to within a few millimetres of its end was sealed off. The heating of the tube was effected, as in my previous work, in a large volume of water, and was very slow.

In the first tube submitted to experiment the air-bubble disappeared at 63·50° C., which may be described as the "closing" temperature, and reappeared with the characteristic click at 59·10°. Three other observations were made with this tube. All four agree in indicating that the sap withstood a tension of over 45 atmospheres before rupture (cf. Expts. 1, 2, 3, and 4 in the Table).

A second tube was charged with some of the same sample of sap; it was found to become completely filled at 66·25° C., and ruptured at 59·50° C. Calculating the tension developed in this case, the result is over 70 atmospheres (see Experiment 5 in the Table). In another experiment with this tube a tension of about 50 atmospheres was produced (see No. 6).

It was thought that possibly, by keeping one of these tubes after closing at a temperature close to that at which the bubble disappeared, greater tensions might be attained. This surmise was not realized. The tube used in the first experiments described above was kept for two days at a temperature of about 61° C. However, when ultimately allowed to cool slowly, the rupture occurred at 59·20°, a temperature not quite so low as had sometimes before been successfully passed. This experiment is recorded as No. 3 in the Table.

It may be noted that there is no reason to believe that the tensions produced in these experiments are indications of the maximum cohesion of boiled sap. The results quoted happen to be the first obtained. Other experiments were not made, as these are sufficient to demonstrate that the boiled sap possesses cohesive properties of the same order as those of water.

Having found that sap, free from unwetted nuclei, but saturated with air, is able to sustain considerable tensions, it seemed worth while trying if unboiled sap could be put into the tensile condition. The consideration that heating the enclosed sap in the glass envelope until the last visible bubble disappeared would probably completely remove all invisible bubbles encouraged me in this attempt. Accordingly a quantity of sap was collected from a branch of *Ilex Aquifolium* by means of centrifuging; and this after exposure to air and without any special treatment was introduced into several capillary tubes, which had been prepared in a manner similar to those used in the other experiments.

The first tube closed at a temperature of 78·20° C., and ruptured on cooling to a temperature of 72·00° C. (see Experiment No. 7 in the Table). This rupture occurred simultaneously with a slight shock accidentally dealt it by the stirrer of the vessel of water in which it was immersed. Had it not been for this, probably a lower temperature would have been attained without rupture. Taking these figures and the dimensions of the tube into account, the tension developed must have been about 75 atmospheres.

Another tube containing some of the same sample of sap completely filled at a temperature of 91·10°C. On one occasion rupture took place only when a temperature of 76·20°C. was reached; on another occasion a rupture developed at some temperature below 81·50°C. In the latter case, when the tube had fallen to 81·50°C., it was withdrawn from the water for examination, and rupture occurred some seconds after it was lifted from the water. In the first instance the tension must have approximated to 207 atmospheres; while in the second a tension of about 132 atmospheres was attained before rupture occurred.

The former of these is the highest yet recorded, I believe, for the cohesion of any liquid. Possibly this very good cohesion possessed by unboiled sap is due to the presence of colloids in it. It seems probable that when the tension is just adequate to start a rupture, if the latter remains sufficiently small, its surface tension will be able to withstand the stretching action due to the contraction and cohesion of the liquid. Thus, if the rupture at its first inception can be delayed in spreading, it may be obliterated and cohesion re-established. The presence of the colloid may bring about the necessary delay.[1] The appearance exhibited occasionally in these sap-containing tubes may be interpreted as favouring this view. The click of rupture is not, in these cases, attended by the development of a single bubble becoming surrounded by a group of small visible bubbles, but, at the moment of rupture, a milky semi-opaque region develops in the tube. This slowly rises and clears away as it turns into a mass of excessively minute bubbles. Here apparently at the destruction of cohesion countless numbers of minute ruptures have been simultaneously produced.

No. 8 gives the details of a third observation with this tube.

---

[1] The fact that the presence of colloids leads to a large volume-contraction of the solvent may also increase the cohesion of the sap.

TABLE.

No. of Experiment.	No. of Tube.	External Radius R.	Internal Radius r.	Closing Temperature t_2.	Temperature of Rupture t_1.	Coefficient of Compressibility $\beta \times 10^7$.	Coefficient of Expansion $\alpha \times 10^5$.	Tension in Atmospheres.
1	S_1	3·57	0·50	63·50°	59·10°	455·9	54·1	47
2	S_1	,,	,,	63·50°	59·20°	455·9	54·1	45
3	S_1	,,	,,	63·50°	59·20°	455·9	54·1	45
4	S_1	,,	,,	63·40°	59·00°	455·9	54·0	46
5	S_2	,,	,,	66·25°	59·50°	457·0	55·1	73
6	S_2	,,	,,	66·25°	61·25°	457·6	55·7	54
7	S_3	3·50	1·00	78·20°	72·00°	464·4	63·1	75
8	S_4	,,	,,	91·10°	77·25°	453·0	68·6	192
9	S_4	,,	,,	91·10°	81·50°	454·5	70·0	132
10	S_4	,,	,,	91·10°	76·20°	440·5	68·3	207

For the calculation of the results displayed in the table the formula kindly given to me for my previous work by Mr. J. R. Cotter has been used, viz. :—

$$T = \frac{(a - g)(t_2 - t_1)}{\beta[1 - a\,(t_2 - t_1)] + \dfrac{1}{R^2 - r^2}\left(\dfrac{r^2}{k} + \dfrac{R^2}{n}\right)}$$

in this

T = tension in atmospheres.
a = coefficient of expansion of water over the range.*
β = ,, compressibility of water.†
g = ,, cubic expansion of glass = $2{\cdot}4 \times 10^5$.
t_2 = temperature when the tube is full, viz. a 'closing' temperature.
t_1 = ,, ,, rupture occurs.
R = External radius of tube.
r = Internal ,, ,,
k = Compression modulus of glass (volume elasticity) = 4×10^5 atm.
n = Torsion modulus of glass (torsional rigidity) = 3×10^5 atm.

The tubes S_1 and S_2 were filled with boiled sap of *Fagus sylvatica*, which

---

* Landolt-Börnstein, Physikalisch-Chemische Tabellen, Berlin, 1905, pp. 38 and 39.

† *Idem*, p. 60.

was, however, subsequent to boiling, exposed in a thin layer to the air; whilst the tubes S_3 and S_4, on which Experiments 7, 8, 9, and 10 were performed, contained unboiled sap of *Ilex Aquifolium*.

In Experiment 3, after the bubble had been "closed" at a temperature of 63° C., the tube was maintained at about 61° C. for two days. During this time no rupture appeared.

The foregoing shows that the sap of trees has considerable tensile strength, and in this respect does not differ from water. In the few experiments made, the ease with which tension was generated and its magnitude before rupture occurred possibly indicate that sap is somewhat more stable under tension than pure water.

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NOTES ON THE SPECIMENS OF *BORBORIDÆ* AND SOME *EPHYDRIDÆ* IN THE HALIDAY COLLECTION AT THE NATIONAL MUSEUM, DUBLIN.

BY

J. E. COLLIN, F.Z.S., F.E.S.

[COMMUNICATED BY PROFESSOR G. H. CARPENTER, B.SC.]

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XVII.

NOTES ON THE SPECIMENS OF *BORBORIDÆ* AND SOME *EPHYDRIDÆ* IN THE HALIDAY COLLECTION AT THE NATIONAL MUSEUM, DUBLIN.

By J. E. COLLIN, F.Z.S., F.E.S.

[COMMUNICATED BY PROFESSOR G. H. CARPENTER, B.SC.]

[Read February 24. Published April 17, 1914.]

In the Entomological Magazine for 1836, Haliday published a Monograph of the *Sphæroceridæ* or *Borboridæ*, and in the Annals and Magazine of Natural History for 1839 one of the *Hydromyzidæ* or *Ephydridæ*, describing with his usual conciseness and accuracy many new species. A large number of the specimens from which these descriptions were made still exist in Haliday's Collection in the National Museum of Ireland, Kildare Street, Dublin; and though unfortunately in many cases (nearly all the *Borboridæ*) the original labels are missing, still the fact that these specimens are undoubtedly the identical ones studied by Haliday, while the periodicals in which the descriptions appeared are not easily accessible, may make the publication of the following notes of some value to students.

Borboridæ.

The species are numbered as in Haliday's Monographs.

Sphærocera.

1. *Sphærocera subsultans* F.—Several specimens of this very common species.

2. *Sphærocera monilis* Hal.—Originally described under Section "A. *Thoracis lineæ scutellumque hispidulæ*," as follows:—

> "Sp. 2. Sph. monilis. *Pedibus simplicibus, annulo tarsorum anticorum albo.*

> "Head and thorax as in No. 1: abdomen as in *Sph. denticulata*: hind legs longer and more slender than in this last, and the 1st joint of the feet less dilated: fore feet rather thick, with the end of the first joint and the entire second white: the hind legs are scarcely thicker in the male than the female. (Length 1½; wings 3 lines.)
>
> "Found in the New Forest by F. Walker, Esq.; also near London."

This species is not to be found in the Collection; apparently Haliday described it from specimens in Walker's Collection. It, however, is a most distinct species which I have found in various localities near Newmarket, England.

3. *Sphærocera vaporariorum* Hal.—Originally described under Section "AA. *Thorax et scutellum granúlati, hujus margo denticulatus*," as follows:—

> "Sp. 3. Sph. vaporariorum. *Capite thoraceque nigris*; *femoribus posticis parum incrassatis* fem., *clavatis* mas.
>
> "Lordatia coprina, *Rob. D.* 809. No. 4.
>
> "Resembles the following species, but the hind thighs of the male are nearly as large as in No. 1, and a little thickened in the female also: the 1st joint of the hind feet is less dilated than in No. 4, the wings shorter: abdomen attenuate behind: thorax generally without impressed lines, but is more irregularly and thinly shagreened about the middle than elsewhere. (Length 1; wings 2 lines.)
>
> "I find it commonly on deliquescent cucumbers. Mr. F. Walker also takes it near London."

Five specimens, four bearing a label "Cucumbers." The species is very closely allied to *pusilla* Fln., the abdomen is not always attenuate behind, the groundwork of thorax is not very shining, and there are faint indications of two smooth lines on thorax; its shorter wings and stouter femora distinguish it. The fact that Haliday did not use Desvoidy's name of *coprina* for this species seems to prove that he was doubtful of the synonymy, moreover the species does not agree with Desvoidy's generic description of "*tarsi postici duobus primis articulis dilatatis*"; it therefore appears inadvisable to revive the name *coprina*.

4. *Sphærocera denticulata* Meig.—Several specimens of what I consider to be *S. pusilla* Fln., which may have represented Haliday's idea of *denticulata*; he found them "in the same localities with the first species, but much less abundant," and this applies well to *pusilla*. It is quite possible that Meigen's *denticulata* was only *pusilla*, for he originally recognized only *subsultans* and a small species (his *denticulata*).

5. *Sphærocera scabricula* Hal.—Originally described as follows:—

> "Sp. 5. Sph. scabricula. *Brunnea, abdomine nigro.*
>
> "Head and thorax chestnut-brown, opaque, very thickly shagreened, and set with minute white points, lying flat: head very long: eyes small: arista whitish: teeth of the scutel very sharp, decurved: abdomen black: legs short, set with very minute whitish bristles, light brown, with the knees and feet paler: hind thighs of the male not thickened: feet very short; 1st joint of the hind pair as long as the next three together: wings whitish hyaline, with pale ferruginous nerves; the costal brown: they are shorter than in No. 4, and the rib is finely ciliate. (Length $\frac{3}{4}$; wings $1\frac{1}{2}$ line.)
>
> "Found near London by Mr. Walker."

Two specimens in the box containing the *Borboridæ* and some more in another part of the Collection. A remarkably distinct species, easily recognized from the description. Haliday's description was made from rather immature specimens, as stated in Ann. Nat. Hist., 1838, p. 188. He placed it as a synonym of *pusilla* Fln. in the Errata to Walker's Ins. Brit. Dipt. III., and it is so dealt with in the Palæarctic Catalogue, but this must be a mistake. Rondani redescribed it in 1880 under the name *pallidimana*. I caught two females on a stable window here at Newmarket (England) on April 8th, 1896.

BORBORUS.

1. *Borborus nitidus* Meig.—Several specimens of this not uncommon species.

2. *Borborus suillorum* Hal.—Described under Sections "A. *Tibiæ posticæ calcari instructæ*," and "B. *Tibiæ mediæ extrinsecus setigeræ*," as follows:—

> "Sp. 2. B. suillorum. *Niger nitidus, halteribus albidis*; *alis ferrugineis, nervis transversis infuscatis.*
>
> "Mycetia tibialis, *Rob. D.* 806. No. 2.
>
> "Very like the last: the bristles on the outside of the middle shanks are much finer; the legs more slender; the thighs of male unarmed, and only the fore pair thickened; the 2d joint of the hind feet slender: the posterior coxæ, the trochanters and feet, and the extreme base of the shanks, are rust-brown, the fore and hind feet darker: the cross-nerves of the wings are constantly suffused with brown. (Length $1\frac{1}{2}$; wings 3 lines.)
>
> "Inhabits fungi in England and Ireland, but is rather uncommon. I cannot determine whether Macquart's 9th species may not be the same,

though the great difference of size makes it less likely. In any case the name *punctipennis* will have to be dropped, as it is already used by Wiedemann.

"Var. β.—Shanks and feet ferruginous; end of the fore shanks and base of the fore and hind feet brown.

"Mycetia communis, *Rob. D.* 805. No. 1.

"Taken by Mr. F. Walker near London."

Four specimens of the type form. This is given in the Palæarctic Catalogue as a synonym of *glabrifrons* Mg., which is incorrect; in *suillorum* the hind tibiæ are hairy, but there is no anteroventral bristle, while in *glabrifrons* (according to a note made some years ago when I examined the type) there is a distinct anteroventral bristle. Stenhammer appears to have recognized the species correctly. Haliday's *var.* β is evidently our *Roseri* Rnd., but I could find no specimen in his collection; probably he only knew it from Walker's specimens. Haliday evidently considered the identity of Desvoidy's species open to considerable doubt, or he would have used the names; and in the absence of any indication by Desvoidy as to whether his species had the venation of *Borborus* or of *Limosina* it does not appear advisable to revive his names.

3. *Borborus niger* Meig.—One specimen labelled "niger," and five others, were correctly named. This species has only one pair of dorso-central bristles developed on thorax.

4. *Borborus equinus* Fln.—Many specimens of this very common species.

5. *Borborus nigrifemoratus* Macq.—I could find no specimens in the Collection; therefore probably Haliday only knew it from Walker's collection. *B. stercorarius* Meig. and *tibialis* Zett. appear to be synonyms of this species.

6. *Borborus flavipennis* Hal.—Originally described as follows:—

"Sp. 6. B. flavipennis. *Niger; facie, coxis anticis et genubus testaceis; halteribus albidis; alis flavescentibus pallido-nervosis.* Fem.

"Black: the frontals dull; the triangle glossy: face and palpi testaceous: thorax shining: abdomen dull black: 2d segment not longer: legs hairy: the fore coxæ and the extreme base of the shanks rust-yellow: poisers whitish: wings yellowish: nerves scarcely darker; the small cross-nerve placed about the first third of the discoidal cell: resembles the next species, but the fore and hind thighs are thick; the 1st joint of the hind feet almost triangular; the 2d very little longer;

and the cross-nerves are much less distant. (Length 1½; wings 2¾ lines.)

"Found by Mr. Walker near London."

One female specimen in the Collection. This must be identical with *pallifrons* Fln., as suggested by Stenhammer, though there are no distinct bristles above the middle tibiæ, as might be inferred from Stenhammer's remarks—"Tibiæ intermediæ ut in *C. nitida* et *C. suillorum*, tantummodo ob minutiem speciei exilius spinosæ." The species certainly exhibits relationship to this group in the presence of a distinct bristle beneath the hind femora towards the tip, and in having a second vibrissal bristle curving up towards the eyes in addition to the usual bristle.

7. *Borborus longipennis* Hal.—Originally described as follows:—

> "Sp. 7. B. longipennis. *Niger; pedibus ferrugineis; femoribus et tibiarum apice fuscis; halteribus albidis; alis pallido-nervosis; nervis transversis remotis.*
>
> "Black: pubescent, with little gloss: frontals opaque: segments of the abdomen nearly equal: the extremity in the male but little thickened: hairy: the underside and sometimes the incisures pale: legs hairy; in the male pitchy brown, with the fore coxæ, and knees and the base of the shanks, rust-brown: in the female, either of the same colour, or rust-yellow, with the fore and hind feet, the end of the shanks and of the posterior thighs, brown: the spur springs before the extremity of the hind shank, and is very slender and long: the 2d joint of the hind feet is one-half longer than the 1st, and a little thickened: in the male, the first joint of the fore feet is very distinctly unguiculate; poisers whitish: wings hyaline with pale nerves; the small cross-nerve usually at the first fifth of the discoidal cell. (Length 1½; wings, 3 lines.)
>
> "On the sea coast of Ireland; in various parts of England; not rare."

Two males and three females in the Collection. The usual row of spines in front of middle femora is entirely absent, not even a single spine, as in *flavipennis*, being present; but in the male there is a short, strong posteroventral spine; the bristles about tip of middle tibiæ are short and weak, and the anteroventral bristle on hind tibiæ is very weak and inconspicuous. It is practically certain that the *vitripennis* of Zetterstedt and Stenhammer, but not of Meigen, is identical with the above.

8. *Borborus vitripennis* Meig.—Three specimens in the Collection, and a fourth, very immature, probably representing the variety with legs less hairy

and much longer, and the second joint of hind feet not thickened, mentioned by Haliday. This species is smaller and darker than *longipennis* and the abdomen duller. The first joint of the front tarsi is distinctly unguiculate at tip in the male, and there is one short spine in front of the middle femora as in *flavipennis*; the pre-apical bristle to hind tibiæ is very distinct, and there is another smaller bristle near it, a little nearer the tip and placed slightly more anteriorly. I feel little doubt but that *costalis* Zett. and Stenh. is a synonym.

9. *Borborus ater* Meig.—A number of this common species, more correctly known as *geniculatus* Macq., for, the face being greyish-black, Meigen's description of *ater* as having "Untergesicht und der vordere Stirnrand rostgelb," hardly applies to this species.

APTERINA.

1. *Apterina* (*Borborus*) *pedestris* Meig.—One male of this very distinct insect.

LIMOSINA.

1. *Limosina silvatica* Meig.—Several specimens of this distinct species.

2. *Limosina limosa* Fln.—The only specimens in the Collection answering to Haliday's description are four *L. lutosa* Stenh. It should be noted that Fallen included at least two species under his *limosa* (at any rate, *limosa* and *lutosa*, as distinguished by Stenhammer, exist in his Collection at Stockholm), and these two species were not differentiated until Stenhammer described *lutosa*, and limited the name *limosa* to the blacker insect with the smaller number of bristles to the scutellum given by Stenhammer as four, but there being in reality six, the basal pair very small.

3. *Limosina humida* Hal.—Described as follows under Section "D. *Halteres nigri capitulo albido*":—

> "Sp. 3. L. humida. *Nigra, facie albida*; *scutelli setis quaternis.*
>
> "Form of the last, but with much fewer and slighter bristles on every part; one only at each end of the scutel, which is not so long: the face is hoary: thorax with dull blue reflections: abdomen of a glaucous tinge: legs and base of the costal nerve simply pubescent: wings obscure hyaline: nerves as in the last.
>
> "Not rare about muddy drains, near Holywood. Mr. Walker has taken it in England also."

A number of specimens of this common species, which has been generally considered a synonym of *pumilio* Meig.

4. *Limosina arcuata* Macq.—Three specimens, two labelled *arcuata*, are *curvinervis* Stenh. (*roralis* Rdi); five other specimens are *fontinalis* Fln. Haliday in his description said, "size of *L. limosa*; sometimes but half the size": so he obviously included *fontinalis*, which are always larger than *curvinervis*, and in addition have some acrostichal bristles strongly developed. In the errata at the end of vol. iii of Walker's Ins. Brit. Dipt., he placed the name *arcuata* as a synonym of *fontinalis*.

5. *Limosina geniculata* Macq.—I found a pair of this species in the Collection; it belongs to the group with bristly base to costa, a strong bristle on middle trochanters and incurved bristles on front of thorax. It is exceedingly closely allied to *breviceps* Stenh., but appears to have a less projecting keel between the antennæ.

6. *Limosina crassimana* Hal.—Originally described as follows:—

> "Sp. 6. L. crassimana. *Nigra alis infumatis*; *halteribus fuscis*; *tarsis crassis*; mas, *tibiis anticis clavato-compressis.*
>
> "Nerea stercoraria, Rob. D. 803. No. 2?
>
> "Black; the front sometimes with a narrow reddish margin: arista finely pubescent: scutel scarcely so long as the metathorax (with but four bristles, as in all which follow to the end of this section): legs more pubescent than in any of the following; spines or bristles of the middle shanks scattered: feet thick; fore pair evidently dilated in the male, in which also the fore shanks are clavate and furrowed, and the hind feet have two joints dilated: poisers brown or blackish: wings rarely hyaline, generally dusky: nerves darker; base of the costal ciliate with short hairs; the 2d ending nearer to the 3d than 1st: interval of the cross-nerves generally one-half longer than the principal one. (Length 1; wings $2\frac{1}{3}$ lines, sometimes less.)
>
> "In profusion everywhere on dunghills and hotbeds, more rarely on fungi."

A number of this very common species.

7. *Limosina ochripes* Meig.—Eight specimens. Haliday described the antennæ as "black, or red at the base," and there is one specimen in the collection which has the antennæ distinctly yellowish on the first two joints; this might pass for *fulviceps* Rnd. were it not that it could hardly be called much smaller than *ochripes*, and the last costal segment is not "manifeste longiore, non subæquale penultimo."

8. *Limosina scutellaris* Hal.—Described as follows:—

> "Sp. 8. L. scutellaris. *Nigra scutello aterrimo*; *facie, coxis, genubus que testaceis; halteribus albidis; tarsorum posticorum articulis duobus incrassatis.*
>
> "Like the last in character: head black, face and fore margin of the front pale testaceous: thorax glossy black: scutel elongate, opaque, deep black: abdomen dull black: shanks and feet dusky: the fore coxæ, the base of the shanks, often the entire of the middle shanks and feet testaceous or rust-brown: 2d joint of the hind feet twice as long as the first, and thickened: poisers whitish: wings hyaline, with pale brown nerves, the costal darker; 2d terminating much nearer to the 3d, which does not quite reach the tip of the wing: smaller than No. 6.
>
> "With No. 6, but not common; north of Ireland. Near London; Mr. Walker."

Three specimens of this easily recognized species. There are two smaller dorso-central bristles in front of the strong prescutellar, and the long basal joint of middle tarsi has a small anteroventral spine at middle, while in the male there is no bristle beneath the middle tibiæ, but there are two small spines at the base of the middle femora beneath.

9. *Limosina nivalis* Hal.—Originally described (1833) as follows:—

> "B. nivalis. *Niger, hypostomate ferrugineo, alis abbreviatis.* (Long. ·08.) Dull black: face rusty yellow: legs rufescent: thighs and hind shanks dusky: 2d joint of hind feet twice as long as 1st, scarcely thickened: wings shorter than the abdomen.
>
> "About the roots of trees during the winter; leaping far."

I failed to find this species in the box containing the *Borboridæ* of the collection, but it is well known to me from specimens taken in Chippenham Fen (Cambs.). It much resembles *erratica* Hal.; but the abbreviated wings with the outer cross-vein missing make the species an easy one to recognize.

10. *Limosina quisquilia* Hal. Originally described as follows:—

> "Sp. 10. L. quisquilia. *Nigra alis infumatis*; *halteribus fuscis*; *tibiis* mar. *simplicibus.*
>
> "Resembles *L. crassimana* both in size and character, but the feet are slender, and the fore shanks not clavate in the male: from most of the small species which follow, it differs by the longer scutel and more pubescent legs: I consider it as distinct, though not satisfactorily characterized.
>
> "Has occurred once or twice along with *L. crassimana*."

Unfortunately there are no specimens labelled *quisquilia* in the Collection; and, the description being unsatisfactory, it is difficult to say with any degree of certainty which of the specimens are those from which Haliday described his species. There are, however, six specimens of *coxata* Stenh. in the Collection, to which Haliday's description of *quisquilia* might be said to apply; but there appears to be little justification for placing Stenhammer's name as a synonym, the more satisfactory way being to consider Haliday's species unrecognizable.

11. *Limosina fungicola* Hal. Described as follows:—

> "Sp. 11. L. fungicola. *Nigra nitida, fronte opaca*; *halteribus nigris*; *alarum lineola costali nigra.*
>
> "Glossy black: the pubescence very fine: front opaque, deep black, with a glossy triangle: face elevated between the antennæ, rather hoary: legs slender, scarcely pubescent: fore knees and middle feet brown: middle shanks with only a pair of bristles on the outside: 2d joint of the hind feet one half longer than 1st, and somewhat thickened: poisers black: wings ample, blackish, rarely hyaline: nerves dusky; the costal pubescent at the base; black from the 1st to the 2d main nerve; the latter extends scarcely halfway from the 1st to the 3d: the sub-marginal cell is wider than usual; the interval of the cross-nerves almost twice as long as the principal one: smaller than No. 6.
>
> "Inhabits fungi, Hollywood. North Devon, and near London; Mr. Walker."

Haliday must have included two species under this name; one with a dull black frons, longer and more pubescent arista, ample wings, more distinct keel between antennæ, only one pair of small pre-scutellar dorso-central bristles, and more numerous minute bristles on thorax, abdomen shining black without any indication of reddish-brown or red, and middle femur in male with a posteroventral row of 4–5 small bristles near base; the second species having a frons extensively greyish, wings not so ample, and veins rather less distinct, a smaller second pair of dorso-central bristles in front of the pre-scutellar pair, and less numerous minute bristles on thorax, abdomen brownish or obscurely reddish, with shining black genitalia, and no bristles at base of middle femora in male. This latter is undoubtedly *vitripennis* Zett., and the name *fungicola* may be restricted to the former.

12. *Limosina erratica* Hal. Originally described thus:—

> "Sp. 12. L. erratica. *Nigro-fusca fac pedibusque ferrugineis*; *halteribus fuscis*: *alis infumatis.*

> "Approaches the last in character: the marginal and sub-marginal cell of the wings are much narrower, the cross-nerves less distant: the legs sometimes are entirely ferruginous; in others the thighs and the middle of the shanks are pitchy; or the legs are blackish, with the knees and feet ferruginous: wings brownish, with distinct brown nerves, the costal not incrassate: from the following it differs by the wings, the 2d joint of the hind feet not thickened, &c.; but I am not satisfied that all these varieties belong to one species, or that some of them may not connect the present with the last."

The only specimens in the collection at all answering to this description are two males of *rufilabris* Stenh., which would represent Haliday's dark legged form; true *erratica* I feel convinced must be a synonym of *fenestralis* Fln, as defined by Zetterstedt and Stenhammer.

13. *Limosina clunipes* Meig.—This species with its reddish-yellow base to antennæ, reddish-brown pleuræ and cubital vein straight (almost bent downwards towards tip), can hardly be confused with any other; *puerula* Rnd. might well be considered a synonym, were it not for Rondani's statement, "Halteres pallidissimi." There were five specimens in Haliday's Collection.

14. *Limosina spinipennis* Hal.—Originally described as follows:—

> "Sp. 14. L. spinipennis. *Nigra pubescens halteribus nigris*; *alis denigratis, costa incrassata, basi spinigera.*
>
> "Rather dull black: face elevated between the antennæ: arista with thick black pubescence: thorax thickly pubescent: more bristles on the middle shanks than in *L. fungicola*; 2d joint of the hind feet scarcely thickened: poisers black: wings blackish: costal nerve thickened along the middle, somewhat bristly at the base, with a long erect spine springing near the root: 2d nerve ending half way between the 1st and 3d; interval of cross-nerves rather longer than the principal one: size of No. 18.
>
> "Occurs but rarely, in company with No. 6."

Very distinct by reason of the spine at base of costa. Three specimens in the Collection.

15. *Limosina heteroneura* Hal.—Described as follows:—

> "Sp. 15. L. heteroneura. *Nigra, facie pedibusque ferruginosis*; *alis infuscatis, nervis transversis fere contiguis.*

"Black, pubescent: face reddish: arista thickly pubescent: legs nearly naked, dusky; the fore pair, the knees and shanks rust-brown: middle shanks with a pair of bristles only on the outside: poisers brown: wings brownish: the costal nerve a little bristly at the base; 2d nerve as in the last: interval of the cross-nerves not longer than the small one. (Less than No. 18.)

"In the same situations."

The comparatively small distance between the two cross-veins renders this species easy of identification. Several specimens in the collection.

16. *Limosina fuscipennis* Hal.—Originally (1833) described as follows:—

"B. fuscipennis. *Niger, pedibus piceis, thorace scutelloque ferruginosis setosis, alis fuscis, halteribus luteis.* (Long. ·09.)

"Resembles *B. limosus*, but is smaller, and the wings darker: the disk of the scutel, as well as the thorax, set with bristles; both have a very dull ferruginous tinge: 1st joint of hind feet very broad, 2d twice as long, scarcely at all thickened: seta of antennæ black.

"Common on marine rejectamenta."

This description might be said to apply to *ferruginata* Stenh., but in reality was made evidently from somewhat immature specimens of a quite distinct species, as may be gathered from Haliday's redescription of the species in 1836; moreover there were no specimens of *ferruginata* in the box containing Haliday's *Borboridæ*, but there were six specimens of a species near *limosa* with the same bristly base to costa, distinct spine on middle trochanters and incurved bristles on front of thorax, but having, in addition to the 8 marginal bristles on scutellum of *lutosa, fontinalis*, &c., two other stout bristles surrounded with minute bristles and situated on the disc, one at each side; the middle femora of the male also bear a small distinct spine at the base beneath.

17. *Limosina vagans* Hal.—Originally (1833) described as follows:—

"B. vagans. *Niger, opacus, scutello pubescente, alis denigratis, halteribus luteis.* (Long. ·06.)

"Resembles *B. Zosteræ*, but the disk of the scutel is pubescent and not so flat: feet short, fore pair a little dilated."

This description was elaborated considerably in 1836, and as the species has not been recognized on the Continent, and the work in which it was redescribed is difficult to obtain, it may be as well to quote the redescription in full.

"BBB. *Scutellum pubescens.*

"Sp. 17. L. vagans. *Nigra opaca, alis infumatis, halteribus flavidis.*

"Borborus vagans. Ent. Mag. I. 178.

"Dull black: eyes small: arista finely and thickly pubescent: scutel as long as the metathorax: legs pubescent, dusky, with the fore coxæ and knees, and the middle feet rust-brown; sometimes the legs are entirely of the latter colour: middle shanks with numerous bristles; 2d joint of the hind feet twice as long as the 1st, not thickened: poisers yellowish: wings brownish yellow; nerves of the same colour; costal more dusky, bristly at the base, rather thick: 2d nerve extending over ⅔ of the interval between the 1st and 3d: interval of the cross-nerves longer than the principal one. (Length 1; wings 2 lines, or less.)

"Not rare on sea-weed."

There are only four strong bristles round margin of scutellum, and only one pair of dorso-central bristles on thorax; the only species with which it might be confused is the female of *acutangula*, but that has less infuscated wings with still shorter ciliation at base of costa, shorter bristly hairs on hind margins at sides of abdominal segments, and shorter hairs on legs.

There were four specimens in the collection.

18. *Limosina lugubris* Hal.—Described as follows:—

"Sp. 18. L. lugubris. *Nigra pubescens, alis denigratis*; *halteribus fuscis.*

"Face piceous: eyes larger than in the last; scutel shorter; colour deep black: middle shanks and feet dusky: middle shanks with fewer bristles; 2d joint of hind feet shorter: wings blackish: base of the costal nerve less bristly, 2d ending half way between the 1st and 3d; cross-nerves not so distant. (Length ¾; wings 1½ line.)

"Common in the same situations with No. 6."

This species has in the male a yellowish face, jowls, and 3d joints of antennæ beneath, while in the female those parts are much darker. There is only one pair of dorso-central bristles on thorax. The description of *pusio* Zett. applies very well to this species, but no mention was made either by Zetterstedt or Stenhammer of the bristly disc to scutellum; however, the specimens in Zetterstedt's Collection at Lund and the Swedish General Collection at Stockholm under that name possess this character, and I have no doubt represent Haliday's species. In the Trans. Linn. Soc., London, 1912, I suggested the possibility of *L. Thalhammeri* Strobl. being also a synonym. There are a number of specimens in Haliday's Collection.

19. *Limosina zosteræ* Hal.—Originally (1833) described as follows:—

> "B. Zosteræ. *Niger, tarsis luteis, alis denigratis, thorace scutelloque opacis planis, antennarum seta albida.* (Long. ·06.)
>
> "Thorax with an obsolete depressed line down the back: scutel with only about two pair of bristles at the sides and tip: feet short, yellowish, 2d joint of the hind pair somewhat thickened: wings of an uniform opaque smoky tint: knob of the poisers deep brown.
>
> "Common upon Zostera, drying on the shore."

This description was elaborated in 1836 as follows:—

> "Sp. 19. L. Zosteræ. *Nigra opaca alis infumatis.*
>
> "Borborus zosteræ, Ent. Mag. I. 178.
>
> "Opaque black: front gibbous, bristly: face much elevated between the antennæ, which are turned in opposite directions, lying close to the eyes; their 2d joint is very bristly, and larger than the 3d: the arista thickly pubescent, the pubescence whitish: thorax scarcely pubescent, very flat, with an impressed line down the middle: scutel not as long as the metathorax; glabrous, with four bristles, as also in those which follow: legs rather short, thinly hairy, piceous, with the knees and feet tawny, or entirely tawny: middle shanks armed with numerous bristles: 2d joint of hind feet not very long, scarcely thickened: poisers with a deep brown knob: wings of a brownish yellow, the nerves of the same colour; costal more dusky, rather thick, bristly at the base; 2d nerve extending little more than half way between the 1st and 3d: interval of the cross-nerves considerably longer than the principal one. (Length 1¼; wings 2 lines.)
>
> "There is a variety scarcely a third that size, but differing so little in other respects, that I cannot consider it a distinct species.
>
> "Common on seaweed: Mr. Walker has found it near London; and also in the Isle of Wight, Cornwall, and North Wales."

There are four pairs of dorso-central bristles on the thorax, the two front pairs being incurved, while one of the humeral bristles, one notopleural bristle and a bristle quite close to the root of wing are also incurved; there are four prescutellar bristles, the two middle ones being close together and as strong as the outer ones; the hind tibiæ have two distinct bristles above; there is no bristle beneath the middle tibiæ about the middle; costal ciliation strong, but scanty at base of wing.

Four specimens in the Collection.

The small variety mentioned by Haliday is represented in the Collection by seven specimens of *L. brachystoma* Stenh. which, in addition to being much smaller than *zosteræ,* have much shorter bristles on thorax, the bristle near root of wing being the strongest; while the notopleural bristle is not incurved, the middle pair of prescutellar bristles are very small, and there are no distinct bristles above hind tibiæ.

20. *Limosina leucoptera* Hal.—Described as follows under Section "AA. *Antennæ in latera aversæ*":—

> "Sp. 20. L. leucoptera. *Nigro-fusca, alis albis, costa nigricante.*
>
> "Dusky with paler legs: eyes small: arista with thick whitish pubescence: scutel short, nearly semicircular: middle shanks bristly; 2d joint of hind feet long and scarcely thickened: poisers brown: wings whitish; the costal nerve and those next to it dusky, the rest colourless; the costal region dusky towards the end: costal nerve with a few bristles at the base, a little thickened from the 1st to the 2d main nerve; the latter ending much nearer to the 3d; marginal cell long and very narrow; submarginal broad, not extending quite to the tip of the wing: interval of the cross-nerves equal to the principal one. (Rather less than No. 18.)
>
> "The examples which I have before me are not in good order, but the small eyes, the 2d joint of the antennæ, which is very bristly, and the wings satisfy me that the species is better placed in this section than in A. Taken by Mr. Walker, near London."

Two specimens in the Collection. It is a dull species with only one pair of dorso-central bristles developed on thorax. The cubital vein is evenly curved upwards to costa, and the wings distinctly whitish.

21. *Limosina nigerrima* Hal.—Originally described in Ent. Mag. i. (1833), where on p. 150 it is given as *Borborus nigerrimus* N.S., while by a *lapsus calami* the description on p. 178 is given under the name *aterrimus* as follows:—

> "Borborus aterrimus. *Ater, holosericeus, alis albis, seta antennarum albida.* (Long. ·04.)
>
> "Feet short, fore pair a little dilated: seta pubescent whitish: wings opaque, milk-white: rib blackish, the other nervures inconspicuous."

This description was elaborated in 1836 as follows:—

"C.C. *Areola marginalis perparva.*

"Sp. 21. L. nigerrima.	*Atra velutina alis albis.*
"Borborus nigerrimus.	Ent. Mag. i. 178.
" ,, ,,	Curt. B.E. 469. No. 29b.
"Limosina minima	Macq. S. à B. ii. 573. No. 9.

> "Deep black without gloss: pubescence of the arista abundant, whitish: the feet short: middle shanks almost naked: poisers black: wings white hyaline; nerves colourless, the costal blackish, not thickened: the 2d nerve scarcely reaches to the middle of the rib, the 3d is arched and terminates before the tip of the wing; the marginal cell is therefore exceedingly small, the submarginal wide and oblong ovate: the cross-nerves are almost contiguous. (Length not $\frac{1}{2}$, wings 1 line.)
>
> "Occurs along with No. 6, but very rare: Mr. Walker has taken it near London."

Seven specimens in the Collection of this very distinct, though minute, species.

22. *Limosina melania* Hal.—Originally described as follows:—

"BB. *Oculi hispiduli.*

"Sp. 22. L. melania. *Atra opaca alis hyalinis.*

> "Resembles the preceding very much: deep black, opaque: eyes small, with minute erect hairs; arista thickly pubescent: legs piceous, middle shanks almost without bristles: poisers black: wings hyaline: nerves darker, very delicate, the costal blackish; 2d extending nearly half way between the 1st and 3d; the latter scarcely arched, nearer to the tip of the wing than in the last species: interval of the cross-nerves equal to the principal one. (Length not $\frac{1}{2}$ line.)
>
> "Found with the last, but still more uncommon."

Three specimens in the Collection. *L. atoma* Rnd. also has pubescent eyes, but the cubital vein is strongly curved upwards to costa as in *nigerrima.*

Limosina acutangula Zett.—This species was known to Haliday under the name *Heteroptera pusilla* Fln., in which he followed Meigen's and Macquart's interpretation of Fallen's species. There were two males and one female in the collection, but the female was probably included by Haliday under his *vagans* as it does not possess the sloping cross-vein of *acutangula* ♂. Zetterstedt's name must be used for this species, because no one recognized the fact that Fallen's *pusilla* was different from Meigen's *pusilla* until Zetterstedt differentiated the latter under the name *acutangula.*

Ephydridæ.

Hydrellia cardamines Hal.—Original description :—

> " Sp. 1. *cardamines*, H. nigro-æenescens, antennis subtus facie ore palpis coxis et tibiis anticis totis tibiis posterioribus apice tarsisque basi fulvis ; *m.f.* $\frac{3}{4}$–$1\frac{1}{4}$ lin.
>
> " *Var.* β. Facies albo-micante.
>
> " *Var.* γ. Antennis et mento nigris.
>
> " Among aquatic plants, Hollywood ; local but not rare."

The specimens in the Collection evidently had been rearranged by Haliday under the names *flavilabris* and *laticeps* in accordance with his note in the Errata to Walker's Ins. Brit. Dipt. iii. p. 344–345. There are eleven specimens of *flavilabris* (one male bearing two labels " cardamines " and " flavilabris," and one female a label " flavicoxa,") and three specimens of *laticeps* (one female labelled " laticeps "). The white-faced variety mentioned by Haliday is present in the collection, while his *var.* γ. probably referred to the female, in which sex the clypeus and third antennal joint are darker than in the male. It is obvious from the labelling of the specimens that if Haliday's name be retained it must be for *flavilabris* Stenh. and not for *laticeps* Stenh.

Hydrellia hydrocotyles Hal.—Original description :—

> " Sp. 3. *hydrocotyles*, H. obscure viridis tibiis anterioribus et posticarum apice palpis tarsisque fulvis, facie albissima : *f.* 1 lin.
>
> " *Hydrellia communis.*—Desv. Myod. 791 ?
>
> " Hollywood ; extremely rare."

The female specimen labelled " hydrocotyles " appears to be a female of *discolor* with darkened base to hind tibiæ, and as usual the third antennal joint darker than in the male. Two specimens (♂ ♀) labelled " communis " are also *discolor*, while there are two males labelled " discolor " which belong to the same species, and probably represent that described by Haliday as *flaviceps* Mg. for in the Errata to Walker's Ins. Brit. Dipt., vol. iii, he quotes *discolor* Stenh. as a synomyn of *flaviceps* Mg.

On a card containing four females of *discolor* is a note in Haliday's handwriting, " O. intense virides," probably referring to the colour of the eyes in life.

Hydrellia porphyrops Hal.—Original description :—

> " Sp. 4. *porphyrops*, H. nigricans antennarum articulo 3° tibiis apice tarsis basi fulvis, facie ore palpis flavis, puncto frontali albo, oculis hyacinthinis ; *m.* $\frac{4}{5}$ lin.

> "This distinct and beautiful species has occurred but once at Hollywood among *Mentha sylvestris* in a ditch. The eyes are large and of an exquisite purple tint, and the face remarkably small. The eyes are dark-green or brassy in most other species."

There is a single male labelled "porphyrops" which is identical with a species occurring not uncommonly at Snailwell (Cambs.) at the end of May; additional characters lie in the yellowish front coxæ, the deep black triangular frontal stripes, the dull black patch visible on the upper part of pleuræ beneath the notopleural suture when viewed from in front, and the presence of a distinct presutural dorso-central bristle on thorax. The tarsi are extensively pale, and the fifth abdominal segment in the male is half as long again as the fourth, and truncate at the tip; in the female the third antennal joint is dark reddish-brown, and the abdomen broader, with the fourth and fifth segments equal.

Hydrellia thoracica Hal.—Original description:—

> "Sp. 5. *thoracica*, H. thorace cinereo obsolete lineato, facie alba, palpis nigris, tarsis posterioribus ferrugineis; *m. f.* 1⅓ lin.
>
> "On the seacoast, Hollywood; June; rare.
>
> "A very distinct species, of robust form, and the only one which has any vestige of markings on the body. The middle and hind tibiæ are evidently thicker than the fore pair. The discoidal recurrent nerve is very near the margin."

One female labelled "thoracica," and three others, represent the species generally recognized under this name. Schiner (Fauna Austr. Dipt. ii, 249) makes a curious mistake in his reference to this species, quoting characters as attributed to it by Haliday, which were really those upon which Haliday founded his species *tarsata*.

Hydrellia ranunculi Hal.—Original description:—

> "Sp. 6. *Ranunculi*, H. nigro-olivacea facie alba, tarsis posterioribus basi palpisque ferrugineis, nervo transverso subobliquo: *m.f.* 1⅓ lin.
>
> "Abundant in meadows and marshes. This is probably the variety of *H. griseola* with a white face, of which Fallen makes mention, but he is mistaken in considering it as a sexual distinction."

Only a fragment remains of the specimen labelled "ranunculi," but this fragment appears to be identical, owing to the slope of the outer cross-vein with a number of other specimens, one of which (a female) is labelled "incana Stn.," and they all represent a species which is *not* the *ranunculi* Hal. of Loew, Schiner, or Becker, nor the *incana* of Stenhammer, and which does not appear to have been redescribed since Haliday's time, though it is not uncommon on the sea-coast of the east and south of England according to my own experience. The species generally recognized as *ranunculi* (= true *incana* Stenh.) is also present in the Haliday Collection, and was probably included by him under the species of that name, but the upright outer cross-vein appears to prevent it being considered the type of that species.

The following additional characters may help to distinguish the true *ranunculi* Hal.:—Third antennal joint yellowish in male, brownish red in female (nearly all Haliday's specimens are females); face broad below, thorax with three pairs of dorso-central bristles, one being in front of suture; fifth abdominal segment of male long, but not truncate at tip; sixth visible from above; legs strong in male, front femora stout, middle tibiæ distinctly dilated, front tibiæ with rather more distinct bristly hairs than usual, last joint of male front tarsi somewhat dilated, pleuræ and sides of abdomen distinctly greyish, outer cross-vein decidedly oblique.

The *H. chrysostoma* Mg. described by Haliday, is a not uncommon variety of above, with the face yellowish, at least this is the case with one specimen so labelled in the Collection; a second specimen also labelled "chrysostoma" is *griseola.*

The placing of *incana* Stenh. as a synonym of *ranunculi* by Haliday himself in the Errata to Walker's Ins. Brit. Dipt., vol. iii, probably accounted for the confusion that has arisen over this species; Loew (N. Beitr., vii, p. 23) obviously slurred over the character of the oblique cross-vein in an endeavour to make his specimens of *incana* fit Haliday's description of *ranunculi,* and subsequent writers have followed his lead. The fact that Haliday makes no mention of the colour of the antennæ does not necessarily imply that those of his species must have been black, for we know that most of his specimens were females in which the antennæ are darker than in the males; and also he expressly stated that he considered the colour of the antennæ in species of this genus liable to variation.

Hydrellia griseola Fln.—A large number of this very common species.

Hydrellia tarsata Hal.—Original description:—

> "Sp. 9. *tarsata,* H. nigro-olivacea facie palpisque flavis; femoribus anticis validis, tarsis iisdem subtus flavo-tomentosis, onychiis longiusculis rufescentibus; *m.* 1¼ lin.
>
> "Distinguished particularly by its onychii; those of the other species being short and white in both sexes.
>
> "Found but once at Hollywood."

The male specimen labelled "tarsata" is closely allied to *ranunculi* Hal., having strong legs, three pairs of dorso-central bristles, and a somewhat oblique cross-vein, but the third antennal joint is dark, the facial bristles still longer, and the reddish ventral plate of genitalia very differently shaped, resembling somewhat that figured by Stenhammer for *plumosa*. I associate with it two females in Haliday's Collection, and consider it identical with a male in my collection taken by Col. Yerbury at Nairn (Scotland), on July 2nd, 1904, though in that specimen the pulvilli are not reddish.

Hydrellia cochleariæ Hal.—Original description:—

> "Sp. 12. *Cochleariæ*, H. nigro-aenescens facie flavicante, palpis nigris alis obscuris, halteribus basi nigris; *f.* 1 lin.
>
> "Very like the last,[1] but I am inclined to consider it a distinct species.
>
> "Hollywood; June; rare."

Nothing but a leg and one wing is left of the specimen labelled "cochleariæ," with an additional label "P. nigr" (probably meaning *palpis nigris*); but there are three other specimens with a label "P. nigr," which almost certainly represent the same species, and they are the *H. nigripes* Zett. of Becker, who mentions the darkened palpi, though Zetterstedt in his original description wrote "*pălpi flavi.*"

Two specimens labelled "erythrostoma" in Haliday's Collection are males of the above (*nigripes* Zett.), and have reddish-brown palpi, described by Haliday as "fulvis."

Hydrellia albilabris Meig.—Five specimens of this pretty little species, with velvety black frons and silvery white face.

The synonymy of Haliday's species of *Hydrellia* would appear at present to be as follows:—

CARDAMINES Hal.
  flavilabris Stenh.
LATICEPS Stenh.
    cardamines Hal. p.p. (var. major).
  ? *aurifacies* R. Desv.
  ? *flaviceps* Meig.
HYDROCOTYLES Hal.
    flaviceps Hal. (? nec Mg.).
    discolor Stenh.
  ? *obscura* Mg.

---

[1] *erythrostoma* Meig (= *flavicornis* Stenh. = *nigripes* Zett., according to Haliday in Walker's Ins. Brit. Dipt., iii, 345.)

PORPHYROPS Hal.
THORACICA Hal.
RANUNCULI Hal. (*nec* Lw. Schin. Beck.)
    var. *chrysostoma* Hal. (*nec* Mg.).
TARSATA Hal.
COCHLEARIÆ Hal.
    nigripes (? Zett. &c.) Beck.
    erythrostoma Hal. (*nec* Mg.).

Cœnia defecta Hal.—Original description :—

> " Ephydra defecta. *Nigro-œnea, scutello violaceo nitido, alis obscuris hyalino-guttatis, antennis subtus luteis.* (Long. ·08.)
>
> " (To division B.) Seta of antennæ pectinate : face silvery : semicircle of the vertex steel blue : markings of the wings nearly as in *E. noctula* : base of the metatarsi obscure yellow."

The " division B." referred to is that of Meigen, Syst. Beschr. vi, 115. In 1839 Haliday re-described it as follows :—

> " ** *Antennæ articulo 3° subconico.*
>
> " Sp. 21. *defecta*, E. Cæn. nigro-ænea scutello cyanescente, antennis subtus tarsisque luteis, alis obscuris hyalino-guttatis.
>
> " Ent. Mag. i, 174.
>
> " Common in swampy spots."

There are five specimens in Haliday's collection without label, which can only be the above species, because of the pectinate antennæ and steel-blue or violet vertex and scutellum; they have three pairs of strong dorso-central bristles and acrostichals extending irregularly to the scutellum, two pairs of vertical and two pairs fronto-orbital bristles, and undoubtedly belong to the genus *Philotelma*, as I suggested in 1911 (Ent. M. Mag., p. 186). They appear to differ from *nigripennis* Mg. by the more greyish-white face, the paler third antennal joint beneath, and in the male by having a less distinct posteroventral row of bristles on middle femora. The hyaline spots on wings are not very conspicuous.

Parydra hecate Hal.—Original description :—

> " E. hecate. *Nigro-œnea, alis fuliginosis, nervis transversis obscuris utrinque hyalino guttatis.* (Long. ·11 ; dilat. ·22.) (To division C. b.) Resembles the last ; but the wings are much shorter and darker, at the end of the second nervure a dusky spot, and a distinct white one above and below each transverse nervure : the nervures of the tip bordered with brown : hind feet brown."

The "division C. b." referred to is that of Meigen Syst. Beschr., vi, 117, and the "last" species *fossarum* Hal.

In 1839 Haliday re-described it as follows:—

> "Sp. 16. *hecate*, E. N. fusco-ænea tarsis basi ferrugineis, alis fuscanis, nervis transversis obscuris utrinque hyalino-guttatis.
>
> "Ent. Mag. i, 175.—*Eph. fuscipennis*, Macq. S. à B. ii, 540.
>
> "Hollywood: very rare."

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One specimen labelled "hecate" and two others in Haliday's collection.

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ON THE INVESTIGATION OF THE DEEP-SEA DEPOSITS.

BY

J. JOLY, D.Sc., F.R.S.

(PLATES XIX, XX.)

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The "division C. b." referred to is that of Meigen Syst. Beschr., vi, 117, and the "last" species *fossarum* Hal.

In 1839 Haliday re-described it as follows:—

> "Sp. 16. *hecate*, E. N. fusco-ænea tarsis basi ferrugineis, alis fuscanis, nervis transversis obscuris utrinque hyalino-guttatis.
>
> "Ent. Mag. i, 175.—*Eph. fuscipennis*, Macq. S. à B. ii, 540.
>
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XVIII.

ON THE INVESTIGATION OF THE DEEP-SEA DEPOSITS.

By J. JOLY, D.Sc., F.R.S.

(Plates XIX, XX.)

[Read January 27. Published April 27, 1914.]

In 1897 I communicated to the Royal Dublin Society a suggested method of boring into such rocks as might be exposed on the sea-floor. The boring-machine then described involved a motor to drive the drill and an insulated wire from the surface. This machine I subsequently improved, but the features just referred to still remained as essential.

There are difficulties and much expense involved in transmitting electric power from the surface to the bottom at great depths. To develop power below from storage cells or wound-up springs presents even greater difficulties. These considerations have prevented me from hitherto embarking on the construction of any machine intended for the purpose of submarine exploration.

Recently, however, it occurred to me that the pressure of the water prevailing at great depths might itself be utilized to provide the necessary power *in situ.* The principle is simple. Suppose an empty vessel, of sufficient strength to resist the pressure, lowered to the bottom. This provides a receptacle into which the working substance—that is the water—may be discharged after it has done work in a hydraulic engine. This engine may be of the ordinary reciprocating type with the usual directions of the pressure reversed and acting from without inwards. Or the motor may be of the Pelton-wheel form; the wheel being protected from the pressure, and water directed from without upon it, the spent water finding its way to the receptacle.

The dynamical principles are easily stated. We may trace back the work to the sinking of the receptacle under the influence of gravity—a general rise in the level of the ocean occurring in consequence. In this operation we do no work. We, in fact, borrow the work at the expense of the conditions of gravitational potential obtaining. If we desire to recover the receptacle, however, we must pay back what we have borrowed. It is in raising the filled receptacle to the surface that work has to be expended equivalent to what has been done at the bottom of the sea. The elevation of the land above the floor of the ocean and the relatively low density of water supply the conditions essential to the localization of power in the manner proposed.

The power available is very considerable. Let us suppose a sufficiently strong cylinder fitted with a piston 1 square centimetre in area, and having a stroke of, say, 30 centimetres. The volume swept out in one stroke is 30 cubic centimetres. Now at 1000 fathoms the pressure is about 220 kilograms per square centimetre. Hence if the piston describes one stroke under the influence of this pressure, the force on it is 220 kilograms (484 lbs.), and the work done 66×10^5 gram-centimetres (484 foot-pounds about). At 500 fathoms the work done in one stroke is about half this, and in 2000 fathoms it is, of course, about double this.

In the next place, let us assume that the receptacle into which the water is discharged holds 10 litres. The volume of water entering it at each stroke is 30 c.cs. Hence the number of strokes required to fill the receptacle is 333. In practice the force at 1000 fathoms on the piston imagined above would be found inconveniently large. A piston or plunger of one-half a square centimetre in area, used along with the imagined receptacle, would give 666 strokes at a force of 110 kilos. (242 lbs.). If one stroke is accomplished in one second, this amounts to about 0·4 horse-power for eleven minutes. A receptacle one cubic foot (28·3 litres) in volume would enable such an engine to run for thirty-one minutes. It is evident, then, that quite small receptacles will suffice to work an engine developing considerable power, the motion of which is sustained over a period sufficient to carry out useful drilling or boring operations. For the greatest depths the volume of the receptacle may be diminished and the piston area correspondingly reduced. These conditions are convenient, as they involve for the greater depths a lighter machine.

A working drawing of a hydraulic engine suitable for the conditions described, and intended to bore into the soft sediments and oozes on the ocean-floor, accompanies this paper (Plate XIX). A description of this will now be given.

The plan shows four cylinders *A*, *B*, *C*, *D*. Each couple of cylinders, *A-B*, *C-D*, constitutes a separate engine. The fittings permit the ready suppression and removal of one of the engines when, at considerable depths, the one engine is sufficient to operate the drill. Or we may, in this way, secure a more sustained drilling action; the time required to fill the receptacle being, of course, halved when two engines are used.

A plunger, *P*, of uniform section, plays from the one cylinder to the cylinder *vis-à-vis* to it. This plunger is forced to the left when the left cylinder is connected through the valve V_A with the receptacle (not shown in the drawing), and the right cylinder is in connection with the external water through the valve V_B. At the completion of the stroke the valves are suddenly turned through such an angle as will place the left cylinder in connection with the sea and the right in connection with the receptacle. The plunger now moves to the right.

The motion of the plunger is conveyed to the drill by means of an attached rack, *R*, which moves back and forth with the plunger, and engages with the cogged wheel, *W*, giving a reciprocating circular motion to it. The drill-tube feeds down through a sleeve, *H*, so recessed as to engage with projecting ribs upon the outer surface of the tube. The drill is in this way rotated one revolution alternately in either direction, and feeds down into the mud under the influence of its weight, which, if necessary, may be augmented by an added weight placed at its upper extremity. The rollers, *r*, maintain the racks in position against the cogged wheel.

Referring now to the more detailed vertical longitudinal section and the transverse section through the centre, and also to the section on *YY*, it will be seen that the cylinders are carried on U-shaped beds continued right across and preserving the alignment of the opposing cylinders; and that the rack attached to the plunger enters the recess beneath the cylinders, and is engaged with the plunger by a forked projection which embraces a stop upon the plunger. This latter cannot, therefore, be strained laterally by forces reacting through the rack. It is important to notice that the plunger fits loosely in the cylinder, entering it through a leather collar of the form usual in such cases. At its extremity a guide-piece is attached, which permits the water to freely pass it.

The valves are of stop-cock pattern, and rotate back and forth through about 90° at each reversal of the stroke. A detailed figure shows their construction. It, however, conveys a somewhat exaggerated idea of the dimensions of the ways through the valve. A bore of one millimetre is sufficient for the free passage of the water. The water from without enters through a port at the top of the valve. The perforated piece surrounding this port is for the attachment of a cage-like strainer of wire, covered with a fine fabric, so that no coarse particles can enter the cylinder. Connection with the receptacle is made below through a coned coupling. The manner in which the valve is actuated is shown fully in the longitudinal section on *XX*. (Compare this with the transverse section at centre.)

The object aimed at is to secure a *rapid* turnover of the valve through

90° at the moment of conclusion of the stroke in either direction. This is accomplished by two strong springs, *S*, *S*. These are coiled loosely round the valve-rod *T*. As depicted, this valve-rod is just about to be released, throwing both valves over to the left, and so opening cylinder *A* to the external water, and cylinder *B* to the receptacle. The force acting to bring this about is contained in the compressed spring *S*. The loading of this spring has occurred through the engagement of the plate *Q* with the fork projecting from the rack, which in turn is moved by the stop attached to the plunger, as already explained. The plate *Q* is attached at the end of a sleeve *c*, sliding on the valve-rod. The movement of this sleeve to the left has compressed the spring against the stop *x*, which is firmly fixed on the valve-rod. This stop has, however, been retained from moving to the left by catching on the notched trigger *t*. When the sleeve is moved yet a little further to the left by the motion of the plunger, the trigger is lifted by the piece *n*, and the valve-rod released. The spring is sufficiently strong to rapidly throw over both valves to the left through 90°. A similar action occurs at the other end of the valve-rod when the stroke to the right is being completed.

The entrance from valve to cylinder is narrowed to an aperture of about 0·06 cms., which is the calculated aperture (assuming the formula $V^2 = 2gh$, and a value for the coefficient of efflux of 0·6) required to confer a velocity of one stroke per second on the engine at a depth of 1000 fathoms.

This small aperture is conferred by means of a screwed-in nipple, which is not shown in the drawing. Alternative apertures may be substituted according to the depth, their dimensions for a given velocity varying with the square root of the depth.

As shown in the drawing, each of the four cylinders is fitted with a valve. These valves are, however, coupled across, so that when both engines are in operation the one valve-rod actuates all four without any possibility of loss of phase. It is evidently possible to work with one valve only, suitably perforated, and connected with distributing tubes. This system, however, although it lessens the risk of leakage, involves trouble in installing the engines, as well as difficulty in locating the single valve. There is much to be said for having two valves only and controlling the auxiliary engine from these.

The general construction of the drill-sounder is as follows:—The four cylinders already described are installed in a wrought-iron bowl-shaped vessel, shown in vertical section in Plate XX. This vessel, which contains also the receptacles for the spent water, is about 90 cms. in diameter (say, 36 inches), and is constructed of strong boiler-plate. The engines are supported from the deck of the vessel, being carried beneath a diametral segment of the deck upon a base-plate (lettered *J*, *J* on Plate XIX), which is rigidly attached to the deck-plate by the double *T* girder *p* (see transverse section on drawing). The removable deck-plate is lettered *K*, *K* on the drawing, and the bolt-holes for attaching it in position are indicated by the lines *o*, *o*. It is thus easy to lift out the engines after a sounding, for the purpose of cleaning and drying.

The receptacles for receiving the water from the cylinders are in the form of steel bottles. For depths up to 1000 fathoms the steel bottles upon the market, and used for holding gases at high pressures, would be of ample strength; for greater depths special bottles must be provided. In the present machine it is purposed to use four bottles, each of about 2½ litres capacity and having a length of about 60 cms. (24 inches), and each weighing when empty about 9 kilograms (20 lbs.). With these bottles the cylinders communicate by high-pressure steel tubing, the connections with the engine-valves being made through port-holes provided in the top sides of the containing vessel. The bottles are located in the lower part of the sounder, as shown in Plate XX.

The drill-tube feeds down through the collar revolved by the engine, as already stated. It is intended to give the tube a length of about 1½ metre (or about 5 feet). The tube will occupy the position shown in the figure when the sounder is being lowered. Upon reaching the bottom the engine automatically starts into operation by the slackening of the wire suspensions attaching the sounder to the sounding-wire. These wires are four in number. Two of them pass through the deck, as shown, and until the bottom is reached keep the stopcocks *h*, *h*, connecting the valves with the bottles, open, against the pull of two strong springs *c*, *c*. The other two suspensions pass outside the vessel through guides and actuate a slip-door, closing the opening in the sounder for the emergence of the drill-tube. This slide-door, during descent of the sounder, is held closed by the very considerable tension on the suspensions; on reaching the bottom it opens under the pull of a strong spring, allowing the drill to descend upon the mud. Similarly, when the drill is withdrawn from the mud, and the sounder is being raised (in the manner to be described), the slip-door closes over its lower end, protecting its contents from being washed out during the ascent of the sounder to the surface. The details of these doors, being readily filled in, are not shown in the figure.

The drill-tube may be weighted by a lead and iron topping, as shown. The lower or cutting edge of the drill is coarsely saw-toothed. The upper end is closed by a non-return valve opening outwards. This readily permits of the escape of the water as the drill enters the mud. When the drill is being withdrawn, the valve closes, and thus preserves the ooze within the drill from falling out or remaining behind. This can only happen if the water makes its way past the ooze to the top of the tube.

The drill-tube may be made in various forms adapted to different conditions of the bottom materials. It is intended in the first instance to use a plain, drawn-steel tube, with projecting ribs running longitudinally for engagement with the driving-collar. The lower cutting extremity may be recessed outwards by a groove which receives a ring sprung into its place, and which serves to retain in position a lining of oil-silk, which extends throughout the entire length of the tube, or the oil-silk lining may be cemented to the tube at its lower extremity. The function of this lining is to enable the contents of the tube to be readily removed for examination without mixing its contents longitudinally. The entry of the drill is a little narrowed in order to lessen the friction of firm materials entering the tube.

When the sounder is about to be raised from the bottom, the haulage first comes upon the drill-tube, and not until this is brought home, and its lower extremity brought within the sounder, is the pull transferred to the latter. An examination of Plate XX shows how this is effected. The sounding-line is passed through the floating tube *l.* Within this tube it is nipped by the steel nippers *g*, *g*. These act only so long as the weight of the sounder is on the lowering wire, being then jammed by friction with the wire in the position shown. When the weight is removed, upon the sounder reaching the bottom, the grip-jaws open. During descent a certain amount of slack wire is preserved coiled upon the upper deck *d*. This is for the downward feed of the drill into the ooze. When the sounding-wire is hauled from above, it passes freely through the open jaws of the nippers *g*, *g*, and the drill is lifted from the mud. The lifting wire runs through the floating tube till the stop *p* is arrested against the aperture of the tube, when the stress is transferred to the suspenders leading to the sounder. In the event of the drill-tube being for some cause so tightly fixed in the mud as to endanger the lowering wire, the connection with the tube below the stop *p* first ruptures, leaving the drill in the mud. This is accomplished by removing some of the component strands from the lifting wire between the stop and the attachment to the top of the drill-tube.

The drill-tube is guided in its descent by the two guides, *n*, *n*, which are stayed by the four stays, *s*, *s*. These guides and stays are readily unbolted from the deck for convenience of stowing.

It is intended to use a sounding-wire of sufficient strength to ensure the safety of the sounder. The weight of the whole apparatus may amount to about 250 lbs. (114 kilos.). The sounding-wire should have a breaking stress of about 1500 lbs., and, of course, be stranded and of galvanized steel. If serious sticking of the drill in the mud is found to occur, the use of two concentric tubes does not appear to present any difficulty. In this case the outer or active drill-tube would be left behind in the mud, and only the inner tube brought up. But I may point out that the effect of the longitudinal ribs on the outside of the drill will be to loosen a surrounding part of the bottom materials, and that in consequence we may expect that the drill will be easily withdrawn under the influence of a steady pull. The best method of providing such a steady pull from the surface will be mentioned later.

It is very desirable to be able to follow the operation of the drill from the boat above. To this end I propose to use the well-known acoustic properties of water. Above the deck of the sounder a large bell is attached. A hammer is so placed that the raised ribs on the drill-tube lift the hammer and again allow it to fall forcibly on the bell four times in a revolution of the drill, a strong spring occasioning the blow. A telephone suspended beneath the surface of the water above enables these strokes of the hammer to be heard. There is no reason to anticipate any difficulty in this. Submarine bells are heard under much more disturbed conditions of the water and at a far greater distance than would obtain even at the greatest depths of the ocean. With this arrangement it is easy to arrange such distinctive characters for the succession of hammer-strokes as will enable the position of the drill to be known by the listener above, for if the raised pieces are notched at intervals so that one or more strokes are missed in a revolution, or none occurs for an interval, the vertical position of the drill will be recognized by the character of the sounds. It is not difficult even to reckon up the strokes so that the exhaustion of the bottles can be anticipated and the operation of withdrawing the drill proceeded with while there is still power in the engine, so that the drill is reciprocated back and forth while it is being withdrawn, and its release from the ooze in this way facilitated.

A point of some interest may be mentioned here. There is considerable difficulty in providing a float which will act at the great depths of the ocean. It is possible that a vessel made of hard steel or of aluminium might be designed to resist the pressure and retain some buoyancy. It is very doubtful, however, if at the greatest depths any appreciable buoyancy would remain after strength was secured. At any rate, such a float would be expensive to construct. The use of a light liquid appears to be the only feasible method of surmounting the difficulty. Petrol at a specific gravity 0·7 would make an efficient substance. A volume of 14 litres ($\frac{1}{2}$ cubic foot) would give a lifting force of about 4·5 kilos. (10 lbs.). The liquid would be, of course, enclosed in a thin-walled vessel permitting the pressure of the water to act freely upon it. Judging from the compressibilities ascertained for other spirits, there would be little loss of buoyancy with increasing depth. The float *f*, shown in Plate XX, is intended to be made of thin copper, and to be buoyed with petrol.

The form given to the body of the sounder is not without purpose. A flat-bottomed vessel might adhere to the bottom by a "sucker" action, and be brought up only with difficulty. Moreover, as designed, the sounder is mud-tight. The tube permitting the passage of the drill rises from the bottom without opening till it nearly meets the rotating collar. The drill is, therefore, not likely to sink in soft mud if such existed at the surface of the ooze. The four projecting fins are for giving so much bite in the ooze as will resist the reaction of the machine to the torque upon the drill.

Experience only can decide as to what is best regarding the rate of working of the engines and the weight placed on the drill. Experiments made upon a small scale on a sample of globigerina ooze wetted to the consistency of a thick mud showed that above a certain velocity of spin an immersed vertical cylinder experienced relatively little resistance to turning. Thus, a smooth brass cylinder, immersed to a depth of 10 cms., and having a diameter of 2·6 cms., and urged round in one direction by a tangentially applied force as given in the second column, rotated with the velocities given in the third column.

Surface of mud free from water.

No.	Tangential force in grammes.	Revolutions per second.
1	200	0·14
2	300	0·20
3	400	0·25
4	500	0·31
5	600	0·40
6	600	0·39

Surface of mud flooded.

No.	Tangential force in grammes.	Revolutions per second.
7	400	0·3
8	500	1·0
9	500	Very rapid.
10	400	,, ,,
11	200	0·31
12	240	0·50
13	280	2·0
14	300	Slow, changing to very rapid.

There appears to be a critical velocity when the mud is water-covered, beyond which the ooze rapidly loses its hold upon the drill, and lets it run away. When this is once reached, a lesser force serves to produce the rapid rotation, as No. 10 seems to show, and again No. 14, made shortly after. But it is to be remembered that the mud flooring the ocean, left for ages undisturbed, is probably in a much more compacted condition; if, indeed, it is not in a state approaching that of chalk at a little depth from the surface.

It might be found desirable to give the drill a rotary motion in one direction only. This can readily be accomplished by the addition of two free wheels on the driving-collar, an idle wheel connecting both by bevelled gearing, and both free wheels driving the collar, or running free, when moving rotationally in the same direction. Suppose one rack gears on the upper free wheel and the other on the lower. Then when the upper rack moves from right to left, the free wheel geared with it directly drives the collar, and when it moves from left to right this free wheel runs free; but as it transmits its motion to the lower wheel, turning it in the contrary direction, the latter now drives the collar. The second engine, geared on the lower wheel, acts in the same manner. This mechanism gives an intermittent rotation in the one direction. A double set of free wheels, each set separately driven with a difference of phase of one-half a stroke, will give a practically continuous uniform motion in one direction. Of course any desired arrangement of phase may be arranged for and maintained with certainty by the coupling of the valves.

The problem of rock-drilling calls for a fast and light stroke of less power, as well as special arrangements for grasping and cutting off the drilled-out core. I shall not here consider these conditions otherwise than to observe that I believe an engine of the type already described is best for the purpose, but of lesser plunger-area and more rapid stroke.

Finally, it is desirable to describe certain modifications of the customary methods of raising sounding-machines in deep water. It is impossible to operate always in still water. The effect of a swell when loosening the drill might result in putting serious stresses upon the lifting wire—stresses which no convenient arrangement of absorption springs could guard against. To avoid this danger the stress is first applied through the arrangement shown in figure 1. A pulley-block is let run down the wire, and when it has gone down a few fathoms a nipper makes it fast. Beneath the sheave of the pulley a rope leads to a float, the other end being hauled from the ship. Now, in this case one-half the actual lifting force is applied from the ship, and the effect of this force is to partly submerge the float. The safe limit of stress is conditioned entirely by the dimensions of the float, and may be easily calculated. The effect of the lifting of the ship on a swell is only to increase the submergence of the float. In this manner a stress may be applied which cannot exceed a certain calculated amount, and cannot vary abruptly. When the drill is released, the behaviour of the float indicates the fact, as it will not then be submerged by hauling in the rope.

Although the pull from the ship is by this arrangement reduced to one-half the lifting force, it might be that even this amount of winch-power was not available when the extraction of the drill was involved; for it is desirable that a considerable force be at command for this purpose. I refer now especially to operations carried out on a small boat. A simple mode of raising the drill may, however, be resorted to, which has at once the advantage of being adequate and of guarding against suddenly applied stresses. A nipper as before is let down a few fathoms on the lifting wire, and then locked. To the nipper a block-and-sheave is attached in the manner already described. A rope as before passes under the sheave. One end of it is on board; the other is made fast to a strong waterproofed canvas bag of cylindrical or balloon shape. This bag is hauled beneath the surface, and is kept from sinking by a little contained air. The mouth of the bag is inverted, and a rubber tube leading from the boat enters the bag from beneath. A foot-blowers is now used to increase the volume of air in the bag; and this increase is continued till the drill is released. A little calculation shows that this method presents no difficulties as regards the

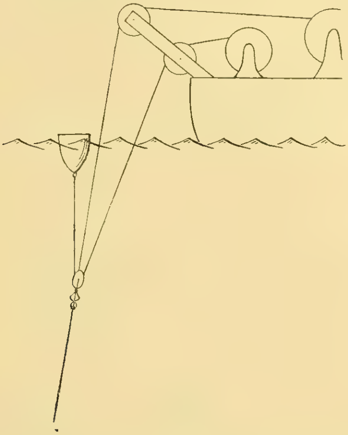


FIG. 1.

dimensions and quantities involved. Every cubic foot of air increases the lifting force by over 64 lbs. A cubic yard of air exerts a force of buoyancy amounting to 1728 lbs.—a force far in excess of what would be required. The float need possess a volume no greater than that of a sphere 3 feet in diameter.

There would be many advantages in carrying out the operations in a small boat, preferably a motor-boat or steam-launch. It would be found easier to approximate to a position vertically above the drill; for, in fact, there may be a considerable component of the force of haulage tending to bring the boat into this position. This horizontal component depends on the distance of the boat from the point vertically over the sounder and upon the depth. If θ be the angle with the horizontal at which the drill would be seen from the boat, and the lifting wire be supposed to be stretched taut from boat to drill, then $f \times \cos \theta$, where f is the stress in the wire, gives the horizontal force urging the boat towards the point above the drill. If f is 200 lbs. and $\theta = 70°$, the force urging the boat towards this point is nearly 70 lbs. Now in the case of a small boat this force would readily move the boat; but in the case of a ship there would, of course, be no effect. Even at an angle so large as 84° there would be a horizontal component of over 20 lbs., which would at least certainly indicate the proper direction in which the boat should be urged in order to approximate to the vertical position. I think the sound of the bell will also help in finding the vertical position above the drill. I may observe that wind-drift is easily guarded against by the use of a mark dropped at the moment at which the sounder reaches the bottom. This mark must present only a small area above water, and expose a considerable surface below water. A good plan is to let a lightly weighted rope depend from it say to 10 or 12 fathoms. Such a mark will only be moved by currents.

If the operations are carried out from a small boat provided with power, the following method of drawing the sounder to the surface would probably be found at once safe and expeditious, and one which would avoid many difficulties arising from raising so considerable a weight by the limited mechanical resources of a small vessel. The drill having been lifted from the ooze in the manner described, the lifting wire is taken in on the winding-engine, and the float released. To the lower extremity of the float a block-and-sheave with attached nipper is now fastened. The block and nipper are opened and closed again round the lifting wire. Just above the block a sea anchor is now attached. This sea anchor consists of one or more conical bags of canvas of the usual form. They are tied in series if more than one are required. This anchor must be of sufficient resistance in the water to move but slowly under a considerable horizontal traction. The boat is now backed away from the float (fig. 2), it being assumed that the winding machinery is forward in the boat. This manœuvre results in pulling the lifting wire over the sheave, and, of course, in correspondingly raising the sounder. The sea anchor only slowly drifts in the direction taken by the boat. When the boat has run back about one or two hundred fathoms, she is brought to rest, and her motion reversed. The lifting wire is now gripped by the nipper, which seizes it just below the pulley: it cannot, therefore, run back again over the sheave. The wire is taken in on the boat as the boat approaches the buoy. It may for safety be kept stretched by a weight running on a block and sheave sent out from the boat, and left upon the wire, throughout the whole of the operations. This operation is repeated till the sounder is brought to the surface.

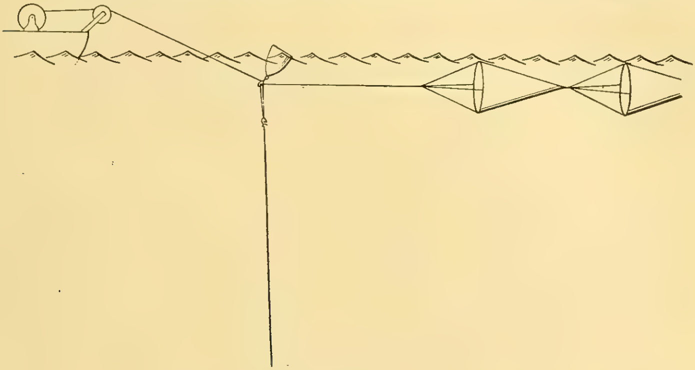
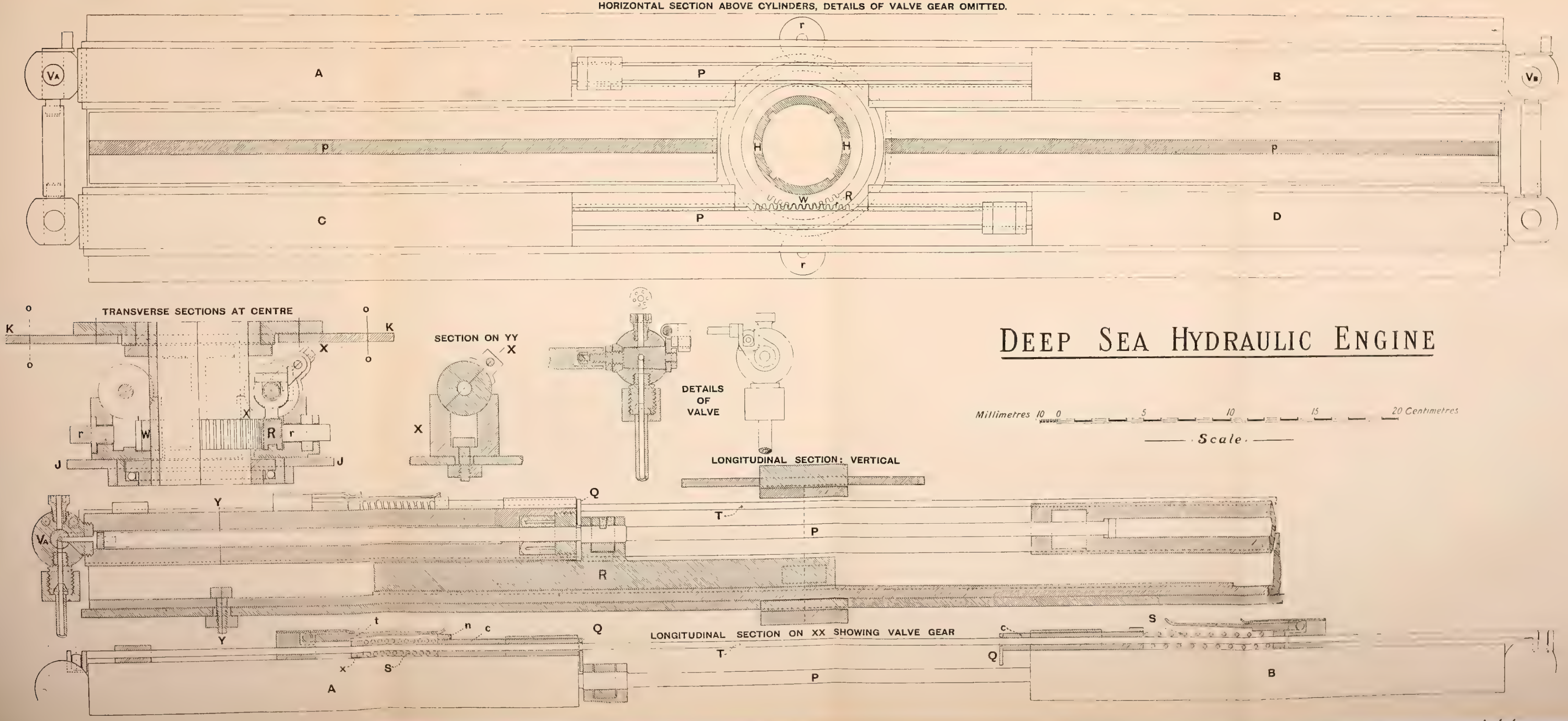


FIG. 2.

The advantages of this mode of raising the sounder are many. There can be no sudden stress put on the wire due to heaving of the boat, and the heaving of the float is mitigated by its ready submergence. The speed of raising the sounder may be very considerable without risk either to winding-engines or to lifting wire. The float acts as a spring, absorbing excessive stresses, and revealing them by its submergence. The wire, finally, is brought on to the winding-drum in a state of low tension, and all danger of bursting the drum is avoided.



DEEP SEA HYDRAULIC ENGINE

HORIZONTAL SECTION ABOVE CYLINDERS, DETAILS OF VALVE GEAR OMITTED.

TRANSVERSE SECTIONS AT CENTRE

SECTION ON YY

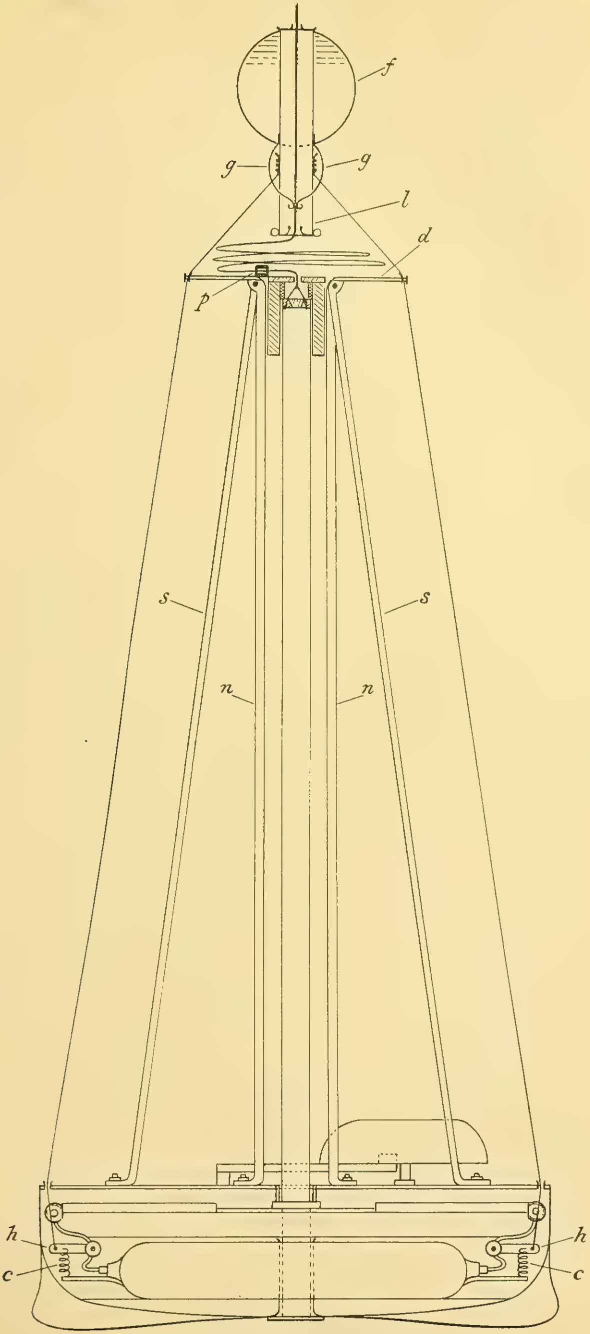
DETAILS OF VALVE

LONGITUDINAL SECTION; VERTICAL

LONGITUDINAL SECTION ON XX SHOWING VALVE GEAR

Millimetres 10 0 5 10 15 20 Centimetres

Scale



Scale $\frac{1}{10}$.

M'Farlane & Erskine.

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THE REPRODUCTIVE ORGANS AND THE NEWLY HATCHED LARVA OF THE WARBLE-FLY (HYPODERMA).

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(PLATES XXI–XXVI.)

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away from the float (fig. 2), it being assumed that the winding machinery is forward in the boat. This manœuvre results in pulling the lifting wire over the sheave, and, of course, in correspondingly raising the sounder. The sea anchor only slowly drifts in the direction taken by the boat. When the boat has run back about one or two hundred fathoms, she is brought to rest, and her motion reversed. The lifting wire is now gripped by the nipper, which seizes it just below the pulley: it cannot, therefore, run back again over the sheave. The wire is taken in on the boat as the boat approaches the buoy. It may for safety be kept stretched by a weight running on a block and sheave sent out from the boat, and left upon the wire, throughout the whole of the operations. This operation is repeated till the sounder is brought to the surface.

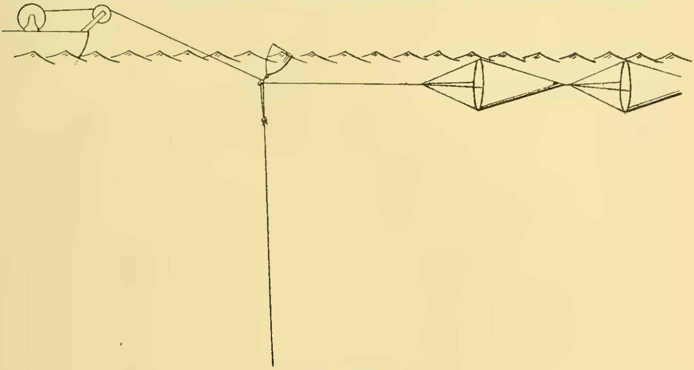

FIG. 2.

The advantages of this mode of raising the sounder are many. There can be no sudden stress put on the wire due to heaving of the boat, and the heaving of the float is mitigated by its ready submergence. The speed of raising the sounder may be very considerable without risk either to winding-engines or to lifting wire. The float acts as a spring, absorbing excessive stresses, and revealing them by its submergence. The wire, finally, is brought on to the winding-drum in a state of low tension, and all danger of bursting the drum is avoided.

XIX.

THE REPRODUCTIVE ORGANS AND THE NEWLY HATCHED LARVA OF THE WARBLE-FLY (HYPODERMA).

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(Plates XXI–XXVI.)

[Read February 24. Published April 29, 1914.]

For nearly ten years past observations and experiments designed to complete our knowledge of the life-history of the Warble-fly—of which there are in Ireland as in Great Britain and on the Continent two species: *Hypoderma bovis* (De Geer) and *H. lineatum* (Villers)—have been carried on at Ballyhaise Agricultural Station, in Co. Cavan, under the auspices of the Department of Agriculture and Technical Instruction for Ireland. Attempts have also been made to ascertain the most effective means for exterminating these destructive insects, or at least of considerably reducing their numbers. Three reports containing the results of several years' work have already been published (Carpenter, and others, '08, '09, '10). During 1913 these researches have been continued on an extended scale of operation, and one of us (T. R. H.) has had the opportunity of spending the whole summer on the farm at Ballyhaise, so as to study the insects in their various stages, and to watch the effect of the flies and their egg-laying on the cattle. It is remarkable that the problem of the Warble-fly is being simultaneously attacked in several countries at the present time; we would call attention to the recent publications of Hadwen ('12) and Gläser ('13), who are working respectively in Canada and in Germany on lines very similar to our own.

The mode of life of the stages of the Hypoderma larvæ within the bodies of cattle has long been known; the behaviour of the larvæ hatched from the eggs laid on the beasts' hairs, and the mode of their entrance into the host's body, are the points still requiring investigation. According to one view the young maggot must bore through the skin. According to others the egg or the larva must be swallowed by the cow or calf, and the latter work its way through the gullet and the tissues to its final position beneath the skin of the back; maggots in the second stage have been repeatedly found in the sub-mucous coat of the gullet and elsewhere. For facts and discussions bearing on this question, reference may be made to the recent writings of Imms ('06) and others.

In the investigations at Ballyhaise the attempt was made, by muzzling and otherwise, to prevent cattle from licking themselves, under the belief that they should thus be protected from Warbles, if the parasites really enter by the mouth; so far it has been found that the practice furnishes, at most, incomplete protection. Experiments on the same lines are being continued, and a further report will shortly, it is hoped, be issued. Last year's work, however, enabled us to obtain a fairly large number of flies, reared from maggots caught by "sleeving" over the holes in the cattle's backs, and we—as well as Gläser in Germany—were fortunate enough to observe the newly hatched maggots of *Hypoderma bovis*, which had previously been unknown. Material was thus furnished for anatomical study of general entomological interest, the results of which are now published with the Department's approval. The reproductive organs of Hypoderma, except the ovipositors, have never, we believe, been described; and we are especially glad to be able to make a comparison of the male genital armature in the two species of Warble-fly, and, further, to compare the condition of the structures as we observe them with what has been described by other students in allied genera of Diptera. It is now recognized that the Warble-flies are nearly related to the group of the House-fly and Blow-fly, whose members have long been favourite subjects for anatomical study. We find that Gläser, in the paper already mentioned, has to some extent anticipated us in his description of the newly hatched maggot—a wonderfully interesting type of insect larva. Our study, however, serves to supplement, and in some respects to correct, his account.

THE FEMALE REPRODUCTIVE ORGANS.

1. THE OVARIES AND OVIDUCTS.

We have studied the internal reproductive organs only in *Hypoderma bovis*. The ovaries (Plate XXI, figs. 1, 2) are very remarkable on account of the arrangement of the *ovarioles*, or ovarian tubes. Each ovary is made up of more than a hundred ovarioles; in the specimen figured we counted 120 in the right ovary and 110 in the left. From the two or three constrictions visible in an ovariole, we conclude that three or four eggs may be developed in each. A single female would thus produce about 800 eggs; Gläser ('13) has observed a female of *H. lineatum* to lay 538 eggs in forty-eight minutes on a calf confined for experiment. The ovarioles open, singly or by twos, threes, or fours, into what may be termed "secondary ovarian tubes," which join together to form larger tubes; ultimately, about five or six main ovarian tubes unite to form an oviduct. This arrangement of the ovarioles gives to the ovary a characteristic tufted appearance, which resembles that of the ovaries of Beetles rather than of other nearly related Diptera in which the fine ends of the ovarioles are continued into converging threads attached, as described by Lowne ('95, p. 668) for the Blow-fly Calliphora, to the dorsal wall of a common ovarian sac. In the Crane-fly (Tipula), however, and in other more primitive Diptera, as described by Dufour ('51), a number of ovarioles open independently into a central chamber continuous with the oviduct.

The egg in the ovariole is already surrounded by the well-known shell with its grooved, flange-like process for attachment to the host-animal's hair. Throughout its progress from the ovariole to the vulva the egg moves with this process in advance. The right and left oviducts (fig. 1, od.), leading respectively from the two ovaries, unite to form a common oviduct (od.′), which merges into the vagina (fig. 1, va.); the latter opens at the end of the ovipositor behind the eighth abdominal segment. We can find no sacculus or copulatory vesicles such as are described and figured from the House-fly (Musca) by Gordon Hewitt ('07, pp. 430–1). The common oviduct and vagina form a sub-cylindrical tube nearly filling the cavity of the elongate terminal abdominal region, which is modified into the ovipositor. With the ovipositor extended, no more than the ovaries can remain in the general abdominal cavity.

2. ACCESSORY GLANDS AND SPERMATHECÆ.

Into the proximal end of the vagina open dorsally the ducts of the two elongate tubular accessory glands ("parovaria" of Lowne) and of the three spermathecæ. The *accessory glands* (Plate XXI, fig. 1, and Plate XXII, fig. 4, a.g.) have the walls composed of granular cells with a delicate fibrous sheath; their ducts (fig. 4, d.′), which are lined with chitin and have a thick muscular wall composed of fibres arranged circularly, open into the dorsal wall of the vagina slightly in front and on either side of the openings of the spermathecal ducts.

The *spermathecæ* (figs. 1, 4, sp.) are three dark brown ovoid chitin-lined capsules, each with a duct, also chitin-lined; the ducts opening close together in the mid-dorsal region of the vagina (fig. 4, d.). In the terminal portion of each duct, the wall is dilated; for the remainder of its course each duct is slender. There are two spermathecæ on the right, with ducts markedly longer than that of the single capsule on the left. This arrangement of two spermathecæ on the right, and one on the left, seems usual among Diptera; we suspect that Gordon Hewitt ('07, p. 430) may be mistaken in describing in the House-fly two on the left and one on the right. In Hypoderma we find that the ducts of the right-hand couple are closely connected together throughout the terminal thickened part of their course; the left-hand duct, except in its furthest distal region, is free from the other two (fig. 4, d.).

On the wall of the spermatheca may be seen transverse striations and a number of dark red spots. When examined with high magnification, the striæ are seen to be thickened ridges of the chitinous coat, and the spots to be minute curved hooks (fig. 4 A), which are on the outside of the chitin, and in contact with the thin outer fibrous sheath of the spermatheca. In a fully extended ovipositor of *H. lineatum* the spermathecæ are visible through the thin cuticle just in front of the sixth abdominal segment (Plate XXIII, fig. 14, sp.).

3. THE OVIPOSITOR.

Sufficient material has been at our disposal for a comparative study of the ovipositor in the two species of Hypoderma. It is well known that the ovipositor of a dipterous insect consists of the hinder abdominal segments, which are modified into cylindrical or sub-cylindrical tubes, their sclerites joined by long tubular tracts of intersegmental cuticle, so that the segments can be successively telescoped into one another from behind forwards, the eighth and ninth into the seventh, the seventh into the sixth, and the whole then telescoped into the fifth segment, when the ovipositor is retracted. In the most highly specialized Diptera, such as the House-fly (Musca) (see Gordon Hewitt, '07), the sclerites of these ovipositor segments are reduced to narrow chitinous rods. It is of interest to note that in Hypoderma the specialization is less extreme; in both species most of the sclerites are plates long and narrow indeed, but not of the excessively attenuated form to be seen in the corresponding parts of Musca. On the other hand, the condition in Hypoderma is more specialized than in Eristalis, whose sixth and seventh abdominal sclerites are comparatively short and broad according to the description and figures of Lacaze-Duthiers ('53, pp. 80–1, pl. v, fig. 6). A short account of the ovipositor of Hypoderma is given in the recent paper of Gläser ('13); his illustrations, however, are mostly photographic, and on too small a scale to bring out the more minute features.

At least four abdominal segments—the sixth, seventh, eighth, and ninth—help to build up the ovipositor (Pl. XXII, figs. 5, 6, and Pl. XXIII, figs. 13, 14, vi., vii., viii., ix.); and we believe that the tenth segment is also represented, by the tip of the terminal dorsal plate. The whole organ when fully protruded measures 12 mm. in length in *H. bovis,* and 8 mm. in *H. lineatum*; the corresponding figures on Plates XXII (*bovis*) and XXIII (*lineatum*) are drawn to the same scale.

In *H. bovis* the *sixth segment* has an elongate dorsal sclerite or tergum (fig. 5, vi.) rounded in front, slightly broadened and truncate behind, and a sternum (fig. 6, vi.) shorter than the tergum, and broader in front than at its hinder edge, which is feebly emarginate. In *H. lineatum* the tergum of the sixth segment (figs. 13, 14, vi.) is deeply cleft in front and much broadened behind, extending to the lateral regions of the segment; the sternum (fig. 15) is parallel-sided and relatively broader than that of *H. bovis.*

The *seventh segment* has in both species two tergal sclerites; possibly the anterior cleft in the sixth tergum of *H. lineatum* indicates a stage leading to this condition. The seventh tergal sclerites in *H. bovis* (fig. 5, vii.) are relatively longer and more slender than those of *H. lineatum* (fig. 14, vii.). In *H. bovis,* there is a single seventh sternal plate (fig. 6, vii.) slightly tapering to the hinder edge, which is feebly emarginate; in *H. lineatum* the seventh segment has two elongate sternal sclerites almost in contact in the median line (fig. 15).

The *eighth segment* is much shorter than those preceding it, and has two tergal plates in both species (figs. 8 and 16, T. 8) with broad anterior and pointed hinder ends; in *H. lineatum* (fig. 16) these plates differ slightly in shape from the corresponding structures in *H. bovis* (fig. 8). The sternal sclerites are in both species a pair of long plates, sinuate in *H. bovis* (figs. 8, 11, St. 8), narrower and straight in *H. lineatum* (figs. 16, 18, St. 8). The hinder extremities of these plates are rounded processes (figs. 8, 11, 16, 18, P. 8) beset with short sharp spines in *H. bovis.* These are described as "blattartige Fortsätze" in Gläser's recent account of the Warble-fly's ovipositor ('13, p. 22). It is barely possible that they may represent the genital processes or gonapophyses of the eighth abdominal segment which are characteristic structures of a typical insectan ovipositor such as is found among the Orthoptera, Hemiptera, and Hymenoptera; but there is nothing in their appearance to suggest that they are anything but prolongations of the sternal sclerites.

These processes form the ventral boundary of the tip of the ovipositor. Dorsally is to be seen a strong tergal sclerite, broad in front, constricted towards the middle, and widening again at the hinder end; in *H. lineatum* (figs. 16 and 17), it is relatively narrower and more constricted than in *H. bovis* (figs. 8 and 10). The front part of this sclerite (T. 9) certainly represents the tergum of the *ninth segment*; from comparison with the tip of the abdomen in the male, we have little doubt that the terminal part (T. 10) in the female belongs to the *tenth segment.* At its extremity this sclerite is bent ventralwards, and ends in a fine point (figs. 11, 18, T. 10). Articulating with the front part of this dorsal plate are a pair of latero-sternal sclerites (figs. 8, 10, 11, 16, 17, 18, St. 9), rounded and somewhat spinose, which doubtless belong to the ninth segment. These are prolonged backwards into a pair of blunt, curved, hook-like processes (P. 9), the "anal scales" of Lowne ('95), or "superior forceps" of Wesché ('06), which must probably also be regarded as outgrowths of the ninth segment; they are beset with hairs and somewhat strong spines, and in *H. bovis* each bears a sharp, inwardly directed prominence (fig. 9). Being present in both sexes, they might be thought to correspond to what have been regarded as cerci by some students of the anatomy of the Diptera. In such primitive insects as the Mycetophilidæ, however, the cerci are jointed in the female and clearly belong to the tenth segment. Together with the tip of the dorsal plate (T. 10) and the two ventral processes (P. 8), these hook-like structures (P. 9) surround the vulva opening beneath the eighth segment, and all five sclerites are joined by membranous cuticle which is stretched when an egg is being laid. Then (fig. 7) the tergal plate and the hook-processes are diverted dorsalwards, the ventral processes remaining almost vertical. We can find no trace of a median ventral sclerite, such as is described by Lowne in Musca. The hindmost dorsal tergum is immediately over the anus. We are interested to find that, although the jaws in Hypoderma are much reduced and feeding appears to be impossible, the food-canal extends through the body; the terminal part of the intestine (fig. 1, in.) leads into a wide rectum (r.), in whose wall characteristic rectal glands (r. g.) like those of Musca and Calliphora can be plainly seen.

The hinder edge of each segment of the ovipositor is fringed with a few hairs. We have been struck with the beautiful reticulate appearance of the segmental cuticle between the hinder sclerites, showing a remarkable pattern in black and white (see figs. 8, 11, 16, 18). The intersegmental cuticle is closely beset with curious spines which have flattened bases and one, two, or three points each; these exquisitely formed structures are stronger in *H. bovis* (fig. 12) than in *H. lineatum* (fig. 19). Their points are directed forwards when the ovipositor is protruded; but when the organ is withdrawn, the spines must point backwards, lying in contact with the cuticle of the segment behind, each tract of intersegmental cuticle becoming necessarily inverted and intervening between the segments respectively before and behind it.

According to Gläser's recent observations ('13) there is a difference in attitude between the two species of Hypoderma when laying eggs: *H. lineatum* holds the ovipositor nearly parallel to the skin of the beast selected as a host, while *H. bovis* pushes her ovipositor almost vertically down among the hairs.

THE MALE REPRODUCTIVE ORGANS.

1. THE TESTES AND THEIR DUCTS.

The internal reproductive organs of the male are of the type usual in the Diptera, and require no lengthened description. We have not been able to dissect a fresh male of *Hypoderma lineatum*. In *H. bovis*, the *testes* are small reddish pyriform bodies (Plate XXIV, fig. 20, Te.), each 1·25 mm. long and ·65 mm. broad at the wider anterior end; they have firm, thick walls. Posteriorly the testis tapers, and passes into a slender *vas deferens* (fig. 20,V.d.), about 3 mm. in length. At the point where the two vasa deferentia unite is situated a small ovoid *accessory gland* (fig. 20, A. G.), 1 mm. long; this would be identified by some anatomists as a seminal vesicle, but Lowne ('95, p. 662) states that the corresponding (paired) structures in the Blow-fly never contain spermatozoa, and calls them *paragonia*, while Brüel ('97) regards them as prostate glands, and describes their secretion. Similarly situated organs in several genera of Diptera are described as accessorial glands by Keuchenius in his recent memoir ('13). From the junction of the vasa deferentia a slender median *ejaculatory duct* (fig. 20, D.e.) passes backwards. After a course of about 5 mm. it enters the *ejaculatory sac* (figs. 20, 22, S. e.), which is ·35 mm. long, and has a chitinous lining, with a remarkable sclerite, the *ejaculatory apodeme*, developed in its wall. This structure has in both species of Hypoderma, when viewed from the side, a heavy, rounded front end, and a more slender, hooked hinder end. In *H. bovis*, however (Pl. XXIV, figs. 21, 22), the front end is slightly swollen, and the hinder end has two prominent recurved hooks; in *H. lineatum* (Pl. XXV, fig. 30, S. e.) the front end is far heavier, and the hinder end has a comparatively feeble single hook. When viewed dorsally or ventrally (see figs. 20, 26, S. e.; the latter is drawn from a specimen in which the ejaculatory sac has been turned on its axis through a right angle), the apodeme in both species is seen to be broad at both ends, and somewhat constricted in the middle; the hooks must, therefore, be shaped rather like scoops. The duct from the testes enters the sac at its ventral face—farther forward in *H. bovis* (fig. 22, D. e.) than in *H. lineatum* (fig. 30). The sac is continued backwards into the terminal part of the ejaculatory duct (fig. 30, D. e.), which traverses the penis.

2. The Genital Armature.

As in the case of the ovipositor, we have been able to make a fairly complete comparative study of the exoskeletal parts connected with reproduction in the male in the two species. So far as we know, these structures have never before been examined in Hypoderma, in which genus their condition throws some light on the vexed question of the segmentation of the abdomen in the higher Diptera.

In the male the hinder abdominal segments are usually hidden, like those of the female, by retraction within the fifth. The fifth tergum and sternum (Plate XXI, fig. 3, v) are broad, especially the former, with an angular notch at the hinder end of each, so that a large sub-quadrangular space is left within which the tail-segments can be withdrawn. Their protrusion is rendered possible by a vast extent of infolded cuticular membrane connecting the hinder edges of the fifth tergum and sternum with the front edges of the sixth (fig. 3, vi). The sixth tergum is a distinct and fairly well-developed sclerite, larger in *H. lineatum* (Plate XXV, fig. 26, T. 6) than in *H. bovis* (Plate XXIV, fig. 22, T. 6); the sixth sternum is much reduced. In *H. lineatum* it is of great interest to find behind the sixth tergum small but perfectly distinct vestiges of the seventh and eighth terga (figs. 26, 28, T. 7, 8; fig. 29, vii, viii). These are absent in *H. bovis*, as one of them apparently is in Calliphora and Musca, so that Lowne ('95, pl. L.) and Gordon Hewitt ('07, pl. 23, fig. 10) reckon the hindmost tergum in the male's abdomen to be the eighth. The existence of reduced seventh and eighth terga in *H. lineatum* shows that there are certainly ten segments in the abdomen of male Muscoidea, the tergum hitherto regarded as the eighth being the ninth (figs. 22, 26, T. 9), and the terminal dorsal plate of the abdomen (figs. 22, 26, T. 10; fig. 26 A.) the tenth tergum. These correlations render needless Brüel's suggestion ('97, pp. 530–1, pl. 42, fig. 8, ix) that minute longitudinal chitinous ridges in front of the anal valves of Calliphora represent the ninth tergum; what he has identified as the eighth tergum is in reality the ninth.

It will be convenient to describe these hinder terga before proceeding to discuss the sternal region and the processes of the genital armature. In front of the ninth tergum in *H. bovis* and also in *H. lineatum* is an extensive intersegmental cuticle, beset with numerous strong hairs. The ninth tergum is, in both species, a fairly large plate, emarginate at its hinder edge, the emargination being shallow and sinuate in *H. bovis* (fig. 25, ix), deeper and more angular in *H. lineatum* (fig. 29, ix). This tergum is prolonged backwards and latero-ventrally into a pair of prominent processes (figs. 22, 24, 26, 28, P. 9), which doubtless correspond with the similarly placed structures in the female, and with the valves of male Lepidoptera assigned by Peytoureau ('95) to the ninth segment. In his figure of the male genitalia of Eristalis, Berlese ('09, p. 327, fig. 395) shows two pairs of processes—"acrocerci" and "mesostyli"; probably the structures now under discussion correspond with the latter. It seems to us that Berlese is mistaken in reckoning the genital segment as the tenth. In *H. bovis* these processes are evenly rounded at their edges, and project inwardly in blunt, spiny tips, which are visible ventrally (fig. 24), but not dorsally (fig. 25). The corresponding parts in *H. lineatum* have a very different aspect, projecting backwards as more prominent lobes, of which the strong chitin is arranged in two bands forming a narrow arch, with membranous cuticle between (fig. 26, P. 9). The tips of these processes are rough, beset with a thick hairy covering, and being more slender and less incurved than the corresponding structures in *H. bovis*, are visible dorsally (fig. 29) as well as ventrally (fig. 28). The ventral edge of the process in *H. bovis* is continued forwards on each side into a slender, delicate ridge (figs. 22, 24, p.), bounding the membranous cuticle which touches the thickened hinder edge of the pre-genital sternum (St. 8), directed dorsalwards and inwards. Also from the ventral edge of the ninth tergum a broad membranous epipleuron (figs. 22, 24, 26, 28, ep.) extends forwards and joins the lateral edge of the pre-genital sternum. In *H. lineatum* this epipleuron is sub-triangular as seen laterally, while in *H. bovis* it is crescentic.

The tenth tergum in both species tapers at the hinder end, which is prolonged into a ventrally directed point. This corresponds to the "uncus" of male Lepidoptera, recognized by Peytoureau ('95, p. 52) as the tenth tergum. In *H. bovis* its edges are rounded, and there is an extensive central white membranous area (fig. 25, x); in *H. lineatum* it is more nearly triangular in form; its front edge produced into a median point and its hinder apex prominent (figs. 29, x, 26 A). The appearance of the tail-region is thus very different in the two species (compare figs. 22 and 26, 25 and 29), but there is no marked difference in size, such as can be observed in the ovipositors, the male's terminal segments in *H. lineatum* being as large as in *H. bovis*.

The most extensive part of the ventral exoskeleton in this region is the *pregenital sternum* (figs. 22, 24, 26, 28, 30, St. 8), which is narrowly rounded in front, broad behind, and bent dorsalwards on either side. As already mentioned, delicate curved processes from its hinder edge meet similar outgrowths from the ninth tergum. This sternal area is called by Lowne the "progenital sternum"; by Brüel the "Gabelplatte"; and by Gordon Hewitt "the body of the penis." Both Brüel and Lowne believe that it belongs to the eighth segment, and, as regards its hinder edge at least, we agree with this opinion. In *H. lineatum* the front and hind edges, especially the former, are definitely stiffened, and the intervening area (except for two converging tongue-like outgrowths of the front edge) membranous; it is thus possible that the seventh, as well as the eighth, sternum may be represented here.

From the hinder edge of this sternum a pair of blunt, stiff, finger-like processes project backwards in both species, one lying on either side of the penis (figs. 22, 23, 24, 26, 27, 28, 30, G. 8). These we consider to be *anterior gonapophyses*, as Lowne does the corresponding structures in Calliphora, called "hook-processes" by Brüel ('97). The "inferior claspers" of the armature of the Tsetse-flies, Glossina, as described by Newstead ('11), are probably homologous structures, and also the "palpi genitalium" of Wesché ('06).[1] The bases of these gonapophyses are united with the chitinous edge of the large sternal plate, and are continued into the strong median *great apodeme* (figs. 22, 26, 30, Ap.)—the "Trägplatte" of Brüel and "inferior apophysis" of Gordon Hewitt—which runs forwards ventral to the ejaculatory duct. The front end of the apodeme is expanded into a spoon-like process, broader and much deeper in *H. lineatum* (figs. 26, 28, 30, Ap.) than in *H. bovis* (figs. 22, 25, Ap.), in which the sclerite tapers to a down-curved apex, as viewed laterally (fig. 22). The apodeme is also continuous with the *theca* or sheath of the penis (figs. 22, 23, 26, 27, 28, 30, Th.), which may be regarded as belonging to the ninth (genital) sternum, to which Brüel would refer also the great apodeme.

The *theca* or *juxta* of the penis is a tubular sclerite surrounding and supporting the membranous glans or vesica (figs. 22, 24, 26, 28, Pe.) which projects beyond it. In *H. bovis* the theca is continuous all around the base of the organ (fig. 23, Th.); but in *H. lineatum* there is a narrow median membranous area on the ventral aspect. The theca is produced dorsally into a strong median *spine* (fig. 22, 30, S.)—the "superior apophysis" of Gordon Hewitt in Musca—relatively shorter and stouter than in the Blow-fly tapering slightly to a point just below its forwardly directed tip in *H. bovis*, but enlarging somewhat in *H. lineatum*, until it narrows suddenly close to its sharp extremity. Backwardly the theca extends along the penis in paired *lateral processes* (figs. 22, 24, 26, 28, 27, 30, Th′), terminating in hooks. These correspond with the *harpes* described by Newstead in the armature of Glossina. In Hypoderma this part of the apparatus is simpler than in Calliphora, which has two pairs of corresponding processes—the "lateral laminae" of Brüel, and the "hypophallus" and "paraphalli" of Lowne.

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[1] It is rather disconcerting to the student of these organs to find that almost every one of his predecessors has elaborated a peculiar terminology of his own.

On either side of the theca, articulating with the membranous cuticle at its base, is a conspicuous hooked process, bearing fine spines, which is broader in *H. lineatum* (figs. 26, 28, G.9; 27, G.9 i) than in *H. bovis* (figs. 22, 24, G. 9; 23, G.9 i). This pair of processes we identify, agreeing with Lowne, as *posterior gonapophyses*; they are termed *paramera* by Brüel. Between them and the anterior gonapophyses are situated another pair of processes (figs. 23, 27, G.9 e; 24, 28, G.9, angled index-line) with the tips somewhat complexly hooked, especially in *H. lineatum*, directed outwardly, and lying partially hidden by the hinder edge of the pre-genital sternum. A forwardly directed extension of the base of each of these processes is fused with the base of the anterior gonapophysis of the same side (figs. 23, 27). Nevertheless, from their position we consider that they belong to the ninth, not to the eighth segment, and that they may reasonably be regarded as *outer posterior gonapophyses*. If this homology be correct, the typical three pairs of gonapophyses are to be recognized in the male genital armature of Hypoderma. This would lead us to consider the organs in these insects as somewhat primitive in type—a conclusion supported also by their perfect symmetry.

In recent systematic work on the Diptera, the importance of the genital armature in distinguishing species has been repeatedly enforced. The comparison of these structures in *Hypoderma bovis* and *H. lineatum* shows the existence of definite specific differences. The male genitalia of the Horse Bot-fly, Gastrophilus (see Schnabl and Dziedzicki, '11, figs. 647–8) show no close likeness to those of Hypoderma.

3. Pairing.

As we have not seen the operation of pairing, we add a summary of the account given by Gläser ('13, pp. 23–5), who observed the behaviour of flies that he kept in cages 40 cms. long. This disposes of a fancy that the Warble-flies pair only in open hill-countries. The male is ripe a day after emergence from the pupa, and flies about; the females usually remain quiet, though some are rather lively, and appear to entice the male. Males were observed to seize their mates sometimes on the floor and sometimes on the vertical walls of the cage. There is a struggle between the paired insects, both partners at times waltzing around the floor, the male beating his wings and humming loudly. During these evolutions the male with his claspers or hook-processes seizes the female's ovipositor, and then the pair remain fairly quiet for three minutes or longer—in one case fifteen minutes. The male stands almost vertically, his front legs holding the roots of the female's wings, his second pair the middle region of her wings, and his hind pair resting on the ground so that he can move in case the female tries to wander away. She rests usually with her abdomen flat on the ground, the ovipositor somewhat protruded, After pairing the female remains quiescent for a while, gently moving the ovipositor in and out.

THE FIRST-STAGE LARVA.

1. ITS MODE OF HATCHING.

The eggs of Hypoderma have been repeatedly described and figured, and it has for many years past been well known that they are attached to the hairs of the cattle by means of the grooved flange-like outgrowth from the hinder pole of the egg (Plate XXVI, fig. 37). Describing the egg-laying habits of *Hypoderma lineatum* in North America, Curtice ('91) and Riley ('92) stated that a number of eggs were attached in a row to a single hair. Hadwen ('12) has recently observed the egg-laying of *H. bovis* in British Columbia (we believe that this species had not before been certainly recorded from America), and he states that the eggs are always laid singly close to the base of the hair, expressing some doubt as to the correctness of Curtice and Riley's description and drawing. We can confirm Hadwen's statement with regard to *H. bovis* from our own observations at Ballyhaise last year; and the position of the eggs, close to the beast's skin, so that in order to see them the overlying hairs must be carefully removed, is doubtless the chief reason why they have been so rarely noticed on the living animal. Gläser, however, who is working at the problem of the Warble-flies' life-history in Germany, has just published ('13) some highly interesting results, and confirms Curtice and Riley's account with regard to *H. lineatum,* whose female clings to the calf or cow better than *H. bovis* does, and is able to lay several eggs in series on one hair, while *bovis* lays only one egg at a time. This difference in habit between the two species in the mode of egg-laying is remarkable. We can fully confirm Gläser's statement that *H. bovis,* like *H. lineatum,* lays her eggs chiefly on the hind limbs, just below the heel-joint or hock. Eggs are rarely laid on the belly, flanks, or breast, and never, under natural conditions, on the back. A female fly "sleeved" on the back of a calf will, however, lay eggs there; and it proves a more convenient place for examination than the heel. We have also induced a captive female *H. bovis* to lay eggs on a calf's hairs in a glass tube. Gläser held some cut-off hairs behind a *lineatum* female's abdomen; she laid eggs on them, and he placed the hairs on earth in a flower-pot, where they hatched in twelve days; eggs laid by the same female on Gläser's trousers hatched in eight days, accelerated by the body-heat. Eggs of *H. bovis* which he observed laid on a calf showed segmentation of the embryo in twenty-five hours, and hatched in three and a half days. We kept eggs of *H. bovis* in an incubator at 40° C., and found that they hatched in four and a half days. Both Gläser and we observed empty egg-shells on the cattle, so that it may be concluded with some confidence that under normal conditions the egg is hatched in about four days while still attached to the host animal's hair.

Gläser has been fortunate in watching the actual operation of hatching, and a summary of his account of the process may well supplement our own observations. The larva, as formed within the egg, was figured by Riley ('92, p. 307), as possessing a spiny armature and strong mouth-hooks. We describe below the newly hatched larva in some detail; for the present it is enough to call attention to the rows of backwardly directed spines on the body-segments (Plate XXVI, figs. 31, 32), and the mouth armature with its sharp median spine (fig. 33, M) and powerful paired hooks (fig. 33, H). Gläser states that the larva, while still in the egg, moves freely, drawing both ends away from the egg-shell, and bending the body. When ready to hatch, the larva draws back its head from the end of the shell, and strikes strongly against the free pole where it will emerge, the paired mouth-hooks then lying parallel to the median spine. As soon as the shell has been pierced, the maggot spreads its mouth-hooks widely apart, and tears loose little pieces of the shell-wall. The larva begins to work at the middle of the egg-pole, and extends its operation to the neighbouring region, till suddenly the envelope gives way, and the maggot's head projects through the opening. It then works its way out, segment by segment, the backwardly-directed spines preventing it from slipping back. The whole operation of hatching takes, according to Gläser, twenty-five minutes.

The empty egg-shell (fig. 37) has a well-marked slit extending around the lateral and front margin, where there is possibly a line of weakness, despite the considerable thickness of the free pole of the egg.

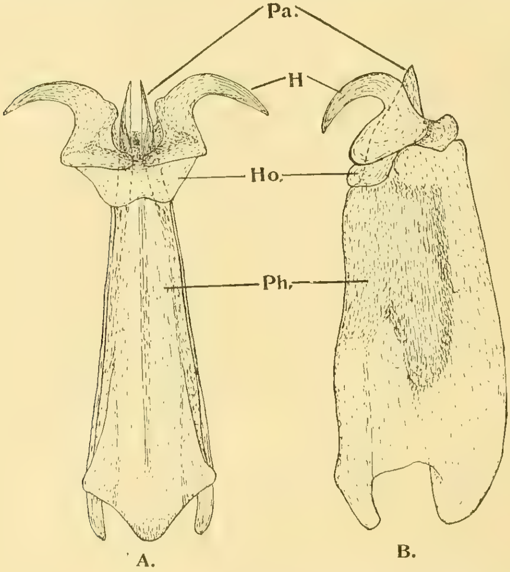
2. The Structure of the newly hatched Larva.

As already mentioned, Curtice and Riley briefly described, and the latter roughly figured, the unhatched larva of *H. lineatum* ('92, p. 307). Until the observations made last year by Gläser in Germany, and by ourselves at Ballyhaise, the first-stage larva of Hypoderma had never been observed in the free state. The "first-stage" larvæ mentioned by various writers as found in the gullets of cattle, are really maggots early in the second stage. For example, the description and figures given by Jost ('07, pp. 672–7, pl. xxxii, figs. 1–9) of the "first-stage" larva of *H. bovis* refer certainly to a small second-stage larva, in which the minute spines and feeble mouth-hooks, recognizable only with a moderate microscopic power, contrast most strongly with the formidable mouth-hooks and spiny armature of the tiny maggot that emerges from the Warble-fly's egg.

This first-stage maggot (Plate XXVI, figs. 31, 32) is ·8 mm. in length. In dorsal or ventral view (fig. 32) both the front and hind ends are rounded, but in side view (fig. 31) the head-end is much narrower than the tail, recalling the form of a typical muscoid maggot, and suggesting that this earliest instar of the Hypoderma larva is far less specialized than those later forms which are adapted for parasitic life within the body of the ox. The segmentation of the body corresponds to the arrangement found in muscoid larvæ; there are the usual twelve obvious segments. According to Lowne and Gordon Hewitt ('08, Pl. 30, fig. 5) the apparent second segment, which usually has the paired anterior spiracles at its front edge, is really the fourth, and the tail-segment on which the large posterior spiracles open is the thirteenth. We believe that this hinder spiracular segment is the ninth abdominal; behind this the minute tenth (anal) segment (fig. 31, A) can be seen ventrally. But this little maggot yields no guidance as to the disputed questions of the segmentation of the anterior region, unless it be in the anomalous position of the front spiracles.

The *anterior spiracles* are usually not recognizable in the first instar of a muscoid larva; later they appear at the front edge of the apparent second segment. But in this first-stage larva of *Hypoderma bovis* we find a pair of spiracles at the far front end dorsal to the mouth (figs. 31, 32, 35, A. Sp., 36), apparently a segment in advance of their normal position. Riley saw these structures in the unhatched larva of *H. lineatum,* and recognized their spiracular nature, which is now questioned by Gläser, who calls them "fühlerartige Vorstülpungen," and says ('13, p. 31) that he can trace no tracheal tubes in connection with them. We have, however, succeeded in finding distinct tracheæ (fig. 35, Tr., 36) which have close to the opening a thickened chitinous lining. In our specimens the spiracles do not project in front of the head; the cuticle around is thickened in the form of radiating ridges, and it is likely that under certain conditions they might be protruded.

The *mouth-armature* has been described and roughly figured by Gläser ('13, pp. 31–2, fig. B). We find that in *H. bovis* the *mouth-hooks* (Plate XXVI, figs. 31, 32, 33, 35, H) articulate directly with the *pharyngeal sclerites* (figs. 33, 35, Ph.), the paired hypostomal sclerites that intervene in most half-grown or full-grown muscoid larvæ (see Banks, '12, pp. 14-15; Lowne, '90, pp. 44-5, fig. 9) not being present; according to Lowne these sclerites are not recognizable in the Blow-fly maggot till after the second moult, so that their absence in the young Warble-maggot might have been expected. The pharyngeal sclerites are of the usual form, the ventral processes being somewhat short and feeble. The mouth-hooks are remarkable on account of their lateral direction, their sharp bifid tips pointing outwards instead of downwards; the strong and prominent basal fulcral processes, which give attachment to the biting muscles, are also directed outwardly. Between the mouth-hooks there projects forward a sharp and prominent *median spine* (figs. 31, 32, 33, 35, M). This is not attached, as Gläser ('13, p. 31) states, "auf einem kurzen Querbalken," but is the prolongation of a pair of slender *parastomal sclerites* (fig. 33, pa.) which lie dorsal to the pharyngeal sclerites; between these parastomals the dorsal pharyngeal wall is feebly thickened, presenting the appearance of a median pointed process directed backwards. It is of much interest to compare this condition with that of the first-stage Blowfly maggot described by Lowne ('90, fig. 9, 1), in which there is a strong median downwardly curved tooth formed also as the prolongation of a pair of parastomal sclerites; in the young Calliphora maggot, however, the paired mouth-hooks are very feeble. Another instructive comparison is to be found in the well-known Bot-maggot (the larva of *Gastrophilus equi*)



Mouth armature of Larva of Horse-bot Fly (*Gastrophilus equi*).
A, ventral, and B, lateral views of pharyngeal sclerites (Ph.), Hypostomal sclerites (Ho.), Mouth-hooks (H), and Parastomal sclerites (Pa.). × 32.

from the Horse's stomach, in which (see Text figure) the mouth-hooks are very strong and divergently directed, while each parastomal sclerite is separately prolonged into a sharp forwardly directed spine.

We have had no opportunity of studying first-stage larvæ of *Hypoderma lineatum.* Gläser states ('13, p. 32) that the mouth-armature is altogether more strongly built in that species than in *H. bovis.*

As already remarked, the *body-segments* of our little larva are remarkable for their strong spiny armature. There are seven or eight irregularly arranged rows of spines around most of the segments, two or three series of strong spines being found close to the front edge of each segment, those behind being feebler, and the posterior margin of each segment showing a strip free from spines. Gläser states ('13, p. 31, Pl. iv) that the *H. lineatum* larva has stronger spines than that of *H. bovis,* and that the dorsal surface of the larva in both species has stronger spines than the ventral. In this latter opinion he is in error, having mistaken the ventral surface for the dorsal. When the larva is examined in side view (Pl. XXVI, figs. 31, 35), no doubt as to the orientation is possible, and the strong spines are clearly seen to be ventral in position. This is the arrangement, as is well known, in muscoid maggots generally, as spines, being of service in locomotion, are naturally best developed on the surface whereon the larva crawls. It is further of interest to find that in the young larva of *Hypoderma bovis,* the dorsal aspect of the two hindmost abdominal segments (fig. 31) is almost devoid of spines, for the unarmed condition of these terga in the fourth-stage maggot has long been recognized as a specific character by which *H. bovis* may be distinguished from *H. lineatum* in the larval stage. (See Riley, '92, p. 311.) We may add that the result of rearing flies from maggots which emerged from the backs of cattle, and were determined by us before pupation, has in all cases confirmed the reliability of the larval characters.

The terminal or hind spiracular segment of the young larva is of especial interest. At its front edge on the ventral surface are four or five rows of very strong spines, which, like all the spines on the preceding segments, point backwards (figs. 31, 32). Behind these are about a dozen weaker spines arranged in a transverse row, and directed forwards. Behind these again, the broad posterior aspect of the larva is covered with numerous very strong curved hook-like spines, most of which point dorsalwards (see figs. 31, 34). In the midst of these are situated the posterior spiracles (figs. 31, 32, 34, P. Sp.) circular openings, close together near the median line, each surrounded by a thickened chitinous margin, and guarded by two or three extra long spines. From the condition of some of our specimens, we infer that the spiracular area can be invaginated so that the openings may be protected by an infolding of the cuticle, as in the case of the larva of Gastrophilus. This fact may have a bearing on the conditions under which the young Hypoderma larva has to live.

We hope that before long these conditions may become well known. The gap in our knowledge with regard to the life-history of Hypoderma is between the hatching of this little spiny maggot and the appearance of the small second-stage maggot in the gullet-wall. Is the maggot's path of entrance by way of the mouth, or does it bore through the skin? Suggestions and observations with regard to this question may be found in the useful summary of Imms ('06).

All the maggots of *H. bovis* which we saw died quickly after hatching amid their unnatural surroundings in the incubator. Gläser ('13, p. 34) states that those under his observation died in one and a-half hours if left in dry air; but that within an hour after hatching, they could be revived by transference to water, in which they would live for two days. He concludes, therefore, that they need moisture for their further development, and that they would obtain this in the gullet. Newly hatched larvæ placed by him on the shaved skin of an experimental calf made no attempt to bore through. One young maggot, however, hatched from an egg laid on his trousers by a female *H. lineatum* in June, bored through the skin of his own leg, and disappeared in one and three-quarter hours, leaving a small round red spot visible externally. Four or five days later the larva could be felt through the skin, having grown to a length of 2·5 mm. Then it apparently worked its way upwards, for early in September swellings were apparent on the hip and abdomen, and at the end of that month a swelling in the lower end of the gullet was indicated by pain when swallowing. This moved quickly up the gullet, and on October 2nd Gläser had the satisfaction of extracting a Warble-maggot 7·5 mm. long (a common size of larva to be found in an ox's gullet) from his own mouth!

This involuntary experiment tends to show that a Warble-larva can bore through the skin of the leg and work its way into the gullet in the human subject, and that the insect might pursue the same course in the ox. As mentioned in the introduction to this paper, the experiments with muzzled calves tried during several years at Ballyhaise show that animals apparently unable to swallow either the eggs or young larvæ of Hypoderma are at the most but partially protected from infection. The strong mouth-hooks and piercer, and the well-developed spiny armature of the newly hatched maggot, suggest that it could, perhaps, bore as readily through the skin as through the mucous coat of the gullet, and we may eventually find the former to be, after all, the usual mode of entrance.

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EXPLANATION OF PLATES.

PLATE XXI. *Hypoderma bovis.*

Fig.

1. The Female Reproductive Organs. × 10. The general view is lateral, but the right ovary has been displaced ventralwards. ov. l., left ovary; ov. r., right ovary; od., paired oviducts; od′., common oviduct; va., vagina; op., tip of ovipositor; sp., spermathecae; a.g., accessory glands; in., intestine; r., rectum; r.g., rectal glands. The ovaries and paired oviducts contain many ripe eggs.
2. Ovarioles and secondary ovarian tubes uniting to form one of the five or six main divisions of an ovary. × 10.
3. Terminal region of abdomen of Male, as seen from behind. × 25. The hinder abdominal (genital) segments (vi, ix, x), are retracted within the large fifth segment (v). The reference figures indicate the terga and (for the fifth and sixth) the sterna of the segments, the intervening cuticle being membranous. The hairy clothing of the segments is not shown.

PLATE XXII. *Hypoderma bovis.* Female.

Fig.

4. The central spermatheca (sp.) with the proximal part of its duct; the distal region of all three spermathecal ducts (d), and of the right accessory gland (a.g.) with its duct (d′), the termination of the left accessory duct being also shown. Dorsal view. × 62.

4a. Chitinous coat of spermatheca with hooks. × 800.

5. The same. Terminal abdominal segments (numbered v–ix) which form the Ovipositor. Dorsal view. × 10.
6. The same. Ventral view. × 10.
7. The same, with the hinder segments partly telescoped, and an egg passing out. Lateral view. × 30.
8. Tip of ovipositor. Lateral view. × 84.
9. A sternum of the ninth segment, with its process. Oblique ventral view. × 84.
10. Tip of ovipositor. Dorsal view. × 84.
11. The same. Ventral view. × 84.

    In figures 8, 10, and 11, the terga (T.), sterna (St.), and paired processes (P) of the various segments are numbered (8, 9, and 10).

12. Spines of the intersegmental membrane in front of the eighth sterna. × 225.

PLATE XXIII. *Hypoderma lineatum.* Female.

Fig.

13. Terminal abdominal segments (numbered vi–ix) which form the ovipositor. Lateral view. × 10.

14. The same. Dorsal view. × 10. sp., spermathecae.

15. The same. Ventral view. × 10.

16. Tip of ovipositor. Lateral view. × 84.

17. The same. Dorsal view. × 84.

18. The same. Ventral view. × 84.

In figures 16, 17, and 18, the terga (T), sterna (St.), and paired processes (P) of the various segments are numbered (8, 9, and 10).

19. Spines of the intersegmental membrane in front of the eighth sterna. × 225.

PLATE XXIV. *Hypoderma bovis.* Male.

Fig.

20. Reproductive organs. General dorsal view. × 10. Te., testis; v.d., vas deferens. A.G., accessory gland; D.e., ejaculatory duct; S.e., ejaculatory sac, below which are seen the terminal abdominal segments.

21. Ejaculatory Sac. Side view. × 50, showing the apodeme.

22. Terminal abdominal Segments and Genital Armature. Lateral view. × 50.

23. Theca of Penis and Gonapopyhses. Postero-ventral view. × 50.

24. Terminal abdominal segments and Genital Armature. Ventral view. × 50.

25. The same. Dorsal view. × 50.

In figures 22, 23, 24, 25, and also in figures 26, 27, 28, 30 (Plate XXV) the terga (T), sterna (St.), paired processes (P), and gonapophyses (G., G.i., internal, and G.e., external) of the various segments are numbered (6, 7, 8, or 9). D.e., ejaculatory duct.; S.e., ejaculatory sac, with apodeme; Ap., great apodeme; Th., theca of penis; S., its median spine; Th′., its lateral processes; Pe., glans penis; p., anterior ridge-procss of ninth segment; ep., epipleuron of ninth segment. In figures 24, 25, and 29, the roman numerals (vi–x) indicate respectively the terga of the segments.

PLATE XXV. *Hypoderma lineatum.* Male.

Fig.

26. Terminal abdominal segments and Genital Armature. Lateral view. × 50. The ejaculatory sac. (S.e.) presenting its dorsal aspect.

26A. Tip of tenth abdominal tergum. Lateral view. × 50.

27. Theca of Penis with Gonapophyses. Postero-ventral view. × 50.

28. Terminal abdominal segments and Genital Armature. Ventral view. × 50.

29. The same. Dorsal view. × 50.

30. The Penis and associated structures. Lateral view. × 50 (Most of the pregenital Sternum (St. 8) has been removed).

In figures 26–30, the lettering corresponds with that of figs. 22–25. See Explanation of Plate XXIV.

PLATE XXVI. *Hypoderma bovis.* First-stage Larva and Egg-shell.

Fig.

31. First-stage Larva. Lateral view. × 125.

32. The same. Ventral view. × 125. The tail-end of the maggot is directed ventralwards.

33. The pharyngeal sclerites (Ph.), mouth-hooks (H), and median spine (M). Ventral view. To the right the median spine (M) from the dorsal aspect, showing its origin from the union of paired parastomal sclerites (pa). × 325.

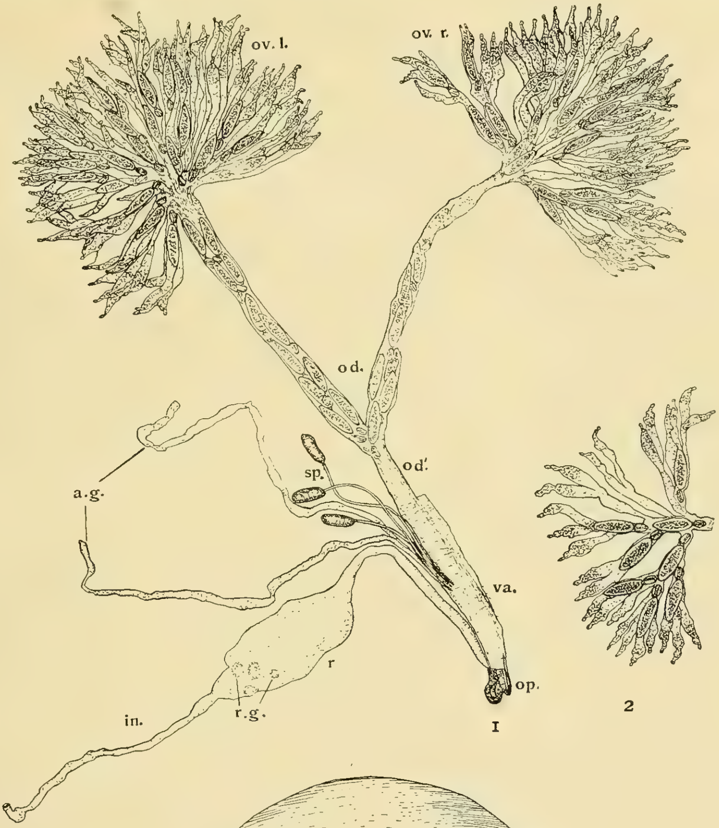
34. Terminal segment, with hinder spiracles (P. Sp.), surmounted by strong hook-like spines. × 325.

35. Front end of Larva. Lateral view. × 325. The bodywall has been partly removed to expose the pharyngeal sclerites and mouth-hooks. × 325.

In figures 31–35, M., median spine; pa., parastomal sclerites; H., mouth-hook; Ph., pharnyngeal sclerites; A. Sp., anterior spiracles; P. Sp., posterior spiracles; Tr., tracheal air-tubes; A., anus.

36. Anterior spiracle. Side view. × 560.

37. Empty egg-shells after hatching of larvae; two views showing slit at anterior pole. × 55.



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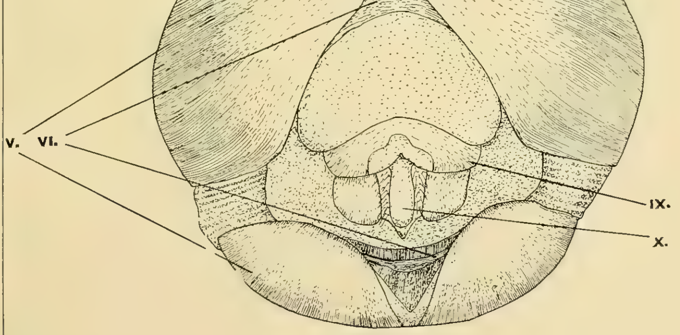

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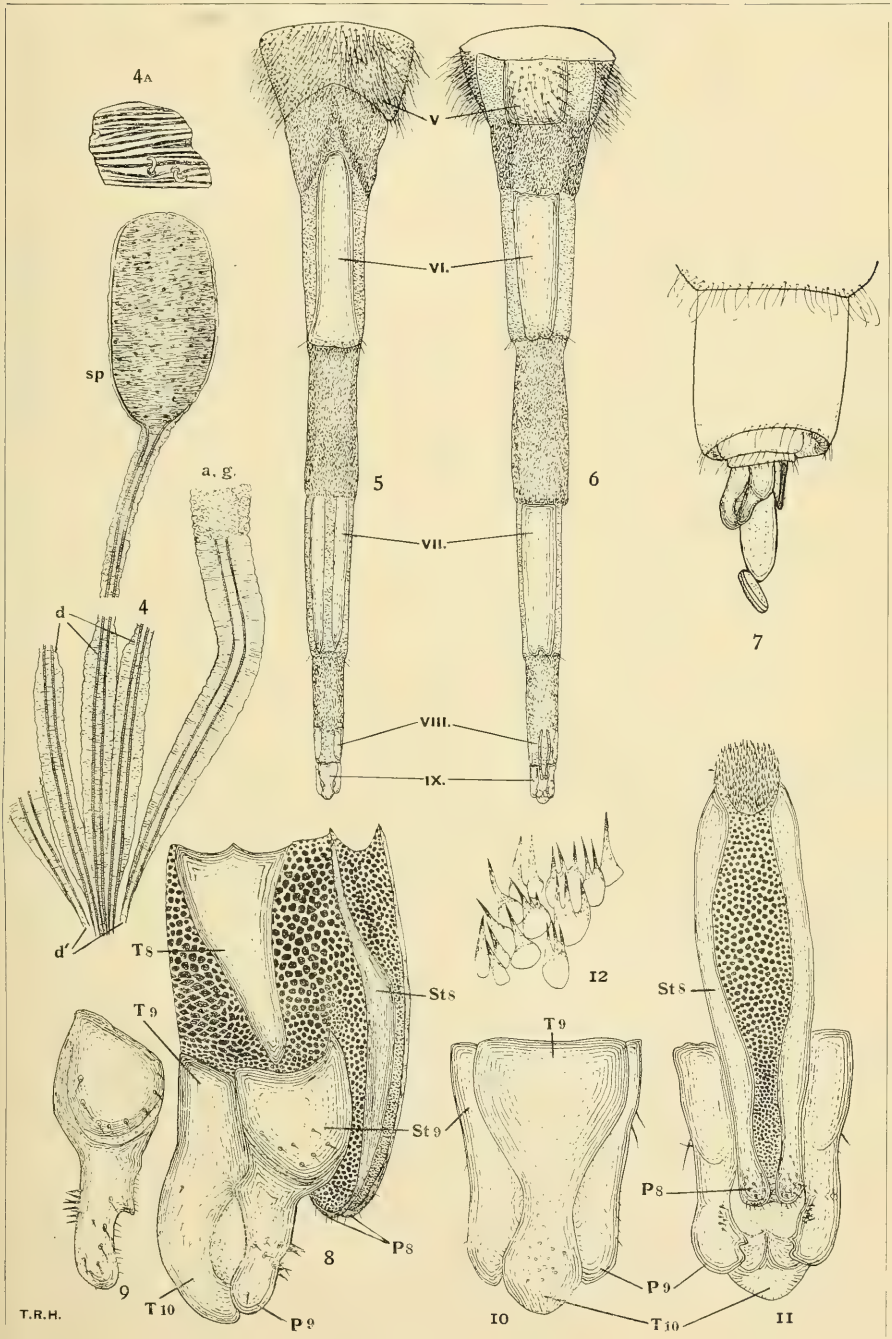

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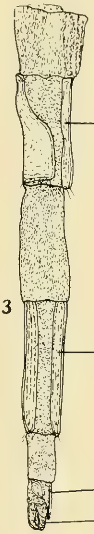

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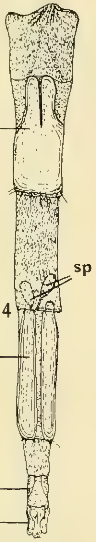

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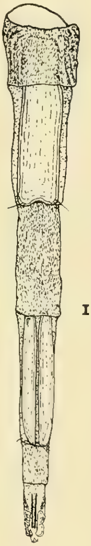

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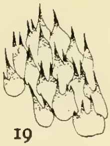

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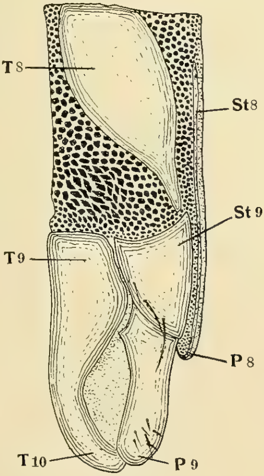

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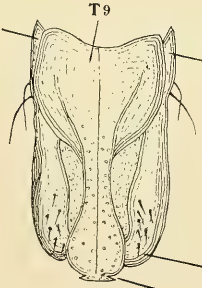

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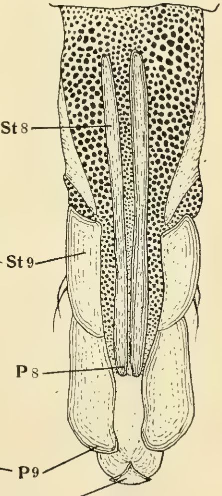

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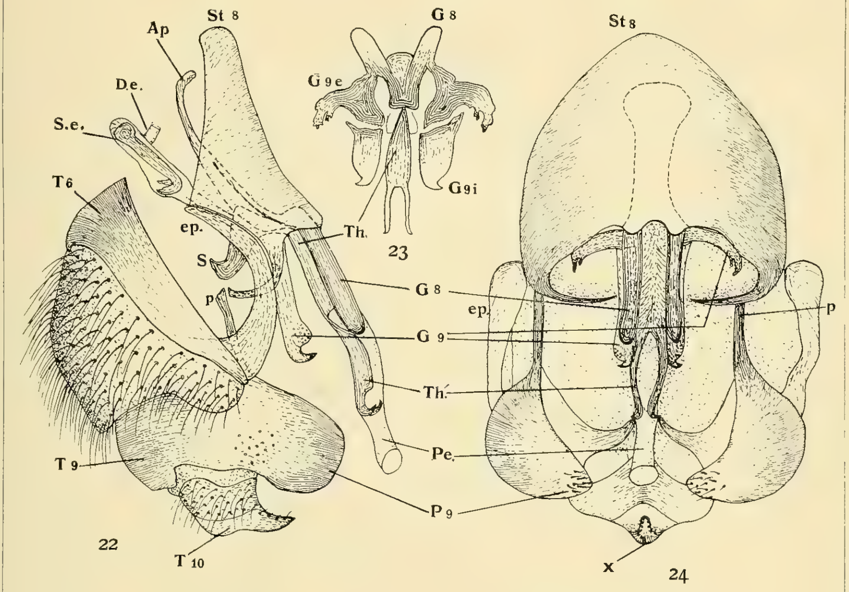

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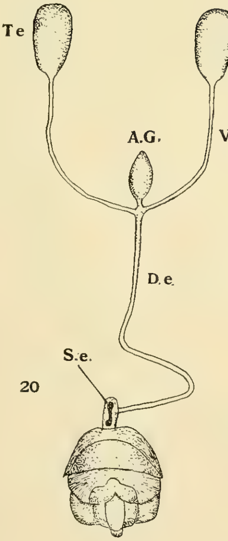

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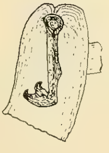

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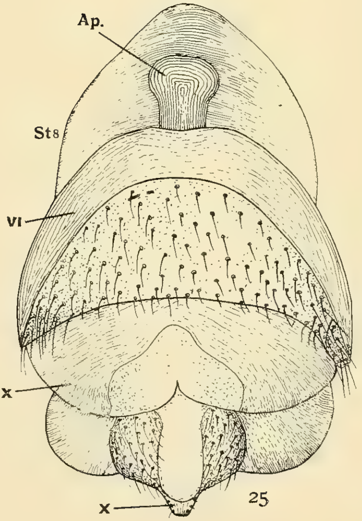

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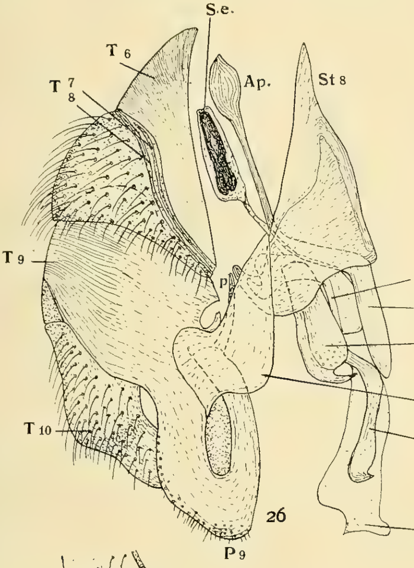

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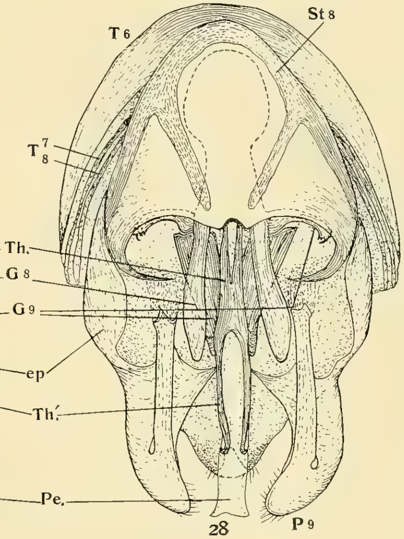

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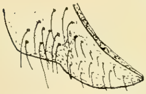

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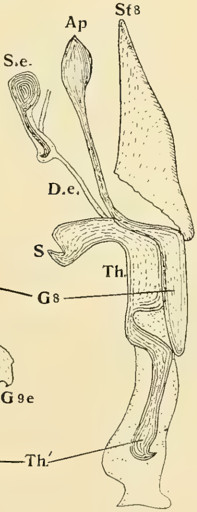

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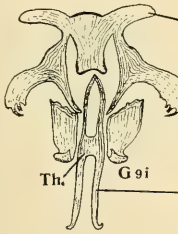

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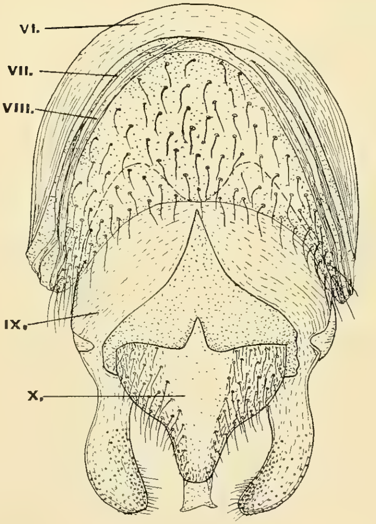

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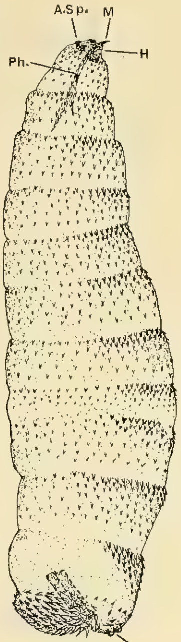

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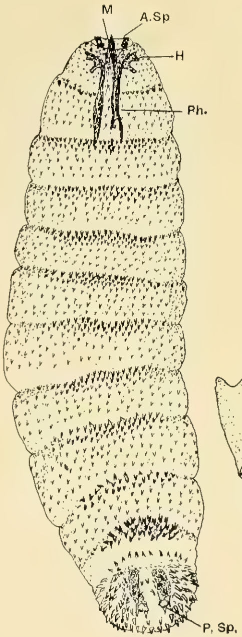

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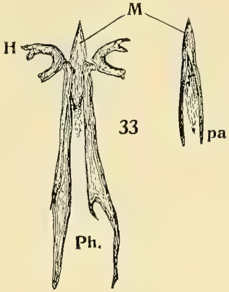

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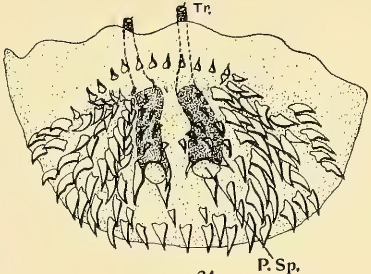

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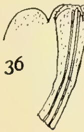

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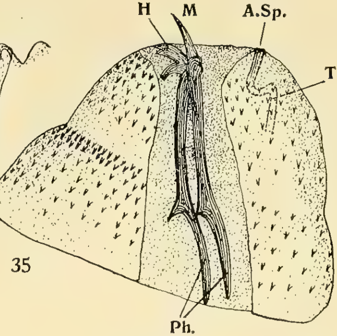

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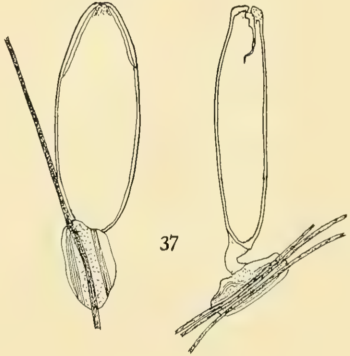

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T.R.H.

M'Farlane & Erskine

SCIENTIFIC PROCEEDINGS

OF THE

ROYAL DUBLIN SOCIETY.

Vol. XIV. (N.S.), No. 20. MAY, 1914.

ON THE LOCAL APPLICATION OF RADIUM IN THERAPEUTICS

BY

J. JOLY, Sc.D., F.R.S.

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1914.

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PLATE XXV. *Hypoderma lineatum.* Male.

Fig.

26. Terminal abdominal segments and Genital Armature. Lateral view. × 50. The ejaculatory sac. (S.e.) presenting its dorsal aspect.

26A. Tip of tenth abdominal tergum. Lateral view. × 50.

27. Theca of Penis with Gonapophyses. Postero-ventral view. × 50.

28. Terminal abdominal segments and Genital Armature. Ventral view. × 50.

29. The same. Dorsal view. × 50.

30. The Penis and associated structures. Lateral view. × 50 (Most of the pregenital Sternum (St.8) has been removed).

In figures 26–30, the lettering corresponds with that of figs. 22–25. See Explanation of Plate XXIV.

PLATE XXVI. *Hypoderma bovis.* First-stage Larva and Egg-shell.

Fig.

31. First-stage Larva. Lateral view. × 125.

32. The same. Ventral view. × 125. The tail-end of the maggot is directed ventralwards.

33. The pharyngeal sclerites (Ph.), mouth-hooks (H), and median spine (M). Ventral view. To the right the median spine (M) from the dorsal aspect, showing its origin from the union of paired parastomal sclerites (pa). × 325.

34. Terminal segment, with hinder spiracles (P. Sp.), surmounted by strong hook-like spines. × 325.

35. Front end of Larva. Lateral view. × 325. The bodywall has been partly removed to expose the pharyngeal sclerites and mouth-hooks. × 325.

In figures 31–35, M., median spine; pa., parastomal sclerites; H., mouth-hook; Ph., pharnyngeal sclerites; A. Sp., anterior spiracles; P. Sp., posterior spiracles; Tr., tracheal air-tubes; A., anus.

36. Anterior spiracle. Side view. × 560.

37. Empty egg-shells after hatching of larvae; two views showing slit at anterior pole. × 55.

XX.

ON THE LOCAL APPLICATION OF RADIUM IN THERAPEUTICS.

By J. JOLY, ScD., F.R.S.

[Read March 24; Published May 8, 1914.]

Within recent years the application of radioactive substances, such as radium emanation, or mesothorium, to malignant and pathological growths, is often carried out by introducing the substance contained in a closed tube into an incision made in the tissues. The tube is screened with lead folded around it to a thickness which is generally about two or three millimetres. The amount of radioactive substance used is seldom less than the equivalent of fifty millicuries of emanation. It may be as much as 300, or even more. The dosage may amount to the application of such quantities for from six to twelve hours, or longer. According to the statements of medical men having special experience in this branch of therapeutics, too feeble a radiation may do more harm than good, actually stimulating the morbid growth.

On the other hand, the screening of the tube is necessitated by the observed fact that too concentrated a radiation may give rise to deleterious effects arising from necrosis of the tissues. Now, close to the tube the rays are much concentrated; and not only is this the case from the mere geometrical conditions, but because the softer gamma rays, if they are permitted to escape, will here be absorbed. These amount to a large part of the total gamma radiation, and their concentration near the tube would give rise to a very intense illumination of the tissues. There are also the beta rays to be considered. These will be completely cut off by a screen of 3 mms. of lead. It is probable that these rays very materially affect the morbid tissues—they *may*, in *all* cases, be the immediate cause of the effects, beneficial or deleterious, observed. It may be recalled here that secondary beta rays are, in any case, powerfully excited by the gamma rays, where these emerge from the lead.

The arrangement described above is a wasteful one. A large percentage of what should be useful radiation is absorbed in the screen or filter. Moreover, it is the worst possible method for securing uniformity of illumination of the morbid tissues. It also fails to provide cross-radiation, a condition which has been found to give the best results. In order to overcome partly the last objection, two, and I believe as many as three, tubes have been used in the case of large tumours.

We can estimate the loss involved in screening, as above described, by reference to recent measurements made by Rutherford and H. Richardson.[1]

When radium, or its emanation, is used, the gamma rays concerned in the treatment are those from Ra B and Ra C. From the first substance three types of rays proceed—a very soft type, for which the absorption coefficient in aluminium is 230 $(\text{cm.})^{-1}$; a soft type for which the coefficient is 40; and a harder type for which the coefficient is 0·51. The rays from Ra C are homogeneous, and for them the coefficient μ is 0·115 $(\text{cm.})^{-1}$ in aluminium.

The softest rays emitted by Ra B are cut off by almost any envelope we choose to employ; they are, in fact, but little more penetrating than alpha rays. The second type will be reduced to ten per cent. of their original value by as little as one millimetre of aluminium. In this calculation we use the equation $I = I_0 e^{-\mu x}$, where x is the thickness of aluminium, and μ has the value 40. The hardest type of rays from Ra B will be transmitted in some degree, even through 0·3 cms. of lead. In this substance the coefficient for these rays has the value of 2·8 $(\text{cm.})^{-1}$. These rays must, therefore, be taken into account in considering the therapeutic application of radium or its emanation, and, along with these, the rays of Ra C, for which the coefficient in lead is 0·50 $(\text{cm.})^{-1}$.

The curves determined by Rutherford and Richardson (*loc. cit.*) are reproduced here. The vertical ordinates represent the ionisation effected in the air of an electroscope after the rays have passed through the thickness of lead plotted along the horizontal axis. The two types of soft rays from Ra B are, however, prevented from entering the electroscope by a suitable screen of lead. The beta radiation is deflected by a strong magnetic field.

Curve B defines the ionisation due to Ra B; curve C that due to Ra C. Curve A is the summation of the effects defined by the two curves C and B. We are only concerned with the summation curve in the remarks now to be made.

We can, from this curve, readily determine the loss due to a screen of 3 mms. of lead, such as is ordinarily placed around the tubes used in therapeutics. This we do by direct measurement of the areas enclosed by the curve and the vertical and horizontal ordinates concerned. The valuable part of the curve not being exponential, we cannot proceed by integration.

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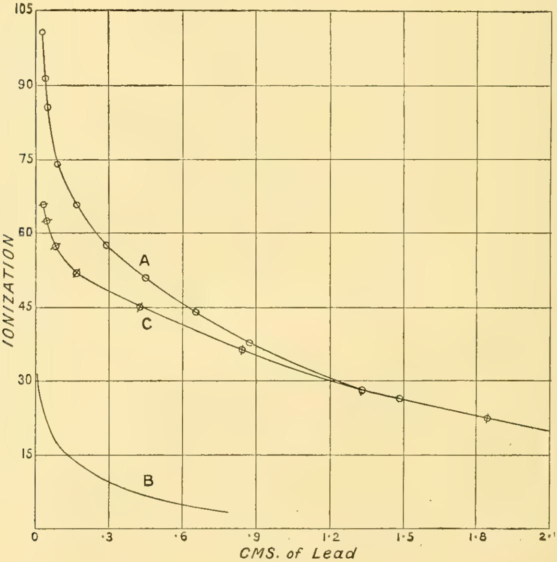
[1] "Phil. Mag.," May, 1913.

We must make some assumptions as to the volume of the tumour being dealt with.

(1) We assume the tumour to be an oblate spheroid, with diameter of 22 cms., and we suppose the tube to be placed axially and centrally, and the rays to be emitted normally to the axis of the cylinder. In this case the rays travel 11 cms. in the tumour. This we take as equivalent to about 1 cm. of lead; an assumption which may considerably depart from the truth, but error in which only involves the assumed dimensions of the tumour.

The area of the curve from 0 to 1·0 cms. of lead (which represents the path in the morbid tissues) is about 11·8 units, reckoning the squares into which the area is divided in the figure as units of ionisation. Now, when a screen is used, that part of the curve extending from 0 to the ordinate proper to the thickness of the screen is not involved, but the curve defining the ionisation now commences at the ordinate proper to the thickness of the screen, and extends to the right for a distance of 1 centimetre of lead, as before. In the present case, then, we find the area of the curve included between the



ordinates 0·3 and 1·3 to be about 9·0 units. The loss has, therefore, been rather more than 23 per cent.

(2). We assume the tumour to be 11 cms. in diameter. The area defining the ionisation from 0 to the radial limit is now 7·2 units. With screening by 0·3 cms. of lead the area is 5·2 units. The loss is in this case 29 per cent.

These figures, we may, of course, also say, define the *gain*, if for the lead screen we substitute one just sufficient to realize the conditions under which the curve is drawn; that is if we enclose the radium or emanation in such a tube as will just suffice to cut off all those rays of Ra B for which in aluminium the coefficient attains the value 40.

We have now to consider the beta rays. Such a screen will permit only a small part of the beta rays to pass through. The value of μ for these rays in aluminium varies from 90 to 13.[1] The softer beta rays proceed, as in the case of the gamma rays, from Ra B. Most of the beta rays from Ra C are much more penetrating. The velocities of the beta radiation from these two substances have lately been analysed by Rutherford and Robinson ("Phil. Mag.," October, 1913). From the coefficients just cited one millimetre of aluminium will transmit 28 per cent. of the fastest beta rays. As these constitute a part only of the total beta radiation, it may be concluded that a screen equivalent to one millimetre of aluminium will suffer only an unimportant percentage of the total beta rays to pass, and will stop, as already seen, practically all those soft gamma rays for which $\mu = 40$.

We have so far considered the mean loss of ionisation within the tissues due to the presence of a lead screen 0·3 cms. thick. The object of the screen is to reduce the intensity of the radiation near the tube. It is easy to estimate the diminution of intensity. In the absence of a screen other than one which cuts off the soft gamma and beta rays, the intensity near the tube is given by the ordinate to curve A at 0. This is 100. If 0·3 cms. of lead are interposed, the intensity falls, as shown by the curve, to about 56.

Now it is evident that a similar reduction of intensity near the tube would be obtained if the quantity of radium in the tube was diminished in the ratio of these figures. We might then dispense with the necessity of screening off some 25 to 30 per cent. of the rays, and at the same time secure immunity from too intense effects near the tube. But if we adopt this expedient, the average intensity of radiation in the morbid tissues becomes insufficient. We must, then, multiply the number of tubes if the illumination is to be sufficient and screening avoided.

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[1] "Practical Measurements in Radioactivity": Makower and Geiger; Longmans. 1912.

It is obvious that many advantages attend the use of several centres of radiation. We may omit wasteful screening. The illumination becomes more uniform. Cross-raying is favoured. The distribution of the illumination is under control. In short, the method possesses all the advantages which a number of small lights possess over one central lamp when a large space has to be illuminated. In the case of the radioactive bodies it is to be remembered that subdivision involves no loss of intrinsic efficiency.

In order to carry out the suggested conditions, I have, in conjunction with Dr. Walter C. Stevenson, worked out in some detail a system of applying radium, or its emanation, contained in ordinary exploring needles. The needles may be about $\frac{1}{2}$ to 1 mm. in internal diameter. They may be made of steel, gilt; or of platinum or gold. The stopping power of such needles and contained glass capillary would not fall short of that of a screen of aluminium 1 mm. in thickness. Their length may be determined according to the requirements of the case. They may be used in surface tumours or, in certain cases, inserted from without into more deeply seated growths, and withdrawn again from outside when the exposure is completed; or a canula may be used in such cases, permitting a repetition of the dose.

The radium bromide, sulphate, or chloride may be directly packed into the needle; or the salt, when fusible, may be sucked into an inner tube while melted. The inner tube may in this case be of refractory combustion tubing drawn down to a thick-walled capillary. But the simplest procedure is the use of radium emanation sealed in drawn-out capillary glass tubes. These glass tubes may, for ordinary purposes, be about 2 or 3 cms. in length. They are placed in the needle and retained in position by a little paraffin wax or sealing-wax closing the tube.

In the case of large growths, a relatively minute subdivision of the charge would probably be advantageous. Thus in place of a central tube containing 200 millicuries of emanation, there might be twenty needles each of 10 millicuries. From what has already been said above it will be evident that there is no reason to anticipate that such a charge contained within an ordinary needle would give rise to injurious effects around it. The intensity near the tube has, in fact, been reduced in the quantities involved from 200 to 10 or 20 to 1. On the other hand, there is increased intensity around the needle, owing to absence of a lead screen, and to the increased convergence of the rays. The increase due to convergence may stand as 1 to 10, or thereabouts.

The charging of the glass capillary tube with emanation presents no special difficulties. I would suggest a method allowing of rapid and accurate work.

The capillary tube is drawn out in one length, sufficient to make the number of separate capillaries required. Into this tube the purified emanation is brought in the usual manner. The whole tube is then sealed off. This operation is best performed by means of radiation from a short length of platinum wire, twisted into a spiral a couple of millimetres in diameter, and heated by a current while it surrounds the tube. This simple apparatus, mounted on a wooden handle, can be easily made. The advantage of this mode of sealing is that there is a perfectly clean closure made; the tube collapsing under the external pressure, without forming either a drawn-out thread or a bent termination which might refuse to enter the needle. The capillary may be broken off at the flattened closure without risk.

The long capillary, sealed at both ends, and containing the emanation diffused through its length, is hung in a small apparatus consisting of a number of platinum spirals placed one vertically above the other, and adjustable as to the distance separating them. This adjustment determines the lengths of the sections into which it is intended to subdivide the tube. A guide plate perforated with a small hole placed above each spiral prevents the glass capillary from touching the heated platinum.

When the long capillary is so hung as to pass through the several spirals, a current, sufficient to heat the spirals to full red, is switched on. In about thirty seconds the sealing is completed and the tube may be lifted out and broken into the small lengths intended for the needles. The simultaneous subdivision of the tube effected by this means secures that the charge is uniformly subdivided, and not driven by unequal heating from one part of the tube to another.

This will be found a very expeditious and simple process. The use of fine capillaries to hold the emanation does not limit unduly the strength of the charge. Thus the volume of emanation in equilibrium with a gramme of radium—that is 1000 millicuries—is but 0·5 cub. mm. While, then, we can load a needle with any required charge, we can also deal with partly spent charges more conveniently than by any other method. By using a needle of somewhat larger bore, and inserting two or more spent capillaries, a charge may be provided having the full value sought for: or, of course, the number of needles in application may be increased as the charge decays.

If strong charges and heavy screening are deemed preferable in any special case, the needle still furnishes the best form of applicator; for lead tubing of any required bore is easily procured, and may be slipped over the needle, furnishing a screened tube of comparatively small diametral dimensions.

I have in these remarks used the term 'illumination' as expressing the effects of the rays. This is justified by the fact now ascertained that the X and γ rays are similar in character to light-waves, although of a wave-length some ten thousand times less. This discovery makes the use of γ rays continuous with that of the Finsen light. They represent a much less easily absorbed luminosity proceeding from the radioactive substance as from a lamp: but a lamp so constituted physically as to meet therapeutic conditions in an extraordinary degree.

IVEAGH GEOLOGICAL LABORATORY.

THE
SCIENTIFIC PROCEEDINGS
OF THE
ROYAL DUBLIN SOCIETY.

Vol. XIV. (N.S.), No. 21. MAY, 1914.

NOTE ON THE CHANGE OF LENGTH IN NICKEL WIRE DUE TO SMALL LONGITUDINAL LOADS AND LOW ALTERNATING MAGNETIC FIELDS.

BY

WILLIAM BROWN, B.Sc.,
PROFESSOR OF APPLIED PHYSICS, ROYAL COLLEGE OF SCIENCE FOR IRELAND, DUBLIN.

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XXI.

NOTE ON THE CHANGE OF LENGTH IN NICKEL WIRE DUE TO SMALL LONGITUDINAL LOADS AND LOW ALTERNATING MAGNETIC FIELDS.

By WILLIAM BROWN, B.Sc.,

Professor of Applied Physics, Royal College of Science for Ireland, Dublin.

[Read April 21. Published May 19, 1914.]

In a very interesting and complete paper by Honda and Terada[1] on "The Change of Elastic Constants of Ferromagnetic Substances by Magnetisation," there are given amongst other data the results of experiments on the change of length in nickel wire under tensions varying from $1{\cdot}54 \times 10^5$ to $5{\cdot}24 \times 10^5$ grammes per sq. cm., while subjected to continuous longitudinal magnetic fields up to about 400 c.g.s. units. The wire employed was 74 centimetres long, and about 1 mm. in diameter, the magnetising coil being 80 cms. long and 5·8 cms. internal diameter.

It occurred to the present writer, when working at the subsidence of torsional oscillations in nickel and iron wires in alternating magnetic fields, that it might be well, before changing the apparatus for further experiments, to find the effects of fairly low alternating magnetic fields on the change of length in nickel wire when it was subjected to small longitudinal loads or tensions—an experiment which, as far as the writer knows, has not hitherto been made.

The solenoid used in the experiments was 236 cms. long and 2 cms. internal diameter, and contained 7707 turns of insulated wire in four layers, giving an internal magnetic field of 41 c.g.s. units per ampere. The effective

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[1] Phil. Mag., Jan., 1907, p. 36.

length of the soft nickel wire employed was 226 cms., its diameter being 1·675 mm., and it was in a uniform magnetic field throughout the entire length.

The magnetic fields applied, both direct and alternating, were up to 200 c.g.s. units as a maximum, and the three loads or tensions employed were $(0{\cdot}1184,\ 0{\cdot}5, \text{ and } 1) \times 10^5$ grammes per sq. cm. respectively. The loads were applied by means of a three-jaw clutch which gripped the lower end of the wire, and then a cord passing over two frictionless pulleys had a scale-pan on its end, in which were placed the weights. This method gave a perfectly steady tension on the wire, and at the same time kept it centrally inside the solenoid. The top end of the wire was caught by a similar three-jaw clutch, and firmly secured to the wall. On account of the great length of the wire under test, the change of length or contraction on application of the magnetic fields could be read off directly, by means of a reading microscope having a fine hair in the eye-piece. The movement of the hair was made by means of a micrometer screw with graduated head or cap; the circumference of the cap was divided into 100 divisions, and one whole turn gave a motion to the hair of 0·0208 cms.; therefore one division on the cap corresponded to a motion of 0·000208 cms.; or $9{\cdot}2 \times 10^{-7}$ per unit length of the wire. By turning the micrometer always in the one direction during the measurements, so as to avoid any small back-lash on the thread, it was found that the same readings for a given magnetic field could be obtained repeatedly.

The temperature of the room during the experiment was kept as uniform as possible, at about 17° C.: it was found, however, that when the longitudinal continuous magnetic field was round the wire, the wire became slightly warmed, as shown by the slight elongation observed in the microscope; and this heating of the wire was more pronounced when an alternating magnetic field was applied. For example, when a direct magnetic field of 166 c.g.s. units was put on for 5 seconds, and then taken off, the wire elongated for about 5 minutes to the extent of 7 divisions on the cap of the micrometer screw, or of value $\frac{dl}{l} = 6{\cdot}44 \times 10^{-6}$ cms.; and when an alternating magnetic field of root-mean-square value 156 c.g.s. units was put on for 10 seconds and then taken off, the elongation of the wire went on for about 5 minutes and then stopped, the amount of the expansion being $\frac{dl}{l} = 14{\cdot}7 \times 10^{-6}$ cms.

In order to avoid as much as possible any error in the readings of the microscope due to this heating of the wire by the magnetic fields, the following method of taking the readings was adopted:—The magnetic field was put on the solenoid, and the hair in the microscope eye-piece set on the given mark; the magnetic field was then taken off, and the hair again moved to the mark; the difference then gave the contraction of the wire for that magnetic field; that is, the contracted length of the wire was taken as the normal length in each case, and the apparent expansion of the wire as seen by the microscope was measured, when the magnetic field was off.

In order to compare the results obtained with alternating magnetic fields, measurements were made with direct magnetic fields, and the values obtained with the latter fields agree very well with those got by Honda and Terada, and show that though the loads or tensions on the wire used in the present experiments were smaller than the loads used by these Experimenters, the softness of the wires used in the two cases was nearly the same.

The alternating magnetic fields used, and the results obtained with them recorded in this paper, were all at a frequency of 50 per second, and are here expressed as root-mean-square values.

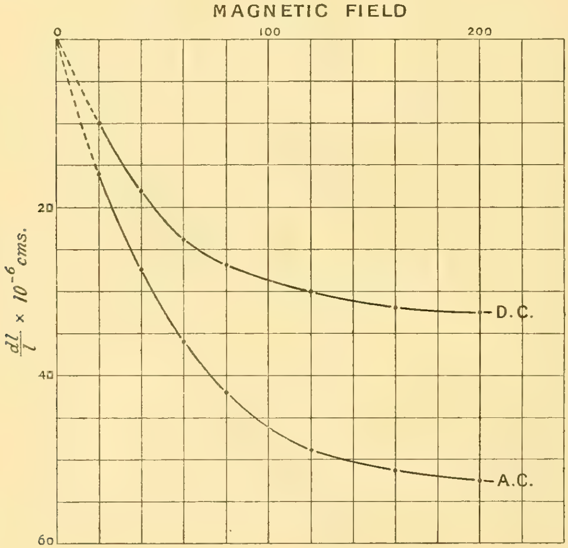
If the results were to be expressed in terms of the maximum values of the alternating magnetic fields, the effect on the A. C. curve in the figure would be to swing it to the right a little, and to raise it slightly.

Alternating magnetic fields of frequency 100 per second were also tried on the wire; the results, however, are not recorded here; but when they were plotted in a curve, it lay slightly below the curve obtained with the same load on the wire, and with fields of frequency 50 per second.

	$\frac{dl}{l} \times 10^{-6}$ cms.					
	$0{\cdot}1184 \times 10^5$		$0{\cdot}5 \times 10^5$		$1{\cdot}0 \times 10^5$	
H.	D.C.	A.C.	D.C.	A.C.	D.C.	A.C.
20	13	18	10	16	10	15
40	22	31	18	27·5	17	26
60	26	40	24	36	23	34
80	29	46·5	27	42	26	39
120	31·5	52	30	49	29	44
160	32·5	53·5	32	51·5	31	46
200	33·5	54·5	32·5	52·5	32	46

The results obtained with the three different loads, for both direct and alternating magnetic fields, are shown in the table, when the D. C. values and the A. C. values are placed side by side under the respective loads on the wire, each load being expressed as grammes weight per square centimetre.

The middle values, or those obtained with the load $0{\cdot}5 \times 10^5$ grammes per sq. cm., are plotted as curves in the figure where the abscissae are the values of H, the magnetic field round the wire, and the ordinates the corresponding values of the contraction of the wire expressed in millionths per unit length.



Comparing the last values in the columns of the table—that is, at the magnetic fields 200 c.g.s. units, or just about where the curves, when plotted, tend to become horizontal, we find that the amount of contraction in nickel wire for the alternating magnetic fields is greater than for the direct magnetic fields by about 63 per cent. for the smallest load and about 44 per cent. for the largest load here employed. Neglecting the values in the table for the low magnetic fields, it will be seen that the contraction for a given root-mean-square alternating field is nearly in a constant ratio to the contraction for the corresponding direct magnetic field, that is, the A. C. contraction is about 1·61 times the D. C. contraction with the smallest load on, 1·58 times for the middle load, and 1·49 times for the greatest load. Again, if we were to plot the A.C. contraction as ordinates, and the corresponding values of the maximum alternating fields as abscissæ, the multiplying factors would be 1·56, 1·50, and 1·37 respectively, in the order of the loads from the low to the high.

For assistance in reading the microscope I am indebted to the Rev. Br. P. V. Ryan, a third-year student in Physics of this College.

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POLYGAMOUS MENDELIAN FACTORS.

BY

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XXII.

POLYGAMOUS MENDELIAN FACTORS.

By JAMES WILSON, M.A., B.Sc.,
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[Read May 26. Published June 17, 1914.]

It is usual for Mendelian factors to mate with one other factor only: that is for dominants to mate with their own recessives and recessives with their own dominants, but with no other. The factors which produce the various colours in horses and cattle are exceptions, however, for any one belonging to either of these species can mate with any other belonging to the same species. This was observed when data for a paper "The Inheritance of Coat Colour in Horses"[1] were being collected in 1910; but at the time it was not realized that the phenomenon was extraordinary and required special demonstration; and two years later, a second paper, "The Inheritance of the Dun Coat Colour in Horses,"[2] was written without it being yet realized that the behaviour of the factors under consideration would have to be demonstrated.

Soon afterwards, however, it was seen, from the conclusions which other workers were arriving at, that the observation would have to be stated again with such proof as could be gathered. Accordingly it was decided to collect more data with regard to the crucial colours; and this was being done, as time permitted, until, when Dr. A. R. Walther kindly sent a copy of his "Beiträge zur Kentniss der Vererbung der Pferdefarben," published in 1912, it was seen that his data together with those which had been collected would be sufficient.

The earliest data pertinent to the question are those collected from the German royal studs at Ivenack and Trakehnen by Dr. M. Wilckens and Dr. H. Crampe and published in the "Landwirtschaftliche Jahrbücher" in

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[1] Sci. Proc. Roy. Dublin Soc., vol. xii (N.S.), p. 331.

[2] *Ibid.*, vol. xiii (N.S.), p. 184.

1887 and 1888. Unfortunately this was before the discovery of Mendel's work, and it is not unlikely that Dr. Crampe, had he known of Mendelism, would have formulated a complete scheme to explain the inheritance of horse colours: for he made two observations which would not only have given him a start but carried him some part of the way.

The first to deal with the question since the Mendelian discovery and to make an important advance was Mr. C. C. Hurst[1] who, from data collected in Weatherby's "General Stud Book," came to the conclusion that chestnut breeds true and is recessive to bay and brown: these two colours being taken together as one. Mr. Hurst dealt of course with Thoroughbreds only, among which there are now only five colours. Chestnuts, bays, and browns are by far the most numerous, but there are also some greys and perhaps a few true blacks; but most of the animals entered as blacks are browns of so dark a shade that they are mistaken for blacks. Mr. Hurst dealt with the first three colours. The figures on which his conclusions were based are these:—

	Chestnut foals.	Bay and brown foals.
(1) 101 chestnut sires with chestnut mares get . .	1095	9
(2) 12 bay and brown sires with chestnut mares get .	347	355
(3) 6 bay and brown sires with chestnut mares get .	0	370

The point to be noted at present is that the twelve impure bay and brown sires get 347 chestnut and 355 bay and brown foals, from which it can be inferred that chestnut differs from bay and brown in one pair of characters.

In the present writer's two papers bay and brown were assumed to be separate colours, and, although it was clear that neither was an intermediate, it could not be determined which was the dominant of the other. It is now quite apparent that the assumption was a mistake and that the two colours are really different varieties or shades of a single colour. For, if either were the dominant, one of the two would not produce the other when mated with chestnut. Yet both colours produce both colours by this mating, and they do so in numbers which indicate that the factor or factors which make bay and brown bay are dominant to those which make it brown: an inference confirmed by the fact that a chestnut sire, Cyllene, gets bays and chestnuts only when mated with browns and that an impure bay sire, Avington, breeds in a similar manner. This point was missed when the 1910 and 1912 papers were written.

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[1] Proc. Roy. Soc., B, vol. lxxvii, 1906, p. 388.

Dr. Crampe's two conclusions referred to above are:—

(1) Chestnuts have chestnut foals only, with chestnut roans and chestnut greys among them, and

(2) Blacks have both black and chestnut foals, and also roans and greys of these colours.

When we remember the liability of roan and grey to the error of misdescription, there can be no doubt that the first of these conclusions is the same as that arrived at independently by Mr. Hurst twenty years later. The second conclusion, however, points to black being a second dominant to chestnut; and this inference is confirmed by Dr. Walther's data. At Trakehnen the desire arose to have a section of the stud consisting of black horses only; and to accomplish this, the black mares which were already in the stud, no matter how they had been bred, were mated with black sires only.

The colours from which the black sires and dams had themselves been bred were as follows:—

			Sires.	Dams.
Black	×	Black	16	149
Black	×	Brown	3	14
Black	×	Chestnut	2	2
Brown	×	Brown	4	8[1]
Brown	×	Chestnut	5	1

These 30 black sires and 174 black dams had 574 foals, of which 506 were black and 68 chestnut. Thus black is a simple dominant to chestnut and differs from it in one pair of characters, for, if it differed in more, some other colour or colours would have been produced.

In Dr. Walther's paper "brown" stands for bay and brown together, and, since the two colours are really one, we may use the same designation in the rest of this paper.

It is the usual experience that if two conditions differ each from a third in one pair of characters, they differ from each other in two pairs, and the three conditions in question form the second, third, and first or fourth groups of a two-pair set of four groups. Thus brown, black, and chestnut should take the places indicated in the following set:—

X		X		x		x
Y		y		Y		y
9	:	3	:	3	:	1
?		Brown		Black		Chestnut

---

[1] From this it can be inferred that black is recessive to brown.

And, in a general population, containing pure and impure browns and blacks, the matings of brown and black should produce all the four colours of the set. This, however, browns and blacks do not do. They produce no fourth colour, but only themselves and chestnut. What is the explanation? Clearly there are not two pairs of factors. Clearly also, since there are three colours, there must be at least three factors. But how many factors are necessary for the production of each colour and how do they combine with each other?

Consider first how brown and black are related. In the Shire Stud-Book a number of sires have been found which produce bay foals only whether mated with brown or black mares; and Dr. Walther found a number of similar German sires. Thus brown is dominant to black. At the same time a number of Shire stallions were found which produced both brown and black foals when mated with brown, and *equal numbers* of browns and blacks when mated with black. Dr. Walther also found a number of German sires of the same kind. Thus brown differs from black in one pair of characters. The following table gives the actual figures. In both cases there are a few exceptions, but they are so few that they may be put down as errors either of description or parentage, and neglected. Horse colours are very liable to such errors for the reason that most of the animals are entered in their stud-books while they are foals, at which age dark brown is frequently mistaken for black and bay for chestnut. In the table the exceptions are enclosed within brackets. The column labelled "doubtful" includes such descriptions as "brown or black" and "bay or chestnut":—

	Colours of foals							
	with brown mares				with black mares			
	Br	Bl	Ch	Doubtful	Br	Bl	Ch	Doubtful
14 Shire sires gave	618	(2)	(2)	(4)	163	(6)	0	(1)
11 German sires ,,	—	—	—	—	200	0	(9)	0
16 Shire sires ,,	366	57	(3)	(2)	51	41	(1)	(1)
10 German sires ,,	141	28	(1)	0	105	98	0	0

By these figures brown is dominant to black and, by the fact that equal numbers of browns and blacks are produced when impure browns are mated with blacks, differs from it in one pair of characters.

Thus we have a series of three characters, chestnut, black, and brown, each of which differs from the other two in one pair of characters and in which chestnut is recessive to black and brown, and black recessive to brown.

From this it follows that each of these three colours is the result of one factor only; for, if not, and each were the result of two factors; then the constitutions would be, say, chestnut $\begin{matrix}xx\\yy\end{matrix}$, black $\begin{matrix}XX\\yy\end{matrix}$, and brown, $\begin{matrix}xx\\YY\end{matrix}$.

But then black and brown would differ from each other in more than one pair of factors, which is not in accordance with the facts.

Thus, since each colour is the result of only one factor, it is impossible for each to mate with more than one other at a time. Otherwise the zygote would obtain three "half" factors at once. This might be stated otherwise: brown is dominant to both black and chestnut, but it can mate with only one at a time, for otherwise it would be impure for both at the same time. Thus brown, black and chestnut are each the result of single factors which are polygamous.

If it were necessary this finding can be confirmed as follows. If brown, black, and chestnut be polygamous and each the result of a single factor, then in a horse population containing all three colours, there should be browns, blacks, and chestnuts of the following constitutions:—

Brown			Black		Chestnut
Br,	Br,	Br	Bl,	Bl	Ch
Br	Bl	Ch	Bl	Ch	Ch

and sires of each of these different kinds of bays and blacks should be found breeding in a definite manner when mated with a general population containing all kinds of mares, thus:—

		With brown mares			With black mares			With chestnut mares		
Br Br	should get	Br	0	0	Br	0	0	Br	0	0
Br Bl	,,	Br	Bl	0	Br	Bl	0	Br	Bl	0
Br Ch	,,	Br	Bl	Ch	Br	Bl	Ch	Br	Bl	Ch
Bl Bl	,,	Br	Bl	0	0	Bl	0	0	Bl	0
Bl Ch	,,	Br	Bl	Ch	0	Bl	Ch	0	Bl	Ch

It is impossible to predict the proportions in which each colour should occur from each mating, because that depends upon the persistence with which each of the three colours has been bred from in the past. The following table gives the numbers and kinds of foals produced by 84 Shire horses when mated with Shire mares of the three colours brown, black, and chestnut. It will be noticed that, among those 84 sires, the five expected kinds occur. The few exceptions in the progeny are again enclosed within brackets:—

	Colours of dams											
	Brown				Black				Chestnut			
Colours of foals	Br	Bl	Ch	Doubtful	Br	Bl	Ch	Doubtful	Br	Bl	Ch	Doubtful
Sires 14 browns .	615	(2)	(2)	(4)	163	(6)	0	(1)	68	0	(4)	(1)
18 browns .	386	57	(3)	(2)	51	41	(1)	(1)	35	9	(1)	(1)
36 browns .	1296	92	134	(5)	167	83	19	0	103	(16)	88	0
4 blacks .	13	12	0	0	(1)	19	0	0	(3)	11	0	0
12 blacks .	266	75	37	0	(5)	53	8	0	(1)	7	17	0

In the one case in which the discrepancies are serious, that namely in which 16 black foals are recorded as the progeny of chestnut mares and brown horses containing recessive chestnut, five of the sixteen are the progeny of a sire which was so very dark brown that he was frequently spoken of as black, and there can be very little doubt that these five foals were the colour of their sire.

But what of the three remaining colours, cream, dun, and grey? Are they also the result of single factors which are polygamous? It is scarcely conceivable they could be anything else, for if they were, a factor or factors in any of them would find no factor to pair with when these colours were mated with brown, black, or chestnut. It can be shown, however, that the six colours: chestnut, black, brown, cream, dun, and grey are each the result of single polygamous factors, and that they form a series in which each can mate with any of the others and each, taken in the order above, is recessive to all set down to its right.

If they do form such a series, then, since no colour can be impure for more than one other colour at a time, it should follow that:—

(1) No colour should produce any other colour than itself and its recessives unless mated with a colour which is its dominant.

(2) When a colour which is impure is mated with the colours lower in the scale of dominance than itself it should produce itself on the one hand and *an equal number of* all the other lower colours together on the other. Impure greys, for instance, should produce greys on the one hand and *an equal number of* all the other colours together on the other. For the grey factor must be carried off by half the progeny, and the recessive factor, whatever it may be, by the other half, and the non-grey colours will be of various kinds, depending upon the factors with which the recessive combines.

(3) An impure colour should produce no colour lower in the scale than

the recessive with which it is accompanied. Grey carrying brown, for instance, should produce no colour lower than brown.

(4) An impure colour carrying any recessive when mated with that recessive should produce itself only and that recessive. Grey carrying chestnut, for instance, should produce greys and chestnuts only when mated with chestnuts.

Perhaps the last of these results brings out the difference between the horse colours and characters of the ordinary nature. Since there are six colours at least, and dominance and recessiveness exist among them, there ought to be at least three pairs of factors, if the factors were normal. Call them X and x, Y and y, and Z and z. Chestnut, being the lowest recessive, should carry the characters $x\ y\ z$, and grey, being the highest dominant, as we shall see presently, should carry the factors $X\ Y\ Z$. Then impure grey might have any one of the following constitutions:—

$$\begin{array}{ccccccc} XX & XX & Xx & XX & Xx & Xx & Xx \\ YY & Yy & YY & Yy & YY & Yy & Yy \\ Zz & ZZ & ZZ & Zz & Zz & ZZ & Zz \end{array}$$

Some of these ought to produce several colours when mated with chestnut and, excepting the last one, no chestnuts at all. Take the fourth for example.

$$\begin{array}{c} XX \\ Yy \\ Zz \end{array} \times \begin{array}{c} xx \\ yy \\ zz \end{array} \text{ should produce } \begin{array}{c} Xx \\ Yy, \\ Zz \end{array} \quad \begin{array}{c} Xx \\ Yy, \\ zz \end{array} \quad \begin{array}{c} Xx \\ yy \\ Zz \end{array} \text{ and } \begin{array}{c} Xx \\ yy \\ zz \end{array}$$

The first of these constitutions would be grey, the other three would each be some other colour, but none of them would be chestnut. This, however, is altogether contrary to experience when grey is mated with chestnut.

Consider, first, the behaviour of grey, because it is easier to get data regarding that colour than the other two, dun and cream. But grey data must be used with care, for the reason that a grey horse is seldom, if ever, born grey; and many misdescriptions result thereby which have to be corrected where possible, or allowed for if not corrected. White foals are occasionally born, but the horse that eventually becomes grey is generally born a dull black. Grey hairs usually appear with the casting of the first coat, most conspicuously about the head. In a year or two the head is distinctly grey and the body well mixed. In seven or eight years the body is quite grey, and the legs, which are the last to be affected, show clear signs of greyness. Eventually all is grey, and if the horse live long enough, white. But the early stages are not the same in all, for some cast several coats before they are noticed to be grey. Thus many a grey, more especially those that die young or are early found to be of little value, remains in the stud book as of some other colour. Consequently we cannot look for the figures being in absolute accordance with expectation.

At one time there were many greys among English Thoroughbreds, but the great successes of some sires of other colours—among them Eclipse—combined with the belief that grey was not a good colour for speed, reduced the number of greys, and made the mating of grey with grey very exceptional. Thus it has been very difficult to find a sire pure for greyness. In the Thoroughbred Stud Book one only—The Coombe Arabian—has been found with enough foals upon which to form a judgment. A few years ago Mr. Robert Bunsow discovered an Arabian stallion, "Celle Amurath," the property of the Prussian Government, which was pure for greyness.[1] This sire got 600 foals from mares of all colours, and every foal was grey. In his researches Dr. Walther found another pure grey horse, Zigeuner, which got thirteen foals from grey mares and forty-six from mares of other colours, and all these foals were grey but one, which died young.

The following table gives the progeny of a number of grey Thoroughbreds (in vols. i to xxi) and Shires (in vols. i and xxxiii) with mares of all colours. The sires are set down with the date of their birth where that is known. The last three are Shires. Obvious exceptions are again put within brackets :—

	Colours of progeny			
Sires	Chestnut	Black	Brown	Grey
Coombe Arabian, 1740 ?	0	0	(1)	19
Panton's Arabian,	4	0	4	15
Crab, 1722	3	3	9	23
Delpini, 1781	10	0	23	46
Blank, 1740	4	0	13	14
Gimcrack, 1760	(1)	0	9	12
Starling, 1727	2	4	14	19
Bordeaux, 1774	2	0	12	29
Mambrino, 1768	4	0	8	19
Strathconan, 1863.	37	0	71	92
Pepper and Salt, 1882	20	0	17	38
Grey Leg, 1891	34	0	22	54
Grey Friars, 1883	8	1	37	48
Buchanan, 1877	(1)	0	29	28
Sir John Falstaff, 1873	5	4	9	11
True Briton, 1858	0	0	35	24
Lincolnshire Lad II, 1872	(1)	(2)	79	64

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[1] See "The Mendel Journal" for February, 1911.

When this table is examined—the figures relating to the Coombe Arabian being left out—it will be seen that in the mating of grey with other colours (there are very few grey with grey matings in the table) the grey foals are equal in number to all the other foals together. The actual figures are 555 grey, and 542 of other colours. It will also be seen that Gimcrack, Buchanan, True Briton and Lincolnshire Lad II get no foals lower in the scale than brown. Thus two of the results expected, on the assumption that the colours dealt with are the result of single polygamous factors, are found actually to occur.

A more detailed analysis of the matings of five of the foregoing sires will bring out a third result, namely that the mating of grey horses carrying chestnut recessive produces greys and chestnuts only. The mating results of the same five horses with other colours may also be put down, as those of the first four show the absence of the black colour among Thoroughbreds:—

	Colours of dams															
	Chestnut				Black				Brown				Grey			
Colours of foals	Ch	Bl	Br	Gr	Ch	Bl	Br	Gr	Ch	Bl	Br	Gr	Ch	Bl	Br	Gr
Sires																
Strathconan . .	15	0	(3)	24	0	0	0	0	22	0	68	68	0	0	0	0
Pepper and Salt .	7	0	0	17	0	0	0	0	13	0	17	20	0	0	0	1
Grey Leg . .	15	0	(1)	13	1	0	0	4	18	0	21	37	0	0	0	0
Grey Friars . .	4	0	(3)	15	0	0	(1)	4	4	1	33	29	0	0	0	0
Sir John Falstaff .	2	0	0	2	0	2	(2)	0	2	2	7	5	1	0	0	4

The first of the expected results can be brought out by the matings concerning dun, and the position of dun in the series can be determined at the same time.

Dun is a very scarce colour, more especially in registered breeds, and data have been very difficult to find. In all only about 200 matings have been met with, collected from the Thoroughbred, Clydesdale, and Pony Stud Books and from private sources. The results are given in the following table:—

Colours of parents	Colours of foals				
	Ch	Bl	Br	Dn	Gr
Dn × Ch	1	0	7	9	0
Dn × Bl	0	6	3	7	0
Dn × Br	2	3	33	39	0
Dn × Dn	0	0	0	8	0
Dn × Gr	1	3	10	21	12
Dn × Unknown	6	0	20	28	2

It will be noticed that, when dun is mated with chestnut black and brown, the total number of these three colours together is equal to the number of duns, but that no greys are produced. On the other hand, when dun and grey are mated, grey foals are produced. Thus dun is dominant to chestnut, black, and brown, and recessive to grey. This finding is confirmed by the fact that seven dun horses were found, none of which had a dun, but each of which had a grey parent. Two were the progeny of grey and grey, three of grey and black, one of grey and bay, and one of grey and brown. And it was confirmed farther by the use of three dun sires sent in succession to Clare Island on the coast of Galway by the Irish Congested Districts Board. There were no other sires on the island, and the mares were bays and browns, with a few blacks and one or two greys and chestnuts. The progeny of the first two sires were all duns, while the last, which had dun mares to mate with, got three quarters of his foals dun, excepting one grey foal, which was out of a grey mare.

In the paper of 1912 the position of cream could not be determined further than to say it was recessive to dun and dominant to chestnut, but Dr. Walther found data which show it to be dominant to black and brown. He found horses which carry what he calls yellow "ground pigment," namely duns and creams, dominant in colour to those carrying red "ground pigment," namely bays and browns and chestnuts. He also found yellow pigmented horses with black "points," namely duns, dominant to similarly pigmented horses without black "points," namely creams.

Thus we have the series: Chestnut, black, bay and brown, cream, dun, and grey in which each of the colours in the order set down is recessive to all those to the right and each is the result of a single polygamous factor.

It may be remarked that Dr. Walther's conclusions, if the "absences" be substituted by what they signify, put the six colours in the same series of dominants and recessives. This will be seen if his conclusions are set down in parallel columns: one containing them approximately as expressed, and the other as altered :—

As expressed.	As altered.
(1) Grey is dominant to its absence.	(1) Grey is dominant to all other colours.
(2) Yellow ground-pigment is dominant to red.	(2) Dun and cream are dominant to brown, black, and chestnut.

As expressed.	As altered.
(3) The presence of black pigment is dominant to its absence.	(3*a*) Duns with black manes and legs are dominant to those without, i.e. dun is dominant to cream.
	(3*b*) Browns and blacks are dominant to chestnut.
(4) The restriction of black to the mane, tail, and legs is dominant to its general distribution.	(4) Brown is dominant to black.

It may also be stated that data are being collected to show that cattle colours are also the result of single polygamous factors, but, as these data have to be collected from cross-bred stock, their accumulation is comparatively slow, and they are still too few for publication.

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THE LARVA AND PUPARIUM OF THE FRIT-FLY.

BY

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RESEARCH SCHOLAR IN THE ROYAL COLLEGE OF SCIENCE, DUBLIN.

[COMMUNICATED BY PROFESSOR GEO. H. CARPENTER.]

(PLATE XXVII.)

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XXIII.

THE LARVA AND PUPARIUM OF THE FRIT-FLY.

By THOMAS R. HEWITT, A.R.C.Sc.I.,

Research Scholar in the Royal College of Science, Dublin.

[COMMUNICATED BY PROFESSOR GEO. H. CARPENTER.]

(PLATE XXVII.)

[Read MAY 26. Published JUNE 18, 1914.]

THIS species (*Oscinis frit* Linn.) is one of the worst cereal pests in Europe. It is common in Great Britain, and in recent years has been reported as doing considerable damage to cereals in several counties in Ireland. (See Carpenter, 1902, 1913.) Accounts of the life-history are given in the memoirs of Bos (1891), MacDougall (1912), and Theobald (1906), referred to at the end of the paper. None of these, however, gives a satisfactory description of the larva, and having material from Cookstown, Co. Tyrone, at my disposal, I have made as full a study as possible of the early stages. I am indebted to the kindness of Dr. Stewart McDougall for mounted specimens of the puparium and very young larva.

The fully grown maggot (Plate XXVII, fig. 1) measures about 3 mm. long, and ·3 mm. in thickness. The body, as is usual among Dipterous larvæ, has eleven segments, is legless and fairly uniform in thickness, except at the head region, which tapers slightly. The head region bears two one-jointed papillæ or feelers (figs. 1, 2, 3 F); below these are two slight prominences, which when highly magnified appear as a number of small outlined areas arranged in the form of a crescent surrounding a circular central area; each area carries a small central spine (fig. 4). These areas appear to be thickenings of the cuticle; and the spines probably serve as sense-organs. The dorsal surface of the head region bears a number of hook-like spines (fig. 2 Sp.) directed backwards; and the cuticle is thickened in transverse ridges (fig. 2, r) from these spines towards the mouth. There are also a few spines on the dorsal and anterior margin of the two foremost body segments (see fig. 1). The cuticle of the body is quite smooth, except for the above hooks and a few very minute spines on the ventral surface. The body is ridged or thickened at the junction of the segments.

Behind the small sensory prominences is situated the mouth (fig. 1 m.), out of which are protruded the mouth-hooks or "mandibles." The cephalopharyngeal skeleton (fig. 3 C. Sk), which lies in the mouth and gullet, consists of a number of paired chitinous sclerites. The lateral plates are long and narrow and deeply bifurcate posteriorly (fig. 3 l. p.). The hypostomal sclerites (fig. 5 h. s.) are broad and short and situated between the mouth-hooks and lateral sclerites. The mouth-hooks (fig. 5 m. h.) are short and strong, each having two blunt teeth behind the prominent apex. In the dorsal wall of the gullet and just above the hypostomals, are situated the pair of parastomal sclerites (figs. 5 and 6 p. s.); each has two parts, a broadened basal, and a pointed anterior, part. In the lateral walls of the pharynx, behind the hypostomals, there are two small hook-like sclerites (fig. 5 s.), occupying the same position as similar structures figured by Banks (1912) in several dipterous larvæ.

At the posterior margin of the foremost body segment are situated the pair of anterior spiracles (figs. 1 and 3 A. Sp.); each has four branches (fig. 7 a), and the spiracular opening has the appearance of a very fine sieve when highly magnified (fig. 7 b).

The large tail-segment bears posteriorly the hind pair of spiracles, situated on prominent outgrowths of the segment which project backwards (fig. 1 P. Sp.). Each posterior spiracle has three branches (figs. 8 and 9); the spiracular opening is bounded by a thick chitinous ring. The adjacent cuticle has four very peculiar sets of radiating thickened ridges (fig. 9 th.), situated between openings of the spiracular branches, and they probably serve as a protection to the spiracles, admitting air and excluding foreign material when the spiracles are retracted. The anus is situated at the extremity of the minute anal segment which projects ventrally from the spiracular segment just described. This anal segment is strongly chitinised, and a semicircular anal proleg (fig. 1 a. p.) can be seen at each side of the medial slit (fig. 10 a).

The puparium (fig. 11) is red-brown, about 2·5 mm. long and 1 mm. broad. The posterior spiracles are very prominent at its posterior end (fig. 11 P. Sp.), and the larval mouth-hooks can be clearly seen near the head end. The outline of the developing fly can be distinguished in the cleared specimen, showing the contour of the body and the rudiments of the wings and legs.

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[EXPLANATION OF PLATE.

EXPLANATION OF PLATE.

Larva and Puparium of *Oscinis frit.*

Fig.

1. Fully grown Maggot. Lateral view. *m.*, mouth ; *f.*, feelers ; *a. sp.*, anterior spiracles. *a. p.*, anal proleg ; *p. sp.*, posterior spiracles. × 30.

2. Head region of maggot. Lateral view. *f.*, feelers ; *sp.*, spines ; *r.*, thickenings of cuticle. × 50.

3. Anterior segments of young maggot. Ventral view, cleared, showing *c. sk.*, cephalopharyngeal skeleton (somewhat displaced) ; *f.*, feelers ; *a. sp.*, anterior spiracles ; *l. p.*, lateral plates. × 100.

4. Small head prominence. × 620.

5. Lateral view of mouth-hooks, and part of cephalopharyngeal skeleton. *m. h.*, mouth-hook ; *h. s.*, hypostomal sclerite ; *p. s.*, parastomal sclerites ; *s.*, small lateral sclerite. × 430.

6. Parastomal sclerites. Ventral view. × 430.

7. Anterior spiracle (*a*). × 210. (*b*) spiracular opening. × 620.

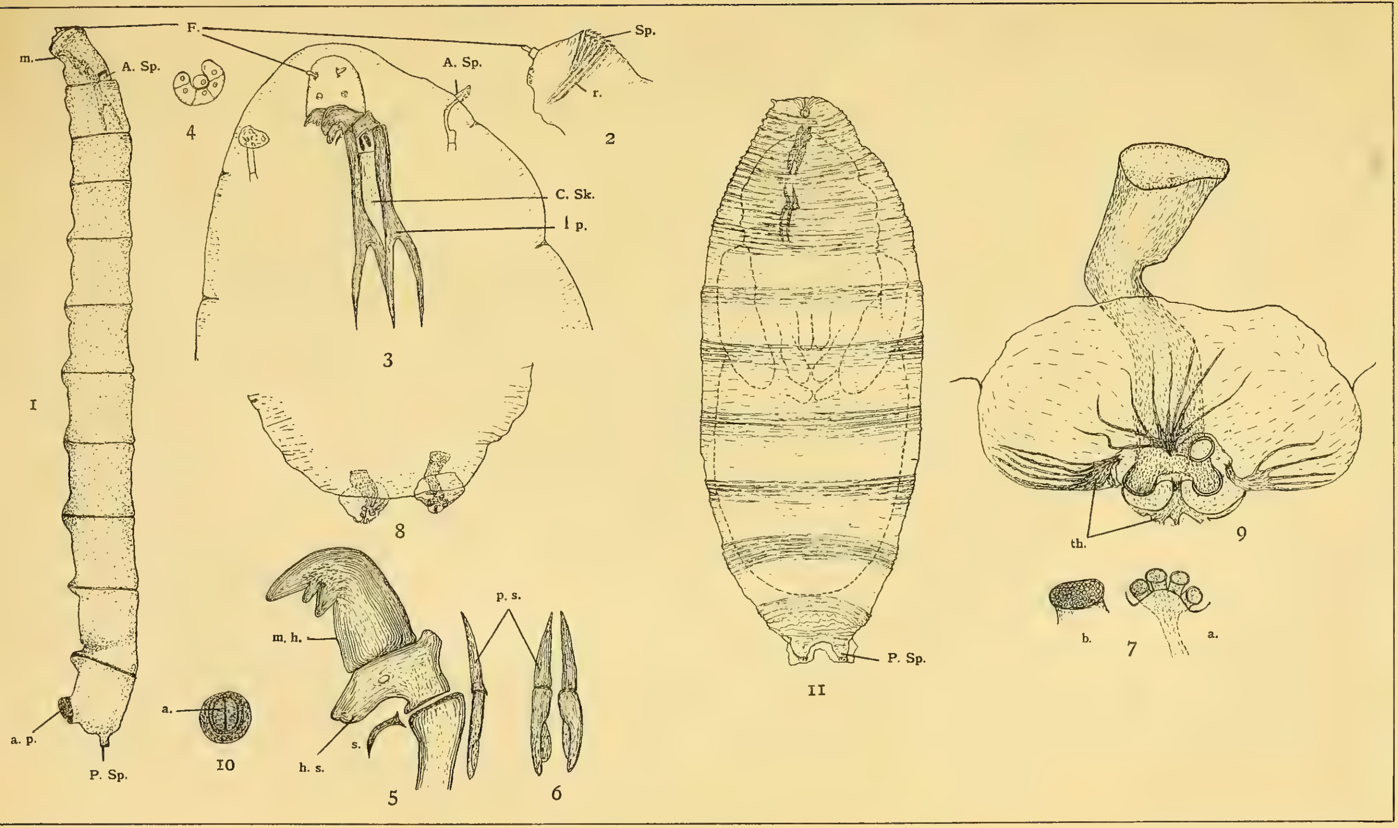
8. Posterior spiracles. × 100.

9. Posterior spiracle. *th.*, thickenings of cuticle. × 620.

10. Anal prolegs and anus (*a*). × 50.

11. Puparium. Ventral view. Outline of pupa visible within. *p. s.*, posterior spiracles. × 45.

PLATE XXVII.



T. R. H. *del.*

M'Farlane & Erskine, Edinburgh.

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OXIDASES AND THEIR INHIBITORS IN PLANT TISSUES.

Part IV.—The Flowers of Iris.

BY

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[COMMUNICATED BY PROFESSOR H. H. DIXON, F.R.S.]

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XXIV.

OXIDASES AND THEIR INHIBITORS IN PLANT TISSUES.

Part IV.—THE FLOWERS OF IRIS.

By W. R. G. ATKINS. Sc.D., F.I.C.,
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[COMMUNICATED BY PROFESSOR H. H. DIXON, F.R.S.]

[Read November 24, 1914. Published January 5, 1915.]

In Part II of this work an account was given of the peroxidase reactions of about thirty Iris flowers of various species. In the present paper the list has been considerably extended, and much of the previous work has been repeated, with a view to finding out how far the activities of oxidising enzymes are dependent upon the age of the flower, and other factors such as illumination.

Undoubtedly unopened buds of forms having an anthocyan pigment are of a deeper colour than the mature flowers. This is due partly to the rapid enlargement of the cells, with consequent dilution of the pigment, and partly to the fading of the colour in sunlight. The results obtained here go to show that the peroxidase reactions in buds and mature flowers differ neither in distribution nor in intensity. Withered flowers, however, give erratic results, as might be expected.

The reagents were applied as described in the previous papers, but Merck's perhydrol, suitably diluted, was employed instead of ordinary commercial hydrogen peroxide, which is slightly acid and requires to be neutralized. In every case the falls, standards, styles, and stamens were tested, but the reactions recorded are those of the falls and standards unless the contrary is stated. It was observed that the stigmatic surface and central vein of the style gave well-marked reactions in almost every case, and in none was there a complete absence of peroxidase activity. The pollen sacs, too, contained active peroxidase. These Iris reactions are all of the indirect or peroxidase type.

Some flowers, such as those of the large "Canadian" variety of *I. pseudacorus*, show intense actions throughout. Accordingly, it was suspected that the foliage leaves of this species might be free from the reducing inhibitor met with in those of *I. germanica*. Such a supposition was found to be incorrect, for the leaves of the above-mentioned *I. pseudacorus*, of *I. Xiphium*, *I. xiphiodes*, and *I. pumila*, all failed to turn guaiacum tincture blue. On testing the tips of the leaves separately it was only in *I. pumila* that an active peroxidase was detected. The blue colour it produced was quickly destroyed when a fragment of the lower portion of the same leaf was added to the solution.

The following tables record the behaviour of the various groups of Iris:—

Table I.

Apogon Group.

No.	—	Benzidine		α-Naphthol	
		Veins	Epidermis	Veins	Epidermis
1	*I. unguicularis*, Poir., mature flower,	+ +	+ +	+ +	+ +
2	*I. unguicularis*, bud, blue colour slightly deeper,	+ +	+ +	+ +	+ +
3	*I. unguicularis*, mature,	+ +	+ +	+ +	+ +
4	*I. unguicularis*, blue colour slightly deeper, bud just opening,	+ +	+	+ +	+
5	*I. unguicularis*, young bud, colour deeper still,	+ +	+ +	+ +	+ +
6	*I. unguicularis alba*,	+ +	+	+ slight	—[1]
7	Ditto,	+ +	+	+ slight	—
8	*I. unguicularis* var.,	+ +	+	+ slight	—
9	Ditto,	+	+ slight	—	—
10	*I. unguicularis speciosa*,	+ +	+	—	—
11	Ditto,	+ +	+	+ slight	—
12	Ditto, unopened bud,	+ +	+	+ slight	—

Discussion of Results of Table I.

The similarity of the reactions of the buds and mature flowers in the different varieties of this Iris are clearly shown in the table. It will be noticed that here, as usual, the α-naphthol reaction is more sensitive to the inhibitor than is the benzidine.

It seems worthy of mention that the first five were examined on February 6. These give pronounced reactions. The remaining seven were tested on February 19, and the α-naphthol reactions were feeble, or negative. It is possible that this may have been due to differences in illumination. The question will be discussed later on.

---

[1] This denotes the absence of any reaction.

TABLE II.

Apogon Group.

No.	—	Benzidine		α-Naphthol	
		Veins	Epidermis	Veins	Epidermis
13	*I. longipetala*, Herb., standards and falls, .	+ +	+	+ +	—
14	*I. sibirica alba*, Linn., standards and falls, .	+	—	+ +	+
15	*I. orientalis*, Thunb. non Miller, ...	+ +	+	+ + +	+
15A	Ditto, ...	+ +	—	+ +	—
16	*I. graminea*, Linn., ...	+ +	+ +	+ +	+ +
17	*I. pseudacorus*, Linn., wild; no markings on claw of falls,	+ +	+	+ +	—
18	*I. pseudacorus*, wild; brown markings on claw, .	+ +	+	+ +	—
19	*I. pseudacorus foliis variegatis*, ...	+ + +	+ + +	+ + +	+ + +
20	*I. pseudacorus*, Linn., large variety from Canada,	+ + +	+ + +	+ + +	+ + +
21	*I. pseudacorus*, like 18, but pale creamy yellow,	+ +	+ +	+ +	+
22	*I. foetidissima*, Linn., mature	+	—	+ traces	—
23	Ditto, ... old, ...	+	—	+ traces	—
24	Ditto, ... mature, ...	+ +	+	+ +	+
25	*I. Monnieri*, DC., α form fairly deep yellow, .	+ +	+	+	—
26	*I. Monnieri*, DC., β form lighter yellow, .	+ +	+	+	—
27	*I. Monnieri* β, young flower just opened, . .	+ +	+	+	—
28	*I. ochroleuca*, Linn., old flower, ...	+ +	+	+ +	+
29	*I. Kaempferi*, Siebold, white, with pink edges, .	+ + +	+ +	+ + +	+ +
30	*I. Kaempferi*, pink red, ...	+ +	+	+ +	+
31	*I. fulva*, Muhl., ...	+ +	+	+ +	—

Discussion of Results of Table II.

It is frequently hard to decide whether a slight colour in the epidermis is really due to oxidase action, such as represented by a single plus sign, or whether it is due to staining, as the benzidine solution is never quite colourless. With α-naphthol this difficulty is not so great.

On the whole, the members of this group of widely divergent forms show well-marked reactions. Of special interest is the behaviour of Nos. 17–21.

No. 20 was brought from Canada to the Botanic Gardens of Trinity College, Dublin, many years ago. It now grows luxuriantly in the shallower part of a pond. Its leaves are 4–6 feet high, and Mr. Dykes kindly identified it as a typical *I. pseudacorus*, which has gone wild in Canada. Nos. 17 and 18 are also quite typical *I. pseudacorus*, and grow in marshy ground on the banks of the Shannon in Co. Clare. With the exception of these two, all the other plants tested came from the College Gardens. The form with variegated leaves, and the creamy variety, are much smaller than the Canadian plant. The cream-coloured flower, according to Mr. Dykes, appears sometimes as a seedling from the normal yellow-flowered plant. The plastids in it are of a much lighter colour than is usual. Inspection of the table shows that the reactions are intense in the Canadian and variegated leaved plants. The cream-coloured flower is No. 5 of Part I, which is incorrectly named there, and is No. 31 of Part II. The Canadian form is No 4 of Part I and No. 30 of Part II of this series.

These reactions, obtained in 1913, agree well with those observed this year. The Co. Clare plants are Nos. 28 and 29 in Part II, and the inhibitor appears to be present in somewhat greater quantity than in the 1914 plants from the same district. However, there is no doubt that there is a difference in the peroxidase activity in these varieties of *I. pseudacorus*. Next year, however, it may be possible to examine a larger number of flowers of the same plant, and so obtain more decisive evidence as to the existence of these physiologically distinct varieties.

It is frequently observed that the severed ends of standards and falls give intense reactions with both reagents, even in cases where the veins themselves give little or no reaction. This has been attributed by Keeble and Armstrong to the production by the injured cells of wound peroxidase. It appears quite probable, however, that the activity of the enzyme is in this case due to the diffusion outwards of the inhibitor. For it has previously been shown that treatment with hydrogen cyanide serves to remove it, and that it can be dialysed away from sap pressed from the leaves, thus demonstrating the presence of the enzyme where it was formerly believed to be absent. Solutions of carbon dioxide were also found by Keeble and Armstrong to remove the inhibitor. Since both hydrogen cyanide and carbon dioxide render the protoplasm more permeable without coagulating it to the same degree as do alcoholic solutions, there seems to be reason for supposing that it is to this physical action, rather than to a chemical change, that the removal of the inhibitor is due. For diffusion is now unchecked.

Table III.

Pogoniris Group.

No.	—	Benzidine		α-Naphthol	
		Veins	Epidermis	Veins	Epidermis
32	*I. pumila*, Linn., purple,	+ +	+	+ claw	—
33	Ditto, purple,	+ +	+	—	—
34	Ditto, purple,	+ +	+	+ +	+
35	*I. pumila*, yellow (*I. Attica*),	—	—	—	—
36	Ditto, yellow,	+	—	+	—
37	Ditto, yellow bud,	+	—	+ claw	—
38	*I. germanica*, Linn., young flower,	—	—	—	—
39	Ditto, young flower,	—	—	—	—
40	*I. germanica major*,	+ traces	—	—	—
41	*I. germanica purpurea*,	+ + in traces	—	—	—
42	*I. germanica* var. *florentina*,	+ in parts	—	+ traces	—
43	Ditto, bud,	+ traces	—	—	—
44	*I. germanica* var. *florentina*, form *albicans* (Lange), viz. Princess of Wales.	+	—	+	—
45	Ditto, bud,	+ traces	—	—	—
46	*I. flavescens*, DC., var. *Munito*,	+	—	+	—
47	*I. flavescens*, var. *Redouté*,	+ traces	—	+ traces	—
48	*I. flavescens* var.,	—	—	—	—
49	*I. pallida*, Linn.,	+ +	+	+	—
50	Ditto,	+	+	+ trace	—
51	*I. pallida*, var. *dalmatica*,	+ +	—	+	—
52	*I. pallida*, var. "Queen of May,"	+ +	—	+ +	—
53	*I. Victorine*, Hort.,	+ traces	—	—	—
54	*I. variegata*, Linn., var. falls,	+	—	+	—
	standards,	—	—	—	—
55	*I. variegata*, var. standards,	—	—	—	—
	falls,	+	—	+	—
56	*I. variegata*, var. falls,	+ traces	—	—	—
	standards,	—	—	—	—
57	*I. variegata*, var., falls and standards,	—	—	—	—
58	*I. Kochii*, Kerner,	—	—	—	—
59	I. "Mrs. Horace Darwin," hybrid,	+ +	+	+ +	+
60	I. "Mrs. Langtry,"	+ +	+	+ +	—

Discussion of Results of Table III.

The outstanding feature in this class is the absence of active peroxidase in all except two hybrids and the purple form of *I. pumila.* One is tempted to regard the yellow form of *I. pumila* as differing from the purple in the possession of an inhibitor in the veins, but more especially in the epidermis of the flower. But if oxidases really do play the important rôle in the production of anthocyan pigments which they are usually supposed to do, it is very hard to explain the absence of reaction in the many deep purple forms included in this group. Possibly the diffusion of the inhibitor from other cells may account for the difficulty. The whole question, however, leaves room for much further work.

TABLE IV.

Xiphion Group.

No.	*I. xiphioides* (Ehrb.)	Benzidine		α-Naphthol	
		Veins	Epidermis	Veins	Epidermis
61	Falls and standards pale blue, ...	+ + +	+ + +	+ + +	+ +
62	Falls and standards dark blue, ...	+ + +	+ + +	+ + +	+ +
63	Falls white, with blue veins and edges. Standards blue,	+ +	+	+	—
64	As in 63, but deeper blue and less white, . .	+ +	+	+ traces	—
65	Deeper purple blue than in 64, and less white in centre,	+ + +	+ +	+	—
66	As in 64, but intense red purple or claret colour,	+ + +	+	+	—
67	As in 66, but very pale red purple or lavender, .	+ + +	+	+ +	—
68	Very like 67, but paler, ...	+ + +	+	+ +	—
69	Very delicate lavender blue, ...	+ + +	+	+ +	—
70	Like 67, but lavender blue, light, with darker spots,	+ +	+ +	+ +	+
71	As in 67, but spots on claw and splashes on blade are red,	+ + +	+ +	+ +	+
72	Standards white, claw of falls tinged with pink, rest white, with pink splashes,	+ +	+ +	+ +	+
73	White, very pale splashes on some standards only,	+ +	+ +	+ +	+
74	Deep blue, almost black at edges of falls and in most of standard; flower just opened,	+ +	+	+ +	+
	I. Xiphium, Linn.				
75	Var. *lusitanica*, Ker., form Thunderbolt, falls, .	+ + +	+ + +	+ + +	+ + +
	,, ,, ,, ,, standards,	+	—	+ +	—
76	Var. *chrysolora*, Hort., falls, ...	+ + +	+ + +	+ + +	+ + +
	,, ,, ,, standards, . . .	+ + +	+	+ + +	+
77	Form yellow fall, blue standard, . . .	+ + +	+ + +	+ + +	+ + +
78	Form blue fall, blue standard, ...	+ + +	+ + +	+ + +	+ + +
79	*I. reticulata*, M. B., deep purple, with orange line on claw of fall, around which is a littl white. Very young bud,	+	+	—	—

Discussion of Results of Table IV.

A general survey of the table leaves one with the impression that there can be little connexion here between the intensity of natural anthocyan pigmentation and that of the peroxidase reactions. Evidently the amount of chromogen is the controlling factor. In *I. Xiphium* the activity of the peroxidase is very great, yet over the yellow "signal" on the claw of the fall there is in every case a sharply marked inhibition area, which remains uncoloured by the reagents, and as the plastid yellow goes into solution in the treatment with alcohol, this region stands out very clearly. One cannot fail to be impressed with the view that in this case the peroxidase is concerned in the production of anthocyan, for the limits of distribution of the former coincide with that of the latter. In *I. reticulata,* however, deep pigmentation occurs together with little or no oxidase activity.

TABLE V.

Miscellaneous.

No.	—	Benzidine		α-Naphthol	
	JUNO				
		Veins	Epidermis	Veins	Epidermis
80	*I. orchioides,* Carrière, ...	+ + +	+	+ traces	—
	HERMODACTYLUS				
81	*I. tuberosa,* Linn., old flower, ...	+ + centrally	—	+ trace	—
82	*Marica gracilis,* mature, ...	+	+	+	—

Discussion of Results of Table V.

In this table, too, there seems to be but little relation between peroxidase activity and anthocyanin. *I. tuberosa,* known as the "Widow Iris" from its almost black colour, contains large masses of solid pigment in the cell vacuoles. Its small oxidase activity may possibly be accounted for by the fact that the flower was a very old one, somewhat withered.

The Effect of Darkness upon the Peroxidase Content of Flowers of Iris.

Before concluding definitely that the reactions shown in the tables have the meanings attributed to them, viz., that they are genuine expressions of the properties of particular species and varieties, it is necessary to inquire into the possibility that they may be in part due to differences in illumination. It was shown by Keeble and Armstrong that both organic peroxide and peroxidase accumulate during darkness in certain plants. It was subsequently found by the writer that the leaves of *I. germanica* showed no change in peroxidase reactions when deprived of light. This, however, is a relatively massive tissue when compared with the blade of a floral leaf.

With a view to testing the above possibility the Irises mentioned in Table VI were picked at 5 P.M. on July 10, 1914, after a hot, sunny day. Of each flower one of the falls was removed and examined immediately. The reactions afforded by these are given in column No. I. The flowers were then placed in total darkness, with their stalks in water. After twenty-one hours the second of the falls was removed, and the third after sixty-six hours. The behaviour of these is shown in Nos. II and III respectively. In the table + refers to the whole of the fall, unless a portion such as the claw is mentioned. Thus, "+ claw" indicates a less general distribution than does + alone. The reagent employed was α-naphthol, as it is more selective in its action than is benzidine.

TABLE VI.

Effect of keeping in darkness upon the peroxidase reactions of Iris flowers.

Number of hours in darkness	No. I. 0		No. II. 21		No. III. 66	
	Ep.	Veins	Ep.	Veins	Ep.	Veins
I. Monnieri, unopened bud,	—	+ claw	++ claw	+[1] (++ claw)	++ claw	+ +
Ditto, slightly withered,	—	+ claw	++ claw	+ (++ claw)	+ (++ claw)	+ + +[1]
Ditto, more withered,	—	+ claw	++ claw	+ +	+ (++ claw)	+ + +[1]
I. xiphioides, like No. 72, but pure white, old,	+ locally	+ +	+ +	+ +	+	+ +
I. xiphioides, like No. 62, dark blue, mature but young,	—	+ trace claw	—	+ +	+ claw	+ +
Ditto, withered,	—	+ +	—	+ +	+ claw	+ +
I. xiphioides, like No. 66, but with darker spots on intense claret colour,	—	—	—	+ trace	—	+ claw
I. xiphioides, Like No. 70, light lavender with darker spots, bud,	—	—	—	+ traces	+	+ +
Ditto, young mature,	—	+ claw and edges	+ traces	+ +	+	+ +
Ditto, older,	—	+ trace	+ +	+ +	+	+ +
Ditto, withered,	—	++ blade (+ claw)	—	+	—	+

[1] These gave a colour without the addition of hydrogen peroxide, and so contained organic peroxide.

Discussion of Results of Table VI.

It is at once evident that the absence of light permits of the accumulation of peroxidase in an active condition, and even leads to the formation of organic peroxide in the veins of *I. Monnieri*. The effect apparently is only brought about slowly, for although it is noticeable after twenty-one hours, it only becomes well marked after sixty-six. It may also be seen that the age of the flower is without influence on the intensity of the reactions, except that in the buds the latter are not so strong as in the more mature flowers. Since these buds opened in the dark, and were tightly folded when placed there, it is clear that the absence of active peroxidase cannot be due to its destruction by light.

In *I. xiphioides*, like No. 66, which was of a dark claret-colour, with spots of deeper hue, even sixty-six hours in darkness failed to bring about the production of peroxidase, except in the veins of the claw. Similar flowers which had been in the dark press for ninety-three and a hundred and fifteen hours behaved in the same manner. The almost complete absence of peroxidase activity from flowers of such a deep colour is hard to reconcile with the view that the pigment is produced as the result of the action of the enzyme. Since there remains the possibility that the anomaly may be due to the presence of an inhibitor, which is set free by the death of the cells, so that it can diffuse into places from which previously it was absent, it was decided to test the point further.

Falls of both *I. Monnieri* and of the claret-coloured variety of *I. xiphioides* were treated for twenty-four hours with 0·2 per cent. hydrogen cyanide, and with water saturated with toluene. The cyanide was then removed by allowing the tissues to steep in toluene water for another twenty-four hours, after which they were well rinsed. Both varieties were found to show a + + reaction in the veins, and in this respect treatment with toluene alone was as effective as with cyanide. Thus an inhibitor was removed, or more probably permitted to diffuse away. This, however, furnishes no proof that the inhibitor was not free to act in the cells before the treatment.

An attempt was made to ascertain whether yellow or blue light was the more injurious to peroxidase production, by placing similar flowers inside double-walled bell-jars filled with potassium bichromate and ammonia copper sulphate, as usually employed in laboratories as light-screens. It was abandoned, since it was recognized that such screens are in no sense capable of affording accurate results, for the total quantity of incident light is also varied as well as the wave-length. As far as the experiments went, however, there was no marked difference in the behaviour of the flowers under the two jars. In both diminution of light caused the peroxidase reactions to become more intense.

SUMMARY.

1. The peroxidase reactions of related species and varieties of Iris are similar, though the distribution and quantity of anthocyanin may be very different.

2. The Pogoniris group is characterized by an absence of active peroxidase; in the Apogon the distribution is variable; while in the Xiphion group the reactions are as a rule well marked, especially in *I. Xiphium.*

3. In *Xiphium* there is a definite inhibition area on the claw of each of the falls.

4. Where peroxidase tests have a negative result when the reagents are applied in the usual way, it is possible to demonstrate the presence of the enzyme by treatment with hydrogen cyanide as advocated by Keeble and Armstrong, or by toluene water.

5. By the above treatment an inhibitor is removed. This is probably not a chemical reaction, but since the reagents render protoplasm permeable, it is apparently to be accounted for by the diffusion of the inhibitor.

6. When Iris flowers are kept in darkness, the quantity of active peroxidase increases, and in one case the production of organic peroxide was recorded. Some varieties, however, show very little increase in the enzyme activity.

7. This alteration in peroxidase activity is not sufficiently rapid to seriously vitiate the results obtained with various species of Iris flowers picked after exposure to unequal periods and intensities of illumination in the garden.

8. Owing, possibly, to complications introduced by inhibitors, it is impossible in Iris to correlate the distribution of peroxidase and anthocyanin, the latter frequently appearing in the absence of the former in an active state.

I take this opportunity to acknowledge my indebtedness to Mr. W. R. Dykes for his kindness in identifying the flowers, and for valuable suggestions and criticism.

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THE PIGMENTS OF FRUITS IN RELATION TO SOME GENETIC EXPERIMENTS ON *CAPSICUM ANNUUM.*

BY

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[COMMUNICATED BY PROFESSOR H. H. DIXON, F.R.S.]

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XXV.

THE PIGMENTS OF FRUITS IN RELATION TO SOME GENETIC EXPERIMENTS ON *CAPSICUM ANNUUM*.

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[Read NOVEMBER 24, 1914. Published JANUARY 6, 1915.]

FOR some time past experiments upon the genetic relationships of varieties of Capsicum have been in progress at the Innes Horticultural Institution. Through the kindness of Professor Bateson we have been provided with material for the following research. The results presented here are only of a preliminary nature, and in a later paper we hope to give a more complete account of the pigments together with their absorption spectra.

Genetics of Capsicum Fruits.

Omitting from consideration the differences in the shapes of the fruits which have at one extreme slender elongated forms and at the other almost spherical ones, we shall confine ourselves to their behaviour with regard to colour only.

The unripe fruits are green as a rule, but pale yellow in one family. In four varieties employed they ripen to red, chocolate, orange, and yellow respectively. In crossing these, red is certainly dominant to yellow,[1] and from results obtained last year appears to be a simple dominant to chocolate and orange. Chocolate and yellow, yellow and orange, and chocolate and orange have not yet been fully examined.

The pigments are located in plastids, but beyond this no further information is to hand up to the present. Ikeno (1913) thinks that yellow is the first stage in the formation of the red pigment, but does not go into the chemistry of the question. In the cross, red × chocolate, a few plants occur with reddish chocolate fruits in F_2.

[1] This was first proved by Ikeno (*loc. cit.*).

One cross of interest as regards the pigments was that between a plant with unripe fruits, primrose yellow, ripening to red, and one which was dark green, unripe, and chocolate, ripe. When crossed together they gave all the combinations in F_2 except yellow (unripe), chocolate (ripe). For instance, there were all the following:—

Unripe.	Ripe.
1. Dark green.	Chocolate.
2. Dark green.	Red.
3. Green.	Chocolate.
4. Green.	Red.
5. Pale green.	Red.
6. Pale green.	Chocolate.
7. Yellow.	Red.

It is not the intention of the authors to treat the genetics more fully in the present paper, as enough has been said to elucidate the reasons for undertaking the study of the pigments.

Microscopic Examination of the Epidermal and Hypodermal Cells of Capsicum Fruits.

As before mentioned, the colours are due to plastid pigments, almost entirely or wholly so. These are contained in both the epidermis and in the deeper tissues. The difference between the dark green, green, and light green fruits appears to be that in the first-mentioned the cells are smaller and more densely packed with chromatophores than in the two other cases. In the former the cell-walls also are seen to be less thickened. Certain differences in the shade of red which appears in the fruits may also be explained in this manner. The thickening of the walls of the epidermal cells is very remarkable. Numerous pits serve to connect the cells. These may be brought out clearly by treatment of a section with a dilute alcoholic solution of benzidine or α-naphthol, with subsequent addition of water and a few drops of neutral three-volume hydrogen peroxide. The reagents are acted upon by oxidases present in the cells, with the production of a deep brown or purple colour, according as benzidine or α-naphthol is employed.

The red fruits contain brilliant red plastids and a colourless cell-sap. In one case a cell was observed in which the plastids were linear and disintegrating. This cell contained a purple sap, having all the appearance of an anthocyan pigment. As it was possible that this might give a clue to the production of the darker shades of red, or even of chocolate, a search was made for other similarly coloured cells, but none were ever found.

The plastids of the chocolate variety appear a deep red under the microscope, hence the possibility has to be examined that they owe their colour to the same pigment as do the red fruits, but in a greater concentration. There is normally a light chocolate or brown-yellow tint in aqueous extracts of this type, but this is too faint to have any importance in affecting the colour of the fruit.

The Peroxidase Reactions of Capsicum Fruits.

At the outset it was imagined that the chocolate colour might be due to an optical combination of red or yellow plastids with a cell-sap pigment as in wall flowers (Keeble, Armstrong, and Jones, 1913), or certain forms of Iris (Atkins, 1913). As the presence of anthocyan pigments has been supposed to be associated with oxidases, it seemed advisable to study the behaviour of sections towards benzidine and α-naphthol. These were applied as before described. It was found that the nuclei were specially deeply stained in cells which showed the reaction. Frequently the benzidine reaction resulted in the deposition of groups of acicular crystals of the oxidation product within the cells. With this reagent the thickened walls of the epidermis became yellow, whereas the cellulose walls of the sub-epidermal cells remained colourless. In some types of fruit there was no reaction at all in these deeper cells. Taking this in conjunction with observations on the behaviour of sclerenchymatous walls, as in *Iris germanica, Catalpa bignonioides,* etc. (Atkins, 1913), it appears probable that oxidases are concerned in the production of modifications in the cell-wall. With α-naphthol the cells are coloured purple, or lavender; but the walls remain perfectly colourless, thus permitting the very ready examination of the pits as before mentioned.

In the following table (p. 331) are recorded the results of some peroxidase tests. The reactions refer to the epidermis only, as in many cases no change took place in the deeper cells. This failure to react was observed both in chocolate and red fruits. The times indicated are those which elapsed after the addition of hydrogen peroxide. The sections had previously been in aqueous alcoholic solutions of the reagents till thoroughly impregnated. V. R. denotes a very rapid action, R a visible reaction inside ten minutes, + a reaction inside forty minutes.

It appears from the table that the benzidine reaction is more rapidly brought about than is that with α-naphthol. In addition it may be concluded that the peroxidase is most active in the unripe fruits, and so may be connected with the changes which occur during ripening. Keeble and Armstrong (1912) found that treatment of flowers, which failed to show oxidase reactions, with 0·2 per cent. hydrogen cyanide was effective in removing an inhibitor. For when carefully washed after treatment with this reagent, the tests gave positive results. Similar procedure with sections of Capsicum fruits demonstrated the presence of inhibitors in them too, intense colourations being now produced in the pale yellow, the yellow, and the round red varieties with both reagents; whereas only the first-mentioned gave them before. These colours were developed not only in the epidermis, but in all the underlying cells. Thus it is evident that the action of oxidases in the ripe fruit is largely checked by the production of an inhibitor.

Fruit	Benzidine	α-Naphthol
Dark chocolate, ripe, ...	R	+ +
Chocolate, ripe, ...	R	+ +
Red chocolate, almost red and very ripe,	– (40 min.) + slight (7 hrs.)	– (24 hrs.)
Red, round, ripe, ...	+ slight (24 hrs.)	– (+ in a few cells after 24 hrs.)
Unripe red, round, viz. green, .	R	R
Long red, ripe, ...	R	– (10 min.) + (7 hrs.)
Unripe long red, viz. pale yellow, .	V R	V R
Orange, ripe, ...	R	– (10 min.) + (7 hrs.)
Yellow, ripe, ...	R	– (10 min.) + (7 hrs.)

Examination of over forty fruits of known pedigree has failed so far to reveal any definite relation beween the oxidase reactions of parents and offspring in the F_2 generation. Further experiments will be made on this point. Surface sections of some of these fruits gave the indirect oxidase (peroxidase) reaction with guaiacum tincture. Others failed to do so. But too much reliance cannot be placed on these tests, as it was subsequently found that the inhibitor in the deeper tissues acted as a strong reducing agent and immediately decolourised the blue solution resulting from the action of the surface section upon guaiacum. This reducing action was even detected in one green fruit. As a rule, however, the oxidase reactions in unripe fruits, both green and yellow, were rapid and intense. In the reds they varied greatly, and were frequently confined to the epidermis, especially when tested with α-naphthol. In the chocolate types the inhibitors appeared to be less active than in the reds as a whole. Again, in the former it was noticed that the cut surface, where a section had been sliced off, gradually became darker till it appeared almost black. In the greens there was a slight browning only, and in the reds no marked change. As a general rule, however, the age of the fruit seems to be the most important factor in deciding the degree of activity of the oxidase.

The epidermal cells gave a dark colour with ferric chloride in almost every instance. This may in some cases extend into the next deeper layer. That this reaction is not due to tannin is shown by the fact that the confirmatory test with potassium ferrocyanide and ammonia always failed to give a positive reaction.

None of the sections darkened a tyrosine solution, either before or after treatment with hydrogen cyanide. Consequently tyrosinase must be absent.

The Plastid Pigments of Capsicum Fruits.

In order to distinguish the chocolate from the red pigment, and to ascertain whether they were mixtures, an examination was made of their behaviour towards various organic solvents.

The results are tabulated beside those for lycopin, carotin, and xanthophyll as given by Willstätter and Escher (1910). From the resemblance of the red of Capsicum to that of tomato, it was at first thought that the colour was due to lycopin. The two fruits are, however, different shades of red, and lycopin occurs not in plastids, but as crystals in the cell-sap or in the cytoplasm.

TABLE OF SOLUBILITIES.

Solvent	Capsicum chocolate	Capsicum red	Lycopin	Carotin	Xanthophyll
Ether, boiling,	....	....	1 g. in 3 l.	1 g. in 900 c.c.	1 g. in 300 c.c.
Alcohol, boiling,	Readily soluble	Readily soluble	Very sparingly soluble	Fairly sparingly soluble	Moderately easily soluble
Alcohol, cold,	Fairly soluble	Fairly soluble	....	....	....
Carbon disulphide, cold,	....	....	Fairly readily soluble 1 g. in 50 c.c.	Readily soluble	Sparingly soluble
Petroleum ether, boiling,	Readily soluble	....	1 g. in 10 l.	1 g. in $1\frac{1}{2}$ l.	Very insoluble
Acetone, cold,	Readily soluble	Readily soluble	....	....	....
Pyridine, cold,	Readily soluble	Readily soluble	....	Readily soluble[1]	....
Benzene, cold,	Readily soluble	....	....	....	....

---

[1] Pyridine was tried as a solvent for carotin, and it gave a deep-brown solution from which glittering orange crystals of carotin separate on cooling slightly. These are never acicular, but are exactly like those figured by Willstätter and Escher (*loc. cit.*) as obtained from ether by crystallization.

These solubilities at once differentiate the Capsicum pigments from the other three. Owing to the small quantities available, it was not possible to measure the solubilities with accuracy. Before extracting with the organic solvents, the fruits were boiled with water till they gave scarcely any tinge of colour to it. The chocolates gave a yellow-brown aqueous extract, and the reds gave either scarcely any colour or a clear yellow. The addition of a little sodium carbonate to the latter neutralized the faintly acid reaction of the solution, and greatly intensified the colour. These water-soluble pigments may possibly be due to disintegration changes in the plastids, but it seems equally likely that they are quite separate substances, as it was possible to remove them almost completely by repeated extraction at the boiling point. The latter view is borne out by the fact that an alcoholic solution of the red fruit, made after prolonged aqueous extraction, when allowed to evaporate slowly in the dark, gave a light red brown ring on the side of the beaker, whilst a light yellow spot was left on the bottom.

Attempts were made to ascertain whether the pigments were mixtures, by extracting the fruits with one solvent after another. Thus chocolates gave a very dark brown to cold acetone, a lighter and somewhat redder brown to cold pyridine, and to cold benzene a still lighter brown. It was, however, found that the acetone solution could be exactly matched with the benzene by suitable dilution. The pyridine employed became slightly coloured on standing, and its solution could not be matched with the acetone or benzene, possibly because of the colour of the solvent. Alcohol, too, gave a light yellow-green. This could not be matched with any of the above.

Extraction of the chocolates with petroleum ether (b. p. 40°–60°) afforded a bright yellow solution, varying with concentration to yellow-brown which could not be matched with the reddish alcoholic extract. The latter was always of a redder hue. The acetone extract was very similar in colour to that made with petroleum ether. By evaporating the alcoholic solution, however, and dissolving the residue in petroleum ether, it was found that the solution was quite similar to that extracted by the latter directly. Accordingly it must be concluded that the difference in colour is only due to an effect produced by the solvent, as in the well-known instance of iodine solutions.

Extraction of red fruits with cold solvents gave the following results:—

Pyridine,	bright red solution.	All exactly matched on dilution.
Alcohol (absolute) Acetone	lighter red, yellower tinge.	
Benzene,	yellow-red, like bichromate solution.	

The evidence here, as far as it goes, is that the red is one pigment.

All the solutions from chocolates and reds gave colourless residues when allowed to evaporate slowly in sunlight. The alcoholic extract of a chocolate which was dark green when unripe, afforded a little bright green residue, which persisted for five days.

Evaporation under similar conditions, but in the dark, resulted in the deposition of red, oily drops from the reds, and dark brown ones from the chocolates. Neither was obtained in a crystalline form.

To prepare the red in a pure form use was made of the fact that hot alcoholic solutions become turbid on cooling, owing to the separation of oily drops. These were separated by decantation and by centrifuging. They were then again dissolved in hot absolute alcohol, and the precipitate on cooling was separated as before, and dissolved in absolute alcohol for spectroscopic examination. Hot acetone solutions yield a similar precipitate on cooling.

Summary.

1. In Capsicum fruits, red is dominant to yellow, and appears to be a simple dominant to chocolate and orange.

2. The differences in colour of unripe, green fruits, viz., light green, green, and dark green are due to variations in the numbers of chromatophores contained in each cell.

3. The colours of the ripe fruits are due to red, chocolate, orange, and yellow plastid pigments. It has not been possible to show that the red or chocolate is due to a mixture of pigments.

4. Some red fruits contain small quantities of yellow pigment soluble in water.

5. The red and chocolate pigments, when pure, are oily liquids which have not been obtained in a crystalline condition. They are distinguished from lycopin, carotin, and xanthophyll by this and by their ready solubility in cold alcohol and in petroleum ether. Such solutions become colourless when allowed to evaporate in sunlight.

6. Carotin is moderately soluble in cold pyridine, and yields crystals from this solvent similar to those from etherial solution.

7. The amount of peroxidase present in all Capsicum fruits appears to diminish as they ripen, and bears no simple relation to the variety of fruit. The enzyme is frequently present only in the epidermis, while the deeper tissues may contain an inhibitor with a strong reducing action.

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THE FATIGUE OF NICKEL AND IRON WIRES WHEN SUBJECTED TO THE INFLUENCE OF ALTERNATING MAGNETIC FIELDS OF FREQUENCY 50 PER SECOND.

BY

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XXVI.

THE FATIGUE OF NICKEL AND IRON WIRES WHEN SUBJECTED TO THE INFLUENCE OF ALTERNATING MAGNETIC FIELDS OF FREQUENCY 50 PER SECOND.

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[Read November 24, 1914. Published January 6, 1915.]

Section I.

Nickel Wires.

In a recent communication to this Society, on the subsidence of torsional oscillations in nickel wires when subjected to the influence of alternating magnetic fields, it was incidentally shown that under certain conditions the wire became *temporarily fatigued*, and a statement was then made that this property of nickel wire would be further investigated.[1]

At that time, the fatigue was observed when alternating magnetic fields of frequencies 100 and 140 per second were used; but since the wave-forms—obtained by means of an oscillograph—of the alternating currents producing these magnetic fields were found to contain the third harmonic, the investigation of the effects produced by high frequency magnetic fields has been held over until high frequency alternating currents of sine-wave form are available. The present communication therefore gives results obtained with alternating magnetic fields produced by currents of frequency 50 per second only, and with current wave-forms of very nearly pure sine-curves.

The results obtained and recorded below are smaller in magnitude than in the two cases referred to above, as may be expected, because the frequencies of the magnetic fields then employed were about *two* and *three* times the frequencies used in the present case.

It is known[2] that the continuous direct magnetic field which must be round a nickel wire in order to obtain the maximum twist or "Wiedemann effect" depends on the longitudinal load on the free end of the wire, for a given constant current *through* the latter.

In the experiments described below these conditions have been followed;

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[1] Scient. Proc. Roy. Dub. Soc., vol. xiv, p. 219.

[2] *Ibid.*, vol. xiii, p. 31.

because it has also been shown[1] that the greatest damping of torsional oscillations in nickel wire takes place under these same conditions, and it follows that the maximum fatigue will also occur in the same circumstances.

The solenoid used in these experiments has already been described.[2] The only important change in the apparatus is an improvement in the method of applying the load on the lower end of the wire. The lead discs formerly employed have been replaced by lead cylinders which can be clamped firmly by means of lock-nuts, so that no change of zero can take place by the vibrations which are set up when the alternating currents are round the solenoid.

The *fatigue* does not occur, or only very slightly, in soft nickel wire. In order therefore to get some idea of the limits between which it does take place, the simple rigidity was measured in the three cases here given. This was done by means of the modified form of Searl's Torsion apparatus previously described.[3]

The nickel wires employed were each 226 cms. long and 0·1675 cms. in diameter, and the millimetre scale for reading off the steady deflection or twist of the free end of the wire was placed at a distance of 167 cms. from the plane mirror on the vibrator or load on the end of the wire. The method of experiment was as follows:—The nickel wire was suspended vertically in the middle of the solenoid; it was firmly fixed to the wall at the top, and the lower end made contact in a vessel of mercury by means of a small iron pin projecting from the under side of the load. Then for a given longitudinal load on the wire, the corresponding direct current was put through the solenoid to give the magnetic field which produces the largest twist of the lower end of the wire when the standard current of *one ampere* was sent through it; this gave a steady deflection of the light spot on the scale which we call D. The direct currents were then taken off both the wire and the solenoid, and the equivalent root-mean-square value of alternating current was for *one minute* sent round the solenoid; this alternating current was then taken off, and separate direct currents of the same values as before were sent round the solenoid and through the wire, and the twist or steady deflection of the light spot on the scale again observed, which we call d. The direct currents were again taken off the wire and solenoid, and the alternating current again put round the solenoid for *one minute more* then taken off, and the direct currents again put through the wire and round the solenoid, and the deflection again observed, and so on until the twist or deflection was no longer diminished by the application of the alternating magnetic field.

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[1] Scient. Proc. Roy. Dub. Soc., vol. xiii, p. 35.

[2] *Ibid.*, vol. xiv, p. 216.

[3] *Ibid.*, vol. xii, p. 481.

Then calling F = the fatigue of the wire,
D = the unfatigued steady deflection or twist,
d = the fatigued steady deflection or twist.

$$F = \frac{D - d}{D}.$$

The nickel wire when first tested was in the physical state in which it came from the manufacturer. Its simple rigidity was about 800×10^6 grammes per sq. cm., and when hung up inside the solenoid, with a certain load on its lower end, and tested as explained above, it gave no deflection or twist when the direct currents were sent round the solenoid, and through the wire; nor was there any humming sound in the solenoid when the alternating magnetic field was applied, that is, the wire was too hard to be magnetised by the magnetic field used.

The wire was then taken down and hung vertically and loosely under its own weight only and heated *once* from the top downwards to a *dull* red heat by means of a broad Bunsen burner; when cool its surface was cleaned with fine emery cloth, its rigidity again tested and found to be about 790×10^6 grammes per sq. cm. The wire was then put up inside the solenoid, and tested for fatigue as explained above; the results obtained are shown in Table I. Three loads were employed for each of the three values of the rigidity; in the tables below the longitudinal loads on the wires are in the top horizontal line, and are of values $1{\cdot}0 \times 10^5$; $1{\cdot}5 \times 10^5$, and $2{\cdot}0 \times 10^5$ grams. per sq. cm., the left hand vertical column gives the time in minutes that the alternating magnetic field was round the nickel wire, and the letters d and F represent the deflection or twist, and the fatigue respectively.

TABLE I.

Rigidity ≑ 790×10^6 grammes per sq. cm.

Loads →	$1{\cdot}0 \times 10^5$		$1{\cdot}5 \times 10^5$		$2{\cdot}0 \times 10^5$	
Minutes ↓	d	F	d	F	d	F
0	4	0	11	0	19	0
1	3	0·250	9·5	0·136	17·8	0·063
2	2·6	0·350	8·9	0·191	17·0	0·105
4	2·3	0·425	8·2	0·252	16·2	0·148
6	2·2	0·450	8·0	0·273	16·0	0·158
8	2·2	0·450	8·0	0·273	16·0	0·158
10	2·2	0·450	8·0	0·273	16·0	0·158

The wire was then taken down and heated as before *once* to a *bright* red heat; it was cleaned, and its rigidity measured and found to be about 770×10^6 grams. per sq. cm. It was put into the solenoid and tested as before; the results are shown in Table II.

TABLE II.

Rigidity $\doteqdot 770 \times 10^6$ grammes per sq. cm.

Loads →	$1{\cdot}0 \times 10^5$		$1{\cdot}5 \times 10^5$		$2{\cdot}0 \times 10^5$	
Minutes ↓	d	F	d	F	d	F
0	14·5	0	19	0	23	0
1	12·5	0·138	17	0·105	21	0·087
2	11·5	0·207	16	0·157	20	0·125
4	10·5	0·274	14·8	0·220	19·8	0·138
6	10·2	0·300	14·2	0·250	19·6	0·148
8	10·0	0·310	14·0	0·260	19·6	0·148
10	10·0	0·310	14·0	0·260	19·6	0·148

The wire was again taken down out of the solenoid and heated *once more* to a *bright* red heat, and when cleaned and tested the rigidity was found to be about 750×10^6 grammes per sq. cm. It was once more put into the solenoid and tested for fatigue, with the results shown in Table III.

TABLE III.

Rigidity $\doteqdot 750 \times 10^6$ grammes per sq. cm.

Loads →	$1{\cdot}0 \times 10^5$		$1{\cdot}5 \times 10^5$		$2{\cdot}0 \times 10^5$	
Minutes ↓	d	F	d	F	d	F
0	25	0	27	0	29	0
1	22·6	0·096	25	0·074	27·5	0·052
2	21·4	0·144	24	0·111	26·0	0·105
4	20·4	0·184	23·2	0·140	25·3	0·127
6	20	0·200	23	0·148	25	0·138
8	20	0·200	23	0·148	25	0·138
10	20	0·200	23	0·148	25	0·138

From the results in these three tables we see that the harder the wire or the greater the rigidity the greater is the fatigue, and, also, that as the longitudinal load *increases*, the *fatigue decreases.* Thus, when the load on the wire is *doubled* the decrease in the maximum fatigue is 65 per cent. for the high rigidity, 51 per cent. for the middle value of rigidity, and 31 per cent. for the lowest rigidity.

In order to show at a glance how the fatigue of nickel wire varies or changes with the rigidity, and also to show the rate of fatigue, the results obtained with the smallest load in each of the three cases are here, in fig. 1, put in the form of curves. The abscissae represent the time in minutes that the alternating magnetic field was applied, and the ordinates represent the corresponding fatigue. The top curve is that obtained with the wire of greatest rigidity, and the lowest curve with the wire of least rigidity.

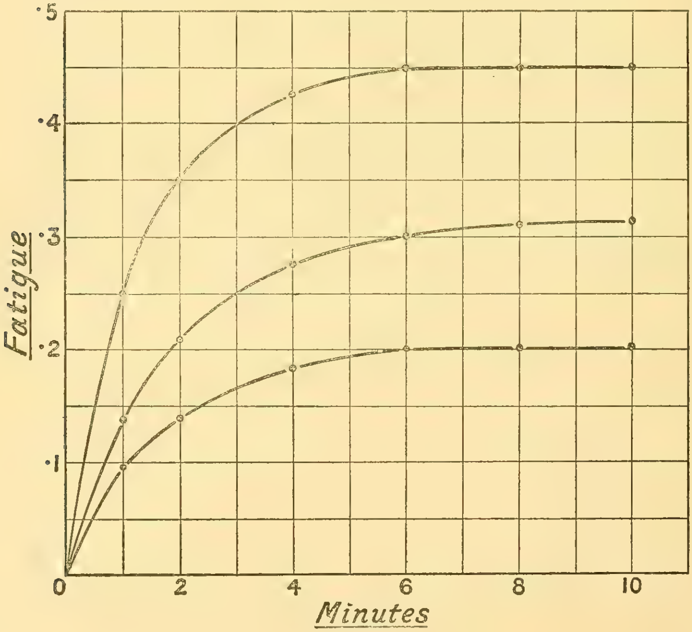

Fig. 1.

The limits between which the nickel wire *is* and *is not* sensitive to fatigue

—that is, between being too hard and too soft—are so narrow, that it is difficult to judge how much the wire should be heated in order to obtain a desired rigidity. In the three attempts here recorded the heating has just happened to give very nearly the rigidities required, and to show the approximate relations between the longitudinal load on the wire, the rigidity, and the corresponding maximum fatigue; the values are here collected in Table IV.

TABLE IV.

Load	Rigidity	Maximum Fatigue
$1\cdot0 \times 10^5$	790×10^6	0·450
	770 ,,	0·310
	750 ,,	0·200
$1\cdot5 \times 10^5$	790 ,,	0·273
	770 ,,	0·260
	750 ,,	0·148
$2\cdot0 \times 10^5$	790 ,,	0·158
	770 ,,	0·148
	750 ,,	0·138

SECTION 2

Iron Wires.

In the case of an iron wire of given diameter it is known—(1)[1] that the continuous direct magnetic field which must be round the wire in order to obtain the maximum twist or "Wiedemann effect" is independent of the longitudinal load on the free end of the wire for a given current through it, (2)[2] that the direct continuous magnetic fields in which the greatest internal friction—or opposition to torsional oscillations of the wire—takes place, are much lower for iron than for nickel wires, and the points of maximum effect are also much less pronounced.[3] From these considerations one would therefore expect that iron wire would not be so easily fatigued by the application of alternating magnetic fields as nickel wire; in fact, the results of experiments here recorded show that the expectation is justified.

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[1] Scient. Proc. Roy. Dub. Soc., vol. xii, p. 484.

[2] *Ibid.*, vol. xiii, p. 41.

[3] The influence of alternating magnetic fields on the torsional oscillations of iron wire is at present under investigation.

The same methods of experiment and observation were carried out with the iron wires as was done in the case of the nickel wire explained above in Section 1. The wire used was at first in the physical state in which it was received from the manufacturer, that is, its simple rigidity was about 810 × 10^6 grammes per sq. centimetre. The effective length of the wire was 226 cms., and the diameter 0·162 cms., the load used being 10^5 grammes per sq. centimetre, and the magnetic field round the wire in order to get the maximum effect was 2·8 c.g.s. units.

The results obtained with this hard wire are given in Table V, and are shown in a curve in fig. 2, where also for comparison is placed a corresponding curve for nickel wire.

TABLE V.

Time Mins.	d	F
0	7	0
5	6·5	0·080
10	5·9	0·155
15	5·5	0·215
20	5·1 +	0·265
25	4·9 +	0·290
30	4·9	0·300
35	4·9	0·300

From these curves it will be seen that the maximum fatigue for the iron wire is about 0·3, and that it took thirty minutes' application of an alternating magnetic field of 2·8 units to give this amount; and also that it takes about three times longer to fatigue an iron wire than it does to fatigue a nickel wire when they are of the same length and diameter, and when subjected to the same longitudinal load.

The nickel wire had a simple rigidity of about 790 × 10^6 grammes per sq. centimetre and the iron wire 810 × 10^6 grammes per sq. centimetre, that is both wires were as hard as they could be without the "Wiedemann effect" being entirely annulled.

The iron wire was now taken out of the solenoid and hung vertically under its own weight only, and by means of a broad Bunsen flame it was heated *twice* to a dull red heat from the top downwards in order to allow it to cool slowly. When cold, the rigidity was again measured, and found to be about 795 × 10^6 grammes per sq. centimetre; it was then placed in the solenoid and tested in the same way as formerly.

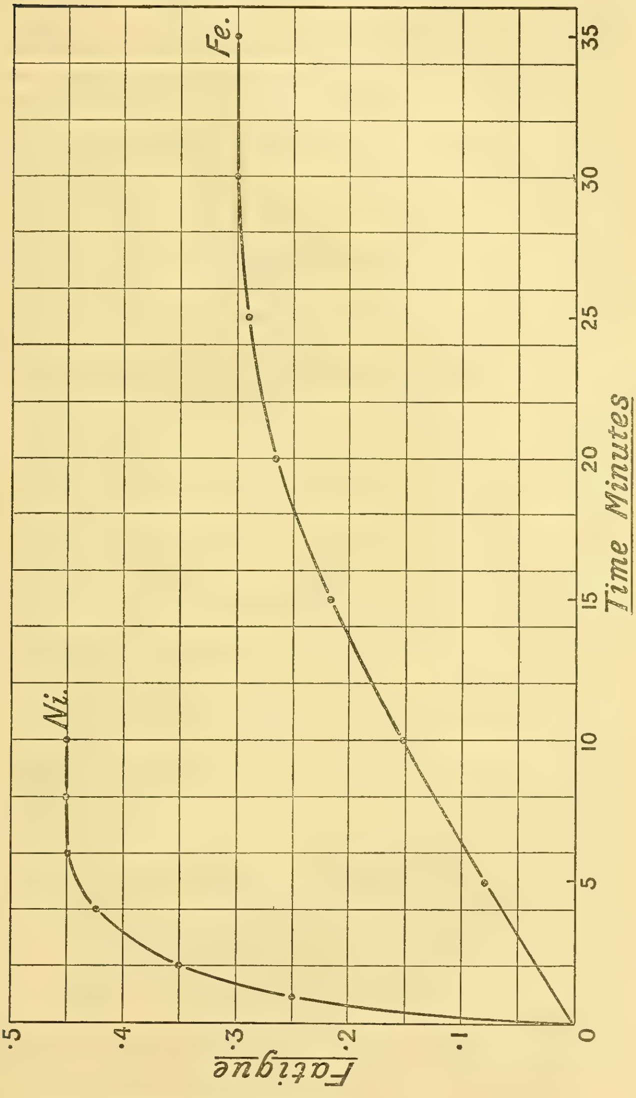

Fig. 2.

The unfatigued or first deflection was now 11 divisions on the scale as compared with 7 divisions when the wire was harder, and it was found that the wire in this comparatively soft state could not be fatigued at all. The alternating magnetic field was put round the wire for a *full hour*, and the wire tested at intervals, when no trace whatever of fatigue was observed. This shows the narrow limits of rigidity between which the fatigue of iron wire occurs, for in the wire under test a difference of about 2 per cent. in the rigidity makes all the difference between fatigue and no fatigue.

This work is being continued with alternating magnetic fields of frequencies from 60 to 240 per second, and it is very probable that with these comparatively high frequencies the fatigue of both nickel and iron wires will be found to take place much more rapidly than with the low frequency of 50 per second employed to obtain the present results; indeed, the two previous cases of nickel wires referred to above would seem to indicate that rapid fatigue is to be expected with high frequency alternating magnetic fields.

THE

SCIENTIFIC PROCEEDINGS

OF THE

ROYAL DUBLIN SOCIETY.

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SEARCH FOR THORIUM IN CANCEROUS GROWTHS.

BY

J. JOLY, Sc.D., F.R.S.

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XXVII.

SEARCH FOR THORIUM IN CANCEROUS GROWTHS.

By J. JOLY, Sc.D., F.R.S.

[Read June 23, 1914. Published January 7, 1915.]

In a recent number of the Proceedings of the Royal Society (vol. lxxxv B, p. 170, and in *The Practitioner* (March, 1914)), Dr. Lazarus-Barlow records observations showing the presence in some cases of abnormally large quantities of radium in morbid tissues. His experiments refer to cancerous and malignant growths of various kinds.

These results may be very significant, for, of course, the radiations from the radioactive derivatives of radium are involved, and it has been shown by observation that feeble γ radiation may accelerate the growth of such morbid tissues. Nor is it difficult to perceive *à priori* reasons for the influence of the rays when their remarkable ionising powers are considered in connexion with the chemical activities involved in metabolism.

Dr. Lazarus-Barlow's results are, however, by no means uniformly positive. The amount of radium present cannot, in every case, be claimed as abnormal. This fact suggested the desirability of seeking for the presence of thorium in such growths.

The element thorium is, so far as we know, much more abundant in Nature than radium. In the sedimentary rocks (which may be taken as covering the greater part of the earth's surface) there is on the average ten million times as much thorium as radium. In radioactivity, however, the amounts present of the two series—i.e. of the radium series of element and of the thorium series—do not differ seriously. Indeed the aggregate energy emitted in transmutation by each series, as determined by the heating effect, is rather greater for the radium series than for the thorium series in average sedimentary material. We may assume, then, that if these elements—radium and thorium—are taken into the body, in amounts proportional to their relative abundance in the surface materials of the earth, their radioactive influence upon growth, etc., will be much alike in importance—be this little or great—and, if by any processes segregation of these elements is equally promoted in any special organ, the effects will be similar in kind and degree. As we know nothing about the causes at work in the observed segregation of radium, it might well be that similar causes operated to segregate thorium: such causes might even operate in the latter case with greater efficiency than in the former. Investigation of the matter is, in short, evidently desirable.

In the observations which follow I have to acknowledge the very helpful directions of Dr. A. C. O'Sullivan, F.T.C.D., and of Dr. Adrian Stokes, without which I could not have ventured on the investigation. Indeed, I only am to blame if the observations recorded are deemed to be too few in number.

The procedure adopted was to break up the tissues by maceration in strong HCl over the water-bath. An almost perfect solution was obtained in this way. This solution was then diluted and treated by a method described by me in the *Philosophical Magazine* for May and July, 1909. This method for measuring the amount of thorium present in a solution is the most sensitive known to me, and is, indeed, the only practical one with which I am acquainted.

The solution being dealt with is brought to such a dilution as to boil freely. It is enclosed in a flask, and during brisk ebullition a steady current of air is drawn through the flask above the surface of the boiling liquid, and thence passes through a condenser, and from this through drying tubes to a gold-leaf electroscope. The condenser removes the steam, etc., returning condensible vapours to the flask; the air is further dried in the short drying tubes, and finally enters the electroscope with much of the thorium emanation still present.

If the normal rate of loss of charge by the leaf is accelerated, the significance of this acceleration is determined by adding to the boiling liquid a small, known amount of a solution of thorium. The acceleration in the rate of collapse of the leaf, produced by this, enables the readings to be calibrated.

In this method the effects due to any radium present may be neglected if the precaution is taken of boiling off the radium emanation before connecting the flask to the condenser. This emanation only very slowly regenerates in the solution, whereas the regeneration of the thorium emanation is very fast. A few recent users of this method substitute mechanical agitation of the liquid—by shaking the flask—for ebullition. For most solutions, however, ebullition offers the most ready and thorough method of agitation.

The sensitiveness of the results now to be recorded is determined by the following experiment:—

Into one of the solutions, which had already been tested, and which possessed a bulk of 900 c.c.'s., a quantity of standard solution of thorianite was put, containing 16×10^{-5} gram of thorium element. The gain in rate of collapse of the leaf was 8 scale-divisions per hour. Hence one scale-division per hour indicates the presence of 2×10^{-5} gram of thorium. If the total weight of tissue dissolved in this solution had contained as much as 2×10^{-5} gram of thorium, this would have accelerated the collapse of the leaf by one scale-division per hour—an acceleration quite determinable.

The following experiments were made :—

I. *Cancer involving stomach and lymphatic glands.*—Examination of sections under microscope showed that about one-fifth part of stomach was actually composed of cancer-cells.

145 grams were heated in 200 c.c.'s strong HCl. The solution was made up to about 800 c.c.'s with distilled water. The whole of this solution was treated in the manner described above.

The electroscope, which had been steady at a rate of loss of 3 scale-divisions per hour before ebullition began, maintained this rate unaltered. Hence thorium, in amount greater than 2×10^{-5} gram, was not present. This amount would be about $1{\cdot}4 \times 10^{-7}$ gram per gram of tissue. The experiment does not bear on the presence of radium, as the solution was vigorously boiled before attaching to the electroscope.

II. *Liver in part replaced by cancer.*—Same case as I.

1189 grams were heated in 1000 c.c.'s strong HCl for 24 hours. Total volume of solution was 2150 c.c.'s. 400 c.c.'s of this solution were taken and diluted to 900 c.c.'s with distilled water. Weight of tissue involved about 236 grams. The electroscope was running at 5, but slowing down a little and nearing 4 scale-divisions per hour when ebullition began. It then rose to 5 scale-divisions, but shortly after fell again, declining finally to 3 scale-divisions per hour.

The conclusion must be that more than 2×10^{-5} gram of thorium was certainly not present, i.e. about 8×10^{-8} gram per gram.

III. *Sarcoma of ovary.*

Weight taken 220 grams: treated with about 290 c.c.'s HCl, and solution diluted to 800 c.c.'s.

Readings of electroscope remained steadily at 2 divisions per hour, whether the boiling solution was in train or not.

Conclusion.—No thorium was present in amount exceeding about 9×10^{-8} gram per gram.

IV. *Myoma of uterus.*

155 grams treated in 150 c.c.'s HCl: diluted to 800 c.c.'s.
Electroscope steady, at 4 scale-divisions per hour.
Conclusion.—No thorium exceeding $1{\cdot}3 \times 10^{-7}$ gram per gram.

V. *Cancer of breast.*—

89 grams in 100 c.c.'s HCl: diluted to 800 c.c.'s.
Electroscope steady at 3 divisions per hour.
Conclusion.—No thorium exceeding $2{\cdot}2 \times 10^{-7}$ gram per gram.

VI. *Sarcoma of knee.*

80 grams treated as in V.
Electroscope steady at 3 divisions per hour.
Conclusion.—No thorium exceeding $2{\cdot}5 \times 10^{-7}$ gram per gram.

It is desirable that the above results—which, as will be seen, are consistently negative—should be extended. They possess a practical bearing; for if it can be shown that thorium introduced into the system is, in every case, again eliminated while radium compounds remain stored, or become segregated, within the body, the use of thorium for internal administration would possess advantages over the use of radium. For this as well as for other reasons the whole subject of the retention and segregation of the radioactive elements in the body deserves full and systematic investigation.

THE
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ON THE ACTION OF PECTASE.

BY

NIGEL G. BALL.

[COMMUNICATED BY PROFESSOR H. H. DIXON, SC.D., F.R.S.]

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XXVIII.

ON THE ACTION OF PECTASE.

By NIGEL G. BALL.

[COMMUNICATED BY PROFESSOR H. H. DIXON, SC.D., F.R.S.]

[Read November 24, 1914. Published January 7, 1915.]

Introduction.—The enzyme which causes the coagulation of vegetable saps containing pectin was discovered by Frémy (9) in 1840. At present there is a certain amount of confusion in the terminology of this and related enzymes. Euler (8) applies the name pectase to the enzyme which converts pectose into pectin, and calls the enzyme which coagulates pectin, pectinase. Other writers retain the original name pectase for the enzyme which coagulates pectin, and in the following account this enzyme will be denoted by this name. With regard to the two other related enzymes, the most generally accepted terminology seems to be that in which the enzyme which hydrolyses pectin to d-galactose and l-arabinose is called pectinase, and the enzyme which converts pectose into pectin, pectosinase.

Previous workers on this subject—Frémy (9), Bertrand and Mallèvre (1), (2), (3), Bourquelot (4), and Bourquelot and Hérissey (5), (6)—simply allowed coagulation of the pectin by the enzyme to take place under various conditions, and noted the time taken before this was complete.

In the present research an attempt was made to study the action of pectase by observing the electrical conductivity of a solution of pectin when acted upon by the enzyme, and also by determining the change in viscosity.

Preparation of Materials.—The pectin used in this research was obtained exclusively from the roots of the carrot, *Daucus carota.* The first extraction was based on a method suggested in Browne's "Handbook of Sugar Analysis" (7) for obtaining pectin from the juice of ripe pears. The chopped-up carrots were put into a can and steamed in a Koch's sterilizer for about forty-five minutes, and the juice was then squeezed out and filtered under reduced pressure. A little oxalic acid was added to precipitate calcium, and a little tannic acid to precipitate albumins. The juice was filtered, and centrifuged until almost clear. The pectin was precipitated by addition of an equal volume of alcohol, and was filtered off and redissolved by pouring a little hot water on to the filter. The pectin was subsequently reprecipitated by addition of alcohol, and the gelatinous precipitate obtained was collected by means of a centrifuge and dried on a watch-glass placed on a water-bath. A horny residue was obtained in this way, and about 0·3 g. of pectin was extracted from about 1100 g. of carrots.

In subsequent extractions the carrot-roots were finely minced, and the pulp covered with water, and heated on a water-bath for one or two hours. The pulp was then squeezed, and the liquid obtained was treated as before. This method was based on one described by Bourquelot and Hérissey (5), who obtained pectin by heating chopped-up gentian roots with water in an autoclave at 110° C.

Browne (7) recommends the addition of tannic acid to juice which has been extracted in the cold. In cases where the carrot pulp had been heated this was found to be unnecessary, as all albuminous substances had been coagulated.

The pectin obtained from these various extractions was dissolved in sufficient water to form a 2 per cent. solution, and a few drops of toluene were added to prevent growth of micro-organisms. The pectin solution thus obtained was in a fairly pure state, but was faintly acid to litmus paper. In all the experiments this solution was diluted with an equal quantity of water.

Sap pressed from the leaves of *Syringa vulgaris* was used exclusively as a source of pectase. This plant was chosen both for the sake of convenience, as the leaves could be obtained easily, and also owing to the fact that Bertrand and Mallèvre (4) state that sap pressed from these leaves is fairly active in coagulating pectin.

The method of extraction was as follows:—Leaves were stripped from fresh shoots of Syringa, the petiole of each leaf being removed. They were then placed in a small steel cylinder fitted with a piston, and provided with a hole at its lower end. The piston was squeezed in by means of a vice, and the expressed sap was centrifuged until clear, and was then ready for use.

Measurement of the electrical conductivity during the coagulation of pectin.—If the product of the action of pectase on pectin is a true gel, there would be little change in electrical conductivity during its formation, as the resistance of a gel to the passage of ions is practically the same as that of the sol from which it has been formed.

1 c.c. of distilled water was added to 1 c.c. of 2 per cent. pectin solution in a test-tube, and 1 c.c. of freshly extracted sap from the leaves of Syringa was put into a similar tube. The two tubes were partly immersed in a large glass tank of water for about ten minutes, in order that their contents might come to the same temperature. At a noted time the contents of the tubes were mixed, and the mixture poured into a Hamburger conductivity tube, which was also immersed in the same tank of water at 13° C. Measurements of the resistance of the mixture were made every ten minutes by comparison with a standard resistance, using a metre bridge with an alternating current and telephone.

The resistance of the mixture remained practically constant for over two hours, and at the end of this time a solid jelly had been formed in the conductivity tube. During the experiment the temperature of the water in the tank was constant within 0·5° C.

The product of the action of pectase therefore consists of a spongy network composed of a more or less solid phase, in the meshes of which a more liquid phase is distributed.

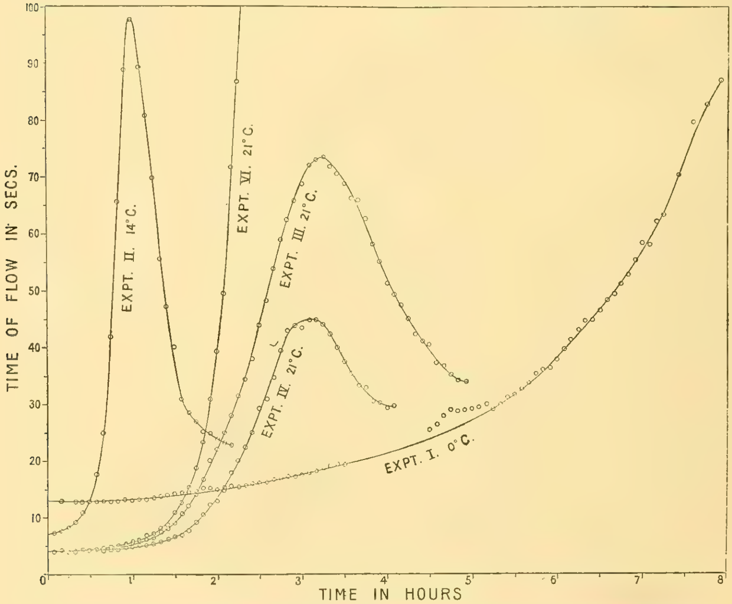
Measurement of the change in viscosity.—In order to measure the change in viscosity, a viscosimeter of the Ostwald type was constructed. This consisted of a U-tube made of glass tubing. Part of one limb was composed of a piece of thermometer tubing, and above this capillary the tube was dilated into a small bulb. 3 c.c. was the amount of liquid which was always used, and this was introduced by means of a pipette. In use the liquid was sucked up to a definite height above the bulb in the limb of the U-tube which contained the capillary, and was then allowed to run back. The time taken by the meniscus in passing between marks on two constrictions above and below the bulb was determined by means of a stop-watch. As this piece of apparatus was not sufficiently accurate to permit absolute determinations of the viscosity being made, the times taken for the meniscus to pass between the two marks were compared with one another.

In the experiments at a temperature above 0° C. the viscosimeter was partly immersed in a tank of water containing about twenty litres. This was kept at a constant temperature by the introduction of either hot or cold water from a tap connected with another tank. The water in the tank was kept well stirred, and a sensive thermometer attached to the viscosimeter was easily maintained within 0·1° C. of any desired temperature.

The viscosimeter was standardized with 3 c.cs. of distilled water at 0° C., and the time for emptying the bulb was 3·0 seconds.

EXPERIMENT I.

In this experiment the viscosimeter was immersed in a large beaker of melting ice, which was kept well stirred. 1 c.c. of freshly extracted sap from the leaves of *Syringa vulgaris* was mixed with 2 c.cs. of 1 per cent. pectin solution, and the mixture immediately tranferred to the viscosimeter. Determinations of the viscosity of the liquid were made every five minutes during nearly eight hours, with the exception of about one hour in the middle. The variation of the viscosity with time is shown in curve No. 1. The time of flow increased from 12·8 seconds to 108·6 seconds, and was still rising when the experiment terminated. The slight irregularity which is observable in the readings taken just after the interval, in which observations were not made, is due to the fact that the mixture of ice and water had been left for about an hour without being stirred, and therefore the liquid in the viscosimeter had become slightly warmer, with a consequent increase in the activity of the enzyme.



The curve obtained shows that the rise in viscosity, which occurs slowly at first, gradually becomes more rapid.

EXPERIMENT II.

The viscosimeter was kept in a tank of water at 14° C. The usual quantities of Syringa sap and pectin solution, which had been kept in separate test-tubes immersed in the tank for half-an-hour, were mixed, and the mixture introduced into the viscosimeter. Viscosity determinations were made every five minutes, and the results are plotted on a curve. The viscosity increases rapidly until a maximum is reached, and then decreases rapidly. Towards the end of the experiment the homogeneous gel which had previously been formed became broken up into clumps of more solid matter suspended in a liquid. These clumps gradually settled down to the bottom of the viscosimeter, so that further determinations of viscosity were useless, as the capillary tended to become clogged, and irregular readings resulted.

EXPERIMENTS III AND IV.

An experiment which had previously been conducted showed that sap, extracted from the leaves of Syringa and saturated with toluene, had practically lost its activity in coagulating pectin after about a fortnight.

In the following two experiments sufficient sap was extracted for use on two consecutive days.

In the first experiment the method adopted was exactly similar to that of Experiment II, but the temperature was kept constant at 21° C. The results obtained are plotted on a curve which shows that a maximum viscosity was reached, but the time taken to reach the maximum was longer than in the previous experiment. The cause of the delay will be discussed later.

In all the experiments up to the present the pectase was acting in a slightly acid medium, due both to the acidity of the cell sap and to the fact that the pectin solution itself was faintly acid.

In Experiment IV the sap was neutralized by the addition of a few drops of sodium hydrate solution.

The sap was accidentally made slightly alkaline to litmus, and more acid had to be added to make it neutral. This resulted in a slight increase in the total quantity of electrolytes present, and also in the substitution of sodium for hydrogen ions. The pectin solution was also neutralized, and the experiment conducted similarly to the last.

The curve obtained from the results is interesting. It will be seen that the rate of increase in viscosity is not very different from the rate of increase in the last experiment, but a maximum is attained at a much lower viscosity. The reason for the decrease in viscosity after the maximum is reached may be attributed to the clumping together of the particles of colloid into aggregates, separated from one another by liquid. This action is plainly visible in the later stages.

It is well known that electrolytes have the power of precipitating colloids; and that the clumping of the gel, which is formed by the action of pectase on pectin, is probably due to the action of electrolytes is shown by the fact that a slight increase in the quantity of electrolytes present has greatly increased the rapidity of clumping. An experiment which will be described later appears to afford conclusive evidence in favour of this view.

Experiment V.

In the last two experiments the rate of increase in viscosity was considerably less than in Experiment II. The optimum temperature for the action of pectase is stated by Frémy to be 30° C., and, therefore, one would expect that at 21° C. the action would have been more rapid than at 14° C. That the opposite was the case seems to show that the sap was less active in the later experiments, or else that the pectin solution had become weaker through decomposition. The sap used in Experiment II was extracted on the 14th of May, soon after the leaves had emerged from the bud, whereas that used in Experiments III and IV was extracted on the 22nd June.

If the sap in the newly formed leaves contained more pectase, it is probable that if only young leaves were used the sap would be more active than that obtained chiefly from full-grown leaves, as was usually the case. On the 22nd of June sap was extracted from young leaves which were less than one-third the size of the full-grown leaf. An experiment was performed in the usual way at 21° C., but the rate of increase in viscosity was found to be about the same as in Experiments III and IV. To avoid confusion a curve was not plotted, as this experiment was carried on for two hours only. The result seems to show that the slower action in these experiments was not due to a decrease in the amount of pectase present, but was due to partial decomposition of the pectin solution. It is probable, however, that further experimental work would show that there is a variation in the amount of pectase present in the sap of leaves of different ages.

Experiment VI.

In Experiment IV an addition of electrolytes greatly increased the rate of clumping of the gel. In this experiment the object in view was to remove the electrolytes as far as possible, and see what effect this would have on the clumping.

Sap was extracted from the leaves in the usual manner, and centrifuged. To 3 ccs. of the sap 15 ccs. of spirit was added, and the liquid containing the precipitated protoplasm and enzymes was centrifuged and the clear liquid poured off. The residue was dissolved in 3 ccs. of distilled water, and 1 cc. of the solution was used instead of the ordinary sap, but otherwise details of the experiment were the same as in the last three.

The viscosity increased slowly at first, and then rapidly. The highest time of flow in the viscosimeter shown on the curve belonging to the experiment is only 100 seconds, whereas readings were taken up to nearly 500 seconds, and after this the mixture refused to flow through the capillary.

This experiment shows clearly that removal of electrolytes removes also the factor which causes clumping of the gel.

The gel was left in the viscosimeter until next day, when it was found that clumping had taken place in the meantime, probably owing to a trace of electrolytes which had not been removed.

DISCUSSION OF RESULTS.

These experiments show that the product of action of pectase on pectin has the structure of a gel composed of a semi-solid reticulum, in the meshes of which a liquid is distributed, and also that the gel, when formed, is broken up in the presence of electrolytes by the clumping of the more solid portion into separate aggregates.

It appears that what has hitherto been described as the coagulation of pectin is made up of two processes, gelification, and subsequent coagulation of the gel.

Bertrand and Mallèvre (2) came to the conclusion that the coagulum formed by the action of pectase is composed of calcium pectate, and not of pectic acid, as previously stated by Frémy (9). They base this conclusion on the fact that the coagulum is completely insoluble in feeble alkaline liquids, but dissolves almost instantaneously after having been macerated in dilute hydrochloric acid, and the resulting solution is found to contain calcium. They also state that if juice, extracted from carrots, which has been decalcified by addition of oxalic acid, be added to a solution of pectin from which calcium has been carefully removed, the mixture remains indefinitely liquid, but the least addition of a soluble calcium salt causes rapid coagulation. They point out that, owing to the very high molecular weight of the pectic compounds, and to the enormous volume which they occupy in the gelatinous state, the amount of calcium required for the formation of a coagulum consisting of calcium pectate would be very small.

In view of the results obtained in Experiment VI it seems possible that the calcium or other metallic ions act in a purely physical manner in causing coagulation. In this experiment sap was extracted from the leaves, and the enzymes precipitated by the addition of five times its volume of alcohol. The precipitated enzymes were redissolved in water, and added to pectin solution, from which dissolved calcium salts were absent. In this way any electrolytes, including calcium salts, would have been almost completely removed. The experiment, therefore, affords no evidence that the gel which is formed is composed of calcium pectate.

If the statement of Bertrand and Mallèvre is correct, namely, that in the absence of calcium salts a mixture of pectase and pectin remains indefinitely liquid, then it would seem that gelification, as well as coagulation, is due to a minute trace of electrolytes. As far as gelification is concerned, the action of the electrolytes is possibly indirect, and it may be that their presence is necessary in order to allow the enzyme to exert its activity.

If this is the case, then possibly the events which take place during the coagulation of pectin are somewhat as follows :—Under the action of pectase some kind of pectic acid is produced from the pectin. If electrolytes are completely absent, the mixture remains liquid; but if any ions are present, and especially if they belong to one of the divalent metals such as calcium, partial precipitation of the colloid takes place. At first these colloidal particles will be free from one another, and while this is the case the viscosity will remain almost constant, thus explaining the first portion of the curves. As the action of the pectase proceeds, and more material is formed, which can be precipitated by the electrolytes, a semi-solid reticulum is gradually built up with a consequent rise in viscosity. As the reticulum becomes denser, the viscosity will increase rapidly; but if electrolytes are present in larger proportion, or if they are allowed to act for a sufficiently long time, clumping together of the particles of colloid forming the reticulum of the gel takes place, and a suspension is formed with a consequent diminution in viscosity. A maximum viscosity will be reached when the rate of gel-formation becomes insufficient to counterbalance the clumping effect.

All this is merely a suggestion, and further experiments would be necessary in order to obtain more definite information on the changes which take place; but as I am unable to proceed with this work at present, it seems better to publish the results which have already been obtained.

SUMMARY.

1. During the action of pectase on a solution of pectin the electrical conductivity of the mixture remains practically constant, showing that a gel is formed, and not merely a very viscous liquid.

2. The changes in viscosity during coagulation were determined by means of a viscosimeter of the Ostwald type. By comparison of experiments at 0 C. and 14° C. the activity of the enzyme was found to be very much greater at the higher temperature.

3. The viscosity was found to increase slowly at first, then more rapidly until a maximum was reached, and this was followed by a rapid decrease. Increase in the amount of electrolytes present lowered the maximum, while a decrease in the electrolytes raised it. The decrease in viscosity is probably to be explained by the action of the electrolytes in clumping together the particles of colloid forming the reticulum of the gel, so that a suspension is produced.

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A QUANTITATIVE EXAMINATION OF THE ELEMENTS OF THE WOOD OF TREES IN RELATION TO THE SUPPOSED FUNCTION OF THE CELLS IN THE ASCENT OF SAP.

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XXIX.

A QUANTITATIVE EXAMINATION OF THE ELEMENTS OF THE WOOD OF TREES IN RELATION TO THE SUPPOSED FUNCTION OF THE CELLS IN THE ASCENT OF SAP.

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AND

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[Read December 15, 1914. Published January 7, 1915.]

The wood of trees, which forms the conducting system for the ascending sap, is composed of lifeless and living elements—the tracheæ and cells. Some of the living elements form vertical sheets or laminæ in the wood, especially surrounding the vessels. These cells constitute the wood parenchyma; the cells of the medullary rays form radiating bands of tissue penetrating between the tracheæ, and connecting together the tracts of wood parenchyma in a radial direction, and putting them in communication with the bark on one side, and often with the pith on the other. In no place do the cells of the wood parenchyma or the medullary rays interrupt the vertical continuity of the tracheæ, which form continuous series running from the roots to the topmost twigs.

It was suggested by one of us (2) that this distribution precluded the intervention of the cells in raising the transpiration stream. It seemed certain that the action of these cells in secreting water laterally into the tracheæ, where it would be free to percolate downwards, could be of no assistance in raising the sap.

Janse (9) took exception to this suggestion, and urged that it is based on the neglect of the resistance to the flow of water offered by the tracheæ. His idea appears to be that if the cells secrete water fast enough into the tracheæ the upward current will only be diminished by the percolation downwards—in fact, that the cells must not only raise the transpiration stream, but must also overcome the leakage backwards due to the permeability of the wood. This view seems to contemplate an amazingly inefficient mechanism for raising the sap.

Janse (9 and 10) has worked out his theory in considerable detail. As was the case of Godlewski's theory, Janse's applies most easily to Conifers. He assumes that the protoplasm in the cells of the medullary rays during transpiration circulates actively—the circulation taking place chiefly in a vertical plane. The protoplasm which is moving across the lower horizontal face of the cell, he supposes, fixes water in its vesicles by means of an enzyme; the water is retained as the protoplasm climbs up one vertical side, but is released from the vesicles by a reversal of the enzymic action, as the stream reaches the upper horizontal wall. The water fixed in the vesicles on the lower side is taken from a lower tracheid, and when it is released from the vesicles on the upper side, it is liberated into a tracheid at a higher level. The one medullary-ray cell is, at least in the Conifers, supposed to serve several tracheids on each side.

According to this scheme, at any one moment half the protoplasm of each cell, at the most, is raising water, while the other half is returning empty. Therefore we may assume that the whole upward transpiration stream is being passed through, as a maximum value, half the lumen of each of the medullary-ray cells in any cross-section. Hence, if we know the average velocity of the streaming, and measure the cross-section of the cells, we will have a major limit of the amount of water passed upwards in this manner. To obtain the actual velocity of the stream in the wood caused by this supposed action of the cells, it will be necessary also to make allowance for the amount of water which will be continually flowing backwards through the tracheæ under the action of gravity.

So far as we are aware no measurements have been recorded on the velocity of streaming in the medullary-ray cells or in the cells of the wood parenchyma. Janse (9) has observed the streaming, but has not recorded its velocity. The average velocity of streaming in the endodermis cells, to which he assigns a similar function, he estimates at 0·03 cm. per min. at 19°. The most rapid streaming in closed cells hitherto observed seems to be 0·2–0·3 cm. per minute (5).

All direct intervention of the living elements of the stem in the lifting of the transpiration stream has been negatived by such a large body of experiment (3) that the refutation of any special hypothesis involving this intervention seems almost superfluous; however, as this consideration of Janse's has led us to make some measurements on the structure of the conducting tracts of some trees which may be of use to other investigators, we have thought it worth putting these measurements on record, and incidentally discussing their bearing on this hypothesis.

Measurements of the Cross-section of the Elements of the Wood of Conifers.

To obtain an accurate measure of the cross-section of the cells in the wood the following method (3) was adopted: a *camera lucida* drawing, or a micro-photograph, was prepared of a transverse section of the wood under investigation. This drawing or photographic print was then carefully cut out, the walls, the lumina of the vessels, of the tracheids and of the cells being kept apart and weighed. From these weights the percentages of the total cross-section occupied by the walls and the lumina of the various elements were deduced.

To estimate the rate of leakage backwards, which must be overcome by the action of the cells before any of the water elevated is available for the transpiration stream, we proceeded as follows:—Pieces of the stems of the trees investigated about 10 cm. × 2 cm. were stripped of their bark, and supported in a vertical position; the upper end of each was kept flooded with water, and the weight of water transmitted thus under unit head in a given time was determined. The water was supplied drop by drop to the upper surface, so that the head did not vary appreciably. The water collecting on the lower surface was drained away by a piece of bibulous paper, so that the discontinuity of drop-formation did not cause an error in weighing. To prevent water passing down on the outside of the woody cylinders a band of vaseline about 2 cm. wide was smeared round the upper end (1).

The comparative simplicity of the structure of the wood of the conifers adapts them peculiarly to Janse's scheme, and the application of his theory to them is more readily comprehended than it is to other trees. Indeed, as was the case of Godlewski's theory (6), it seems doubtful whether the theory under discussion presents any advantages over Westermaier's hypothesis (12 and 13) when it is applied to dicotyledonous trees.

For this reason the measurements mentioned above were first carried out in *Pinus silvestris* (3). A *camera lucida* drawing was made of a cross-section of the wood of this conifer, and then the lumina of each of the elements, viz., medullary ray cells and tracheids, were cut out of the drawing with a sharp knife. The network composed of the woody walls remained over. The weights of the tracings gave the following percentage areas for each category:—

Lumina of medullary-ray cells, . .	6·9	per cent.
,, ,, tracheids, . . .	61·2	,,
Walls of tracheids and cells, . .	31·9	,,

It follows from these figures that the cross-section of the streaming protoplasm effective in raising the sap is not more than 3·5 per cent. of the whole cross-section, and about $\frac{1}{18}$ of the total cross-section of the tracheids. Across this small section we may expect a flow approximating to 0·03 cm. per minute (taking Janse's average figure for the streaming in water-conveying cells), and this might be considered as being capable of producing a flow of about $\frac{1}{18}$ that velocity in the tracheidal channels, or about 0·00165 cm. per minute.

It has been found (1) that water in coniferous wood, when flowing under the action of its own weight, percolates downwards at the rate of from 7–10 cm. per hour, or 0·117–0·167 cm. per minute. It is evident that streaming of the protoplasm producing a velocity in the tracheids of barely 0·00165 cm. per minute could not overcome this leak. Even if we take the highest velocity of streaming which has been observed in any cell, viz., 0·3 cm. per minute (instead of that observed by Janse, viz., 0·03 cm.), and accept the hypothetical enzymic pumping-action, we cannot suppose that the rate of lifting water would nearly balance the leak backwards, much less provide for the raising of the transpiration stream in addition.

Measurements of the Cross-section of the Elements of the Wood and of the Percolation of Water in the Wood of Dicotyledons.

For the sake of testing the applicability of the theory to Dicotyledonous trees, we have made some further measurements. In these cases it is not always easy to distinguish on cross-sections between wood parenchyma and tracheids, so some small error in the estimate of these elements may be anticipated. By making several observations this error is probably eliminated, and can never be large.

In the following measurements microphotographs were employed, and were cut out and weighed in the same way as the *camera lucida* drawing already described.

Where the medullary rays are large the ratios of cells to the tracheæ are very variable in different photographs, as a field, giving sufficient magnification, may be largely or entirely composed of one or other sort of element. This uncertainty has been minimized by multiplying photos of fields taken at random, and so obtaining a more or less characteristic average, and also by determining the ratio of the area of the medullary rays to the rest of the wood on special photos of small magnification. The greatest divergences from the mean were found in *Acer pseudoplatanus*. The results of the measurements are given in Table I. The figures indicate percentages.

TABLE I.

Acer pseudoplatanus.

Section	Lumina of Cells	Lumina of Tracheids	Lumina of Vessels	Total area of lumina of all Tracheæ	Walls
A	44·5	7·6	7·5	15·0	**40·4**
B	10·2	9·7	**40·9**	**50·6**	39·2
C	**62·4**	2·9	0	2·9	34·6
D	11·5	12·5	36·5	49·0	39·5
E	23·4	18·8	20·8	39·6	37·0
F	17·0	**25·2**	18·6	43·8	39·1
Mean	28·2	12·8	20·7	33·5	38·3

Three measurements on photographs of low magnification of the medullary rays (lumina and walls included) alone gave 21·4 per cent., 14·0 per cent., and 7·1 per cent. respectively.

The average area occupied by the lumina of the cells amounts to about 28·2 per cent. of the whole.

The determination of the "leak,' or downward percolation of water in a piece of stem of *Acer pseudoplatanus* under a head of water equal to its length, was made on a cylinder of wood having an average cross-section of 3·70 sq. cm. and a length of 10·4 cm. The "leak" was found to be 0·398 c.c. per minute.

The average cross-section of the upward streaming protoplasm in this stem would be 0·522 sq. cm. The minimum velocity of streaming required merely to overcome the "leak" observed would be 0·763 cm. per minute, or about 25 times that observed by Janse. Furthermore, to give rise to an upward movement of water in the tracheæ of, say, 10 cm. per hour, would require that the streaming of protoplasm in the cells should be at least 30 times as fast as that observed by that investigator.

The foregoing determination of the rate of percolation was made with a piece of stem which had been partially injected with water, as it seemed that the supposed secretion of water upward by the cells would have to overcome the leak through all the tracheæ downward; however, in case it might be urged that the presence of air-bubbles in the tracheæ was a necessary link in the mechanism, another determination was made on a piece of wood cut directly from the tree and without any injection of water. As the experiment was made in November, when the fresh-cut wood is quite dry, and contains so much air that it floats in water, and since no precautions were taken to prevent the formation of air-bubbles in the tracheæ on cutting, it is certain that this fresh bit of wood contained at least the normal amount of bubbles. In the second experiment the area of the cross-section of the wood was 2·35 sq. cm. According to the measurements given in Table I this would include 0·66 sq. cm. of cells, of which 0·33 sq. cm. might be engaged in raising water. We would have to assume protoplasmic streaming through this cross-section with a velocity of 0·970 cm. per minute, merely to overcome the downward percolation, which amounted to 0·321 g. per minute. Such a velocity is 32 times that observed by Janse, and is, of course, quite improbable.

TABLE II.

Cotoneaster frigida.

Section	Lumina of Cells	Lumina of Tracheids	Lumina of Vessels	Total area of lumina of all Tracheæ	Walls
A	7·1	11·9	32·3	44·2	48·7
B	3·4	17·3	38·3	55·6	40·9
C	11·6	8·7	40·9	49·6	38·8
Mean	7·4	12·6	37·2	49·8	42·8

In Table II are given the determinations of the areas of the cross-sections of the elements of the wood of *Cotoneaster frigida*, arrived at in the same manner as those for *Acer pseudoplatanus.*

The "leak" for this wood was also determined in the fresh and in the injected condition, and the amount of downward percolation through a piece of stem having a cross-section of 1 sq. cm. under unit head was found to be 0·080 g. per minute, for a similar piece with the same cross-section, but injected, was 0·103 g. under the same conditions. Of the total cross-section the effective area of the cells would be 0·037 sq. cm., and to overcome the leak in the fresh wood protoplasmic streaming having a velocity of 2·173 cm. per minute would have to be assumed, and in the case of the injected 2·788 cm. per minute. Such a velocity is again of course quite inadmissible.

It should be noticed that in the piece of Cotoneaster stem examined, many of the vessels, perhaps one-sixth of all, were choked with a pale-brown transparent substance. If this had been absent, a still greater rate of "leak" would have been observed.

TABLE III.

Fagus silvatica.

Section	Cells	Tracheids	Vessels	Tracheæ	Walls
A	15·5	25·4	13·0	38·5	46·0
B	15·4	22·1	19·8	41·9	42·7
C	8·6	27·6	14·8	42·5	48·9
Mean	13·1	25·0	15·8	41·0	45·9

Similar measurements were made on the wood of *Fagus silvatica.* The areas occupied by the various elements are recorded in Table III. The percolation, or "leak," was practically the same in the fresh and in the injected specimens, viz. 0·305 g. and 0·294 g. per sq. cm., respectively. This was not surprising, since the cylinder cut fresh from the branch was moist, and sank in water. Evidently injection altered its condition but slightly, since nearly all its tracheæ contained water only. The velocity of protoplasmic streaming necessary to overcome this "leak" is 4·656 cm.–4·496 cm. per minute—a still more exorbitant demand than that of the two previous determinations.

TABLE IV.

Ilex aquifolium.

Section	Cells	Tracheids	Vessels	Tracheæ	Walls
A	13·1	11·7	15.9	27·6	59·2
B	6·5	17·8	29·1	46·9	46·6
C	11·4	21·3	10·8	32·1	56·4
D	9·9	20·5	14·0	34·5	55·5
Mean	10·2	17·9	17·4	35·3	54·4

Table IV shows the determination of the areas for *Ilex aquifolium.* As in the case of Fagus, the "leak" for the fresh and injected wood was practically the same; and here, again, the fresh wood was moist and sank. Measurement of the amount of water which percolated through a cross-section of 1 sq. cm. under unit head gave for the fresh wood 0·055 g. per minute, and for the injected 0·056 g. To overcome this leak a protoplasmic streaming in the cells having a velocity of 1·085 cm. per minute must be admitted.

The fresh wood of *Populus alba* taken in November was very *dry*, and had a small specific gravity; accordingly the amount percolating through a piece of stem having a cross-section 1 sq. cm. is very different in a fresh and in an injected specimen. Through the former 0·158, and through the latter 0·285 g., passed through under unit head per sq. cm. per minute.

Table V.

Populus alba.

Section	Cells	Tracheids	Vessels	Tracheæ	Walls
A	12·8	28·5	22·1	50·6	36·6
B	6·8	23·0	34·0	57·0	36·3
Mean	9·8	25·8	28·0	53·8	36·4

From Table V we see the average cross-section of the upward streaming protoplasm of the cells is 0·049 sq. cm. In this we would require a movement having a velocity of 3·220 cm. per minute, to overcome the leak of the fresh stem or, 5·825 cm. per minute, to counterbalance the leak of the injected stem.

Two other determinations were made on the wood of *Populus alba.* In them two adjacent portions (*a*) and (*b*) of the same branch were used. They were prepared from the branch on the day after it had been cut from the tree. Meanwhile the branch had lain exposed to the air. Owing to this circumstance, it was to be expected that the experimental cylinders would contain comparatively large quantities of air. The first woody cylinder (*a*) had an effective cross-section (*i.e.* omitting pith and stained heart wood) of 4·12 sq. cm., and before injection transmitted 0·177 g. per minute, or 0·043 g. per sq. cm. per minute. To make good this percolation, protoplasmic streaming in the cells having a velocity of 0·876 cm. per minute would be required. When injected (*a*) transmitted 0·210 g. per sq. cm. per minute, necessitating a velocity of 4·289 cm. per minute for the protoplasm. In the case of the cylinder (*b*) the rate of leak before injection was 0·083 g. per sq. cm. per minute; after injection, this rate became 0·277 g. per sq. cm. per minute. The protoplasmic velocities in the cells necessary to overcome these would be 1·687 cm. per minute, and 5·654 cm. per minute respectively.

Of the stems examined by us that of a Prunus—unfortunately the species was not recorded—showed the smallest proportion of cells in the cross-section.

The total area of the lumina of the cells was only 3·1 per cent. of the whole cross-section. That of the tracheae was 44·6 per cent., that of the walls 52·2 per cent.

Table VI.

Salix babylonica.

Section	Cells	Tracheids	Vessels	Tracheæ	Walls
A	4·9	16·3	37·6	53·9	41·2
B	6·4	21·5	39·3	60·9	32·7
Mean	5·7	18·9	38·4	57·4	37·0

Salix babylonica also has a very small proportion of cells, as may be seen from inspection of Table VI. With Salix injection caused no increase in the rate of percolation. This may probably be explained, not because the wood was already full of water—for, in fact, it was dry, and floated—but because the leak took place principally through the large vessels which were full of water, while the air was confined to the tracheids, which in any case transmit water comparatively slowly (11). The amount of leak per sq. cm. was 0·122 g. per minute through the fresh, and 0·115 g. through the injected stem. Protoplasmic streaming in the cells to overcome these leaks would require velocities of 4·299 cm. and 4·032 cm. per minute respectively.

Table VII.

Syringa vulgaris.

Section	Cells	Tracheids	Vessels	Tracheæ	Walls
A	6·3	9·4	36·2	45·6	48·1
B	13·7	10·9	24·1	35·0	51·4
Mean	10·0	10·1	30·1	40·3	49·7

Syringa vulgaris formed our last subject for these measurements; and in Table VII the results are recorded. The wood was sappy, and sank in water; but, notwithstanding this, the injected specimen showed a considerably larger leak than the fresh one. The leak for the fresh was 0·082 g. and that for the injected specimen was 0·129 g. per minute per sq. cm. From these figures Janse's hypothesis would demand a velocity of protoplasmic streaming at least amounting to 1·637 cm. and 2·574 cm. per minute to overcome the leak alone.

From the foregoing records it will be seen that measurements and experiment lend no support to Janse's hypothesis as to the intervention of the living cells in the ascent of sap in stems—the velocity of protoplasmic streaming in the cells demanded by the hypothesis ranging from 0·76 cm. per minute to 5·82 cm. per minute, whereas the most rapid streaming in closed cells, hitherto recorded, is 0·2 cm.–0·3 cm. per minute; while Janse himself records 0·03 cm. per minute in the endodermis cells of the root to which he ascribes a similar function.

No doubt one principal reason why so many investigators have ascribed a direct function in raising the sap to the living elements of the wood, is the fact that no other function has been generally assigned to them. The recognition, however, that the tracheæ not only conduct mineral solutions and water upwards in trees, but also distribute carbohydrates throughout the plant, immediately shows the necessity of living elements in the wood. The function of the wood-parenchyma and the medullary rays is to transmit carbohydrates from the bark into the wood, to transform and store them there, and finally to secrete them into the tracheæ. Not only does this secretion in all probability give rise to root-pressure, and so lead to a translocation of carbohydrates upwards in spring, but even in times of rapid transpiration it charges the sap, as it is drawn upwards in the tracheæ, with carbohydrates, and thus supplies the upper growing regions with the products of assimilation to be used in respiration and growth. This question is discussed more fully in an account of an investigation recently carried out on the contents of the sap of the conducting channels of plants (4).

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THE
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Vol. XIV. (N.S.), No. 30. JANUARY, 1915.

AN EXAMPLE OF THE MULTIPLE COUPLING OF MENDELIAN FACTORS.

BY

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XXX.

AN EXAMPLE OF THE MULTIPLE COUPLING OF MENDELIAN FACTORS.

By JAMES WILSON, M.A., B.Sc.,

Professor of Agriculture in the Royal College of Science, Dublin.

[Read December 15, 1914. Published January 8, 1915.]

The history of the English Campine varieties of fowl, as told in two pamphlets, "The Campine" and "The Production of the English Type Gold Campine," by the Rev. E. Lewis Jones, reveals the coupling of more than two factors in the presence of uncoupled factors having effects similar to those of the coupled factors.

Multiple coupling was revealed in the Cambridge experiments with sweet peas. Two varieties, *Duke of Westminster* and *Painted Lady*, whose flowers differed from each other in three pairs of characters, were mated, and, in the progeny of their hybrids there were only three groups instead of eight, as there should have been, had there been no coupling.

Multiple coupling is undoubtedly common, and is very obvious in regard to sex, since many characters invariably follow either the one sex or the other.

In the Campine fowl the factor for femaleness is coupled with two other factors at least; but, because of the presence of uncoupled factors having similar effects, the same sex is not always followed by the same characters.

A well known case of a similar nature, but in which only two factors are coupled, might be quoted by way of introduction.

It has already been shown that in fowl the males are pure as regards the factors for sex, while the females are hybrid. The two sexes may therefore be represented factorially as—

Male.	Female.
$M M$	$M F$

When pure barred males are mated with plain feathered females the progeny are all barred: from which it can be inferred that barring is dominant to plainness. On the other hand, when pure bred barred females are mated with plain males, only half the progeny are barred, while the other half are plain. From this it can be inferred that barred females, though pure bred, according to the poultry breeders, are not genetically pure for barring, but carry a factor for plainness in addition. Pure bred barred fowl may therefore be represented as follows :—

Males.	Females.
$M\ M$	$M\ F$
$B\ B$	$B\ p$

Nor can the females be bred pure for barring : they carry always a factor for plainness. Males, however, can be bred pure either for barring or plainness. Thus the four following kinds of fowl may be represented factorially as follows :—

Barred males.	Barred females.	Plain males.	Plain females.
$M\ M$	$M\ F$	$M\ M$	$M\ F$
$B\ B$	$B\ p$	$p\ p$	$p\ p$

In addition to this there is the striking phenomenon that the barred progeny of barred females and plain males are all males, while the plain progeny are all females; and of this phenomenon the only possible explanation is that, while the factor M can associate with either B or p, F can associate with p only. The latter two are coupled together. Surrounding the factors F and p with a closed bracket, to prevent confusion, we may therefore represent the barred males and females factorially as—

$$\begin{array}{ccc} \text{Males.} & \qquad & \text{Females.} \\ \begin{matrix} M & M \\ B & B \end{matrix} & & \begin{matrix} M \\ B \end{matrix} \boxed{\begin{matrix} F \\ p \end{matrix}} \end{array}$$

The English Campines are descended from Belgian stock, the first of which were brought to England about a quarter of a century ago. The Belgian stock were of two kinds as regards colour : a silver and a gold, and both kinds were barred. But, while the hens were barred all over, excepting on the hackle, the cocks were unbarred not only on the hackle, but also on the back and tail. Occasionally cocks appeared as fully barred as the hens, but such were not preserved by the Belgian breeders. English breeders, on the other hand, preferred these fully barred males, and, preserving them, eventually produced silver and gold varieties of the breed which were equally fully barred in both sexes. The fully barred fowl are spoken of as of "English type," the others as of "Belgian type."

The fact that the Belgian hens are always of English type, while the cocks, which are usually Belgian, may be of English type occasionally, is clear evidence that the factor for English type is coupled with femaleness.

And the further fact that Belgian cocks have not the power of leaving all their progeny Belgian is also clear evidence that the English type is dominant to the Belgian.

Thus, putting E for English type and b for Belgian, the Belgian breed of fowl may be represented factorially so far as type is concerned, thus:—

Males.	Females.
$\begin{array}{cc} M & M \\ b & b \end{array}$	$\begin{array}{c\|c\|} \cline{2-2} M & F \\ b & E \\ \cline{2-2} \end{array}$

The male birds of English type which appear occasionally among Belgian stock may therefore be represented by $\begin{matrix} M & M \\ b & E \end{matrix}$, and the mothers of such birds must have carried the factors $\begin{array}{c|c|} \cline{2-2} M & F \\ E & E \\ \cline{2-2} \end{array}$.

That the silver colour is dominant to the gold and the factor for gold coupled with that for femaleness is brought out by several results mentioned in Mr. Jones's pamphlets; for, when silver males are mated with gold females the progeny are all silver, but when gold males are mated with silver females only half the progeny are silver, while the other half are gold. Besides, the silver progeny are all males, while the gold progeny are all females. The factor for gold colour is thus coupled with that for femaleness.

The factorial representation of the Belgian and English Campines becomes therefore:—

Belgian silver males.	Belgian silver females.	Belgian gold males.	Belgian gold females.	English silver males.	English silver females.	English gold males.	English gold females.
$\begin{array}{cc} M & M \\ b & b \\ S & S \end{array}$	$\begin{array}{c\|c\|} \cline{2-2} M & F \\ b & E \\ S & g \\ \cline{2-2} \end{array}$	$\begin{array}{cc} M & M \\ b & b \\ g & g \end{array}$	$\begin{array}{c\|c\|} \cline{2-2} M & F \\ b & E \\ g & g \\ \cline{2-2} \end{array}$	$\begin{array}{cc} M & M \\ E & E \\ S & S \end{array}$	$\begin{array}{c\|c\|} \cline{2-2} M & F \\ E & E \\ S & g \\ \cline{2-2} \end{array}$	$\begin{array}{cc} M & M \\ E & E \\ g & g \end{array}$	$\begin{array}{c\|c\|} \cline{2-2} M & F \\ E & E \\ g & g \\ \cline{2-2} \end{array}$

Although it is unnecessary, for the above hypothesis is clear as it stands, it might be well to show how it can be confirmed by a selection from Mr. Jones's experiments.

I.

English silver male.	and	Belgian gold female.	should give	English silver males.	and	English silver females.
$\begin{array}{cc} M & M \\ E & E \\ S & S \end{array}$		$\begin{array}{c\|c\|} \cline{2-2} M & F \\ b & E \\ g & g \\ \cline{2-2} \end{array}$		$\begin{array}{cc} M & M \\ b & E \\ S & g \end{array}$		$\begin{array}{c\|c\|} \cline{2-2} M & F \\ E & E \\ S & g \\ \cline{2-2} \end{array}$

Mr. Jones got 6 English silver males and 6 English silver females

II.

$$\begin{array}{ccc} \text{Hybrid.} & \text{and} & \text{Hybrid.} \\ \begin{array}{cc} M & M \\ b & E \\ S & g \end{array} & & \begin{array}{c} M \\ E \\ S \end{array}\boxed{\begin{array}{c} F \\ E \\ g \end{array}} \end{array}$$

should give

$$\begin{array}{cccccccc} \multicolumn{4}{c}{\text{English silver males.}} & \begin{array}{c}\text{English}\\ \text{silver}\\ \text{females.}\end{array} & \begin{array}{c}\text{English}\\ \text{gold}\\ \text{females.}\end{array} & \begin{array}{c}\text{English}\\ \text{silver}\\ \text{females.}\end{array} & \begin{array}{c}\text{English}\\ \text{gold}\\ \text{females.}\end{array} \\ \begin{array}{cc} M & M \\ E & E \\ S & S \end{array} & \begin{array}{cc} M & M \\ E & E \\ S & g \end{array} & \begin{array}{cc} M & M \\ b & E \\ S & S \end{array} & \begin{array}{cc} M & M \\ b & E \\ S & g \end{array} & \begin{array}{c} M \\ E \\ S \end{array}\boxed{\begin{array}{c} F \\ E \\ g \end{array}} & \begin{array}{c} M \\ E \\ g \end{array}\boxed{\begin{array}{c} F \\ E \\ g \end{array}} & \begin{array}{c} M \\ b \\ S \end{array}\boxed{\begin{array}{c} F \\ E \\ g \end{array}} & \begin{array}{c} M \\ b \\ g \end{array}\boxed{\begin{array}{c} F \\ E \\ g \end{array}} \end{array}$$

Mr. Jones got English silver males, English silver females, and English gold females; and he writes that "the proportion of silver to gold females was generally equal."

III.

$$\begin{array}{ccc} \begin{array}{c}\text{Hybrid}\\ \text{silver}\\ \text{male.}\end{array} & \text{and} & \begin{array}{c}\text{English (apparently}\\ \text{impure) gold}\\ \text{female.}\end{array} \\ \begin{array}{cc} M & M \\ b & E \\ S & g \end{array} & & \begin{array}{c} M \\ b \\ g \end{array}\boxed{\begin{array}{c} F \\ E \\ g \end{array}} \end{array}$$

should give

$$\begin{array}{cccccccc} \begin{array}{c}\text{English}\\ \text{silver}\\ \text{males.}\end{array} & \begin{array}{c}\text{English}\\ \text{gold}\\ \text{males.}\end{array} & \begin{array}{c}\text{Belgian}\\ \text{silver}\\ \text{males.}\end{array} & \begin{array}{c}\text{Belgian}\\ \text{gold}\\ \text{males.}\end{array} & \begin{array}{c}\text{English}\\ \text{silver}\\ \text{females.}\end{array} & \begin{array}{c}\text{English}\\ \text{gold}\\ \text{females.}\end{array} & \begin{array}{c}\text{English}\\ \text{silver}\\ \text{females.}\end{array} & \begin{array}{c}\text{English}\\ \text{gold}\\ \text{females.}\end{array} \\ \begin{array}{cc} M & M \\ E & b \\ S & g \end{array} & \begin{array}{cc} M & M \\ E & b \\ g & g \end{array} & \begin{array}{cc} M & M \\ b & b \\ S & g \end{array} & \begin{array}{cc} M & M \\ b & b \\ g & g \end{array} & \begin{array}{c} M \\ E \\ S \end{array}\boxed{\begin{array}{c} F \\ E \\ g \end{array}} & \begin{array}{c} M \\ E \\ g \end{array}\boxed{\begin{array}{c} F \\ E \\ g \end{array}} & \begin{array}{c} M \\ b \\ S \end{array}\boxed{\begin{array}{c} F \\ E \\ g \end{array}} & \begin{array}{c} M \\ b \\ g \end{array}\boxed{\begin{array}{c} F \\ E \\ g \end{array}} \end{array}$$

Mr. Jones got English silver males, English gold males, Belgian silver males, Belgian gold males, and English silver females and English gold females. Mr. Jones cannot now give the proportions.

IV.

$$\begin{array}{ccc} \begin{array}{c}\text{Hybrid}\\ \text{silver}\\ \text{male.}\end{array} & \text{and} & \begin{array}{c}\text{English (pure)}\\ \text{silver}\\ \text{female.}\end{array} \\ \\ \begin{array}{cc} M & M \\ E & b \\ S & g \end{array} & & \begin{array}{c|c|}\cline{2-2} M & F \\ E & E \\ S & g \\ \cline{2-2}\end{array} \end{array}$$

should give

$$\begin{array}{cccccccc} \multicolumn{4}{c}{\text{English silver males.}} & \begin{array}{c}\text{English}\\ \text{silver}\\ \text{females.}\end{array} & \begin{array}{c}\text{English}\\ \text{gold}\\ \text{females.}\end{array} & \begin{array}{c}\text{English}\\ \text{silver}\\ \text{females.}\end{array} & \begin{array}{c}\text{English}\\ \text{gold}\\ \text{females.}\end{array} \\ \\ \begin{array}{cc} M & M \\ E & E \\ S & S \end{array} & \begin{array}{cc} M & M \\ E & E \\ S & g \end{array} & \begin{array}{cc} M & M \\ E & b \\ S & S \end{array} & \begin{array}{cc} M & M \\ E & b \\ S & g \end{array} & \begin{array}{c|c|}\cline{2-2} M & F \\ E & E \\ S & g \\ \cline{2-2}\end{array} & \begin{array}{c|c|}\cline{2-2} M & F \\ E & E \\ g & g \\ \cline{2-2}\end{array} & \begin{array}{c|c|}\cline{2-2} M & F \\ b & E \\ S & g \\ \cline{2-2}\end{array} & \begin{array}{c|c|}\cline{2-2} M & F \\ b & E \\ g & g \\ \cline{2-2}\end{array} \end{array}$$

Mr. Jones got English silver males and females, and English gold females. He writes that this experiment was carried out by several people, and that taken altogether the proportions were 4 : 3 : 1. The proportion of silver to gold females should have been 1 : 1. The discrepancy is likely to be accounted for by some of the females not having been pure for English type. The English silver breed is only about ten years old, and undoubtedly many hens which breeders consider pure are not pure genetically.

It will be well to bring the foregoing result into line with previous work. Castle and Pearl showed that the American Barred Plymouth Rocks are merely black fowl having portions of their feathers lacking colour, i.e. barred. The "silver" Campine is therefore a black fowl with colourless bars on its plumage; and the black colour is dominant to the golden. Then, putting Bl for black and Bd for barred, a silver Campine hen of the Belgian breed is factorially represented by

$$\begin{array}{c|c|}\cline{2-2} M & F \\ b & E \\ Bl & g \\ Bd & p \\ \cline{2-2}\end{array}\,.$$

NOTE.—It will be noticed that experiments II and IV are apparently the same, but, in the former, the hens, though hybrids, were necessarily pure for E, while, in the latter, the hens were not necessarily pure for E, though "pure bred" from the breeder's standpoint.

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OSMOTIC PRESSURES IN PLANTS.

IV.—On the Constituents and Concentration of the Sap in the Conducting Tracts, and on the Circulation of Carbohydrates in Plants.

BY
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UNIVERSITY PROFESSOR OF BOTANY, TRINITY COLLEGE, DUBLIN;
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IV.

$$\overset{\text{Hybrid silver male.}}{\begin{array}{cc} M & M \\ E & b \\ S & g \end{array}} \qquad \text{and} \qquad \overset{\text{English (pure) silver female.}}{\begin{array}{c|c|} \cline{2-2} M & F \\ E & E \\ S & g \\ \cline{2-2} \end{array}}$$

should give

$$\overbrace{\begin{array}{cc} M & M \\ E & E \\ S & S \end{array} \quad \begin{array}{cc} M & M \\ E & E \\ S & g \end{array} \quad \begin{array}{cc} M & M \\ E & b \\ S & S \end{array} \quad \begin{array}{cc} M & M \\ E & b \\ S & g \end{array}}^{\text{English silver males.}} \quad \overset{\text{English silver females.}}{\begin{array}{c|c|} \cline{2-2} M & F \\ E & E \\ S & g \\ \cline{2-2} \end{array}} \quad \overset{\text{English gold females.}}{\begin{array}{c|c|} \cline{2-2} M & F \\ E & E \\ g & g \\ \cline{2-2} \end{array}} \quad \overset{\text{English silver females.}}{\begin{array}{c|c|} \cline{2-2} M & F \\ b & E \\ S & g \\ \cline{2-2} \end{array}} \quad \overset{\text{English gold females.}}{\begin{array}{c|c|} \cline{2-2} M & F \\ b & E \\ g & g \\ \cline{2-2} \end{array}}$$

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XXXI.

OSMOTIC PRESSURES IN PLANTS.

IV.—On the Constituents and Concentration of the Sap in the Conducting Tracts, and on the Circulation of Carbohydrates in Plants.

By HENRY H. DIXON, Sc.D. (Dubl.), F.R.S.,
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[Read December 15, 1914. Published February 25, 1915.]

So long ago as 1858 Th. Hartig (7) recognized that the soluble products of the reserve materials found in the wood-parenchyma and the medullary rays must utilize the tracheae as their channels of transport to the higher regions of plants. This he demonstrated by the depletion of these stores in ringed branches. He concluded that the materials assimilated in the leaves are passed down in the bark and stored in the wood-parenchyma and medullary rays. In spring these store materials are brought into solution, and passed into the tracheae, where they rise with the upward moving current of water from the roots.

In 1888 A. Fisher (3) demonstrated by chemical means the presence of reducing sugars in the tracheae of a large number of trees at various times of the year. He does not appear to have tested for sucrose.

From this it might be inferred that the conveyance of carbohydrates in the wood described by Hartig, and supposed to occur noticeably only in spring, in reality takes place all the year round, but in spring most markedly.

Notwithstanding this, it is surprising to find how Sachs' (9) statement that the water in the tracheae is "an exceedingly dilute solution of these (nutritive) salts, which may be compared at once to ordinary drinking-water," seems to have won the ear of writers: so that the function of the tracheae in conveying organic substances upward is either ignored in text-books and omitted from the consideration of plant physiologists, or its continuance throughout the year is discredited or left doubtful; as, for example, by Jost (5), and in the cautious statement of Haberlandt (6).

In connexion with our investigations on the osmotic pressures and conductivity of solutions of vegetable origin, it seemed to us desirable to make observations on the sap drawn from the conducting tracts of trees.

With this end in view we subjected wood taken from freshly cut branches and roots of *Acer pseudoplatanus* in the month of August to such pressure that sufficient sap was yielded for our determinations. The results are given in Table I.

TABLE I.

Sap pressed from wood of *Acer pseudoplatanus*, August, 1913.

Expt.	—	Δ	Δ_e	$\Delta - \Delta_e$	P	$C \times 10^5$
643	Roots, ...	0·468°	0·163°	0·305°	5·63	350
644	5-ft. level, small branches, .	0·370°	0·136°	0·234°	4·45	292
645	30-ft. ,, ,, ,, .	0·262°	0·103°	0·159°	3·15	222

In this table under Δ are given the depressions of freezing-point due to all dissolved substances; under C the electrical conductivity, or reciprocals of the resistance, measured in ohms. This, of course, depends upon the concentration of the electrolytes only. Under Δ_e is given the depression which would be caused by a concentration of potassium chloride having the observed conductivity. When this depression is subtracted from the total observed Δ, we obtain an approximate estimate of the depression caused by the non-electrolytes—mostly carbohydrates, as recorded under $\Delta - \Delta_e$. Under P are given the osmotic pressures in atmospheres calculated from the total depressions observed of freezing-point.

It may be mentioned here for the sake of comparison that a—

1 per cent. solution of glucose gives a depression of 0·106°, and a
1 per cent. ,, ,, sucrose ,, ,, ,, ,, 0·054°.

Accordingly the sap must contain 1·5 – 3 per cent. of sugar at least, on the assumption that the non-electrolytes are all glucose: or from 3–6 per cent. approximately if sucrose forms the preponderating part.

A little consideration made it clear that the liquid issuing from the crushed wood must contain some sap pressed from the cells of the medullary rays and of the wood parenchyma. If these cells were burst by the pressure, a more or less concentrated solution liberated from their vacuoles would be set free to contaminate the sap in the tracheae. If, on the other hand, the cells are unbroken, their semipermeable membranes will filter the solutions of the vacuoles, and the escaping liquid will dilute the wood sap with nearly pure water, as previously shown by the authors (1). Evidently, then, the true concentration of the sap in the tracheae may be very different from that of the liquid pressed from the wood.

The possibility of centrifuging the sap from the tracheae of pieces of freshly cut wood subsequently suggested itself, and this method was found very successful.

The buckets of our centrifuge conveniently held cylinders of wood 10 cm. long by 2 cm. in diameter. These were cut from stems and roots, and the amount of sap yielded, even when the wood appeared dry, was often surprising. A cylinder of the dimensions just mentioned rendered as much as 1–5 c.c.

The sap obtained by this method was found to be much less concentrated than that obtained by pressure. In the following table (Table II) are given measurements made on sap derived by centrifuging pieces of the same branches and roots as those which supplied the sap for the determinations recorded in Table I.

TABLE II.

Sap from *Acer pseudoplatanus*, August, 1913.

Expt.	—	Δ	Δ_e	$\Delta - \Delta_e$	P	$C \times 10^5$
646	Centrifuged from tracheae of root,	0·070°	0·032°	0·038°	0·84	69
647	Centrifuged from tracheae of branch, 30-ft.-level,	0·049°	0·019°	0·030°	0·59	41
648	Pressed from leaf-cells treated with liquid air,	1·207°	0·646°	0·561°	14·52	1341

Comparison of the depressions of freezing-point and the conductivities of the liquids obtained by the two methods from the wood shows conclusively that pressure, by bursting the cells in the wood, had contaminated the sap of the tracheae both with electrolytes and non-electrolytes. This contamination was further shown by the fact that while the sap *pressed* from the wood became more or less deeply coloured brown owing to the presence of a chromogen and an oxidase (1), that *centrifuged* from it remained almost colourless, indicating that either or both of these bodies were retained in the substance of the wood, or, more precisely, in the living cells. Moreover, the centrifuged sap is neutral to litmus, whereas that obtained by pressure is acid.

At the same time the interesting fact was made clear that even in the month of August the carbohydrates present in the tracheae are sufficiently concentrated to produce a depression of freezing-point amounting to 0·030° and 0·038° in stem and root respectively. It thus appears that the solution of carbohydrates passing up through the tracheae in the transpiration stream has about the same freezing-point as a 0·25 per cent. solution of glucose, or a 0·50 per cent. solution of sucrose. The concentrations of the carbohydrates of the root approximated to those of 0·3 per cent. solution of glucose, or of 0·7 per cent. of sucrose.

These observations strikingly negative Sachs' view that the stream rising from the roots to the leaves during transpiration is to be regarded as a very dilute solution of salts only.

It is interesting to note how much more concentrated the sap of the vacuoles of the cells of the leaves was at the same time. This sap was extracted by pressure from leaves which had been treated with liquid air to render their protoplasm permeable without affecting the concentration (1).

Contemporaneously with the experiments on *Acer pseudoplatanus* similar measurements were made on the sap of *Populus alba*, and are recorded in Table III.

TABLE III.

Sap of *Populus alba*. August 28, 1913.

Expt	—	Δ	Δ_e	$\Delta - \Delta_e$	P	$C \times 10^5$
650	Centrifuged from tracheae of stem at 40-ft. level,	0·047°	0·016°	0·031°	0·57	34
649	Centrifuged from tracheae of root,	0·072°	0·024°	0·048°	0·87	52
653	Pressed after treatment with liquid air from bark of stem, 40-ft. level,	1·215°	0·218°	0·997°	14·62	453
654	Pressed after treatment with liquid air from bark of root,	1·101°	0·179°	0·922°	13·23	383
651	Pressed after treatment with liquid air from spring leaves,	1·326°	0·438°	0·888°	15·95	909
652	Pressed after treatment with liquid air from summer leaves,	1·487°	0·315°	1·172°	17·88	654

In this case the sap centrifuged from the tracheae of the stem and the root gave no direct reaction with Fehling's solution. Evidently neither glucose nor maltose was present. On inversion, however, a noticeable reduction took place, indicating the presence of sucrose to the amount of about 0·5 per cent. and 1 per cent. respectively in the stem and root.

The sap from the cells of the bark of stem and root, and from the leaves examined at the same time, showed, as appears above, much higher concentrations.

It was then determined to investigate the concentration and constituents of the wood sap at different seasons of the year, and to see if variations of any considerable magnitude occurred in it. With this end in view, pieces were cut from the branches, at the same level on each occasion, and roots of the following trees, and examined cryoscopically, electrically, and chemically: *Acer pseudoplatanus*, *Cotoneaster frigida*, *Fagus silvatica*, *Ilex aquifolium*, *Populus alba*, and *Salix babylonica*.

Under hexose and sucrose is given an approximate estimate of the amount of these sugars present; when 5 drops of the sap decolorized 10 drops of boiling normal Fehling's solution ×××× are set in the hexose column, when equal volumes were required ××, and when 20 drops of the sap had to be used for 10 drops of Fehling's solution × is put down. Under sucrose a similar notation is used, indicating the volume of sap, inverted by boiling with hydrochloric acid, required to decolorize the boiling Fehling solution. Allowance is made for hexose if any was found previous to inversion. In the same way a barely perceptible reduction is indicated by + and a more marked trace by ++. It may be mentioned that the values of these signs approximately correspond ×××× to 1 per cent., ××× to 0·75 per cent., ×× to 0·50 per cent., and × to 0·25 per cent.; + to 0·01 per cent., and ++ to 0·1 per cent.

Where *m* is written in the sucrose column the presence of maltose was detected by phenyl-hydrazine. Of course maltose, being a reducing sugar, contributes to the precipitate observed before inversion. It is somewhat hydrolysed by short boiling with acid, and consequently adds to the precipitate occurring after inversion. Its presence renders the identification of a hexose by the reduction test doubtful.

An asterisk is placed on the date at which the leaves of the buds began to expand.

TABLE IV.

Acer pseudoplatanus. Wood-sap from Stem at 25-ft. level, and from Root.

Expt.	Date	Δ	Δ_e	$\Delta - \Delta_e$	P	$C \times 10^5$	Hexose	Sucrose
716	March 3, stem, .	0·120°	0·022°	0·098°	1·44	47·0	××	×××*m*
726	April 10*, stem, .	0·223°	—	—	2·68	—	××	××××*m*
727	,, root, .	0·076	—	—	0·91	—	0	×××
647	August 25, stem, .	0·049°	0·019°	0·030°	0·59	41·0	—	—
646	,, root, .	0·070	0·032°	0·038°	0·84	69·3	—	—
760	October 7, stem, .	0·041°	0·021°	0·020°	0·50	45·7	+	++
761	,, root, .	0·080°	0·027°	0·053°	0·96	57·7	0	××
703	Dec 19, stem, .	0·091	0·032°	0·059°	1·09	69·0	—	—
704	,, root, .	0·059°	0·035°	0·024°	0·71	74·9	—	—

These observations are graphically recorded in fig. 1, in which the ordinates represent the depressions of freezing-point and the abscissae the months of the year. The broken line is the graph for the concentration of the sap of the wood of the stem; the full line that of the root.

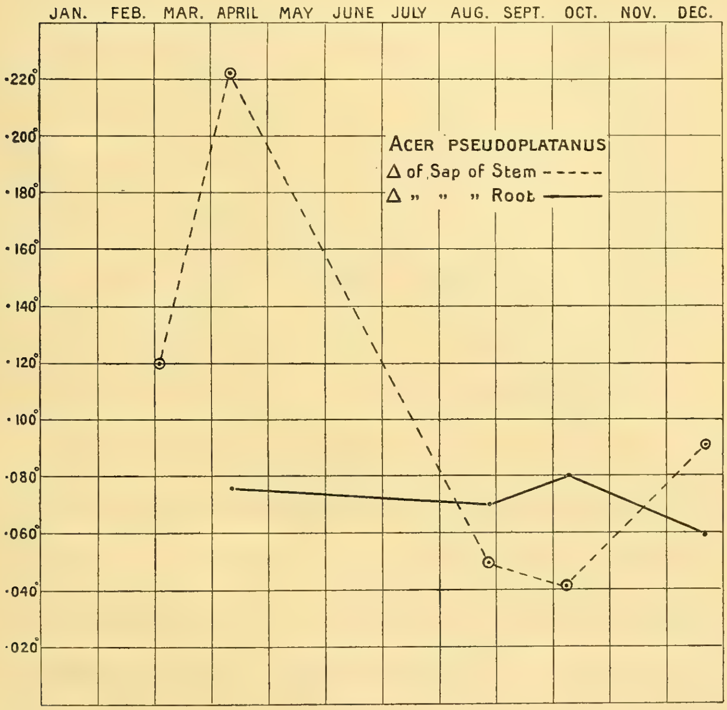


Fig. 1.

Table V.

Cotoneaster frigida. Wood-sap from Stem at 15-ft. level, and from Root.

Expt.	Date	Δ	Δ_e	$\Delta-\Delta_e$	P	$C \times 10^5$	Hexose	Sucrose
713	March 2, stem, .	0·089°	0·018°	0·071°	1·08	38·5	+	++
	,, root, .	—	—	—	—	—	—	—
730	April 10*, stem, .	0·058°	—	—	0·70	—	+	×
731	,, root, .	0·051°	—	—	0·62	—	+	×
754	June 29, stem, .	0·066°	0·027°	0·039°	0·79	58·3	+	××*m*
755	,, root, .	0·045°	0·024°	0·021°	0·54	52·2	0	××
764	October 8, stem, .	0·086°	0·026°	0·060°	1·04	55·2	+	××
765	,, root, .	0·065°	0·031°	0·033°	0·78	67·2	0	××
782	Dec. 5, stem, .	0·053°	0·018°	0·035°	0·64	38·2	0	×
784	,, root, .	0·040°	0·019°	0·021°	0·48	40·1	++	×

See graph in fig. 2.

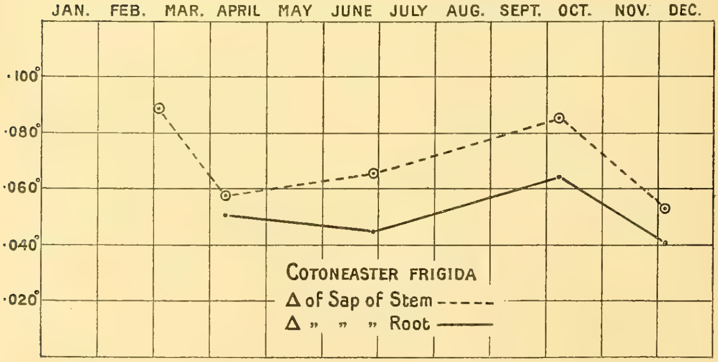


Fig. 2.

TABLE VI.

Fagus silvatica. Wood-sap from Stem at 40-ft. level, and from Root.

Expt.	Date	Δ	Δ_e	$\Delta-\Delta_e$	P	C × 10^5	Hexose	Sucrose
714	March 3, stem, .	0·102°	0·014°	0·088°	1·23	29·9	××	××*m*
	,, root, .	—	—	—	—	—	—	—
728	April 10, stem, .	0·067°	—	—	0·80	—	××	0
729	,, root, .	0·042°	—	—	0·50	—	×	0
737	May 12*, stem, .	0·072°	0·023°	0·049°	0·86	49·5	×	××*m*
739	,, 14, stem, .	0·070°	—	—	0·84	—	×	××
747	June 27, stem, .	0·064°	0·018°	0·046°	0·77	38·3	+	0*m*
750	,, root, .	0·058°	0·030°	0·028°	0·70	63·9	+	××*m*
762	October 7, stem, .	0·022°	0·011°	0·011°	0·26	24·7	+	×
763	,, root, .	0·030°	0·015°	0·015°	0·36	32·4	0	××
781	Dec. 5, stem, .	0·056°	0·015°	0·041°	0·67	31·7	×	×

See graph in fig. 3.

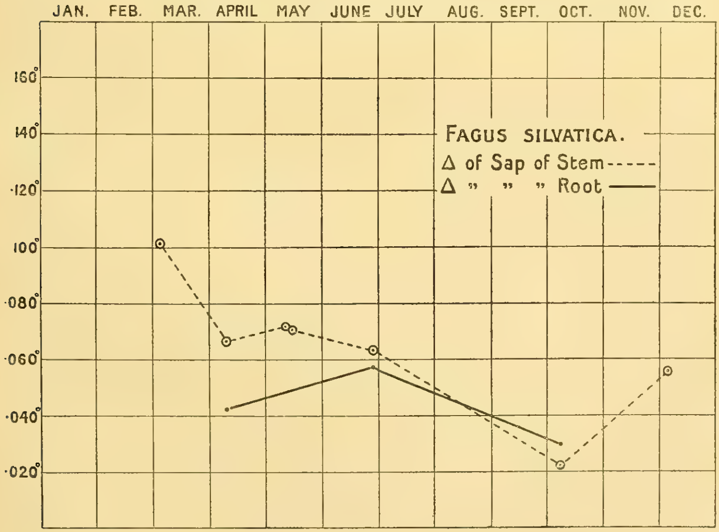


Fig. 3.

TABLE VII.

Ilex aquifolium. Wood-sap from Stem at 3-ft. level, and from Root.

Expt.	Date	Δ	Δ_e	$\Delta-\Delta_e$	P	$C \times 10^5$	Hexose	Sucrose
712	March 2, stem,	0·056°	0·026°	0·030°	0·67	55·9	—	—
721	,, 25, stem,	0·052°	0·025°	0·027°	0·63	54·4	++	××
720	,, 25, root,	0·096°	0·056°	0·040°	1·16	119·4	××	××
738	May 14,* stem,	0·074°	0·035°	0·039°	0·89	76·6	××	××*m*
748	June 27, stem,	0·084°	0·034°	0·050°	1·01	72·4	++	×
663	Sept. 12, stem,	0·082°	—	—	0·98	—	++	—
664	,, root,	0·099°	—	—	1·19	—	×	—
770	October 8, stem,	0·051°	0·029°	0·022°	0·62	61·7	×	××
771	,, root,	0·063°	0·042°	0·021°	0·78	90·4	×	××
708	Dec. 23*, stem,	0·072°	0·030°	0·042°	0·86	63·9	×	×
710	,, root,	0·061°	0·037°	0·024°	0·73	80·3	×	+

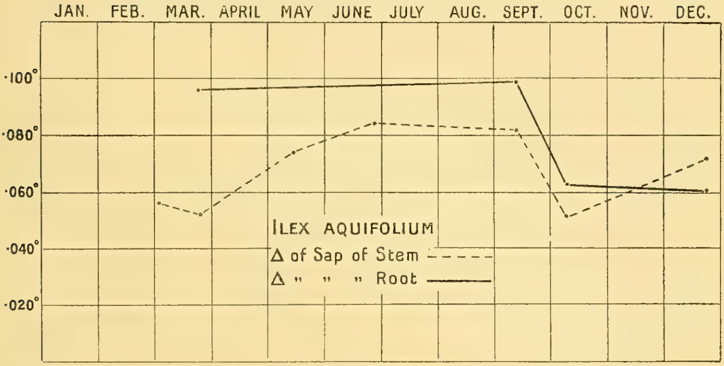
See graph in fig. 4.

TABLE VIII.

Populus alba. Wood-sap from Stem at 40-ft. level, and from Root.

Expt.	Date	Δ	Δ_e	$\Delta-\Delta_e$	P	$C \times 10^5$	Hexose	Sucrose
717	March 3, stem,	0·163°	0·033°	0·130°	1·96	71·5	××	××*m*
724	April 10*, stem,	0·261°	—	—	3·14	—	×××	××
725	,, root,	0·111°	—	—	1·34	—	××	××
746	June 27, stem,	0·065°	0·024°	0·041°	0·78	51·6	+	+
753	,, root,	0·106°	0·028°	0·078°	1·28	61·1	××	×
650	Aug. 27, stem,	0·047°	0·016°	0·031°	0·57	33·9	0	×
649	,, root,	0·072°	0·024°	0·048°	0·87	51·8	0	××
766	October 8, stem,	0·045°	0·024°	0·021°	0·54	51·3	—	—
767	,, root,	0·044	0·020°	0·024°	0·53	43·9	+	××
705	Dec. 20, stem,	0·055°	0·013°	0·042°	0·67	27·1	—	—
706	,, root,	0·053°	0·019°	0·034°	0·64	41·6	—	—

See graph in fig. 5.


JAN.
FEB.
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·100°
·080°
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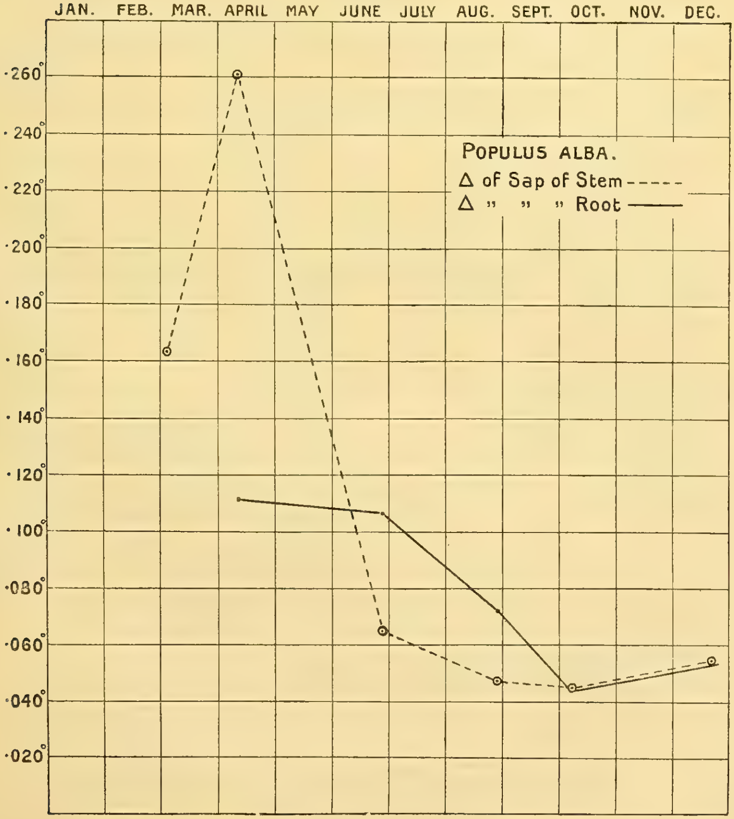
ILEX AQUIFOLIUM
Δ of Sap of Stem - - - - -
Δ " " " Root ———

Fig. 4.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·180°
·160°
·140°
·120°
·100°
·080°
·060°
·040°
·020°
POPULUS ALBA.
Δ of Sap of Stem - - - - -
Δ " " " Root ———

Fig. 5.

TABLE IX.

Salix babylonica. Wood-sap from Stem at 18-ft. level, and from Root.

Expt.	Date	Δ	Δ_e	$\Delta - \Delta_e$	P	$C \times 10^5$	Hexose	Sucrose
715	March 3, stem, .	0·095°	0·028°	0·067°	1·14	59·3	+	××
723	,, 25*, stem, .	0·059°	0·024°	0·035°	0·70	52·2	+	××
722	,, 25, root, .	0·057°	0·036°	0·021°	0·68	77·7	+	××
751	June 29, stem, .	0·050°	0·022°	0·028°	0·60	47·8	+	××
752	,, root, .	0·052°	0·026°	0·026°	0·63	56·6	+	×
768	October 8, stem, .	0·039°	0·015°	0·024°	0·47	32·9	+	×
769	,, root, .	0·038°	0·023°	0·015°	0·46	49·8	+	×
780	Dec. 5, stem, .	0·044°	0·012°	0·032°	0·53	26·1	×	×
783	,, root, .	0·034°	0·014°	0·022°	0·41	31·1	×	×

See graph in fig. 6.

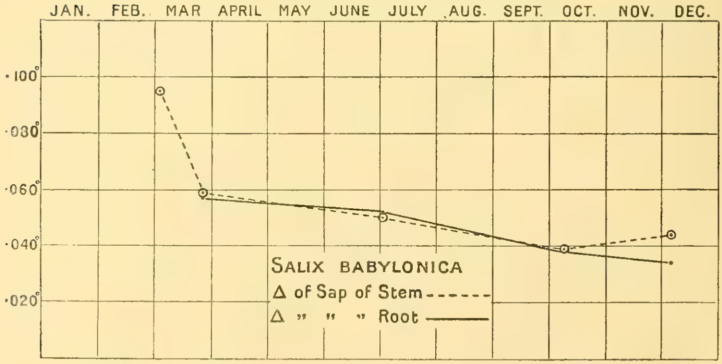

Fig. 6.

Concentration of total Sap Solutes.

The general form of the curves for the concentration of the wood sap in the stem of the deciduous trees (excepting *Cotoneaster frigida*, which will be considered later) examined is similar, viz.:—There is a cusp in the early spring, followed by a rapid fall.[1] Then a period of low concentration in the summer and early autumn,[2] followed by a rise, which is gradual at first, and then becomes steeper as it approaches the spring maximum. So far as the investigation goes it shows that the concentration of the wood sap in the root follows that of the stem, and is generally lower than it, but there does not appear to be such a pronounced rise in the spring, and the succeeding fall is not so rapid, so that during the vernal decline in concentration it is often found that the concentration of the wood-sap of the root is greater than that of the stem. Sometimes this difference persists until the concentration of wood-sap of the stem begins to rise again, e.g. Acer, Populus, and possibly Fagus.

The few observations made upon Salix indicate that the concentrations in the root and the stem are closely similar.

In contrast to the curves traced by the concentrations of the wood-sap of most of the deciduous trees is that exhibited by the sap of the only evergreen examined, viz., *Ilex aquifolium.*

Here the graph for the concentration of the stem-sap has two cusps—one in summer and one in winter, and corresponding depressions in spring and autumn.[3] The relation of the concentration found in the root to that of the stem is reversed, for while in deciduous trees the concentration of the wood-sap is generally greater in the stem than in the root, the converse was found to hold in the case of Ilex. Only in winter was it less in the root than in the stem. During the rest of the year it was greater.

Cotoneaster frigida is also exceptional in having two cusps in its curve—one in spring and one in autumn. This is doubtless connected with the fact that it is a sub-evergreen. Young examples, up to twenty years old or so, keep their leaves on into the winter. In old specimens, which are deciduous, like the tree examined, the opening of the buds in spring is curiously prolonged.

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[1] This sudden rise and fall during the spring in the concentration of the carbohydrates of the sap has been detected by Schröder (10) in his investigation of the bleeding of trees.

[2] A. Fischer (3) also found that there was less glucose in the vessels of several trees in the summer and autumn than in the spring.

[3] It is difficult to correlate the variations in concentration in the sap of Ilex with the periods of bud expansion. The period for bud expansion appears uncertain, especially in pruned or lopped trees; thus in the season 1912–1913 buds expanded from November to January. In 1914 the summer buds were opening as early as April 22, and expansion was complete about the beginning of June. On the whole, however, most buds open during the winter and summer months.

In this plant the only observations made show the concentration of the wood-sap of the root to be less than that of the stem, even during the summer and autumn months. At the same time the curve of the root-sap follows that of the stem-sap.

Concentration of Sugars and other Non-electrolytes.

The form of these curves is not greatly altered if we deduct from each observation the amount of depression due to the electrolytes. Hence the curves given convey a fair impression of the variations in the concentration of the soluble carbohydrates in the tracheae, and it has not been thought worth while to plot special graphs for the carbohydrates in the sap.

From inspection of the tables it is quite clear that, except occasionally in the summer and autumn, the molecular concentration of the carbohydrates in the transpiration stream in deciduous trees is greater than that of the salts. Furthermore, since the sugars have high molecular weights (glucose 180, sucrose and maltose 342) the actual percentage weights of these substances in the sap must be far greater than that of the electrolytes, which have low molecular weights, and are more or less ionised. Hence we must admit that the translocation of carbohydrates is at least as important a function of the transpiration current as the transference upwards of nutritive mineral salts.

In the roots even a similar relative concentration is often found, as a comparison of the columns under Δ_e and $\Delta - \Delta_e$ clearly shows.

The balance of salts and carbohydrates is more evenly kept in the evergreen Ilex, and the concentration of the two throughout the year more closely approximates; so that the function of wood here in conveying the electrolytes may be regarded as being as important as that of transporting carbohydrates.

Concentration of Electrolytes.

With the exception of the two observations made in June on *Cotoneaster frigida* and in October on *Populus alba*, it appears from the experiments quoted that the concentration of electrolytes was greater in the wood of the roots than in that of the stems. This would seem to suggest that while the quantity of dissolved carbohydrates in the transpiration stream may be added to on its upward passage, the amount of dissolved electrolytes is not thus reinforced, but usually is diminished as the stream rises. Doubtless some of the dissolved salts are abstracted and used in various processes of metabolism. The observation on Cotoneaster, however, shows that this does not always hold good, for in it the concentration of electrolytes in the stem was slightly greater than in the roots. And in a number of observations made on *Acer macrophyllum* (see Table X) no steady gradient from below upwards is revealed.

Concentration at different Levels.

The pieces of the stems yielding the sap examined in these experiments were always taken from the same plant and from the same level. These levels above the ground were as follows:—

Acer pseudoplatanus, 25 ft.
Cotoneaster frigida, 20 ft.
Fagus silvatica, 40 ft.
Ilex aquifolium, 3 ft.
Populus alba, 40 ft.
Salix babylonica, 18 ft.

It seemed desirable to compare the sap from the same level on the different occasions in case a difference in level in the tree is associated with a change in concentration.[1]

This point was also separately investigated for one tree, viz., *Acer macrophyllum* (see Table X).

The subject for the investigation was an old tree which had been cut across near the ground many years previously. From the level of the soil three similar branches about 30 cm. in diameter took their origin, and rose to a height of about 10 m. One of these was cut down in the middle of October, and samples of the wood excised at various levels, viz.: (1) ground-level, at (2) 2 metres, (3) 4 metres, (4) 6 metres, and at (5) 8 metres above ground-level, and finally (6) from the small branches about 10 metres above the ground. The following table gives the results of determinations made on the sap centrifuged from the wood at these levels, and shows how the concentration varies from below upwards at that time of year:—

TABLE X.

Acer macrophyllum, Wood-sap, October.

—	Δ	Δ_e	$\Delta - \Delta_e$	P	$C \times 10^5$	Hexose	Sucrose
Stem, 10m. level, . .	0·068°	0·037°	0·031°	0·81	79·0	++	××
,, 8m. ,, . .	0·048°	0·033°	0·015°	0·57	70·9	+	×
,, 6m. ,, . .	0·040°	0·030°	0·010°	0·49	64·8	0	0
,, 4m. ,, . .	0·035°	0·025°	0·010°	0·42	54·2	0	0
,, 2m. ,, . .	0·046°	0·028°	0·018°	0·56	61·0	0	×
,, 0m. ,, . .	0·053°	0·039°	0·014°	0·63	84·6	0	×
Root, ...	0·060°	0·035°	0·025°	0·72	76·4	0	××

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[1] Schröder (10) found this to be the case in bleeding sap of *Acer platanoides* and Betula. The sugar concentration in Acer was found to be greater in the root and in the upper parts of the stem than in the lower parts of the stem. In Betula, on the contrary, the concentration of the bleeding sap is less above in the stem and in the root than it is in the base of the stem.

Starch was present in large quantities in the wood at the time these determinations were made. The cells of the medullary rays, the last few layers of elements formed in each year-ring and the first layer of the next, and the elements in contact with the vessels were densely crowded with starch grains. The sheath of starch-containing elements round the vessels was continuous, and often many-layered, in the root and in the stem at the ground-level. Higher up only some of the elements in contact with the vessels contained starch, but those which did so were densely packed. Also it was noticed that at the higher levels, the first layer of the spring wood was without starch, except where it was in contact with vessels. Generally there appeared fewer starch-containing elements at the higher levels. In the root the number of starch-containing cells is still further augmented by the fact that the vessels are much more numerous, hence the number of elements constituting their starch-bearing sheaths is more considerable. A quantitative estimation of the cross-section of the various elements of the wood in some trees has been given in a previous paper (2).

Function of the Living Elements of the Wood.

Examination of sections for the estimation of the starch content of the wood cannot fail to force on one the remarkably regular and close connexion existing between the starch-containing elements and the vessels. Further, when we take into account that the transference of carbohydrates can no longer be regarded as an occasional and accessory function of the vessels, but is certainly a continual and principal function, the starch layer round each of them becomes evidently a glandular sheath to the vessel for the secretion[1] into it of the carbohydrates to be transmitted upwards. The location of starch in the elements on the borders of the year-rings is clearly connected with the sudden transmission upwards of immense quantities of carbohydrates in the spring. The depletion of the glandular layer of the spring vessels will be made good from the stores massed close by in the outer margin of the year-ring.

We may imagine these carbohydrate glands forming a sheath round the vessels to act somewhat as follows:—In spring their stored starch is rapidly brought into solution, and the resulting sugars secreted into the vessels. The concentrated solution in the tracheae acting osmotically through the semi-permeable membrane formed by the outer tissues of the root determines a flow of water from the soil to the tracheae, and the resulting hydrostatic

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[1] It is quite possible that simple diffusion of sugars from the living cells into the tracheids may be quantitatively sufficient to explain their presence in the latter, since protoplasm is not rigidly impermeable to these substances, but often allows their penetration at a relatively great rate.

pressure is responsible for the exhibition of bleeding and root-pressure characteristic of the spring. This simultaneously forces much of the air in the tracheae into solution, and raises the carbohydrates to the buds. The observations recorded show that the maximum concentration of the carbohydrates is simultaneous with, or just previous to, the expansion of the leaves. The activity in transpiration of the developing leaves is forwarded not only by the opening up of the water-channels on the removal of air-bubbles, but also by the growth of the leaves themselves, rendered possible by the accession of carbohydrates, &c., carried up in the sap. The increase in the volume of the transpiration stream from these causes, and more favourable external conditions, leads to a dilution of the carbohydrates, and is responsible largely for the rapid decrease in concentration at the time of the expansion of the leaves shown in all the curves. Possibly somewhat earlier there is a diminution in the glandular activity of the cells round the vessels, and this initiates and later contributes to the dilution of the carbohydrates. The secretion, however, does not cease at any time of the year; and, consequently, even in summer, we find the enormous transpiration stream possessing a very noticeable concentration of sugars, which is, indeed, greater than that in which the same substances are present in the human blood. To make good this expenditure the glandular cells must be constantly replenished by supplies forwarded from the organs of carbon assimilation through the bark, medullary-rays, and wood parenchyma. The rise in concentration of the sap in the tracheae towards the end of the year is to be ascribed, in all probability, to the more or less uniform continuance of this secretion, coupled with the reduction of transpiration, entailing a diminution in the rate of the current past the secreting cells prior to leaf fall. For it is evident that if the rate of the secretion of sugars remains approximately constant, the concentration of the sap will depend upon the volume of the transpiration stream. In winter the complete cessation of transpiration allows of a further concentration.[1]

Where the wood parenchyma is specially concentrated round the vessels, while the tracheids form comparatively large tracts without living cells intermingled, we may with probability assume that a certain amount of division of labour has come about, and that the vessels function as the principal channels for the transmission of carbohydrates. The tracheids, on the other hand, are chiefly concerned with the upward conveyance of the water. Of course as long as no bubbles are developed in the vessels they will

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[1] In view of this continued secretion of carbohydrates into the tracheae, it is advisable to centrifuge the sap from the wood immediately on its removal from the tree; otherwise the concentration observed may be greater than that actually obtaining during transpiration.

transmit the sap in them, with all its constituents, more rapidly than will the tracheids, when both are under the tension generated by the leaves. But when a bubble arises the stream will be deflected into the tracheidal columns. There, owing to the smaller size of the compartments composing the channels, the tensile column will acquire greater stability. Hence, when the wood is rich in water the most rapid transit upwards will be effected in the vessels, which, at the same time, will be comparatively rich in carbohydrates, while a slower and more dilute stream will pass up in the tracheids. If drought, by causing an increase in tension, produces bubbles in the conducting channels, the major part of the stream, with its solutes, will have to pass up through those tracheids in which no ruptures (bubbles) have developed, thus securing a stable, if slow, supply to the leaves. According to Strasburger's observations (11) during transpiration the majority of the vessels of the spring wood in the conducting zone are without bubbles.

The recognition of the glandular function of the wood parenchyma, the translocatory activity of the medullary rays, and the transmission in the tracheae of the circulating carbohydrates, affords a satisfactory explanation of the presence of living elements among the otherwise lifeless tissue of the wood.

SUMMARY.

1. Sugars (monosaccharides and disaccharides, or both) are found at all times in the sap in the tracheae of the trees examined, and usually in greater quantities than electrolytes.

2. The greatest concentration of the sugars occurs in the early spring; this is followed by a rapid dilution in spring and early summer, so that a minimum occurs in the summer or autumn. A rise in concentration, slow at first, then takes place through the winter, culminating in the vernal maximum.

3. The vernal maximum coincides with the period of greatest root-pressure, and is simultaneous with or just prior to the opening of the leaf-buds.

4. The rise in transpiration, initiated by the expanding leaves and facilitated by the opening of the conducting channels by root-pressure, is largely responsible for the dilution of the carbohydrates. The falling off and cessation of the transpiration stream in the autumn allow the concentration again to rise.

5. The conveyance upwards of carbohydrates, of which sucrose appears to be the most important, is a continual and primary function of the tracheae.

6. The sheath of wood parenchyma round the vessels functions as a gland to secrete carbohydrates into the rising transpiration stream.

7. The relation of the medullary rays to these sheaths supports the view that they convey the carbohydrates from the bark to the glandular sheaths.

8. The presence of large quantities of soluble carbohydrates in the wood-sap of roots is probably responsible for root-pressure and bleeding by producing an osmotic flow across the root-cortex, which acts as a semi-permeable membrane.

9. The curves for the concentration of solutes in the stem of the evergreen Ilex, and of the sub-evergreen Cotoneaster, show smaller fluctuations than do those of deciduous trees; they have two cusps—one about January, and the other about August, in Ilex, and in February and October in Cotoneaster. It is to be noted that in the case of Ilex the buds expanded during the rise preceding each of these cusps.

10. The concentration of the carbohydrates is generally greater in the tracheae of the stem than in those of the root, except during the summer. This rule is broken by Ilex where the concentration in the root is the greater throughout the year, except in winter. The electrolytes, however, are present as a rule in greater quantity in the root.

11. In general the vessels function, in times when water is abundant, to convey rapidly solutions of organic and inorganic substances to the leaves. The columns of tracheids may be supposed to afford a permanent channel for the water and salts, and to a less degree for the organic substances. This is never put out of action, even in times of greatest drought.

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THE SUBSIDENCE OF TORSIONAL OSCILLATIONS OF IRON WIRES AND ALLOYS WHEN SUBJECTED TO THE INFLUENCE OF ALTERNATING MAGNETIC FIELDS OF FREQUENCY 50 PER SECOND.

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XXXII.

THE SUBSIDENCE OF TORSIONAL OSCILLATIONS OF IRON WIRES AND ALLOYS WHEN SUBJECTED TO THE INFLUENCE OF ALTERNATING MAGNETIC FIELDS OF FREQUENCY 50 PER SECOND.

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[Read January 26. Published March 5, 1915.]

Section I.

Iron Wires.

About a year ago there was brought before this Society the results of some experiments on the subsidence of torsional oscillations in nickel wires when they were subjected to the influence of alternating magnetic fields of frequencies from 20 to 140 per second.[1] The present communication gives some results with iron wires and a few alloys, with alternating magnetic fields of frequencies 50 per second only. At present, the alternating currents available for producing magnetic fields of frequency higher than 50 per second do not give pure sine curves when analysed by means of an oscillograph, and experiments with alternating fields of frequencies up to 240 per second have been held over until a machine has been installed for this work which will give high frequency currents the graphs of which are practically pure sine curves.

For a detailed description of the apparatus employed, and of the method of experiment, I would refer to page 216 of the paper mentioned above. The only important changes introduced since that time are :—(1) the longitudinal loads on the end of the wire under test are now shallow cylinders, which can be firmly clamped by means of lock-nuts; (2) instead of the wire being made to oscillate round its own axis by an electrical method, it is now done by means of two properly timed simultaneous puffs of air, which strike tangentially on the sides of the vibrator.

It has been shown for iron wires, that the magnetic field which must be round the wire in order to get the maximum twist of the free end is independent of the load on the end of the wire for a given current through the

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[1] Scient. Proc. Roy. Dub. Soc., vol. xiv, 1914, No. 14, p. 215.

wire[1]; and it has also been shown that the greatest damping of the torsional oscillations in the wire takes place in a magnetic field of the same value.[2] The magnetic field, therefore, employed in the present investigation was 2·8 c.g.s. units, whether continuous or alternating, unless otherwise stated.

The effective length of the wire used was 226 cms., and of diameter 0·162 cms., that is a small No. 16; two different longitudinal loads were employed for the tests on the pure iron wire, namely, 10^5 and 2×10^5 grammes per sq. cm., and the wire was tested in two different physical states: (1) in the condition in which it was received from the manufacturer, (2) after being made as soft as possible. Alternating magnetic fields of frequencies less than 50 per second were tried, but the results are not here recorded, because the differences between the damping curves with direct and alternating magnetic fields are much less for iron than for nickel.

A pure iron wire was taken in the physical state in which it came from the manufacturer, and after having its surface cleaned with emery, and its rigidity measured,[3] it was placed in the solenoid, and the subsidence of torsional oscillation observed when it was surrounded by magnetic fields of value 2·8 units, both D. C. (direct current) and A. C. (alternating current), and also when it was subjected to two different loads.

The results are shown in Table I.

TABLE I.

Rigidity $\doteqdot 808 \times 10^6$ grammes per sq. cm.

Number of Vibrations.	LOADS.			
	1×10^5		2×10^5	
	D.C.	A.C.	D.C.	A.C.
0	300	300	300	300
5	293	290	291	289
10	287	281	283	278
15	281	272	275	268
20	275	263	267	259
30	264	246	252	241
40	251	231	239	223
50	240	217	226	207
60	229	203	214	193
70	218	190	203	178

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[1] Scient. Proc. Roy. Dub. Soc., vol. xii, 1910, No. 36, p. 484.

[2] Scient. Proc. Roy. Dub. Soc., vol. xiii, 1911, No. 3, p. 41.

[3] Scient. Proc. Roy. Dub. Soc., vol. xii, 1910, No. 36, p. 481.

From these values in Table I it will be seen that, for the *hard* wire, the amplitude of the 70th vibration is diminished for the increased load in both the D.C. and A.C. magnetic field, whereas, for the *soft* wire (Tables II and III below), the amplitude of the 70th vibration is *decreased* in the D.C. field and *increased* in the A.C. field for increased load.

The wire was then taken down and hung vertically and loosely under its own weight only, and heated to a bright-red heat three times, from the top downwards, by means of a Bunsen flame, so as to make it as soft as possible. It was then cleaned and its rigidity again measured, and, when replaced in the solenoid, it was put through the same tests as before. The results are shown in Table II, and part of them, in the form of curves, in fig. 1, in which, for comparison, is also put the corresponding curve for nickel wire.[1]

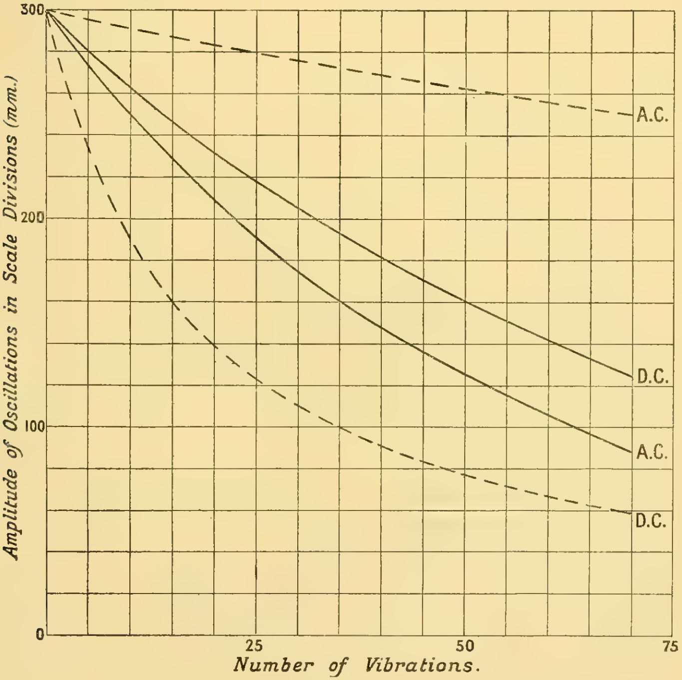

FIG. 1.

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[1] Scient. Proc. Roy. Dub. Soc., vol. xiv, 1914, No. 14, p. 221.

TABLE II.

Rigidity ≑ 790 × 10^6 grammes per sq. cm.

Number of Vibrations.	Loads.			
	1 × 10^5		2 × 10^5	
	D.C.	A.C.	D.C.	A.C.
0	300	300	300	300
5	281	274	280	275
10	263	250	261	252
15	247	228	244	232
20	232	209	228	214
30	205	175	200	181
40	181	148	175	154
50	160	125	153	131
60	142	105	134	111
70	125	89	116	94

The full-line curves are those for soft iron in a magnetic field of 2·8 units, and the broken-line curves are those for soft nickel wire in a magnetic field of 17 units, the load on each being 10^5 grammes per sq. centimetre.

From the tables and curves it will be seen that for iron wires, both in the *hard* and *soft* states, the damping curves or curves showing the subsidence of torsional oscillations obtained with the alternating magnetic fields all lie *below* the curves obtained with the direct longitudinal magnetic fields; whereas in alternating magnetic fields the curves got with *hard* nickel wire lie *below*, and those obtained with *soft* nickel wire lie *above* the corresponding curves got with direct longitudinal magnetic fields.[1]

On examining the figures in Table II obtained with the two loads 1 × 10^5 and 2 × 10^5, it will be noticed that the *difference* in the amplitude of oscillation between the D.C. and A.C. values after 70 vibrations have taken place, *decreases* as the load *increases*; also, the final amplitude is *decreased* in the D.C. and *increased* in the A.C. set of observations for increased load.

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[1] Scient. Proc. Roy. Dub. Soc., vol. xiv, 1914, No. 14, pp. 218–221.

In order, therefore, to test if this would hold with other loads, sets of observations were made for both D.C. and A.C. magnetic fields when the wire was loaded with 0·5, 3·0, and $4·36 \times 10^5$ grammes per sq. cm. respectively. A load higher than the last-mentioned could not be applied with the present arrangement of apparatus, that is, applied in such a way as to keep the radius of gyration of the vibrator constant.

The results thus obtained along with the corresponding values from Table II are given in Table III, and are shown as a curve in fig. 2 (p. 398). In Table III there are given the amplitudes of the *first* and seventieth vibration only for both magnetic fields, and in the lower line are the differences, in scale divisions, of the amplitudes of the oscillations for the D.C. and A.C. fields for each load employed.

Table III.

Number of Vibrations.	Loads in grammes per square centimetre.									
	$0·5 \times 10^5$		$1·0 \times 10^5$		$2·0 \times 10^5$		$3·0 \times 10^5$		$4·36 \times 10^5$	
	D.C.	A.C.	D.C.	A.C.	D.C.	A.C.	D.C.	A.C.	D.C.	A.C.
0	300	300	300	300	300	300	300	300	300	300
70	145	87	125	89	116	94	116	100	115	107
Difference→	58		36		22		16		8	

In fig. 2, the abscissæ represent the longitudinal loads on the wire, and the ordinates the corresponding differences mentioned above ; and it is seen that the resultant curve when produced cuts the axis of abscissæ at the point corresponding to a load of about $5·7 \times 10^6$ grammes per sq. cm. : which means that if the wire were loaded to that amount, the D.C. and A.C. damping curves would be identical.

For loads still higher the iron wire would then behave in the same way as a *soft* nickel wire, that is, the A.C. damping curve would lie *above* the D.C. curve.

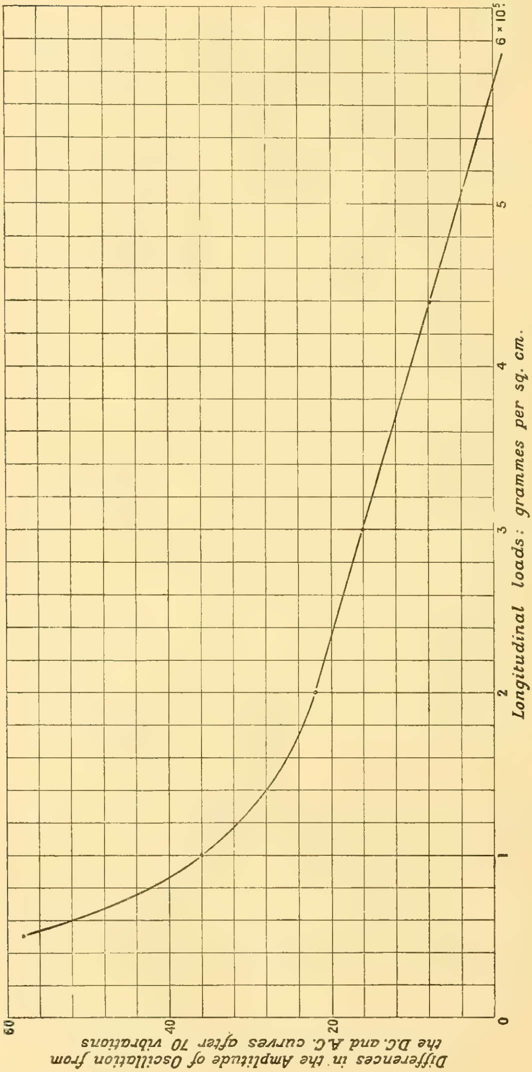
6 × 10⁵
5
4
3
2
1
0
20
40
60
Longitudinal loads: grammes per sq. cm.
Differences in the Amplitude of Oscillation from the D.C. and A.C. curves after 10 vibrations

Fig. 2.

Section II.

Iron Alloys.

The alloy of iron that the present writer wished particularly to test was one whose trade name is *Stalloy,* a silicon iron with about 3·4 per cent. of silicon, and the principal physical properties of which were investigated in the Royal College of Science some years ago.[1]

The material Stalloy could not be conveniently obtained in the form of wire No. 16, which is the most useful size for these experiments, so, for comparison, size No. 20 wires were procured of Stalloy and S.C.I. (Swedish charcoal iron), and put through tests similar to those already described. The two wires were tested under exactly the same conditions as to length, diameter, load, and magnetic fields both D.C. (direct current) and A.C. (alternating current). The longitudinal load on each was 1×10^5 grammes per sq. cm., the magnetic fields 2·8 c.g.s. units, and the distance from the mirror on the vibrator to the scale 167 cms.

The wire S.C.I. in the physical state in which it was received from the manufacturer was too hard to be magnetised sufficiently by the field of 2·8 units, to obtain a curve of fatigue.[2] In the damping curve, or curve showing the subsidence of torsional oscillations, the amplitude of oscillation fell from 300 on the scale to 180 with the D.C. magnetic field, and from 300 to 177 with the A.C. field, for seventy complete vibrations in each case.

The wire was then taken down and heated *three* times to a bright red heat by means of a Bunsen flame, and when cool and cleaned it was again put into the solenoid, and the damping curves obtained for both D.C. and A.C. magnetic fields. The results are shown in Table IV. The maximum fatigue of the wire in this softer state was found to be 0·22, and it took place in 30 minutes' application of the A.C. magnetic field. The silicon iron wire, *stalloy,* in the physical state in which it was received from the manufacturer, was not so hard magnetically as the wire S.C.I., and when put through tests similar to those applied to S.C.I., it was found that in the damping curves the amplitude of oscillation in the D.C. field fell from 300 to 251, and in the A.C. field from 300 to 237 for seventy complete vibrations in each case.

In this comparatively hard state the wire showed fatigue which had a maximum value of 0·2, after 25 minutes' application of the A.C.

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[1] See references to Vestalin below.

[2] Scient. Proc. Roy. Dub. Soc., vol. xiv, 1915, No. 26, p. 336.

magnetic field. This wire was also taken down and heated *three* times to a bright red heat in the same manner as the previous wire, then replaced in the solenoid, and the damping curves again observed for both D.C. and A.C. magnetic fields.

The results for both wires, S.C.I. and *stalloy*, in the soft physical state are shown in Table IV, and also as curves[1] in fig. 3.

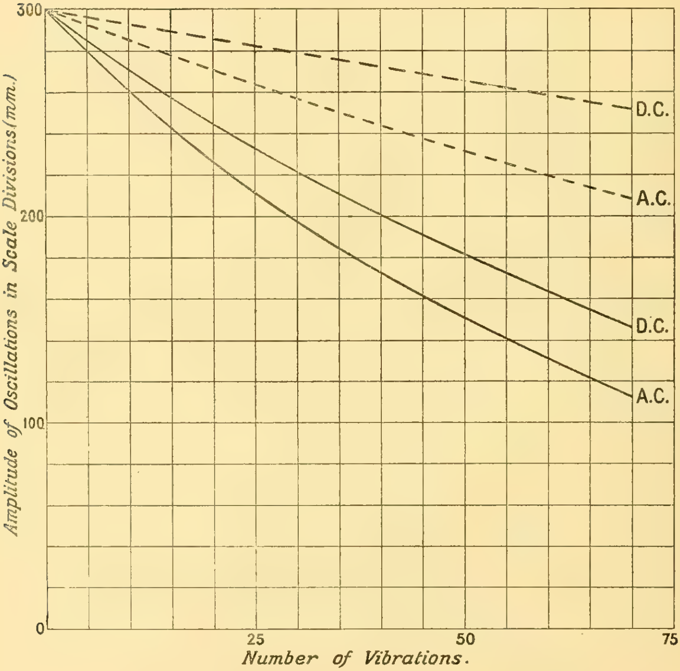

Fig. 3

The simple rigidity of the wires in the soft state was found to be for S.C.I. = 800 × 10^6, and for stalloy = 776 × 10^6, grammes per sq. cm.

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[1] The two wires when tested in the physical state in which they were received from the manufacturer had specific resistance; S.C.I. = 10·5, *stalloy* = 44 microhms, per c.c.

TABLE IV.

Number of Vibrations.	D.C.		A.C.	
	S.C.I.	Stalloy.	S.C.I.	Stalloy.
0	300	300	300	300
5	285	297	280	292
10	271	293	261	285
15	258	288	243	277
20	245	285	227	270
30	222	278	197	256
40	200	271	172	243
50	181	265	150	231
60	163	258	130	219
70	147	251	113	208

The heating of the wires diminishes the amplitude of the 70th vibration of S.C.I. considerably in both the D.C. and A.C. fields; but it has no effect on the stalloy in the D.C. field, though it diminishes the 70th amplitude in the A.C. field by about 12 per cent.

The full-line curve refers to S.C.I., and the broken-line curve to stalloy.

The table and curves show that there is much less damping of torsional oscillations in the *stalloy* than in the S.C.I. wire. The *stalloy* wire in the soft physical state could not be fatigued at all even by the application of an alternating magnetic field for one hour and a half, whereas the S.C.I. wire in the soft state had a maximum fatigue 0·22 with the application of the alternating magnetic field for about half an hour. This silicon iron alloy when submitted to a special heat treatment which prevents it from ageing is used in electrical engineering work under the trade name stalloy; but this specimen of wire under test has not been so treated, as is shown by its ability to be fatigued when in the harder physical state.

About four years ago, Sir R. A. Hadfield, F.R.S., of the Hecla steel works, Sheffield, presented the author with a collection of about a dozen alloys of iron in the form of wires of size No. 19, a few of which were found suitable for these experiments.

The chemical analyses of the specimens were done in the chemical laboratory attached to the Hecla works, and the wires were all subjected to the same heat treatment, namely, they were heated to a temperature of 800° C. and allowed to cool in the air so as to prevent scaling.

The percentage chemical composition of four of the specimens, omitting the iron, is as follows:—

Mark.	Chemical Composition.				
	C.	Si.	Mn.	Cr.	Ni.
1176 F	0·27	0·12	0·21	1·18	—
,, J	0·77	0·50	0·61	5·19	—
,, L	0·71	0·36	0·25	9·18	—
1287 C	0·13	0·23	0·72	—	0·95

These four wires were tested, for the damping of torsional oscillations in the physical state in which they were received from the manufacturer; they were all of the same length and diameter and the longitudinal load on each was $1·5 \times 10^5$ grammes per sq. centimetre. This was the load found most suitable and was used in the comparison of these four wires.

They were too hard magnetically to obtain with them a "Wiedemann curve," or curve relating twist and longitudinal magnetic field, from which can be found the magnetic field corresponding to the peak or turning point on the curve, and therefore the magnetic field which gives the greatest damping to torsional oscillations.[1] In order, therefore, to obtain this particular magnetic field, damping curves were taken for *eight* or *ten* different values of longitudinal magnetic fields, also for the same number of corresponding alternating magnetic fields, and the results plotted as follows[2]:—the values of the magnetic fields employed for both the sets of experiments D.C. and A.C. were taken as *abscissæ*, and for *ordinates* the corresponding values of the amplitude of oscillation after 70 complete vibrations had taken place, starting in each case from the division 300 on the scale. The magnetic field corresponding to the *lowest* points on these two curves was taken and employed in the final experiments on the damping of torsional oscillations in the wires. For the three wires containing chromium this magnetic field both direct and alternating was about 11 c.g.s. units, and for the wire containing nickel, the field was about 16 c.g.s. units. The principal results are shown in the following table, which gives only the amplitude of oscillation—read by means of the light-spot on the scale—after 70 complete vibrations had taken place starting from the division marked 300 on the

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[1] Scient. Proc. Roy. Dub. Soc., vol. xii, 1910, No. 36, p. 484.

[2] Scient. Proc. Roy. Dub. Soc., vol. xiii, 1911, No. 3, p. 37.

scale, that is the division corresponding to an angular twist of the free end of the wire of about *five* degrees.

Table V.

Mark.	D.C.	A.C.
1176 F	300	300
	207	192
1176 J	300	300
	224	233
1176 L	300	300
	244	233
1287 C	300	300
	135	120

The low and high chromium wires and the wire containing nickel behave like ordinary iron wire, that is, the damping curve obtained with the alternating magnetic field in each case lies *below* the curve obtained with the corresponding direct magnetic field, whereas the reverse is the case with the wire containing 5·19 per cent. of chromium. It will be seen also that the A.C. damping curve for this latter wire is identical with the one obtained with the wire containing higher chromium: in fact, added chromium seems to diminish the damping in D.C. fields, but only up to a certain percentage of chromium in A.C. magnetic fields. This wire, however, has a considerable amount (1·88 per cent.) of other impurities, which no doubt masks the effect of damping of torsional oscillations when A.C. magnetic fields are applied.

Vestalin is the trade name of a wire which seems to be practically the same material as an alloy whose physical properties—thermal, electrical, and magnetic—were investigated from seven to fifteen years ago in the Royal College of Science by Sir W. F. Barrett, F.R.S., and the present writer, and was then known as No. 1287L, a nickel steel containing about 25 per cent. of nickel.[1]

The main constituents[2] of the specimen of vestalin under test are Fe = 75·63 per cent., Ni = 23·13 per cent., and it has the peculiar physical property that it cannot be made mechanically softer by heating to a bright-red heat by means of a Bunsen flame, and then allowed to cool slowly. The wire was tested for simple rigidity by three different methods and when in

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[1] Scient. Trans. Roy. Dub. Soc., Series II, vol. vii, pp. 67–126, 1900; vol. viii, pp. 109–126, 1904; vol. ix, pp. 59–84, 1907.

[2] The determinations of the principal *constituents* of Vestalin and Nickelin were done in the chemical laboratory of this College, by Mr. G. van. B. Gilmour, a fourth-year research student in chemistry.

two apparently different physical states, namely—(1) in the condition in which it came from the manufacturer; (2) in its condition after being heated *four* times to a bright-red heat; the rigidity was found to be the same in the two conditions, that is about 685 × 10^6 grammes per square centimetre.

The wire was tested for subsidence of torsional oscillations when in the three conditions: (1) as from the manufacturer, (2) after being heated *once*. (3) after being heated *three* times more. Tests were made in both direct and alternating magnetic fields of values 3, 6, and 32 c.g.s. units, and the rate of subsidence of the torsional oscillations was *identical* in every case; the amplitude of oscillation fell from 300 to 290 after 70 complete vibrations had taken place. This small decrease of 10 divisions only in the amplitude of oscillation after 70 vibrations shows that the wire is remarkably ductile; in fact, it behaves in this matter of damping of torsional oscillations very much like a soft nickel wire when oscillating in a direct longitudinal magnetic field of 200 c.g.s. units.[1] The longitudinal load on the wire during these tests was 1 × 10^5 grams per sq. cm., and when tested for fatigue in a magnetic field of 6 c.g.s. units—the field in which the peak of the Wiedemann curve occurred—the maximum fatigue was only 0·11 after the application of the alternating magnetic field for one hour.

SECTION III.

Other Alloys.

It may be as well to record here the negative results obtained with two wires that are much used in commercial work for resistances, and are known by the trade names Nickelin and Concordin. Nickelin would, at first sight, suggest a material which contained a large proportion of nickel, and this wire was put through the tests for damping of torsional oscillations before being analyzed chemically. It is a copper-nickel alloy, having Cu = 60 per cent., Ni = 40 per cent. This material is perfectly non-magnetic; for, when tested as above for damping of oscillations in various direct and alternating magnetic fields, it gave *exactly* the same damping curves in every case, that is, the amplitude of oscillation fell from 300 to 271 after 70 complete vibrations had taken place.

Concordin, an iron-nickel-chromium alloy, also non-magnetic, was tested in the same manner as nickelin, and gave *exactly* the same damping curves in all the magnetic fields, whether D.C. or A.C., the amplitude of oscillation falling from 300 to 279 for 70 complete vibrations.

For assistance in making some of the observations I am indebted to the Rev. Br. M. C. Wall, a fourth-year Teacher in Training in this College.

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[1] Scient. Proc. Roy. Dub. Soc., vol. xiii, 1911, No. 3, p. 35.

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SOME RESEARCHES IN EXPERIMENTAL MORPHOLOGY.

I.—On the Change of the Petiole into a Stem by means of Grafting.

BY

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[COMMUNICATED BY PROFESSOR J. BAYLEY BUTLER, M.A., M.B.]

(PLATES XXVIII–XXXIV.)

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XXXIII.

SOME RESEARCHES IN EXPERIMENTAL MORPHOLOGY.

(1). On the Change of the Petiole into a Stem by means of Grafting.

By JOSEPH DOYLE, B.A., M.Sc.,
Assistant in the Biological Laboratory, University College, Dublin.

(Plates XXVIII–XXXIV.)

[communicated by professor j. bayley butler, m.a., m.b.]

[Read December 15, 1914. Published March 24, 1915.]

I.—Introduction.

The following is an account of certain attempts made to ascertain if the petiole of a plant can be made to function as a stem, and to study any anatomical changes that may take place. The differences between the petiole and the stem are well known. The differences are essential, and would almost seem to prevent the former ever taking on the function, not to mention the form, of the latter. Thus the petiole has limited growth, and usually a very restricted length of life. Coupled with this limited growth and length of life, we find that the cambium loses its activity as soon as the leaf has reached its full size, so we never find in the petiole that well-developed secondary growth so characteristic of the stem. Finally, the development of periderm—except pathologically—is in far and away the vast majority of plants entirely absent from the petiole. It is these differences that lend the interest to the work.

The attempt has been made by other workers, but in only one instance in any detail, and then by a method different from that adopted in this paper.

The question as to whether the petiole can function as a stem was answered by Knight (1) as long ago as 1803 in a positive manner. Thus Knight grafted a sprout on a petiole of a vine plant. The graft took, the scion grew well, attaining a height of 9 feet to 10 feet. The petiole in the autumn, he notes, had developed wood below the graft. He gives no further details.

Carrière—quoted by De Vries (2)—rooted leaves of the orange. He grafted small sprouts on the petiole at the base of the lamina. The sprouts grew well, and he maintained the petioles growing for four years. Beyond saying that the petiole had enlarged to a firm stalk 1·5 cms. in diameter, no details are given.

Vöchting (3) grafted a sprout of the beetroot on a petiole. He gives no further details.

More recently—1909—Kny (4) records some results with *Begonia rex.* He did not use the grafting method. It is well known that adventitious buds grow readily on the leaves of this plant. The leaf was rooted, and an adventitious bud at the base of the lamina was allowed to develop. At the conclusion of the experiment the petiole carried a fine plant. He found in this petiole by comparison with petioles from normal plants that the area of the vascular bundles had greatly increased, and that the old cambium had re-awakened and had become active. The cells of the neighbouring ground-tissue were also in a state of division.

"Es machte ganz den Eindruck dass hiermit der Beginn der Anlegung eines interfascikulären Kambiums gegeben war, das bei weiterer ungestörter Fortbildung die peripherischen Bündel zu einem Kreise zusammengeschlossen haben würde." But he gives us no more information or discussion.

In 1907 Winkler (5) published an account of his work on the same problem. He made use of the existence of adventitious buds on the leaves of *Torenia asiatica.* Though with some difficulty, they were induced to grow just as Kny's buds on *Begonia rex* grew, and considerable secondary tissue developed in the petiole. Normally a simple bow-shaped bundle, an abnormal cambium appeared in the ground-tissue opposite the bundle, and formed with the original a complete cambium ring, and eventually a thick cylinder of wood. He gives a very careful description of the origins of the cambium, and ends with an extensive criticism of the possible causes of the secondary wood. His paper will be frequently alluded to in the following pages.

Finally, we have a short paper by Löhr (6) in 1909. He rooted leaves of many plants—including *Pelargonium zonale*—and grafted sprouts on them. He gives comparatively few anatomical details of secondary increase in the petioles.

So much for the historical account. It shows us that the petiole can function as a stem with remarkable changes of structure. But when we compare the masterly paper of Professor Hans Winkler with the other contributions, we see at once that his is the most important one. He has realized the importance of the answer to the further query: Whence these changes? That answer he has himself attempted, but more and more extended and detailed work is necessary to verify that answer. Hence these experiments, which were begun at his suggestion. The first half of the work was carried out under his supervision in the Botanical Gardens and Institute at Hamburg. To his sage advice and direction as to the larger methods of the experiment and the smaller but hardly less important details of technique—such as grafting and culture and microscopy—is it due that the work has gone so far. I wish to put on record my heartfelt appreciation of his kindness to me.

If we recall now the various experiments, we notice that the petiole has been got to function as a stem in two ways.

(*a*) A sprout has been grafted on it, and the subsequent development of that sprout has induced the changes in the petiole.

(*b*) In the other cases—*Begonia* and *Torenia*—we find adventitious buds already growing on the leaf, almost inviting the attempt to root the leaf and allow the adventitious bud to grow.

At first sight then it would appear that method *b* should be chosen, but very little consideration showed the advantages of the grafting method. Thus :—

1. Leaving out of account the Pteridophytes, where the presence of buds on leaves is comparatively common, we shall find that the number of plants at our disposal is limited. Thus, *Malaxis paludosa*, *Drosera rotundifolia*, *Cardamine pratensis* (and many of its relations), *Bryophyllum calycinum*, *Tolmiea Menziesii*, species of *Lycopersicum*, *Pinellia tuberifera*, many *Begonias*, *Torenia asiatica*, *Nymphaea Daubenyana* would about exhaust the list—(4, 5, 7, 8).

2. The captious critic can object to the use of any of these plants, on the ground that they are abnormal plants. Their structure is exceedingly plastic, and it could be argued that any response in the petiole to change or increase of function is but a peculiarity of these, and could not be taken as indicating anything of deep importance in plant structure. Of course, the argument is speciousness itself. The more plastic the plant, the more it is sought after by those interested in functional hyperplasies and kindred phenomena.

3. But there is a final argument which eventually settled the question, and it is the difficulty of the proper culture of these adventitious buds. In most cases the influence of correlation is very manifest. Thus, to note one—*Bryophyllum* (Goebel, 9 and 10; Winkler, 5). This is a well-known plant belonging to the Crassulaceae. It has an ovate, somewhat dentate, leaf. At the bases of the small bays at the edge of the leaf, there are nearly always found small buds. As long as the leaf remains in connexion with the mother plant, so long do the buds remain dormant. If the apex of the mother plant, as well as all the normal axillary buds, be removed, the adventitious buds on the edges of the leaves develop. Not all of them do so. The largest do so at once; those that are beaten in the race remain still dormant. They may also be

induced to develop by cutting the leaf at right-angles in such a way as to sever the main nerve. Again, by the exercise of no little gardening skill, isolated leaves may be rooted, the buds on the edge develop. But now comes the difficulty. Invariably, as soon as the buds have got beyond the bud rudiment stage, they begin to develop roots. Remove these roots laboriously day by day, and for a few days the young sprout will grow, but always drops off before it has reached even a few centimetres in height. It is possible that methods might be devised to overcome these difficulties, but unfortunately the time could not be spared to deal with them. The same difficulties crop up in the others, save perhaps *Begonia* and *Torenia*, and they have been used before. Perhaps something could be done with that *Solanum Lycopersicum* mentioned by Lütz (7), which developed buds in the axils of the secondary leaves, which buds developed freely on plants whose main stem and branches had been removed. But I do not know the variety he used, nor did I ever meet with a specimen of any of the ordinary Lycopersicums with such adventitious buds.

For these reasons, then, it was decided to make use of the first method, namely, the grafting of sprouts on petioles. Here we can try any plant that can be grafted, and this gives us a wide choice. We can make use of the most ordinary plants of the greenhouse or garden, and thus silence the critic referred to above. Thus it is a poor botanical garden, indeed, that cannot supply the experimenter with Pelargoniums, and the more detailed portion of the work was carried out on one such—*Pelargonium zonale* v. *meteor*—the despised geranium of the suburban window. The technique is simple, and results can be obtained with the minimum of delay.

II.—Material and Methods.

Material.—The plants used were:—

1. *Pelargonium zonale* v. *meteor.*
2. *Solanum Richardi.*
3. *Solanum Balbesii.*
4. *Sanchezia nobilis.*
5. *Phytolacca dioica.*

Not all of these were investigated as closely as *meteor*. Even of the results given by them only such will be recorded as are necessary for the argument.

Method.—The following methods were used:—

A healthy plant was first chosen, and a large, well-developed leaf having been decided upon, its lamina was removed by a transverse cut some little way down the petiole. A large petiole was chosen, as the differences in size between stock and scion at the grafting-place would have been most awkward to deal with had a younger and smaller leaf been chosen. This is especially so in *Pelargonium zonale*, as a glance at any specimen will show.

An incision was then made with a very sharp razor into the cut-end of the petiole. This was made in different planes. In some cases it was horizontal, in some cases vertical, in some cases oblique. A small apical bud was then selected, one with the little internodes as long as possible. A bud two centimetres long, however, was looked on as quite a large bud. All the leaves were removed from it, save about one. This is to leave the scion with actively metabolic leaf-tissue. If one leaf was too small, a second with one-half removed was also left. The cut-end of the bud was then carefully cut to a thin wedge shape, with the edge of the wedge as fine as possible. This was inserted on the petiole of the stock, bound, as usual, with bast, and the bud and the bast carefully sprinkled. The mother-stem above the petiole selected was removed, as also were all the buds on the plant. Usually one or two leaves were also left on the stock, so as to ensure a continuous supply of food material to the mother-plant while the graft was taking. In *Pelargonium zonale*, however, such was not essential.

Obviously it is essential to keep down the transpiration of the scion till the graft-union is effected. The most convenient way of keeping the grafts in a very damp warm atmosphere is to cover them with a bell-jar, embedding its rim in the sand-bed.

All the plants had to be gone over every day to remove the adventitious buds, which grew with great rapidity. Even after the graft had taken, these adventitous buds grew continually.

The grafts took quickly—the bast binding could usually be removed after ten days.

Plate XXVIII, fig. 1, shows a graft of *Pelargonium zonale* a short while after the graft was thoroughly established. The mother-stem in the bottom right-hand corner, the petiole attached to the actively growing scion, and the grafting zone are clearly distinguishable. The difference mentioned above between the bud thickness and the petiole thickness is also seen. Attention is also directed to the very definite difference in size between the petiole and the mother-stem.

Plate XXVIII, fig. 2, is more interesting. It was photographed about a fortnight after the graft had well taken, shows the wedge very clearly, and gives a good idea of the size of bud used. But the label shows the method of numbering. When many plants were in use, some system was necessary. We see then on the label the name *P. dioica.* The capital A denotes that it is a sprout grafted on a petiole. Plants dealt with in other ways were referred to as B, C, D, as we shall subsequently see. This was for identification when the petioles had been removed for section-cutting. The number 13125 shows that it was the 125th plant grafted during 1913. Then follows the date and month. The Egyptian-like hieroglyphic shows how the slit had been made in the petiole—horizontally, vertically, or obliquely.

Once the initial stages were past, the grafted sprouts grew well. A large number of grafts had been made, so that some were sacrificed at regular intervals, i.e., when 2, 6, 8, etc., leaves had become expanded. In this way the changes taking place in the petiole could be consecutively followed.

Controls.—The following controls were set up:—

1. That portion of the petiole still attached to the lamina, when that was removed just before the grafting operation, was in every case examined—in many cases in paraffin section, in the others in hand section. As well many petioles of many other individuals of *Pelargonium zonale* v. *meteor* were examined. In all at least 50 petioles from as many different plants were tested. In this way variations from the normal petiole structure could be noted. In passing it may be remarked that these variations were singularly few.

2. Leaves were grafted on the main stem of the plant. All the other leaves and buds were removed from the plant, and kept removed. These leaves grafted just as well as the sprouts on the petioles. These grafts were referred to as B for identification purposes.

3. Some plants were entirely debudded, and all the leaves removed except a single one. These leaves grew long and well. They were called D.

4. Many attempts were made to root single leaves removed from the plant. The conditions for successful rooting were difficult to determine, but still some were successfully rooted.

Thus we can study the structure of the leaf petiole under these conditions:—

1. Normally growing with all the ordinary correlational influences of the mother-plant being exercised on it.

2. With the correlational influence of the growing apices of the mother-plant removed. In D we have this state exactly; in B we have it free from these influences, but with the wounding stimulus of the grafting acting on it. In both we have it still in intimate connexion with the mother-plant.

3. With all the influences of the plant removed—in the leaf-cuttings.

4. With the correlational influences of the growing points of the mother-plants removed, but subject to the influence of the abnormal sprout growing on itself.

In this way it was hoped to determine, if possible, how far the changes resulting in the petiole were due to the influence of the grafted sprout, how far due to changes in the relation of the petiole with the mother-plant, how far due to increased duration of life of the petiole.

III.—THE CHANGES IN THE PETIOLE.

A.—*Macroscopic.*

As the grafted sprout grew and enlarged, distinct changes were apparent in the petiole. Week by week it increased in diameter, and finally assumed for all intents and purposes the diameter of the stem above or below. In *Pelargonium,* be it noted, the diameter increase, save in one instance, was much greater in the vertical than in the horizontal direction. By the time the small grafted bud had become a shoot about two feet high, the petiole had assumed the proportions as well as the functions of a stem. The increase in diameter was obviously due to an internal tissue increase, as in every case the outer tissue was burst in slits, the slits becoming protected by well-developed cork masses. A series of pictures will demonstrate this increase in size more clearly than any description.

Plate XXVIII, fig. 3, is *Pelargonium zonale* v. *meteor,* A, 1390, 3/5. It was photographed on January 7th, 1914. It is fairly weak, but was selected for photography as showing the petiole-become-stem very clearly. What appears to be the branch leaving the top at right angles is the petiole, a short distance along which the swelling at the grafting-place can be seen. All above this is the growth of the small grafted bud.

Plate XXVIII, fig. 4, is a near view of the petiole seen in Plate XXX, fig. 2. The whitish mass on the upper side of the petiole is the cork mass which developed where the upper corticle tissues became split. The V-line of the wedge-graft can be clearly seen. The grafting zone is singularly free from graft callus.

Plate XXIX, fig. 1, is *Solanum Richardi,* A, 1301, 10/4. It was photographed on June 21st, i.e., little more than two months from the date of grafting. The main stem is at the bottom in the middle; the small lateral branch on the right is the petiole. As we see, its dimensions all but equal that of the mother stem. If we follow up the swollen petiole, we come to a swelling. This is a mass of graft callus, and indicates the position of the grafting zone. All above this zone was developed from the grafted buds. This shows with what rapidity results may be obtained by this grafting method.

Just where the petiole leaves the stem we see a swelling like a very large pulvinus. This was very well marked in all the Solanums, and to a less extent in the Pelargoniums. It is the place where the wood of the petiole is being gathered together preparatory to fusion with the vascular ring of the stem. I shall have occasion to refer very briefly to it later.

Plate XXVIII., fig. 5, is a photograph on January 7th, 1914, of the petiole of *Solanum Balbesii*, A, 13119, 24/5. The cortical splits with cork development are clearly shown. But it shows how thoroughly the petiole in certain cases becomes included in the branching system of the plant.

Plate XXIX, fig. 2, is a photograph taken on January 8th, 1914, of *Pelarg. zonale* v. *meteor* A, 13104, 6/5 taken to show the size of the plant for which the petiole can act, as a supporting and conducting organ. This plant was about 2 feet high—the main stem below being about ¾ inch thick. The grafted petiole can be seen running in between the two supporting sticks. This plant grew well till it was cut down early in March to provide cuttings of the same variety for further investigation. It shows then to what extent the length of the petiole's life is increased. It would seem that a petiole which normally has only a limited length of life may have its life-duration indefinitely prolonged when thus included in the branching system of a plant.

At the risk of wearying I would like to include Plate XXIX, fig. 3. It is the root-system developed from a petiole of *Sanchezia nobilis*, of which a leaf had been rooted. On the petiole of this leaf a sprout was then grafted. The roots are attached to the petiole about 1 inch, along which can be noticed a small bend. Here the V of the wedge-graft can be seen; farther on, running out of the picture, is the stem developed from the grafted sprout. The approximation in size of stem and petiole is here very marked. The root-system is comparatively small, as the plant grew weakly.

Plate XXIX, figs. 2 and 3, were specially included to emphasize under what different conditions the petiole is after the development of the grafted sprout, being the only support for a large plant, and the only conductive passage between a large root development and a large foliage development.

B.—*Microscopic.*

Before going on to the microscopical history of these changes, and indeed to understand them at all, we must first study the structure of the normal petiole. The changes have only been followed in *Pelargonium zonale* in detail—in the other cases we must content ourselves with the final form.

1. *The Structure and Variations of the Normal Petiole of* Pelargonium zonale *var.* meteor.

While there are many references in the literature to the anatomy of the order Geraniaceae and even of Pelargonium [see, for example, Brunies (11), and the literature cited in Solereder (12)], there is only one description of the petiole of *Pelargonium zonale.* Jännicke (13) shortly says that it contains a peripheral ring of bundles, some large and some small, which lie against a comparatively weak mechanical ring (Bast ring). In the medulla there lies free a central bundle. "Dasselbe besteht aus einer runden Cambiumgruppe, die in ihrer Mitte eine kleine Bastgruppe und beiderseits im Durchmesser zweier grösserer Mestombündel eine Gefässgruppe erzeugt, von denen die eine stärker ist wie die andere" (p. 18).

Let us look now at Plate XXX, fig. 1.

It is a photograph at 20 diameters of a normal, well-developed petiole of *Pelargonium zonale* var. *meteor*. It is dorsiventral, (*a*) being the upper side or the side turned towards the stalk, (*b*) the lower side. It is thus flattened from above downwards. In fact, its greatest diameters are—horizontally 3·6 ms. vertically, 2·7 mms. The peripheral ring of large and small bundles as mentioned by Jännicke, appear clearly. One can see, too, that at the middle of the upper side there is one large bundle with two smaller ones closely approximated to it. Opposite them on the vertical diameter of the petiole is another large bundle with two small bundles on each side. This appearance is quite constant in the variety, and will be of importance in the subsequent pages. These bundles show nothing of importance, consisting of primary wood and phloem. No tracheides are developed, though traces of the primary cambium can be seen in the large bundles until the leaf is quite old. Its presence can be detected even when the bundle gives every appearance of a complete cessation of cambial activity. These bundles lie against a mechanical ring which is but poorly seen, as at C. The nature of this ring—whether we are to refer to it as sclerenchyma fibres, as Strasburger (14) would, or as bast used in Haberlandt's (15) sense, whether we are to regard it as developed from a special tissue which we ordinarily call phloem, or with Morot (16) insist that it is derived from an equally special tissue which we call the pericycle, may indeed provide us interesting and important questions from the standpoint of comparative anatomy, but need not detain us here. But very definitely free in the medulla there lies the largest bundle of the petiole. It is worthy of note that, contrary to Jännicke, the wood of this bundle is obviously developed on one side only. It is equally worthy of note that, contrary to the normal position of wood in, say, a large bow-shaped bundle in a petiole, the wood is on the lower surface. Its origin is very simple. Without regarding details, the vascular strands of the leaf leave it in three aggregates of bundles arranged in a crescentic form. The horns of the crescent gradually fuse, but some of the vascular tissue before the fusion leaves the periphery to form the central bundle. On entering the stem the central bundle splits a portion going to each side to fuse with the upper and lateral bundles, which have all come together, forming a large lateral bundle on the under side of the petiole. As it advances to the fusion it twists on itself and completes the fusion with the wood on the more normal upper side. There is little else of importance in the petiole, save that there is a single sub-epidermal layer of collenchyma which can be made out with difficulty in the photograph, owing to aberration caused by the use of a low-power objective.

Plate XXX, fig. 2, shows the centre bundle at a magnification of some 45 diameters. It shows the wood (*a*) on one side only. A complete ring of small-celled phloem is on the upper side of this, (*b*) being one layer, (*c*) being the other. The ring surrounds a larger-celled mass of parenchyma with thin walls—in no specimen did there appear bast used in Jännicke's sense. Jännicke makes no mention of the presence of phloem (i.e. sieve tubes and companion cells). The wood consists merely of primary elements, that is tracheae and wood parenchyma.

Plate XXX, fig. 3, shows us that traces of cambium are still to be found, even in well-matured petioles, between the xylem and that part of the phloem lying adjacent to it. Thus (*a*) shows two small groups of dormant cambium cells, (*b*) are the masses of small-celled phloem. The tissue lying between (*c*) and (*c*) is the large-celled parenchymatous tissue which the phloem ring surrounds.

Plate XXX, fig. 4, shows that part of the phloem ring farther from the wood which lies just at (*a*). (*b*) (*b*) is the phloem nearest to the wood, (*c*) (*c*) is the central cortical tissue, (*d*) (*d*) the phloem removed from the wood. This phloem is clearly marked off from the medullary tissue which abuts against it—in many petioles even more clearly than here. (This one was, however, photographed, so as to have all the petiole structure thus studied from the same petiole.) As will be seen then, the outer or medullary side of this phloem shows no trace of that cambium which Jännicke refers to as forming a definite ring. And this is the typical appearance. In one petiole A, 1374, however, at one corner the divisions had so followed in the phloem parenchyma as to constitute a definite cambium for a very short length indeed of the phloem. In one other petiole A, 1386, this cambium had developed along the whole length of what we may term the "xylem-free" surface of the phloem, and even formed a few isolated vessels on this side (Plate XXXI, fig. 1).

These small vessels are shown at (*a*). This is the only instance of the appearance of lignified tissue or conducting vessels of any sort on the xylem-free surface of the central bundle out of more than 50 petioles examined. Plate XXX, fig. 4, is the type of the rest. We may mention, too, that the grafts on this petiole, A 1386 and A 1374, above, both died as a result of their atmosphere having been kept too warm and moist.

Plate XXXI, fig. 4, shows the further fact we must learn, that the central bundle does not always remain one, but in many petioles is found split. Thus (*a*), (*b*), (*c*) all constitute the central bundle here. When, however, we remember that when coming from the leaf lamina the central bundle is formed nominally from the fusion of several bundles, this appearance is not surprising. Indeed, if we follow the central bundle down along the petiole, we find very frequently that the bundle will split and unite, and then split again a couple of times. The phloem is remarkably well shown indeed in (*b*)—its demarcation from the cortex almost gives one the impression of its being surrounded by a bundle sheath.

We see then that the detailed structure of the petiole is very constant and differs from Jännicke's description in the following points:—

1. Wood is developed on the under-side of the central bundle only, and not on the upper-side as well.
2. There is no complete ring of cambium in the central bundle.
3. There is no "bast" formed within that cambium.
4. A large quantity of phloem is formed.

These divergencies from Jännicke seemed so great as to require further investigation. And so it was considered necessary to look into this point just a little, although its connexion with the taking on by the petiole of the function of stem is meagre.

And this for two reasons—

1. Because Jännicke does not describe what variety he used.
2. Because the petioles A 1374 and A 1386 gave one the idea that perhaps the tendency to form a complete ring of wood was latent in the petiole—a sort of Mendelian recessive perhaps.

These combined suggested that *meteor* was conceivably a hybrid between the form used by Jännicke and a form which never developed cambium on the xylem-free side of the central bundle—the latter condition being dominant.

I forthwith made inquiries as to the history of *meteor* from Sir Frederick Moore, Keeper of the Royal Botanic Gardens, Glasnevin. For his information, so gladly given, as also for his ready supply of plant material, at times of rare plant material, which he forwards at a moment's notice, I wish to return my sincerest thanks. These inquiries elicited the information that in all probability the garden race of *meteor* arose from a cross between *Pel. zonale*—the true specific Cape form—and *Pel. inquinans*. I then hand-sectioned about half a dozen petioles each from *P. zonale* (true) and *P. inquinans,* as well as fourteen different varieties of *P. zonale,* with the following results :—

1. In both *P. zonale* (true) and *P. inquinans* wood and cambium were not developed on the xylem-free side, so that the little speculation as to the Mendelian origin of A 1374 and A 1386 was shown to be specious.

2. The varieties varied very much thus :—

(*a*) With no wood on xylem-free side of central bundle in even old petioles.	Manteau rouge, single crimson. Mrs. Gibson, single pink.
(*b*) With a little wood occasionally present (cf. *meteor*).	Nipheta, single white. Constance, single salmon. California, double brick-coloured. Wilhelm Pfitzer, brick-red.
(*c*) With a few vessels—but only a few—always on xylem-free side.	Gus Enick, semi-double scarlet. Paul Blondeau, double pink. E. Isemberg, pink-tinged single. Madame Carnot, double white. Athlete, single scarlet. Nora, single flesh-coloured.
(*d*) Well-developed wood mass on xylem-free side.	Julius Caesar, double salmon. Dagata, double pink.

This tabulation shows us the variability of the anatomical nature of this central bundle in the different varieties. It is unfortunate that the origins of most of even the well-known garden races of Pelargonium have been almost scrupulously lost, because it is possible that this variability might have proved an interesting problem in genetics. We have, say, *P. inodorum,* on the one hand, with a central bundle just like an ordinary collateral bundle, and *P. zonale* v. *Julius Caesar*, with a well-developed wood cylinder, on the other, and a whole gradation of forms between. The behaviour of this anatomical character in the various crosses from which the many races sprang might thus have been after Mendelian laws.

But, whatever our regrets might be, this digression—already much too long—has shown us (cf. *Julius Caesar* and *Dagata*) that, in spite of the essential differences between the structure of the petiole described by Jännicke and that one till recently under consideration by us, Jännicke was quite accurate, having apparently used some such variety as the said *Dagata*.

2. *The Successive Microscopic Changes.*

After so long a wandering we get on now to a consideration of the microscopical changes that take place in the petiole after the grafting of the sprout upon it.

The first change is the re-awakening of the old dormant cambium in many of the already existing bundles. This always appeared in that aggregate of bundles which we mentioned as situate above and below the central bundle, less frequently, however, in the lateral bundles, possibly due to the fact that these bundles never made a satisfactory union with the vascular cylinder of the grafted shoot, though this was not investigated. The behaviour of the central bundle was interesting. Just as in the peripheral ones the dormant cambium became very active, but as well there appeared on the xylem-free side of that bundle a series of cambial divisions (Plate XXXI, fig. 3).

Here we see *a–a* the band of cambial cells appearing on that side of the phloem ring abutting on the cortex *d*. *b–b* is the internal parenchymatous mass already often referred to. *c–c* is that portion of the phloem which lies against the primary wood.

This layer very soon multiplies to form a typical cambium band (*a*), Plate XXXI, fig. 4. (*b*) is the cortex. (*c*) internal parenchyma.

Though the figure is somewhat hazy, it shows clearly that this cambium layer has been derived not from the surrounding cortex, but from the phloem band.

Both this cambium and the bands of cambium which became active again elsewhere build up secondary wood.

Plate XXXII, fig. 1, shows this extremely well.

(*a*) is the primary wood, (*b*) is the wood formed from the re-awakening of the original cambium. Its characteristic close texture is very visible, consisting of large vessels scattered in a compact mass of thick-walled tracheides. To what extent fibres are developed has not yet been investigated. (*c*) shows the beginning of secondary wood of a similar nature on the formerly xylem-free side.

The portions (*a*) and (*b*), with the cambium and phloem appertaining thereto, are typical of the larger peripheral bundles at this stage.

It should be carefully noted that this is a picture of one of the B plants—that is, taken from a leaf which had been grafted on a stem.

The wood portion (*c*) gradually extends till a complete ring of secondary wood has been formed in the central bundles. But after the first rapid development the cambial activity becomes quite slow. I have no figure showing the maximum development of the central bundle, even when a plant nearly a yard high was growing on the petiole; but if we imagine at (*b*) as much secondary wood again as is now present, and at (*c*) a band about as large as (*b*) is at present, we have a good idea of its maximum. Why this should be so in every case is hard to explain, because such a development in comparison with the secondary thickenings elsewhere is extremely small.

Referring to the fig. of the normal petiole, Plate XXX, fig. 1, we remember that on the upper side above the central bundle there was an aggregate of bundles.

Plate XXXII, fig. 2, shows the development of these bundles. Here we see a compact band of secondary tissue, with only one gap at (*a*), and even here we notice that the cells are small and the divisions regular, reminiscent of a cambium formation, (*b*) and (*b*) are the primary wood of two adjacent bundles, the rest is secondary development. The figure also shows how with the increased development of wood the mechanical ring (*c*) and (*c*) becomes ruptured, cortical cells growing into the gaps as at (*d*).

Plate XXXII, fig. 3, shows how the compact band is formed. After the fascicular cambium had been active for some time there appeared, as seen in this figure, a series of divisions in the interfascicular cortex. These were not so mathematically regular as the interfascicular divisions in the typical Dicotyledon stem, but they eventually formed a complete line joining up two, three, or in some cases even four bundles of the upper surface. This line of cambium gave rise then to a large mass of secondary wood along its whole length, and hence the appearance in Plate XXXII, fig. 2. In this figure we may see a series of divisions at (*e*) as if the cambium were spreading to other bundles, but it never did so. Never more than four of the upper bundles lying close together were joined up by this interfascicular cambium.

We have further the peculiar fact that in comparatively few cases was there ever seen a similar joining up of the lower aggregate of bundles, these bundles, as we shall presently see, lagging in their development very much behind the upper ones in the majority of cases.

It was hoped when this interfascicular cambium was first noticed in A, 1353, that all the peripheral bundles would eventually be linked together to form a complete ring resulting in a solid cylinder of wood. But even though limited in its position, the development of an interfascicular cambium at all from the cortical cells of a mature petiole, is quite an interesting phenomenon. In a mature petiole, in its normal relations with the plant-bearing the leaf, the dead cambium, and, above all, the cortical cells, are as differentiated as they ever will be. This development of an interfascicular cambium especially shows that we must not regard a differentiated tissue as necessarily a permanent tissue. As long as the cells contain living protoplasm, and are bounded by unthickened cell-walls, so long will they retain the potentiality of manifold development if the proper stimulus is acting.

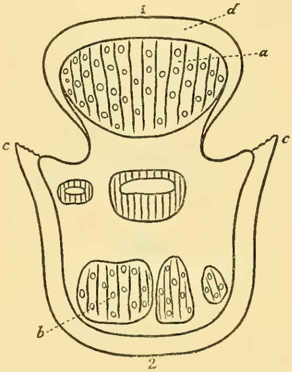

FIG. 1.—*P. zonale.* Old grafted petiole. A 13103 6/5—3/3/14. Very diagrammatic.

The final stage in the old petiole is easy to understand. Four individuals were grown to quite a large size. The final structure of the petioles was, however, not the same in all four.

Fig. 1 shows the structure of one of the four. It is a diagrammatic sketch drawn very roughly to scale. (1) is the upper surface, (2) the lower surface. We see thus that the greatest increase has been in the vertical diameter. Thus Plate XXX, fig. 1, is a good-sized petiole, it measures 3·6 mms. horizontally and 2·7 vertically. Fig. 1 measured 4·2 mms. by 6·9 mms. in the same planes.

We see, however, that the upper and lower bundles have developed wood to about the same extent .The upper bundles fused perfectly, the lower bundles not so well. The wood occupies about half the area of the section. Now, the total area has been increased more than four times. So the wood developed from the small bundles in Plate XXX, fig. 1, is now at the very least equal in area to the complete section itself. The comparatively weak development of the central bundle already referred to is also seen; (*c*) and (*c*) are the jagged ends of the old epidermis which was first split by the growing volume of (*a*) and then thrown off by the development underneath it of a thick cork layer (*d*). The under-surface epidermis was still intact and no cork formed.

Fig. 2 shows a different arrangement. Here, though the upper bundles have completely fused and developed a large quantity of secondary wood, the lower bundles have remained comparatively tiny.

This figure shows the formation of two of the remaining petioles. The fourth was about midway between the two. The upper bundles were fused and well developed. The lower bundles and one or two of the lateral had developed fairly well, but were still completely separated by large masses of medullary tissue. The area was hardly equal to one half of that of the fused upper bundles. In the other cases those lateral bundles which had not been crushed out by the upper mass of wood had remained very small indeed.

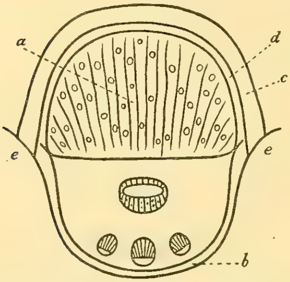

FIG. 2.—*P. zonale.* Second type of old grafted petiole. Diagrammatic.

(*a*) is the upper wood mass.

(*b*) the original cortex which remains unruptured on the lower surface.

(*c*) a thick layer periderm derived as appears at the points (*e*) and (*e*) from the sub-epidermal collenchyma layer. In this respect the periderm development in the petiole corresponds with that in the stem where it also develops from the single sub-epidermal layer of collenchyma.

(*d*) a crushed mass of old cortex and phloem.

To recapitulate the changes:—

1. The old cambium re-awakened; being more active in the upper and lower than in the lateral bundles.

2. New cambium appeared on xylem-free side of central bundle, this eventually resulting in a ring of secondary wood whose development, however, never proceeded very far.

3. The upper bundles are linked by an interfascicular cambium which proceeds to very great secondary activity.

4. In the mature petiole a large amount of wood is formed. This is due in three cases mainly to the upper bundle. In one case only it is almost equally contributed to by the upper and lower bundles.

5. A thick periderm layer is developed on the upper surface where the wood developed has ruptured the epidermis. The petiole thus has taken on the function of the stem, and, with this, those properties characteristic of the stem—indefinite life-duration, development of secondary wood, and periderm-formation.

The results in the two species of *Solanum* are no less remarkable, Thus, Plate XXXII, fig. 4, is a section of a well-developed petiole of *Solanum Balbesii.* The single bow-shaped bundle is prominent; (1) is the upper, (2) the lower, surface.

Fig. 3 shows very diagrammatically the result of grafting the sprout on it. The old cambium awakens and forms a mass of secondary wood (*b*). But as well as in the region marked (*a*) in Plate XXXII, fig. 4, a cambium appears which gives rise to the heavy mass of wood on the upper side of the petiole. The two cambiums unite at *e* and *e*, and in this way a complete cylinder of wood is formed. The wood from the new cambium presents peculiarities which unfortunately have not yet been studied. But at places such as *f* and *f* we find not normal secondary wood but very large tracheides, so large as to appear not like tracheides cut across, but as if the tracheides had their longitudinal increase horizontally instead of vertically. It was as if the tracheides had been developed in layers—here a column of vertical tracheides, against it a column heaped high of tracheides lying horizontally. Of course we might expect tracheides varying from the normal as they are derived from a cambium which has abnormally arisen in large differentiated cortical cells; but why one part should give rise to normal secondary wood, and another part to this formation, seems difficult to explain. It might be suggested, however, that it is in some way a response to mechanical stimuli, as we shall later discuss. As to its mechanical efficiency, no opinion, of course, can be expressed.

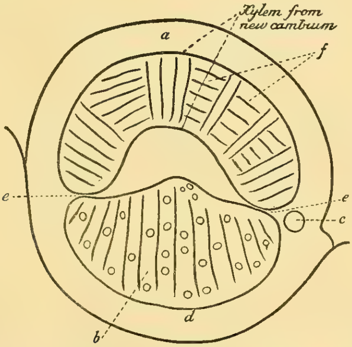

Fig 3.—*Solanum Balbesii.* Old grafted petiole. Diagrammatic.

(*a*) is a thick cork layer developed where the outer layers burst with the internal expansion. The cortex at (*b*) is still intact.

The increase in diameter is marked. Thus the normal petiole measures in its horizontal diameter 3·1 by 3·0 mms. in its vertical. The corresponding measurement of the petiole after grafting was 6·4 × 7·7 mms.

Plate XXXIII, fig. 1, is a photograph of the secondary development in the bow-shaped bundle of Plate XXXII, fig. 4. It is taken at the very same magnification. The primary wood of fig. 3 is seen here at (*a*). The presence of well-developed thin medullary rays consisting of one or two layers of cells can be seen. It gives us a good idea of the mass of wood developed in these petioles.

Similar results were recorded for *Sol. Richardi*, but in both species one out of the three or four grown to maturity had no development of wood from the abnormal cambium.

The changes in the other two leaves must next come under consideration. In those plants B where a leaf was grafted on the main stem there always appeared a re-awakening of the fascicular cambium. Thus, Plate XXXII, fig. 1, shows us such in Pelargonium. But this development never approached even remotely that of the petiole-made function as a stem. Thus the greatest increase in any such was seen in a leaf of *Solanum Richardi* which was grafted on the stem of *Solanum Lycopersicum*, *Gloire de Charpenne*.

The normal petiole of *S. Richardi* is shown in Plate XXXIII, fig. 2.

Plate XXXIII, fig. 3, shows the leaf after growing for three months on the Tomato stem. It was only removed when it began to die. This is at the same magnification as Plate XXXIII, fig. 2.

Comparing Plate XXXII, fig. 4, and Plate XXXIII, fig. 2, at the same magnification, we see that *S. Richardi* and *S. Balbesii* are very similar. Looking now at Plate XXXIII, figs. 1, 2, and 3, all at the same magnification, we can see how the maximum secondary wood development observed in these B plants, compares with the normal primary wood and with the wood in the petiole-made function as a stem. Remember, too, that Plate XXXIII, fig. 1, is only part of one of the wood masses in the petiole. Interfascicular cambium never appeared in this group. The petioles were always packed with starch.

In the D groups, i.e., leaves left on the stem whence the other leaves and all the other buds had been removed, very little change was observed, although the leaves grew for months before they died. In one case out of at least a dozen Pelargoniums a development up to about the same extent as in the B group took place, but in only one. In the others never more than a few tracheides were secondarily developed. In a few cases, however, active divisions took place in isolated places in the cortex similar to those to be described in the rooted leaves. Here the outer layers became burst, and a covering of periderm protected the exposed tissue. In all cases the cells of the medulla were so packed with starch as to give the appearance when treated with Iodine of a section of a potato tuber so treated.

The leaves which were rooted and grown so for some time were of interest. Only *Pelargonium* was investigated. In every case there was a re-awakening of the old cambium, but the resulting activity was less than in the B groups. Again, it was much greater near the petiole base than near the lamina attachment. Its extent can be gauged from the portions of bundles appearing in the next three figures. But most peculiar of all was that without exception there was a very close demarcation indeed between the portion above the ground and that below, because below the ground an elaborate and considerable periderm was formed.

Plate XXXIII, fig. 4, shows a complete view of the cross-section. The small dark patches all over the slide are starch grains. But we see the remarkable periderm surrounding the petiole. This periderm is shown on a larger scale, Plate XXXIV, fig. 1.

It is derived from the sub-epidermal collenchyma layer of the petiole. Thus, 1 is the epidermis intact; 2 is the internal half of that collenchyma layer. Here it is very clearly standing out from the cortex. The periderm is definitely divided into two layers—(*a*) an outer one with all the characteristics of dead suberized cells, and giving the staining reaction of these; (*b*) an inner layer of living parenchyma with thin cellulose walls and round in form like cortical cells. Close examination will show the presence in some of these cells of minute starch grains. The cork cambium would seem to be situated between the two layers—see the suggestion of cell-divisions at 3—cutting off true cork tissue on the outside and this parenchymatous tissue on the other. The large starch grains in the medulla and the smaller grains in the cortex are clearly visible. At 4 the small increase of secondary wood is seen.

But these leaf-cuttings showed other changes. More often on the above-ground parts, but occasionally also usurping a portion of the petiole from the periderm mentioned above, a fierce activity of medullary cells would take place, resulting in the appearance of a split filled with cork.

Plate XXXIV, fig. 2, shows this mad activity of these cells. A scrutiny of the photograph shows the presence in them all of large numbers of small starch grains. The whole gives the impression of a tendency towards tuber development. The cork-protective mass is very evident, but is not derived from the typical sub-epidermal layer, as a glance at (*a*) will show. It is a purely protective cork developed over those parts exposed by the splitting of the cortex; (*b*) shows us secondary thickening in a bundle.

To recapitulate briefly:—

1. The A group develops a very large quantity, indeed, of secondary wood.

2. The B group develops a small quantity of secondary wood.

3. The leaf-cuttings develop a small quantity of secondary wood, but also an elaborate periderm below ground, and most frequently above ground an active local division and multiplication of many medullary cells.

4. The D group develops practically no secondary wood, but occasionally a multiplication of medullary cells is to be seen, as in 3.

Interesting though it be to observe these changes in an organ which had reached its normal full differentiation, it is no unique case. That cells in their normal adult condition can become active again, and even like the medullary cells which gave us our interfascicular cambium, and thence tracheides and tracheae, can give rise to tissue which in the normal course of their development they never form, is a widespread phenomenon. A few examples selected from the literature of the subject will let us realize how widespread it is. Many of the examples will be referred to later in our discussion of the possible causes of the changes in the petiole.

Thus De Vries (17) mentions a case of the adventitious development of a vegetative bud on the flower-head of *Pelargonium zonale* in place of a flower bud. Such an appearance is comparatively common. I have one myself in a very small stock of Pelargoniums. De Vries, however, preserved the bud, which he kept growing for three years. The flower-stalk, which is even more transitory than the petiole, developed changes similar to those in our petiole.

Vöchting (18) gives us quite a series in his work on tubers. 1. In one case he planted tubers of *Oxalis crassicaulis*, so that they were only half buried. In some examples sprouts developed from the over-earth portion and roots from the underground part. In this way he made the tuber part of the branching system of the plant. He describes then the quick and large development of the vascular bundles in the tuber bundles, which normally are exceedingly small. The typical secondary elements—tracheides and fibres—are described by him. 2. Again, the plant *Boussingaultia baselloides* normally develops tubers from underground nodes. If, however, an internodal piece be rooted, the lower end swells into a tuberous formation. This occurs in young and old stems indifferently. The swelling in the main is due to divisions in the cells of the medulla, although the cambium cells also become active, and give rise to much parenchyma. Needless to say, the whole tissue is packed with storage material. 3. The formation of tubers in *Oxalis crassicaulis* is brought about by the development of stolons, which run out, the apices forming tubers. These stolons carry very small buds and leaves, so small, indeed, that only with a good lens can the petiole and lamina be distinguished. If the apex is removed, and also the buds, the cut end of the stolon swells; but likewise all that part of the small leaves which corresponds to a petiole develops into a tuber. 4. A leaf of *Raphanus sativus* v. *radicula* when rooted developed no buds, and but few roots. The base, however, swelled to a tuberous formation, mainly derived from the old cambium. Here we see tubers functioning as stems and contra tubers, resulting from the active division of the parenchymatous cells of old stems and leaves.

For such apparently stable organs as leaves, there are a large number of instances of abnormal tissue development in them. Thus Mer (19) mentions an ivy leaf which he rooted, and kept growing for seven years. The petiole bundles enlarged radically to three or four times their original size, due to the activity of the old cambium. We may also mention that he observed an increase in the palisade tissue.

Recently Mathuse (20) describes a large number of very similar results with rooted leaves, using about twenty from fifteen different orders. His results varied, but in most cases he got increased secondary thickening in the petiole, and very frequently increased palisade tissue. As far as can be deduced from his sketches, his secondary development never exceeded our *Sol. Richardi* leaf grafted on *Lycopersicum* (Plate XXXIII, fig. 4). In one case, however—*Achryanthes Verschaffelti*—he describes how the base of the petiole hypertrophied, due to active divisions of the parenchyma, giving a mass of small cells starch-packed, just like the leaves of *Pelargonium zonale*, or those of Raphanus, mentioned by Vöchting.

Interesting is the work of Freundlich (21), who cut the main nerves of the cotyledons and foliage leaves of various Dicotyledons. Not every plant was suitable, but in several cases—most neatly in *Fittonia argyroneura hort.*—leaving the cut end nearer the apex, bridges of tracheides grew out, being formed in the parenchymatous tissue round the end of the bundle. These actually grew out round the cut, and formed anastomosis with the bundle end below the cut, or with neighbouring large branches. Simon (22) has given us a most interesting paper on similar connexions formed in stems and roots in the medullary tissue. In one of his most interesting experiments he cut across a plant again, placed the parts in apposition, with a sheet of mica perforated in the centre between them. In this way only at the medulla were the two parts in contact. But tracheide bridges were formed at the cut end of the bundle, near the apex, grew into the small hole in the mica, grew through it, and out to join the other end of the cut bundle. The bridge followed the course of a procambial strand, which first appeared.

Finally, we may mention the case of galls, quoting as an example the *Spathegaster* gall on the male inflorescence of some oaks, where the gall-bearing axes have their life duration greatly increased, and show increase of the vascular bundle beneath the place of inflection (Kuster 23).

This series of examples shows us that the further differentiation in the petiole of *Pelargonium zonale* is no isolated phenomenon, but merely one example of a general characteristic of plants. Because a tissue remains normally at a certain development stage we cannot regard it as an absolutely permanent stage—as Jost (24, p. 444) says: "Wir werden nicht umhin können, anzunehmen, dass die Befähigung zu derartigen Lebensäusserungen [speaking of the phenomena of Regeneration] in jeder protoplasmahaltigen Zelle vorhanden ist und nur für gewöhnlich durch die Beziehungen der Teile untereinander unterdrückt wird." Let the proper stimulus act on such cells, and "redifferentiation" is certain to follow.

And thus it is naturally the object of the next section of this paper to determine as far as possible what are the causes which have operated to produce the remarkable changes which have been described as appearing in the petiole as a result of the grafting of a sprout upon it.

IV.—The Possible Causes of the Changes in the Petiole.

When we endeavour to analyze the causes which produce any physiological reaction, we must remember the dictum of Pfeffer (25), to the effect that "it is evident that no theory can be correct which ascribes a phenomenon of complex origin to the action of a single factor," and so we shall have to refer to many factors. Luckily we have a masterly analysis of the possible causes in Winkler's paper (5). While we may not agree with him in all points, we must thank him for that summary. To his paper reference must be made for fuller discussion of most of the points. Only such points as are absolutely necessary for completeness, or such points as those on which one cannot quite agree with Winkler, will be introduced here.

Wound stimulus. — Undoubtedly the petiole when undergoing the grafting process is subjected to a very severe traumatic stimulus. But its effect would appear to be of no very great direct importance in our petiole, even in spite of the well-known development of so-called wound wood in a wound callus (see Kuster (23)). It does not seem that we need go beyond our own experiments for proof. Thus the leaf grafted on the stem was also subjected to severe traumatic stimulus, but the secondary thickening in it is by far less than that in the petiole upon which the sprout was grafted. An observer of Plate XXXIII, fig. 3, might maintain, however, that here we have indeed a large wood development, perhaps due to a wound stimulus. But, remembering Freundlich's (21) experiment in severing the main nerve of a leaf in which he even got bridges of tracheides growing round the cut to the lower end of the bundle, we fail to notice any marked increase in that bundle either above or below the cut. Again, Jost (28) describes an experiment in which he cut through the bundle of one of the primary leaves of *Phaseolus multiflorus* in a very young plant. The leaf grew well, but there was no increase in the bundle either above or below the cut. Until, then, we get some positive evidence of the extent to which a wound stimulus can increase the development of wood in a vascular bundle, we can hardly admit that that stimulus has, at any rate, any great direct importance in the secondary developments in the petiole.

Loss of Correlational Influences.—To measure the effect of such a factor as correlation seems futile, since we know so little of the real causes of plant development. That it has a widespread effect we know well. The whole phenomena of regeneration are bound up with it. Remove the apical buds of Bryophyllum, and the buds on the leaves develop. Remove the lamina from the petiole, and the petiole soon dies. But it does not seem that the mere correlational changes brought about by the suppression of the buds on the parent plant have any direct effect on the large secondary development. The same loss of correlational influences was a factor in those plants on which only one leaf was left, the plant having been completely debudded. In this D group there was less change than in any other. In the leaf cuttings, where *all* correlational influences had been removed, the secondary vascular changes were very slight. But that these influences have some powerful and fundamental effect we cannot deny. But it is an indefinable indirect effect. Thus, for the successful taking of the graft, all the parent buds must be kept removed. It is the removal of the retarding influences exercised by them which allows the so-called "permanent" tissue of the petiole to react as it does to the other profound physiological stimuli which act upon it after the grafting. It is like exploding a mine by releasing a spring. The spring is no direct cause of the violent explosion, but without its release no explosion occurs. Similarly the petiole is released from the correlational influences (the spring), under the influences of the graft (i.e. the spark firing the mine), it "explodes" into a very much thickened petiole. Only from this point of view, I think, can we consider the correlational influences as working at all.

Increased life duration.—Since petioles can be kept carrying large plants for at least 15 months, it is possible that this increased duration of life may in some way be bound up with the increased development. Thus Kuster (26), speaking of such a secondary development as we saw in the tubers of *Oxalis crassicaulis*, denies that it is an activity hyperplasy. "Durch die eigenartige Versuchsanstellung," he says, "wurde die Lebensdauer der Kartoffelknollen weit über das normale Zeitmass verlängert die verschiedenen Gewebe der Knolle wurden sehr viel länger als unter normalen Verhältnissen in Anspruch genommen. Nun ist offenbar fortgesetzte Inanspruchnahme an sich noch nicht gleichbedeutend mit gesteigerter Inanspruchnahme, es wäre sehr wohl möglich dass auch fortgesetzte Inanspruchnahme unter Umständen schon genügt, um die abnormale Bildung sekundärer Gewebe, wie sie Vöchting beobachtete, anzuregen." Similarly in regard to Mer's seven-year-old *Hedera* leaf he speaks of "die infolge der abnorm verlängerten Lebensdauer fortgesetzte Inanspruchnahne." It is true that more recently (23) he leaves his position with regard to Mer, referring his results now to the "Nährstoffstauung die bei Fortgang der Assimilation in den Blättern zustande kommt und an die vielleicht abnorm reichliche Wasserversorgung die das neu gebildete Wurzelsystem der Blätter diesem zukommen lässt," but still he seems, in spite of Winkler's criticism, to retain his idea about *Oxalis crassicaulis.* But his argument of the effect of increased life duration has no force at all in these experiments. If we refer to Plate XXXII, fig. 2, we see that the graft was made on the 21st April, and cut down on the 2nd June—a bare 6 weeks. Surely there is no question of increased life duration here to account for such development—the petiole diameters increased from 3·6 by 2·8 to 4·7 by 4·0 mms. I think that without any further evidence this figure shows that the secondary developments occur before the question of the effect of prolonging the life duration comes in at all, and that so this factor can be ignored completely.

Increased Storage of Nutritive Material.—If we recall Plate XXXIII, fig. 4, the section of a leaf-cutting, we remember that a considerable quantity of starch was to be seen in the petiole. The same phenomenon appeared in the B group. It might now be suggested that this excess of carbohydrate was responsible for the secondary thickening. Such is suggested by Mathuse (20) to account for similar changes in his rooted leaves. That it is not the only cause, or even a powerful factor, is shown by the fact that B and D petioles, although, when removed, always so packed with starch that it seemed impossible for them to hold more in their cells, showed such very feeble secondary development when compared with the A petioles. But that it may be a factor in the B and D petioles is a consideration which cannot be ignored. This would mean, however, that permanent tissue can be induced to become active and to give rise to highly differentiated vascular elements, if an excess of foodstuff be present. This is contrary to the usually accepted view of the relation between nutrition and development as expressed for example by Jost (24, p. 447), "wir sind der Meinung dass er [i.e. foodstuff] durch das Wachstum und den Verbrauch Bedingtes und nicht die bedingende Ursache der Gestaltung ist." But that this is not universal is shown by Vöchting's experiments of producing tubers abnormally in *Boussingaultia* and on petioles of *Raphanus*. He himself attributes this development to a nutritive stimulus. But the result of that stimulus is storage parenchyma, and not vascular tissues. It develops tubers, and not masses of tracheides. If now we look back again at the peculiar periderm on the underground portion of our rooted leaves, we have such parenchyma immediately suggested to us. But Plate XXXIV, fig. 2, shows—I think indubitably—a tuberous nature. Again, if the Plumule is removed from a growing seedling of *Phaseolus multiflorus*, the hypocotyl enlarges greatly, the cambium having given rise to thick layers of parenchyma—this parenchyma being full of starch. There is also a large amount of reserve starch deposited in the medullary cells, such reserve being derived from that originally present in the seed, since the embryo has none, and the experiment was conducted in the dark (Jost 27 and 28). Here again, we have storage parenchyma as the result of nutritional stimulus. It may be objected that Wieler and Hartig maintain that a nutritional stimulus is the origin of the secondary growth of trees. Wieler's works I have been unable to obtain, but Hartig only claims as a result of nutrition the different thicknesses of the cell-walls of the tracheae and tracheides at different seasons. He does not claim that vascular tissue owes its origin to a nutritional stimulus (29). Until, then, we have evidence that storage of food material can induce the appearance of secondary vascular elements we can decide that this factor is of no importance in the development of the secondary tissue in our petiole.

The next factors cannot be disposed of so easily. Indeed they are also so intimately connected that they must be taken together—they are the stimulus of *increased mechanical strain*, and the *stimulus of increased conduction.* If we glance again at Plate XXIX, figs. 2 and 3, we realize this very clearly. Plate XXIX, fig. 2, shows us the heavy weight of foliage which must be borne by the petiole; the strains and stresses to which it is subjected must be enormously increased. It shows, too, that a great activity of assimilation is in progress, and that thus a large transpiration current and a large stream of plastic substances must be passing in the petiole between this foliage and a large root-system such as in Plate XXIX, fig. 3. Here we meet enormous and fundamental changes in the physiological conditions of the petiole, and here consequently the main causes of the secondary thickening must be sought. We take the increase in conduction of plastic substances first. Thus De Vries (17) claims that the secondary developments in the tuber of *Oxalis*, caused to function as a stem, are due to a current of such nutritive material—the cause is to be sought "in der Bewegung der Nährstoffe im Xylem und in den Siebröhren, sowie dem benachbarten Parenchym." He particularizes this Nährstoff later as Eiweissstoffen and Kohlehydraten. Now, apart from the fact that it seems hard to see why cambium cells should be stimulated to such activity by a "Bewegung" of these materials when a more passive storage, although an equal nutritive excess, should merely give rise to storage parenchyma, we find De Vries vigorously combated by Jost (27) and (28). He demonstrates that in seedlings of *Phaseolus*, as already mentioned above, with plumule removed, there is a continual stream of such material passing from the cotyledons to the epicotyl, and thence after a short time to the developing roots while vascular development is all but absent. He adduces, too, such facts as that secondary thickening in trees stops before autumnal leaf-fall, and thus while a nutritive stream is still passing in the neighbourhood of the cambium, concluding that the nutritive stream "ist nur eine Bedingung niemals aber die Ursache des Wachstums." Lately Snell (30) has re-opened the question of the effect of the nutritive streaming on vascular development, but he has not made any criticisms of Jost's objections to the nutritive current theory, still less does he adduce any further experimental evidence in its favour. So that the movement of large masses of food material in the neighbourhood of a cambium would seem to have no influence on the development of secondary tissue. From *a priori* reasons it does not seem improbable that changes of turgor in the cambium cells should result from such a nutritive stream, and that cell-wall thickenings might in some way result therefrom; but pending further general research along these lines, we certainly cannot claim a positive influence for this factor. An active upward movement of such material, apart from the parenchyma of the bundles, would be so closely bound up with the transpiration stream which will presently be discussed as not to need discussion here.

The Stimuli of increased Mechanical Strain, and of increased Water Conduction.

These two factors can be more conveniently treated together.

Winkler (5) says, p. 57, speaking of mechanical strain increases—"Ich möchte es als wahrscheinlich bezeichnen dass der mechanische Faktor dabei eine Rolle spielt, wenn sich diese vorerst auch nicht näher präzisieren lässt.

Sicher erscheint mir nur, dass er an Bedeutung weit zürücksteht hinter dem letzten Faktor—den geänderten Stoffleitungsvorgängen." He goes on then to make a strong case in favour of the proposition that the transpiration increase which gradually follows the increasing size of the sprout is responsible to a much greater extent than any other factor for the secondary developments in the petiole. Reasonable as such seems, are we yet in a position to decide so definitely as Winkler has done? Are we not liable to ignore the mechanical factor too much, and be too sure of a direct effect of transpiration on vessel development, tending to regard this vascular development as a much more simple process than it really is? A short discussion of the points at issue will, perhaps, indicate why it seems undesirable to take up so definite a position.

Thus the question of the effect of mechanical strains on the development of the mechanical tissue of a plant has produced some interesting results. Hegler, quoted by Pfeffer (25, p. 127), says that petioles of *Helleborus niger*, with an original breaking strain of 400 grammes, were able to bear a breaking strain of 3·5 kilogrammes, after they had been subjected to increasing loads for five days. He describes, too, an increase in the mechanical tissue. These results, however, have not been subsequently confirmed. Again, Ball (31) was unable to find any response either by mechanical tissue increase, or by increase in the breaking strain in a number of plants grown with a continual tension acting on them. Wiedersheim (32) subjected weeping forms of certain trees to continued tension, with no response save in one case *Coryllus avellana* v. *pendula*, where he describes an increase in the bast-fibre masses. Hibbard (33), too, found that tension had no effect on the formation of mechanical tissue in stems, save in *Vinca major*, where there was an increase in the amount of xylem, and in the thickness of its cell-walls. Vöchting (34), however, by growing a gourd in such a way as to make the fruit-stalk support the weight, describes considerable mechanical increase both in bast fibres and in wood-cells. This he describes as due to a mechanical stimulus. "In diesem Beispiele zeigt sich also der Einfluss der Belastung sobald sie als Eigengewicht in die Verkettung der korrelativen Vorgänge eingreift. Hätte der hier gewonnene Schluss weitere Geltung, dann liesse sich das Entstehen der mechanischen Gewebe in den Knollen der Oxalis [already often referred to] auf den Druck zurückführen, den die sich entwickelnden scheitelständigen Triebe auf die sie tragenden unteren ausüben, einen Druck, der nun korrelativ wirkte, d.h. gänzlich verschieden von dem, welchen er als fremde, den objectiven angehängte oder aufgesetzte Last verursacht. Das Eigengewicht äusserte einen functionellen Reiz in dem angegebenen besonderen Sinne auf die Organe und veranlasste sie so zur Erzeugung mechanischer Zellen." So Vöchting at any rate considers the mechanical factor of importance in a case very similar to ours—abnormal secondary tissue in a tuber bearing a large vegetative development above and roots below. Indirectly he might be adduced against Ball and Wiedersheim and Hibbard as contending that their methods of applying a mechanical stimulus were inaccurate, and that positive results should result from a properly acting stimulus. Such results were recently obtained by Bordner (35) in 1910. He subjected plants—the same species it may be remarked as were used by Ball and Hibbard—to longitudinal tension for prolonged periods. The work was carefully and accurately carried out with many individuals under tension and many controls. He concludes thus—"The results of the experiments have convinced the writer conclusively that actively growing stems of the herbaceous plants used, and of *Vinca major*, respond to traction along their longitudinal axis by increasing their breaking strength, and also by increased development of bast, or of xylem, and in most cases by an increase of both these mechanical tissues." Thus an average of about thirty plants of *Helianthus annuus* loaded on November 21st with 25 grammes, gradually increasing to 300 grammes on December 4th, the experiment ceasing a few days afterwards, gave the following results :—

Breaking strength increased	57·6 per cent.
Cross-section of walls of hard bast increased .	16 per cent.
No. of hard bast elements increased . . .	12·8 per cent.
Total xylem area increased	40 per cent.

This is a remarkable positive result which cannot be gainsaid. And when we remember that one of the functions of the xylem is undoubtedly support, we cannot afford to ignore the mechanical factor if it may cause as great an increase in the xylem as 40 per cent.

Again, it has long been known that tendrils after attachment to the support show an increase in mechanical tissue and in resistance to breaking strains (see Worgitsky 36). How far this is a response to the stimulus of contact, and how far to that of tension, has been long disputed. Thus Fitting (37) claims that the stimulus of contact is active to the very base of the tendril, while Newcombe (38) and McDougal (39) affirm that its effect is much more localized, hardly extending at all beyond the tip. Quite recently, however, Brush (40) had made an endeavour to analyze the effects of tension and contact in the tendrils of *Passiflora caerulea*, coming to the conclusion that considerable increase in the breaking strength is produced by contact alone; but if the stimulus of tension is also present, there is a still further marked increase. Thus, without explaining his methods, he measured tendrils which had remained free, tendrils stimulated by contact alone, and tendrils stimulated by both contact and tension. The average breaking-strengths of the three were:—

Free tendril, ...	190 grms.
Tendril subject to contact only, ...	651 ,,
Tendril subject to contact and tension, .	1007 ,,

Again, by other methods, he measured the results with tendrils subject to tension only.

Breaking strength of tendril under no tension, .	862 grms.
,, ,, ,, with tension, . .	1239 ,,

This result, coupled with Bordner's, seems to indicate very positively, indeed, that tension can have very marked effect on tissue. And so it seems probable that the increase of mechanical strain has played a very important rôle in the development of the secondary xylem. There is one fact which, I think, speaks in favour of it. Unfortunately it was not at first considered that mechanical effects would be of any large direct importance, and so it was only at the very last moment that it was thought fit to make such measurements as those below. The material necessary, which might otherwise have been preserved, was then found to have been in most cases sacrificed. However, the following was accurately done. The area of the wood in the petiole *Sol. Balbesii*, A, 13119, 24/5–26/1/14, was carefully drawn with the Abbé camera lucida at a magnification of 40 diams. The area of wood in the stem below was similarly drawn, and the two areas compared by weighing, with the remarkable result:—

Area of wood in petiole. . . .	24·2 sq. mms.
,, ,, ,, stem below, . .	16·4 ,, ,,

Further in the stem below there were two rings of wood quite distinctly to be seen, indeed nearly every section was split at parts along the line between the two rings. If, as seems to be plausible, the two rings are explained by the fact that the vegetative portion had been completely removed from the plant at the time of grafting, that thus the plant rested for a fairly prolonged period without any marked foliage development, and that then with the active growth of the small scion once the union was firmly established, a second vegetative period for the plant began just comparable to the appearance of the Johannis branch in the ash;—if this is so, the outer ring only in the stem corresponds to the wood-development in the petiole.

This would reduce the stem-wood to about 8 sq. mms. or only one-third the quantity of wood developed in the petiole. And a possible suggestion to me at any rate is that the petiole running out at right angles to the stem, and carrying a very large plant upon it, is subject to most abnormal conditions of mechanical strain. The large wood-development is the result of this stimulus. Now if we glance back at Plate XXVIII, fig. 5, we see a swelling at the base of the petiole running into the stem. The wood here was also measured —although very roughly—and was found to be in area more than twice that in the petiole. This is surely a large development, hardly to be accounted for by any claims of water conduction. The closer anatomy of the secondary wood in all the petioles, as well as the abnormal wood in the Solanums, must be carefully investigated as soon as possible.

As a result of this survey it must be admitted that there is sufficient evidence in favour of the proposition that mechanical stimuli are a very important factor indeed in the development of the secondary wood in our petiole, to indicate the necessity of further and immediate investigations to confirm the point.

Even if we grant the mechanical factor is not only more important than admitted by Winkler, but may even be of considerable importance absolutely, yet there is still some other powerful factor at work. Indeed, its effect may well be much greater than that of the mechanical factor, but the relative importance of the two can hardly be decided in our present state of knowledge. However, this other factor is certainly intimately connected with the large foliage development. Winkler decides very definitely that the xylem increase is directly dependent on the gradually increasing transpiration of the growing shoot. He denies that the transpiration effect is brought about through any changes in the water-content of the plant, but that it is a functional stimulus acting indirectly on the cambium. The stimulus is carried to the cambium by the living tissue of medullary rays, and apparently originates in the vessels—that is, if a vessel, owing to increasing transpiration, has to carry a larger quantity of water than usual, then a stimulus passes to cambium to form more vessels. In his own words, the actual factor is "der Grad der Inanspruchnahme der Gefässe." Now this is an interesting theory, but it would seem to have been built on too few facts. It is, indeed, quite possible that it expresses with some accuracy the state of things, but surely our accurate knowledge of the actual *causes* of secondary xylem development is too incomplete to really warrant the building-up of any cut-and-dry theory.

In the first place, one can readily admit that there is some connexion between leaf-development and that of secondary xylem. The work by Jost

(27 and 28), Snell (30), Winkler (41), and Scherer (43) have shown that the further development of the leaf-trace depends on its intimate connexion with the developing-leaf. "Organbildung," says Jost, "ist in vielen Fällen eine notwendige Bedingung fur die Gefässbildung." Snell and Winkler have also shown that it is not necessary to remove the leaf—if its function is removed, we have the same result. Again, secondary thickening in trees seems to go normally hand in hand with leaf-development. This is most strikingly seen in those of our common trees which may bear "Johnnistriebe"—that is, branches upon which the buds suddenly open and develop in late summer. When this happens, a second yearly ring is commonly seen (Jost. 27). Jost himself cannot bind himself to anything more definite than the statement that it is a correlation; but Winkler plumps for transpiration and, presumably, "der Grad der Inanspruchnahme der Gefässe" as the factor. Now there is much evidence to show that a general connexion exists between transpiration and vessel-development. The greater quantity of vascular tissue in plants grown in dry air than in moist, the greater vascular reduction of water-plants, may all be adduced in favour of the contention. (For references to a literature which it is unnecessary to quote here, see Winkler (5), and Schimper (42)).

But admitting a general connexion between transpiration and vessel-development, we have no accurate knowledge of the closeness of this connexion. In the first place, quantitative measurements on any extended scale seem lacking in the experiments referred to above. We do not know in most cases by how much the transpiration increased in the dry chambers, still less how far the vascular increase bore any close relation to such transpiration increase, nor do we know the relations between transpiration and absorption. Again, assimilation and respiration increase also in dry air and light, and the effects of such metabolic changes, acting with transpiration, have never been analysed; indeed, it is difficult to see how they could be. Again, granting that transpiration is the factor, what evidence have we for the "Grad der Inanspruchnahme" theory? Jost (24, p. 430) says in this connexion: "Bei starker Transpiration wird die Kutikula verstärkt, Kollenchym und Sklerenchym gefördert, die Gefässe werden weiter und zahlreicher, in den Blättern tritt reichlich Palisadenparenchym auf. Es fehlt aber zurzeit noch an einer kritischen Untersuchung der ganzen Frage, wir wissen nicht, wieviel von den beobachteten Erfolgen einfach auf Kosten von Differenzen im Wassergehalt der Pflanze, wieviel auf Verschiedenheiten in der eigentlichen Transpiration zu setzen ist, und im letzteren Fall wäre weiter zu untersuchen, ob die Wasserabgabe als solche einen Reiz ausübt oder ob die mit der Transpiration in naher Beziehung stehende Versorgung mit Nährsalzen von massgebender Bedeutung ist." And this fact just mentioned of the increase under conditions favouring transpiration of mechanical tissue—collenchyma, bast, sclerenchyma—seems to speak very directly against the "Grad der Inanspruchnahme" theory, and in view of a more indirect working of transpiration.

Again, transpiration itself, not to mention the "Inanspruchnahme" theory, seems to have no bearing on such phenomena as the appearance of small rings for some years in stumps of firs, pines, and larches. (See Kuster (23) and Jost (28) for literature, most of which was inaccessible to me.) Here, too, may be mentioned the development of wound wood, and especially such developments of it as are referred to by Simon (22). Thus a large callus with wound wood was formed on the outer surface of a *Populus canadensis* twig. Some mms. down a horizontal hole was bored into the pith. This hole soon filled with callus. The wound wood in this callus joined with that of the external callus by bands of vessels which were formed in the normally differentiated medulla. Such phenomena still require an explanation.

Again, Winkler supports his contention of the all-importance of transpiration with an appeal to the formation of annual rings. To go fully into the present state of knowledge as to the origin of annular rings is hardly in place here. The inaccessibility of the literature in any case would make a critical discussion impossible, but the following three quotations seem to me to mean that the question is still very much *sub judice*, and that indeed one brings it up in a transpiration question at one's own risk. Thus Haberlandt (15), in spite of the fact that he has been mentioned by Winkler as supporting the transpiration theory of annular rings, says (p. 617):—"Eine entwicklungsmechanische Erklärung der Jahresringbildung halte ich mit Krabbe und Jost derzeit fur unmöglich. Die verschiedene Ausbildung von Frühlings—und Herbstholz ist in dieser Hinsicht mit anderen periodisch-wechselnden Wachstumserscheinungen auf gleiche Stufe zu stellen, in deren Mechanik uns vorläufig nicht der *geringste* Einblick gegönnt ist." Pfeffer (25) says, p. 216: "The annual series of changes in the structure of the wood is the result of a complex correlative reaction which we are not at present able to resolve into simpler factors. Indeed, to do this, we must first know the causes which determine the differentiation of equipotential meristem cells." And Jost (24, p. 476): "Die 'Beziehungen,' die zwischen Jahresring und Jahrestrieb bestehen, brauchen aber nicht, Korrelationen zu sein derart dasz die Blattbildung direct auf Frühjahrsholzbildung hinarbeitet, es können auch beide Erscheinungen auf gemeinsamen Ursachen beruhen, Ursachen die bewirken das nach einer gewissen Ruheperiode ein neuer Trieb mit Laubblättern eine neue Holzzone mit weiten Gefässen beginnt. Zweckmässig ist jedenfalls dieses Zusammentreffen denn mit dem neuen Laub tritt erhöhte Transpiration ein, zu deren Deckung weitere Wasserbahnen nötig sind." The very same remarks might, indeed, apply to all the dry-air experiments, and to our own petiole. Again, Winkler makes an appeal to ecology. Desert plants have, he says, a much reduced vascular system, due to a reduced transpiration as a result of protective devices. This is a somewhat startling generalization, but it allows one point to be made with regard to the ecological aspect of transpiration. For the literature seems very poor indeed with regard to such points as comparisons between the absolute transpiration of Xerophytes and desert plants, and that of temperate mesophytes, as well as any statistics as to the relation existing between the transpiration of xerophytes and the woody development in them.

A critical exposition of such would much aid us in our inquiry into the causes of the development of secondary wood. We are often inclined to look upon Xerophytes as plants whose transpiration is absolutely small. If so, we find—in spite of Winkler's opinion above—that "in contrast to Hydrophytes, most Xerophytes and alpine plants have highly developed conductive systems with large, thick-walled elements." (Cowles 44, p. 686.) Schimper (42, p. 349), too, says that "the majority of trees of xerophilous woodland (i.e., in tropical rain forests with dry seasons) and savannah are of low stature with a relatively thick stem; the volume of wood in comparison with the foliage is greater than in hygrophilous trees." And again (p. 612): "Desert plants, dependent upon subterranean water, mostly have lignified axes." When, however, we remember with Delf (45 and 46) that many succulents—especially halophytes—have a high transpiration, i.e., Transpiration of *Salicornia* bears to that of *Vicia* the ratio of 36 to 19 in one case; when we remember with Holterman (47) that the transpiration of plants in the tropical Ceylon forest may equal, if not exceed, that of temperate mesophytes, and with Schimper (42, p. 308) the brittle structure with a few vessels of herbaceous plants of the tropical rain forest, then we feel the need of that critical discussion on vascular development from the ecological standpoint. But we may mention that Cowles (44) seems largely of the opinion that desiccation, whether brought about by increased transpiration or by diminished absorption, appears to stimulate increased vascular development, the important point being a relatively great transpiration to absorption.

What it is sought to convey in the last few paragraphs is that the foliage development bears some large part in the production of the secondary thickening in our petiole; that transpiration, or rather the water change in the plant, is the most probable factor; that, with our incomplete knowledge, it is useless at present trying to settle the exact mechanical "how" of transpiration; and that the branch of botany—ecology—which could yield us most assistance really makes us more confused. Much research by many workers must be carried out before the question of the origin of secondary wood will be definitely settled. For these reasons it is difficult to associate oneself with Winkler's very definitely expressed opinion.

SUMMARY.

(*a*) A petiole, by grafting a sprout on it, can be made to assume the functions of the stem.

(*b*) The properties of a stem, viz., long life-duration, indefinitely active cambium, interfascicular cambium linking up bundles, periderm development, and considerable secondary thickening, all appear in the petiole.

(*c*) The causes of this secondary thickening are to be sought:—

1. In the removal of correlational influences.
2. Increased mechanical strain.
3. Some influence exerted as a result of foliar development. This influence is probably bound up with the water economy of the plant—particularly transpiration—but its precise nature must still be determined.

POSTSCRIPT.

Much work remains to be done.

1. The nature of the ordinary secondary wood in the petioles and the peculiar wood mentioned in the Solanums must be studied.

2. It is intended to carry on exactly similar researches with the flower stalk of *Pelargonium zonale.*

3. It is intended to repeat these experiments in detail with *Phytolacca dioica*, only one or two preliminary graftings having been made. The interest of this plant lies in the fact that the secondary thickening of the stem—as in most Phytolaccaceae—is abnormal. It forms a normal ring whose cambium soon dies. Well outside the phloem of this ring a new cambium is formed which gives rise to a new ring. In this way a series of xylem rings are formed separated by bands of parenchyma (Kruch 48). The secondary thickening in the petiole, and the position of abnormal cambiums, if any, should prove interesting.

4. It is intended to investigate as carefully and as accurately as possible—as it will be done on a larger scale—the changes in the B and D leaves and in leaf-cuttings. I am anything but satisfied with this portion of the work. Also a comparison between the transpiration of the B and D leaves and leaf-cuttings with the average transpiration per unit area of the *Pelargonium*, will be carried out, for the purpose of seeing how far the small changes in them may be accompanied by increase of transpiration.

So much claims immediate attention.

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EXPLANATION OF PLATES XXVIII–XXXIV.

PLATE XXVIII.

1. Young graft of *Pelargonium zonale* var. *meteor.*
2. Young graft of *Phytolacca dioica.* Shows method of numbering.
3. *Pelargonium zonale* var. *meteor*, A, 1390, 3/5. A well-grown graft photographed on January 7th, 1914, i.e. about eight months after grafting.
4. The same. A near view of the petiole.
5. Near view of petiole of *Solanum Balbesii*, A, 13119, 24/5. Photographed June 7th, 1914.

PLATE XXIX.

1. *Solanum Richardi*, A, 1301, 10/4. Complete plant photographed on June 21st, i.e. a little over two months from date of grafting.
2. *P. zonale* var. *meteor*, grafted 6/5/13, photographed 8/1/14—much reduced, i.e. main stem below was about $\frac{3}{4}$ inch thick.
3. A leaf of *Sanchezia nobilis* rooted on which a sprout had been grafted 15/4/13. Photographed to show root system 8/1/14. This plant never grew well, mainly because it was not cared for well.

PLATE XXX.

1. Cross-section normal petiole of *P. zonale*. *a* = upper, *b* = lower side; *c* = mechanical ring. × 20.
2. Central bundle of I. × 45. *a* = wood; *b* = part of phloem near wood; *c* = part of phloem away from wood.
3. Portion of same to show quiescent cambium remains between *a* and *b* of 2. × 200.
4. Portion of 2, showing outer phloem mass. × 200.

PLATE XXXI.

1. *P. zonale.* Isolated occurrence of vessels on xylem-free side of central bundle. × 45.
2. *P. zonale.* Splitting of central bundle. × 45.
3. *P. zonale.* First appearance of cambium on normally xylem-free side of phloem of central bundle. × 45.
4. *P. zonale*, B, 1375, 24/4–2/7. × 200. Proliferation of this cambium. Note this is a *leaf* grafted on a *stem.*

PLATE XXXII.

1. *P. zonale*, B, 1375, 24/4–2/7. × 45. Shows secondary increase in central bundle. Note length of time grafted—about ten weeks.
2. *P. zonale.* Secondary increase and fusion of upper bundles of normal petiole. This was grafted on 21/4/13, and cut down 2/6/14—just six weeks.
3. *P. zonale.* Interfascicular cambium development in already differentiated cortical cells preparatory to giving stage shown in last fig. × 200.
4. *Solanum Balbesii.* T. S. normal petiole. × 25.

PLATE XXXIII.

1. *S. Balbesii.* Secondary wood developed in grafted petiole. × 25.
2. *S. Richardi.* T. S. normal petiole. × 25.
3. *S. Richardi.* T. S. petiole of leaf grafted on Tomato 10/4/13, removed 29/7/14. × 25.
4. *P. zonale.* Leaf-cutting. × 20. Shows starch and abnormal periderm.

PLATE XXXIV.

1. *P. zonale.* Leaf-cutting. Shows periderm. × 45.
2. *P. zonale.* Leaf-cutting. Shows activity (tuberous ?) of medullary cells. × 45.

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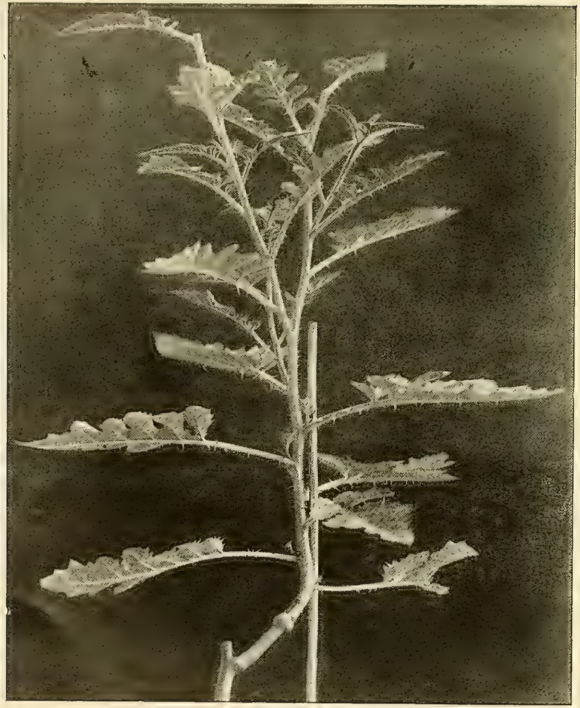

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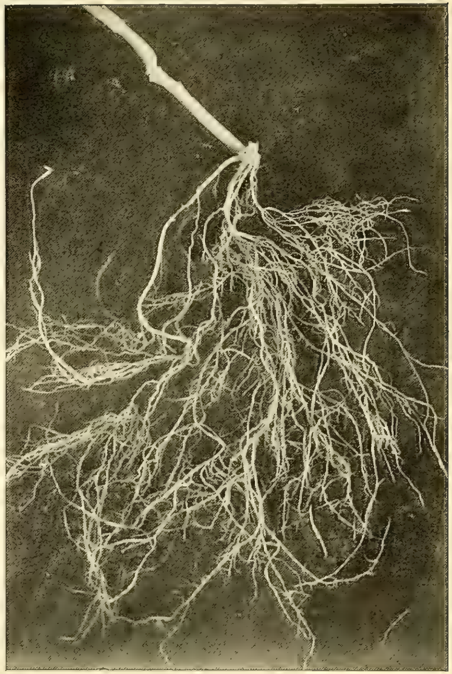

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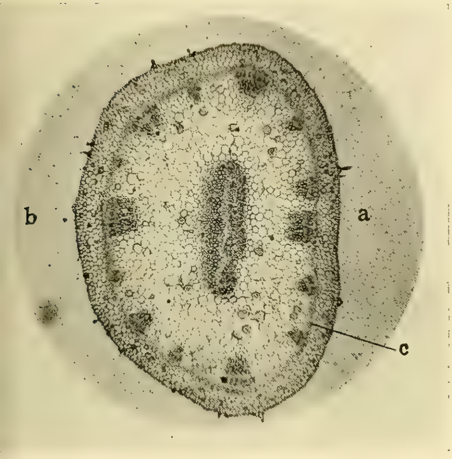

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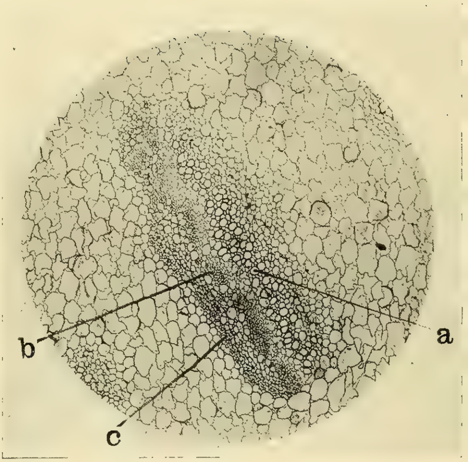

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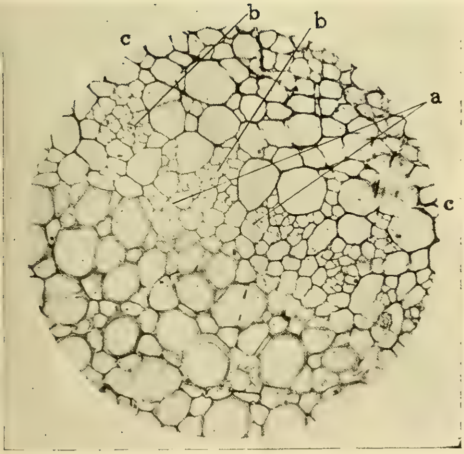

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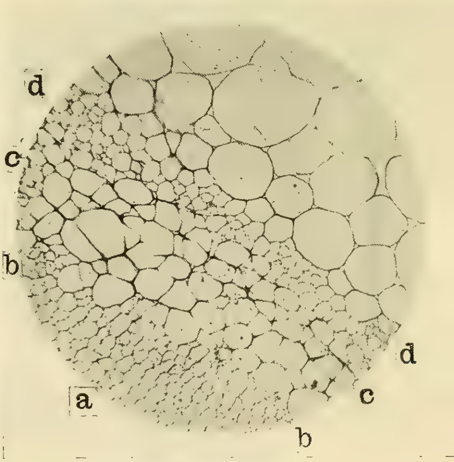

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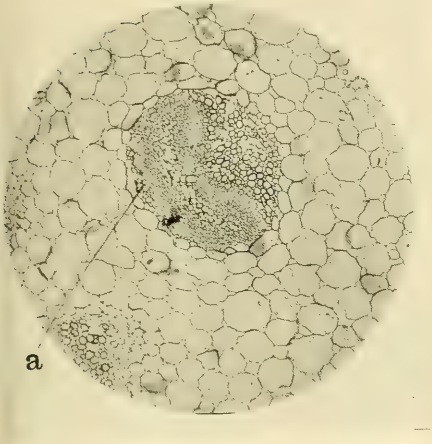

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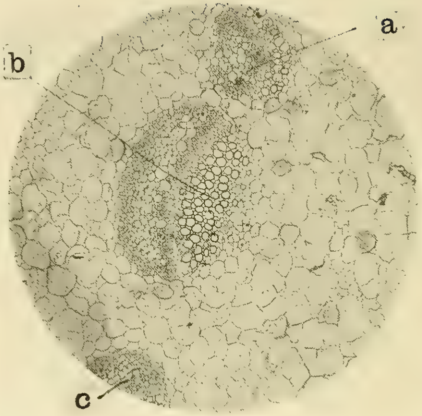

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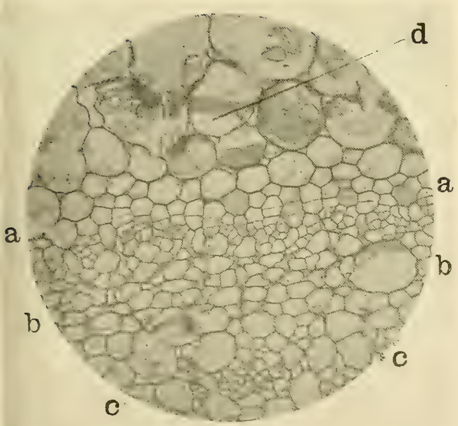

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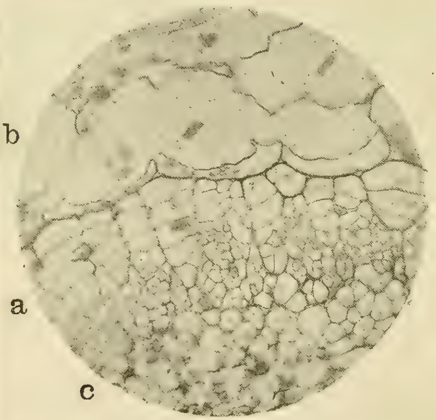

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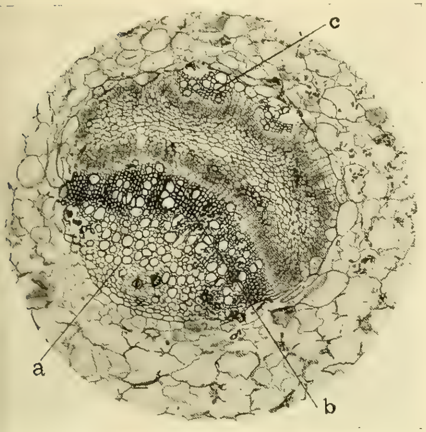

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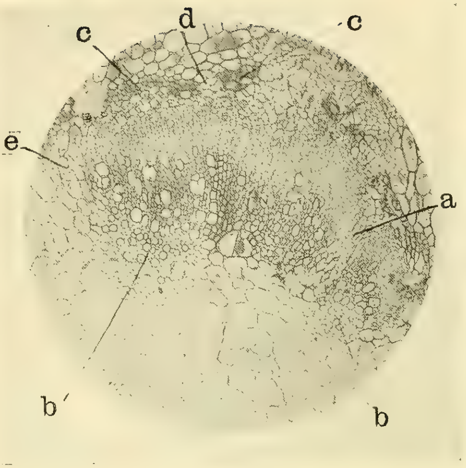

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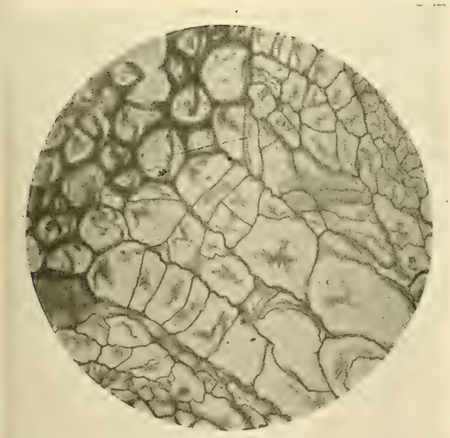

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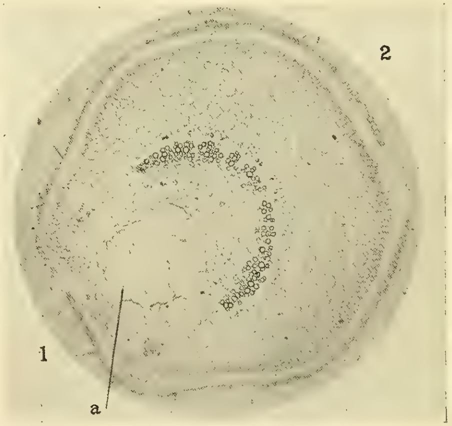

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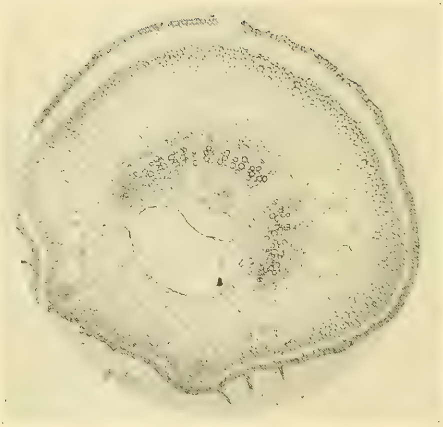

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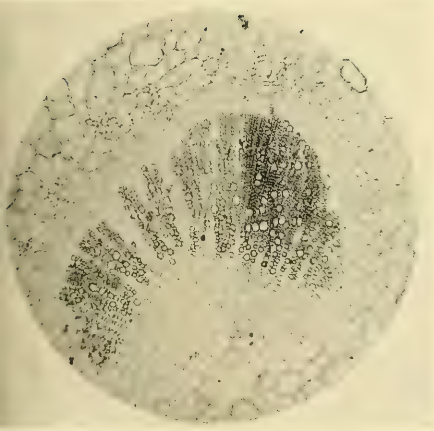

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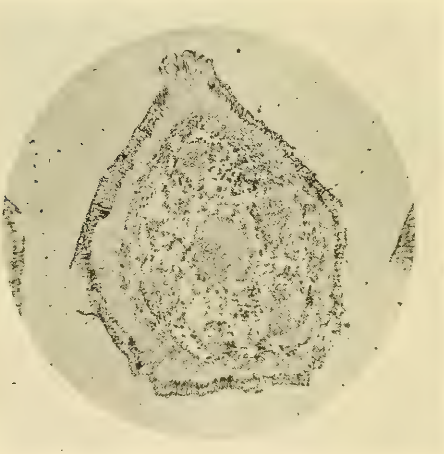

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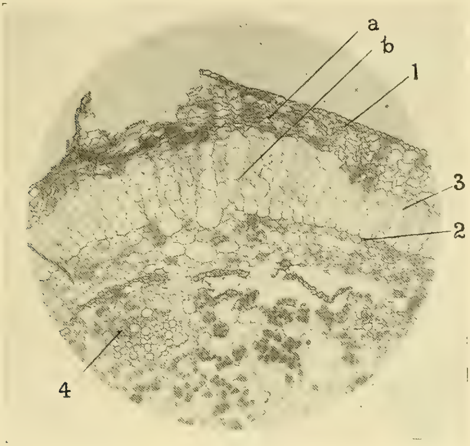

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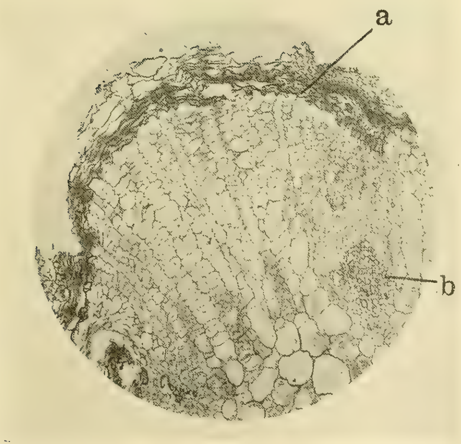

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THE
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OF THE
ROYAL DUBLIN SOCIETY.

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OSMOTIC PRESSURES IN PLANTS.

V.—SEASONAL VARIATIONS IN THE CONCENTRATION OF THE CELL-SAP OF SOME DECIDUOUS AND EVERGREEN TREES.

BY

HENRY H. DIXON, Sc.D. (Dubl.), F.R.S.,
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XXXIV.

OSMOTIC PRESSURES IN PLANTS.

V.—SEASONAL VARIATIONS IN THE CONCENTRATION OF THE CELL-SAP OF SOME DECIDUOUS AND EVERGREEN TREES.

By HENRY H. DIXON, Sc.D. (Dubl.), F.R.S.,
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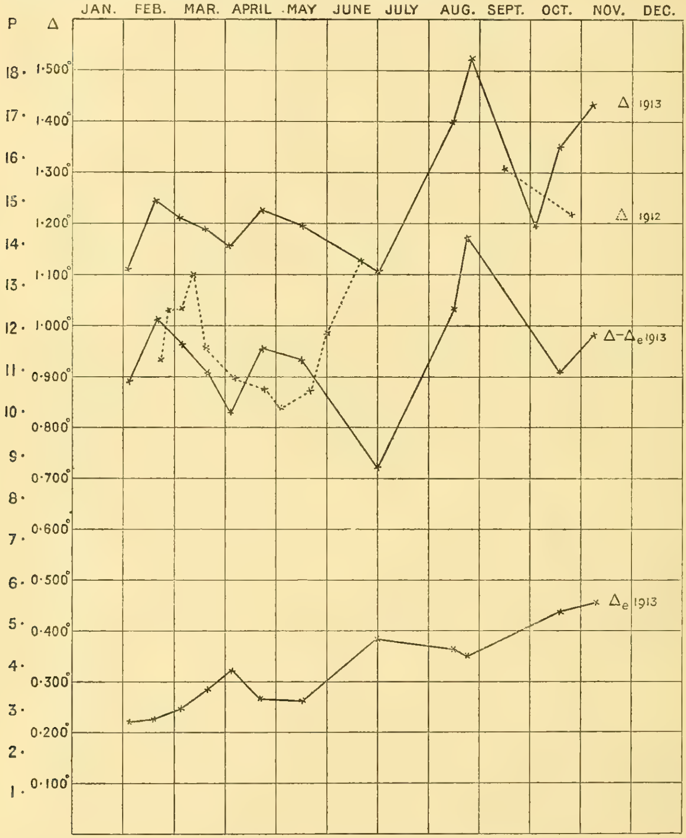
[Read January 26. Published March 26, 1915.]

A knowledge of the concentration of the cell-sap of plants is essential for the solution of several fundamental problems of vegetable physiology. Several years ago we (2, 3, 4) attempted to investigate the periodic changes during the year in the concentration of the sap of the leaves of *Syringa vulgaris*, *Ilex aquifolium*, and *Hedera helix*. At the time, in common with other investigators, we experimented on sap pressed from the tissues not subjected to any previous treatment. Subsequent investigation showed us (6) that the sap thus obtained is not so concentrated as that in the vacuoles of the uninjured cells, and that the unaltered sap may be obtained by pressing tissues previously killed by freezing. This discovery rendered necessary the revision of our previous work on the seasonal variations in the concentration of the sap; and in the present paper the results of this revision are recorded, together with certain additions.

Method.

The sap for freezing-point determinations (1, 5) and conductivity measurements was pressed from tissues previously frozen in liquid air. The depression of freezing-point Δ is defined by the concentration of all the dissolved substances, and hence gives a measure of the total osmotic pressure. These depressions and the calculated pressures in atmospheres are recorded in columns under Δ and P in the tables, and in the ordinates of the graphs. Under C are given the conductivity measurements in mhos. These are proportional to the quantities of electrolytes dissolved in the sap. From these figures are calculated those under Δ_e, the depressions of freezing-point of solutions of potassium chloride having the same conductivity as that observed for the sap. Under $\Delta - \Delta_e$ are the depressions due to the non-electrolytes, which are mainly sugars. In this manner not only are the osmotic pressures obtained, but also the parts played by carbohydrates and electrolytes in producing the pressure are indicated.



SYRINGA VULGARIS.—Leaves.

Fig. 1.

Syringa vulgaris.

The leaves of *S. vulgaris* formed the first subject of investigation. The results of the experiments are displayed in Table I. The leaves of three individuals were investigated—one in an exposed position in Trinity College

Botanic Garden, and two growing in a garden outside Dublin, and removed from the smoky atmosphere of the city. Most of the experiments were made on the first-mentioned tree, and are denoted in the table by an asterisk. These results are plotted in the graphs of fig. 1. Where two experiments were made on the same date—one on the inner leaves and one on the outer leaves of a bud—the mean is plotted. In Table I the observations marked Sw and Se were made on the two country plants having respectively a western and eastern aspect.

TABLE I.

Syringa vulgaris: Leaves.

Expt.	Date, &c.	Δ	Δ_e	$\Delta - \Delta_e$	P.	$C \times 10^5$
581*	1913. Feb. 4, buds, ...	1·109°	0·220°	0·889°	13·34	456
590*	,, ,, 18, ,, ...	1·247°	0·228°	1·019°	15·00	474
604*	,, Mar. 4, inner leaves of buds,	1·186°	0·248°	0·938°	14·27	514
605*	,, ,, 4, outer ,, ,,	1·239°	0·243°	0·996°	14·89	504
619*	,, ,, 19. inner ,, ,,	1·188°	0·282°	0·906°	14·29	586
618*	,, ,, 19, outer ,, ,,	1·200°	0·284°	0·916°	14·44	589
624*	,, April 3, outer ,, ,,	1·152°	0·327°	0·825°	13·86	678
732*	1914. ,, 22, young leaves still expanding,	1·223°	0·269°	0·954°	14·71	559
740*	,, May 15, full-sized leaves still soft,	1·198°	0·263°	0·935°	14·40	546
758*	,, June 30, mature leaves, .	1·105°	0·385°	0·720°	13·29	800
625	1913. Aug. 13, Se., . . .	2·004°	0·222°	1·782°	24·10	461
626	,, ,, 13, Sw., . . .	1·816°	0·222°	1·594°	21·84	461
629*	,, ,, 14, . . .	1·397°	0·365°	1·032°	16·81	757
634	,, ,, 22, Se., . . .	2·119°	0·215°	1·904°	25·50	446
635	,, ,, 22, Sw., . . .	1·945°	0·229°	1·716°	23·40	476
638*	,, ,, 23, . . .	1·524°	0·350°	1·174°	18·33	726
655	,, Sept. 9, Sw., exposed, . .	2·032°	—	—	24·45	—
656	,, ,, 9, Sw., shaded, . .	1·614°	—	—	19·42	—
681	,, Oct. 4, Sw., exposed, . .	1·643°	—	—	19·75	—
678*	,, ,, 4, ...	1·191°	—	—	14·32	—
689	,, ,, 18, Sw., margin yellow,	1·661°	0·221°	1·440°	19·96	476
691*	,, ,, 18, still green, . .	1·349°	0·437°	0·912°	16·23	998
695*	,, Nov. 8, becoming yellow, .	1·437°	0·455°	0·982°	17·30	944
698	,, ,, 8, Sw., yellow, . .	1·722°	0·265°	1·457°	20·70	549

Reference to the graphs shows that the osmotic pressure rises sharply in February, viz., from 13·3–15·0 atm. Little of this rise is due to the electrolytes, for the graph Δ_e shows only a small elevation in concentration. Consequently by far the larger part is to be attributed to an increase of sugars. As the buds are only just beginning to expand, this rise in concentration of the carbohydrates can scarcely be attributed to photosynthesis. With much greater probability it is to be assigned to the translocation of stored carbohydrates from the lower parts of the tree. It has already been shown that such a transference takes place from below upwards through the wood, especially in the early spring (7). The sugars in the sap have been in part previously stored as such in the wood parenchyma, and have in part arisen from colloidal storage carbohydrates.

The irregular fall in the osmotic pressure taking place between the end of February and the end of June may be in a large degree assigned to the expansion of the cells of the leaves, leading to an increase in the size of the vacuoles, with which the production and translocation of carbohydrates do not keep pace. It is to be observed that the greater part of the irregularities in the graph for the depression due to the total solutes is to be attributed to variations in the carbohydrate content of the vacuoles, probably connected with the erratic nature of the external conditions upon which photosynthesis depends. Also the diminution in the osmotic pressure during this period is due to the diminution in concentration of the carbohydrates only; for the lowest curve plainly indicates that during the same time the concentration of the electrolytes has increased.

From June till September the osmotic pressure rose, attaining the maximum observed in this town plant at the end of the latter month, viz., 18·3 atm. Here, again, the carbohydrates are the important factor. Less than one-quarter of the total pressure is attributable to the electrolytes. Fluctuations in concentration also characterize the carbohydrate content of the vacuoles during the end of the season, while the concentration of the electrolytes steadily grows. Disregarding the fluctuations, we see the osmotic pressure rises during the period the leaves are on the tree, from about 13·5 to 16·5 atm. Although the carbohydrates maintain the major part of the osmotic pressure, the *rise* seems just what is due to the increasing concentration of the electrolytes, viz., 2·7–5·7 atm. It is to be noted that when the leaves are falling from the tree they contain quite a large amount of non-electrolytes.

The dotted graph indicates the concentration of solutes in sap pressed from untreated leaves in our previous research. It is inserted here and in the other figures to institute a comparison between the results obtained by the liquid air method and the previous one in which the tissues were pressed without preliminary treatment.

Individual variation in plants belonging to the same species is brought out in the table. Evidently the specimen SE has usually a somewhat higher osmotic pressure than SW, and the sap of the latter is in turn considerably more concentrated than that of the tree growing in the Botanic Garden. That these differences may be largely or entirely due to the external conditions is shown by the two observations on September 9th in SW. Here the exposed leaves had a pressure of 24·4 atm., while those growing in a shaded position had only 19·4 atm. The difference between the concentrations observed in SE and SW may be due to the fact that the leaves were gathered in each case about 10 a.m., and consequently the illumination of the tree growing in the eastern aspect immediately before gathering was more intense than that of the tree exposed to the western sky. Although less variable with conditions of illumination, the electrolyte content also is possibly dependent on other external conditions. Thus we find the two plants SE and SW growing close together in similar soil have similar electrolyte concentration, and this concentration differs from that of the tree grown in the Botanic Garden, where the soil is more moist, and conditions are not so favourable to evaporation.

Ilex aquifolium : Leaves.

The tree which was used in the observations on *Ilex aquifolium* was a well-grown plant about six metres high growing in an open place in Trinity College Botanic Garden.

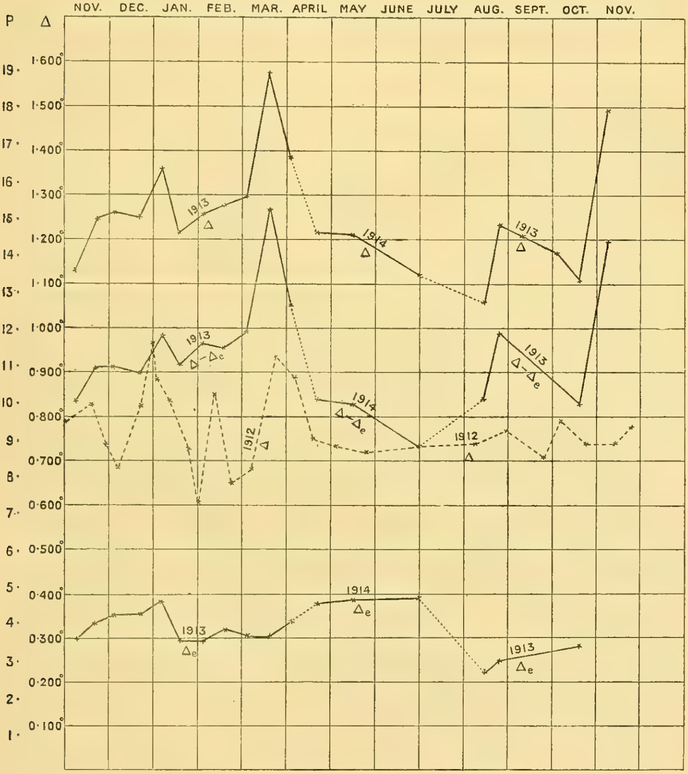
Previous observations (3) on sap pressed from unfrozen leaves showed that we might expect a variation in osmotic pressure in the leaves of the succeeding growths. *I. aquifolum* usually makes two growths in the year. All the shoots do not elongate at each period of growth, and on some shoots the opening of the buds is much delayed. The leaves remain attached during four or five such periods. Owing to the erratic nature of the elongation, it is impossible to be certain of the exact age of the leaves on the older growths. The observations recorded in Table II are classified into those made on the leaves of the ultimate, penultimate, antepenultimate, and pro-antepenultimate growths. Those on the ultimate growths are further subdivided into mature and immature leaves. Only the experiments made on mature ultimate leaves, or on the previous growths of rapidly elongating shoots, are plotted in the graph (fig. 2). The numbers of these experiments are marked with an asterisk in Table II.

TABLE II.

Ilex aquifolium : Leaves.

Expt.	Date, &c.	Δ	Δ_e	$\Delta - \Delta_e$	P.	$C \times 10^5$
485	1912. Nov. 8, immature, . .	1·072°	0·199°	0·873°	12·89	428
487*	,, ,, 8, penultimate, . .	1·130°	0·298°	0·832°	13·60	619
488	,, ,, 8, antepenultimate, .	1·132°	0·352°	0·780°	13·62	731
501*	,, ,, 19, penult. and antepenult.	1·244°	0·335°	0·909°	15·03	696
503	,, ,, 19, pro-antepenult., .	1·305°	0·406°	0·899°	15·70	844
535	,, Dec. 4, immature, . .	1·218°	0·199°	1·019°	14·65	427
536*	,, ,, 4, antepenult., . .	1·259°	0·352°	0·907°	15·14	730
557	,, ,, 21, immature, . .	1·289°	0·248°	1·041°	15·50	516
558*	,, ,, 21, antepenult., . .	1·246°	0·350°	0·896°	14·98	726
562	1913. Jan. 6, immature, . .	1·246°	0·314°	0·932°	14·98	653
563*	,, ,, 6, antepenult., . .	1·359°	0·380°	0·979°	16·35	789
569	,, ,, 17, antepenult., . .	1·344°	0·447°	0·897°	16·15	928
571*	,, ,, 17, ultimate, mature, .	1·213°	0·298°	0·915°	14·60	618
578*	,, Feb. 4, ,, . .	1·256°	0·290°	0·966°	15·10	603
588*	,, ,, 18, ,, . .	1·273°	0·320°	0·953°	15·31	664
600*	,, Mar. 4, ,, . .	1·290°	0·301°	0·989°	15·52	626
617*	,, ,, 19, ,, . .	1·572°	0·305°	1·267°	18·91	633
622*	,, Apr. 3, ,, . .	1·385°	0·335°	1·050°	16·65	696
733*	1914. ,, 22, penult. buds opening,	1·216°	0·377°	0·839°	14·62	784
742*	,, May 16, ,, ,, .	1·208°	0·383°	0·825°	14·53	794
759*	,, June 30, penult., . . .	1·119°	0·389°	0·730°	13·47	807
630*	1913. Aug. 14, ult. mature, . .	1·054°	0·216°	0·838°	12·68	448
639*	,, ,, 23, ,, . .	1·231°	0·246°	0·985°	14·81	510
659*	,, Sept. 10, ,, . .	1·206°	—	—	14·50	—
680*	,, Oct. 4, penult., . . .	1·170°	—	—	14·07	—
692*	,, ,, 18, ult. mature, . .	1·104°	0·279°	0·825°	13·27	580
696*	,, Nov. 8, ,, . .	1·491°	0·299°	1·192°	17·96	621

On comparing the curves in fig. 2 with those of Syringa in fig. 1, one is immediately struck with the complete difference in the nature of the two. In the first place, there appears in the lowest graph no steady rise in the concentration of the electrolyte such as is seen in that of Syringa. We would, however, fall into grave error were we to interpret this as indicating that there is no concentration of electrolytes with age in Ilex. As we shall see, the opposite is the case. The explanation of the uniformity is obviously the fact that the leaves examined in each case are on the whole about the same age—owing to the more or less continuous growth of the evergreen, and consequently possess approximately the same concentration of electrolytes.



ILEX AQUIFOLIUM.—Mature Leaves.

FIG. 2.

The uppermost curve—that tracing the changes of the total concentration of solutes and total osmotic pressure—is also quite different from that of Syringa. In fact, while the osmotic pressure of Syringa is at a maximum in

August–September, the osmotic pressure of Ilex is very low at that period, reaching its observed minimum in the former month. Its maximum is in spring, and there is another period of high pressure in autumn.

The graph traced by the concentration of carbohydrates in the leaves of Ilex differs in its form from that of Syringa, but it shows, as in Syringa, that the major part of the solutes are carbohydrates, and that consequently they are chiefly responsible for the osmotic pressures in the cells. Further, to the fluctuations in the amounts of these substances are due the large variations in the osmotic pressure. The fact that the greatest concentrations are found in spring and autumn may indicate that the photosynthesis of this evergreen is most efficient in light of a moderate intensity, whilst the leaves of a deciduous plant, like Syringa, are most active during the period of more intense illumination; some such an adaptation for each is quite conceivably advantageous where a maximum amount of carbon assimilation is desirable. These considerations also possibly suggest an explanation of the greater irregularity of the curves in the winter sections. The fluctuations then probably correspond to fluctuations in the intensity of the light. In the summer the optimum intensity is nearly always exceeded, and the limit of activity residing in the leaves themselves determines a more uniform production of carbohydrates. The smaller fluctuations then may be referred to want of uniformity in consumption and translocation.

The dotted graph which traces the osmotic pressure of sap pressed from untreated leaves follows, in a general way, the graph obtained from the frozen leaves, but is, as is usual, lower than it.

The figures in Table III demonstrate plainly that, just as in the case of Syringa, there is a concentration of electrolytes in the sap of the leaves with age. In this table the means of all the observations on the leaves of the different growths are entered, and the number of observations thus averaged is given in the top line. It may be seen that, while there is a decided increase in the concentration of the electrolytes, from the youngest to the oldest, the concentration of the total solutes does not show such a rise, nor does that of the carbohydrates.

The osmotic pressure, of course, is dependent on the fluctuations of the total solutes.

TABLE III.

Ilex aquifolium: Leaves. Concentration and Ages. Mean Values.

Age	Immature ultimate	Mature ultimate	Penultimate	Ante-penultimate	Pro-ante-penultimate
No. of observations taken for averages,	4	10	2	5	1
Δ	1·206°	1·286°	1·124°	1·268°	1·305°
Δ_e	0·240°	0·289°	0·339°	0·376°	0·406°
$\Delta - \Delta_e$	0·966°	0·998°	0·781°	0·888°	0·899°
Osmotic Pressure, . .	14·51	15·48	13·53	15·27	15·70

Ilex aquifolium: Roots.

There is considerable difficulty in examining the sap of the roots of most plants throughout the year. The amount of roots required for each set of measurements is considerable, and unless the plant is very vigorous it will not survive this deprivation very often. Of the plants examined we found *Ilex aquifolium* the most resistant to such treatment, and, consequently, we selected it for this work.

With regard to the preparation of the roots, it is necessary that they should not carry on their surface any of the unabsorbed water of the soil; at the same time, care must be taken that water is not abstracted from them by drying. The method of bringing the roots to a uniform condition before freezing and extracting the sap was to immerse them for a few minutes in a large quantity of air-dry soil, and change the soil round them a few times. In this way moisture and extraneous substances were removed from their surfaces, while the soil, not being very dry, did not tend to extract water from the tissues.

The results of the experiments are shown in Table IV. In this the thin roots, under 2 mm. in diameter, are marked C, those over 2 mm. in diameter A, while, when the sample from which the sap was extracted was a mixture of roots of 4 mm. and under, it is denoted by B. These letters are also attached to the points on the graphs in fig. 3, and indicate the nature of the sample from which the results plotted were obtained.

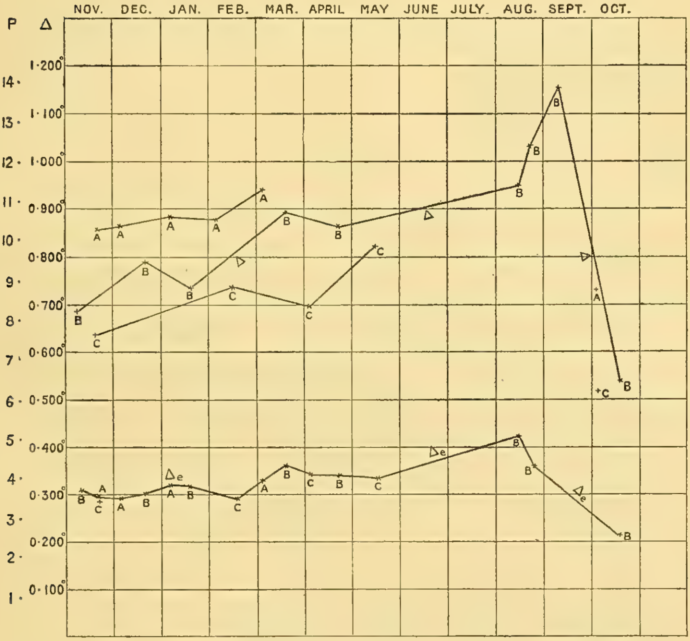
Table IV.

Ilex aquifolium: Roots.

Expt.	Date, &c.	Δ	Δ_e	$\Delta - \Delta_e$	P.	$C \times 10^5$
484*	1912. Nov. 8, mixed, B,	0·682°	0·303°	0·379°	8·21	629
504*	,, ,, 19, thin, C,	0·635°	0·287°	0·348°	7·64	596
505	,, ,, 19, thick, A,	0·858°	0·295°	0·563°	10·32	613
537	,, Dec. 4, thick, A,	0·862°	0·291°	0·571°	10·38	603
559	,, ,, 21, mixed, B,	0·788°	0·301°	0·487°	9·47	624
564	1913. Jan. 6, thick, A,	0·884°	0·320°	0·564°	10·64	664
570	,, ,, 17, mixed, B,	0·734°	0·317°	0·417°	8·83	659
577	,, Feb. 4, thick, A,	0·876°	—	—	10·54	—
589*	,, ,, 18, thin, C,	0·735°	0·289°	0·446°	8·84	601
601	,, Mar. 4, thick, A,	0·940°	0·331°	0·609°	11·31	687
616	,, ,, 19, mixed, B,	0·897°	0·358°	0·539°	10·78	742
623*	,, Apr. 3, thin, C,	0·698°	0·341°	0·357°	8·40	708
734	1914. ,, 21, mixed, B,	0·861°	0·339°	0·522°	10·35	704
743*	,, May 16, thin, C,	0·823°	0·335°	0·488°	9·90	694
631	1913. Aug. 14, mixed, B,	0·949°	0·423°	0·526°	11·42	878
640	,, ,, 23, ,, B,	1·030°	0·359°	0·671°	12·39	746
660	,, Sept. 10, ,, B,	1·156°	—	—	13·91	—
684	,, Oct. 4, thin, C,	0·516°	—	—	6·20	—
685	,, ,, 4, thick, A,	0·728°	—	—	8·76	—
693*	,, ,, 18, mixed, B,	0·534°	0·218°	0·316°	6·42	453

The three upper graphs in the figure show that the depression of freezing point and the osmotic pressure of the sap of the thicker roots are constantly greater than those of the thinner ones. The graph for the mixed samples shows that the pressure rises fairly steadily through the winter, spring, and summer. At the end of the summer it rises abruptly to a maximum (nearly 14 atm.) in September, and then drops even more suddenly to its winter condition (6–7 atm.). No concentration with age could be discovered in the electrolytes. As will be seen, the concentrations of the electrolytes determined in the three sets of samples cannot, as in the case of the total solutes, be separated into three groups. Apparently the concentration of electrolytes is the same in the thick and thin roots. A steady rise in this concentration up to August is indicated, and a decline from that into the winter.

As in the leaves, the major part of the osmotic pressure is due to the carbohydrates. The great rise in the late summer is also due principally to the storage of carbohydrates, but in part to a concentration of electrolytes.



ILEX AQUIFOLIUM.—Roots.

Fig. 3.

As is often the case with the leaves, age leads to an increase in osmotic pressure; but, whilst this increase in the case of the older leaves is due to the accumulation of electrolytes, all the evidence goes to show that the higher pressure of the older roots is due to the storage of carbohydrates.

Hedera helix, leaves.

The next object of study was the evergreen *Hedera helix.* Two specimens were investigated concurrently; one from a northern and one from a southern aspect. Both grew trailing upon the ground; that from the north aspect was close under the north side of a building, and never received direct sunlight; the other was situated under high trees, which rendered the ground it grew in comparatively dry, but did not cut off the sunshine. The latter position was open to the sun almost as long as it was above the horizon.

The results of the cryoscopic and conductivity measurements on these two are recorded in Tables V and VI. From these the graphs given in figs. 4 and 5 are plotted.

Table V.

Hedera helix: Leaves from north aspect.

Expt.	Date	Δ	Δ_e	$\Delta - \Delta_e$	P.	$C \times 10^5$
489	1912. Nov. 12,	1·239°	0·262°	0·977°	14·90	545
530	,, ,, 29,	1·289°	0·257°	1·032°	15·51	533
544	,, Dec. 9,	1·171°	0·287°	0·884°	14·09	596
555	,, ,, 21,	1·246°	0·282°	0·964°	14·97	588
561	1913. Jan. 6,	1·322°	0·279°	1·043°	15·91	580
567	,, ,, 17,	1·392°	0·274°	1·118°	16·74	569
579	,, Feb. 4,	1·306°	0·297°	1·009°	15·70	616
587	,, ,, 18,	1·361°	0·280°	1·081°	16·38	582
602	,, Mar. 4,	1·357°	0·279°	1·078°	16·32	579
614	,, ,, 19,	1·468°	0·340°	1·128°	17·66	705
620	,, Apr. 3,	1·432°	0·341°	1·091°	17·22	708
735	1914. ,, 22,	1·311°	0·267°	1·044°	15·78	554
744	,, May 19,	1·231°	0·583°	0·648°	14·81	1210
757	,, Jun. 30,	1·107°	0·250°	0·857°	13·31	518
628	1913. Aug. 13,	1·092°	0·255°	0·837°	13·14	530
637	,, ,, 22,	0·962°	0·283°	0·679°	11·58	587
658	,, Sept. 9,	1·020°	—	—	12·27	—
690	,, Oct. 18,	1·249°	0·254°	0·995°	15·09	527
699	,, Nov. 8,	1·172°	0·260°	0·912°	14·10	540

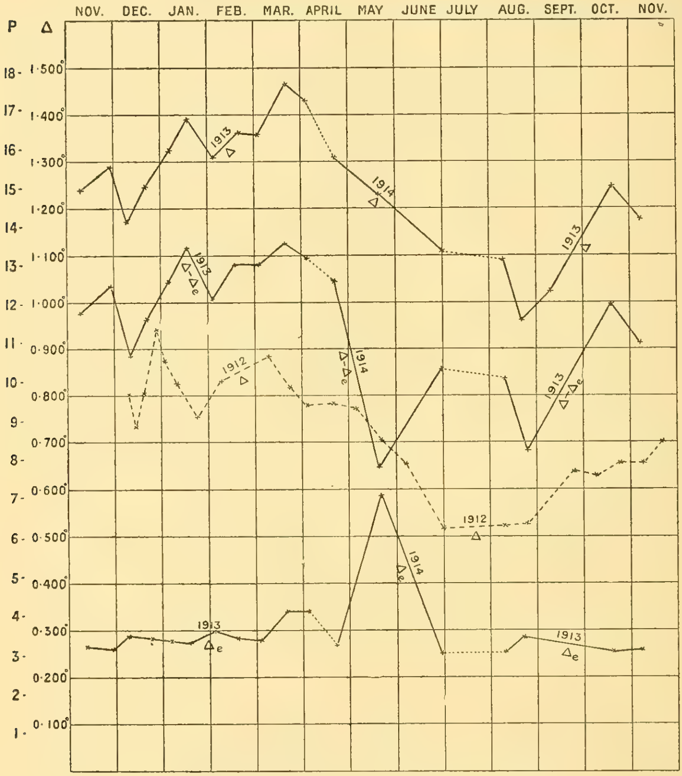
TABLE VI.

Hedera helix : Leaves from south aspect.

Expt.	Date	Δ	Δ_e	$\Delta - \Delta_e$	P.	$C \times 10^5$
474	1912. Nov. 4,	1·258°	0·287°	0·971°	15·13	601
477	,, ,, 5,	1·239°	0·269°	0·970°	14·90	558
490	,, ,, 12,	1·309°	0·267°	1·042°	15·74	556
493	,, ,, 13,	1·315°	0·271°	1·044°	15·81	562
529	,, ,, 29,	1·437°	0·247°	1·190°	17·29	512
543	,, Dec. 9,	1·289°	0·251°	1·038°	15·51	521
556	,, ,, 21,	1·293°	0·283°	1·010°	15·55	588
560	1913. Jan. 6,	1·414°	0·290°	1·124°	17·00	603
568	,, ,, 17,	1·561°	0·274°	1·287°	18·77	569
580	,, Feb. 4,	1·348°	0·295°	1·053°	16·21	613
586	,, ,, 15,	1·417°	0·344°	1·073°	17·04	714
603	,, Mar. 4,	1·335°	0·368°	0·967°	16·05	765
615	,, ,, 19,	1·555°	0·326°	1·229°	18·70	678
621	,, Apr. 3,	1·473°	0·326°	1·147°	17·72	678
736	1914. ,, 22,	1·262°	0·327°	0·935°	15·18	679
745	,, May 19,	1·250°	0·583°	0·667°	15·03	1210
756	,, June 30,	1·236°	0·303°	0·933°	14·86	628
627	1913. Aug. 13,	1·136°	0·299°	0·837°	13·67	621
636	,, ,, 22,	1·063°	0·287°	0·776°	12·78	595
657	,, Sept. 9,	1·164°	—	—	14·01	—
683	,, Oct. 4,	1·096°	—	—	13·18	—

Considering that the specimens grew under such totally different conditions of illumination and moisture, the seasonal variation in the concentration is remarkably similar. The osmotic pressure of the leaves of both, like those of Ilex, shows a decided average increase in autumn, winter, and spring, and a corresponding drop in the summer. The plant in the northern aspect had its maximum in March and its minimum in August. The specimen from the south aspect was extremely similar, except that the crest in March was slightly exceeded by a rise in January. The magnitude of the osmotic pressures also approximated to those of Ilex.

The concentration of the electrolytes also was similar to that of Ilex, and except for a very remarkable rise in May remained approximately uniform throughout the year. The two observations—on the specimen from the north and from the south aspect—made in May indicate an extraordinary

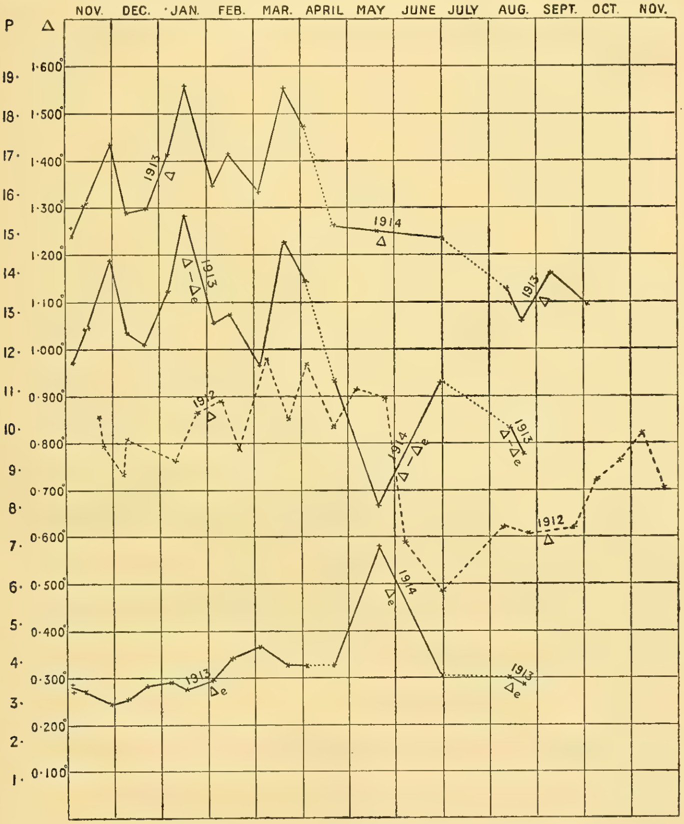


HEDERA HELIX.— Mature leaves, N. aspect.

Fig. 4.

rise in the concentration of the electrolytes. No external conditions were observed to account for this rise, but at the same time the measurements were quite unequivocal, and so the observations are recorded while no explanation can as yet be offered.

The concentration of the electrolytes accounts for about 4 atm. of the osmotic pressure, the remaining 8–15 atm. are due to the soluble carbohydrates. The principal fluctuations also are due to variations in the carbohydrates content, with the exception of the erratic records just alluded to.



HEDERA HELIX.— Mature leaves, S. aspect.

FIG. 5.

The two dotted graphs in the figures show that the sap pressed from the untreated leaves has, as is generally the case, a lower osmotic pressure, but at the same time exhibits a marked diminution in concentration during the summer months.

SUMMARY.

1. The major part of the osmotic pressure of tissues is due to dissolved carbohydrates.

2. The variations in the osmotic pressure are due to a large extent to fluctuations in the carbohydrate content of the cells, and to a smaller degree to changes in the concentration of the electrolytes.

3. A progressive average rise in the osmotic pressure has been found during the development and life of each organ examined.

4. This progressive rise is due in the case of leaves to the accumulation of electrolytes with age. In the case of the only root examined, viz., that of *Ilex aquifolium*, it was due to the concentration of carbohydrates.

5. The osmotic pressure of the deciduous tree, *Syringa vulgaris*, reached its maximum (about 18 atm.) in August, rising irregularly from about 13 atm. at the opening of the buds. No very pronounced diminution was observed before the fall of the leaf.

6. The concentration of carbohydrates in falling leaves is considerable.

7. Two specimens grown in the country possessed higher osmotic pressures than the specimen grown nearer town. The maxima for the town and country specimens were 18·3 atm. and 25·5 atm. respectively.

8. The leaves of the evergreens examined, viz., *Ilex aquifolium* and *Hedera helix*, possess higher osmotic pressures during the winter months than during the summer months.

9. The curve representing the seasonal variations of osmotic pressure in the leaves of *H. helix* is similar for specimens growing in a sunny and in a shaded position. But on the whole the osmotic pressure in the insolated is higher than that in the shaded leaves, the mean pressure being 15·0 atm. and 15·7 atm. respectively.

10. The osmotic pressure of the sap of the roots of *I. aquifolium* rose from a minimum of about 6 atm. in October to a maximum of 14 atm. in September.

11. No concentration of electrolytes with age was observed in these roots, the higher osmotic pressure in the older roots being due evidently to increased concentration of the carbohydrates.

12. In each case the concentration of the total solutes of the sap pressed after freezing was greater than that of sap pressed from the same tissues untreated. The seasonal variations in concentration of the sap obtained by the two methods showed a remarkable similarity.

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A PRELIMINARY ACCOUNT OF A NEW OEDANOMETER FOR MEASURING THE EXPANSIVE FORCE OF SINGLE SEEDS, OR SIMILAR SMALL BODIES, WHEN WETTED.

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XXXV.

A PRELIMINARY ACCOUNT OF A NEW OEDANOMETER FOR MEASURING THE EXPANSIVE FORCE OF SINGLE SEEDS, OR SIMILAR SMALL BODIES, WHEN WETTED.

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The question of the absorption of water by seeds has hitherto been studied mainly by investigation into the quantity absorbed, and the volume changes which the seed undergoes.

The fact that seeds, and other organic bodies, during the swelling which accompanies this absorption of water, can exert a considerable pressure has long been recognized. This phenomenon has been put to practical use in the splitting of rocks by wooden wedges wetted with water; by the disarticulation of skulls through the swelling power of peas; and by the action of Laminaria tents in dilating the Os uteri.

It appeared to us that it might prove to be of interest to attempt to measure the maximum pressures which are generated by swelling organic bodies; and later, if possible, to determine the influence of altered conditions such as temperature, saline solution, or absence of life in seeds on these pressures.

In the following paper we give a brief account of an instrument, which we may term "Oedanometer" (*οἰδάνειν* = to cause to swell),[1] which we have designed to record the pressures generated by single large seeds or similar bodies.

Only a few records are given; further records and a discussion of the results will form the subject of a subsequent paper.

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[1] Reinke (13) uses the term "Ödometer" for his apparatus, which measures primarily the volume changes of swelling bodies rather than the force with which the body swells. It appeared to us that it would avoid confusion if we used the term "Oedanometer" for this apparatus, which measures the force rather than the volume of swelling.

We also include descriptions of two simpler forms of Oedanometers more suitable for class demonstration.

Hales recorded the fact that peas swelling in water could lift a weight of 83·5 kilos. Since the time of Hales a number of such records are to be found scattered through botanical literature. Further, a number of papers, principally of a physico-chemical nature, discuss the theoretical aspects of swelling organic bodies: for example, those of Riecke (14), Schwendener (17), Rodewald (15); and in some cases estimate theoretically, from thermodynamic considerations, the pressures under which water enters swelling bodies—for example, starch.

Of the experimental determination of the pressures Reinke (13) gives the most complete account, using an instrument which he terms an "Ödometer," which consists of a massive metal cylinder, bored perpendicularly for several centimeters. In this boring a piston travels. The piston is pierced by numerous fine holes serving to admit water. The upper end of the piston is continued as a strong rod, terminating in a platform, on which various weights can be placed. The movements of the table-top are registered by a lever travelling over a divided scale. The object to be tested is placed in the hollow cylinder under the piston. A heavy weight is placed on the table, and water is then admitted. The rise of the piston is registered by the pointer on the scale, a portion of the load is removed, and when equilibrium is again established, a second reading is taken, and so on. In this way a curve is plotted, showing the increase of volume under a diminishing load.

This apparatus is very satisfactory in dealing with bodies of a cylindrical shape, which can completely fill the hollow cylinder; it is not so suitable for registering the pressure of irregular bodies like seeds. As will be shown later, it is essential to fill the pore spaces with some incompressible, or slightly compressible, yet porous body such as sand. If one looks at the mass of seeds under the piston in figure 1, it will be seen that a rise of the piston through a distance H will allow a total expansion of $\frac{H}{L}$, where L is the length of the cylindrical mass of peas.

Now the seeds, as will be shown later, only occupy $\frac{2}{3}$ of this volume, so that their increase in volume is $\frac{3}{2} \times \frac{H}{L}$. Further, the layers of seeds near the piston will be freer to swell than the others, owing to the friction of the compacted mass of seeds. On both these accounts such an apparatus is not likely to give very consistent results in the case of seeds.

Gréhant (8) used an apparatus consisting essentially of a strong cylindrical vessel filled with seeds, and closed by a strong lid bolted or screwed on. In the centre of the mass of seeds he placed a bag containing liquid, which was connected by a tube with a pressure-gauge, indicating pressures on a dial.

Water was admitted by a second tube entering the mass of seeds. The figures were variable even with the same species of seed.

His experiments were repeated by Coupin (4), who states : "Les chiffres obtenus par M. Gréhant sont variables avec une même espèce de graine. J'ai répété quelques-unes des expériences de M. Gréhant, et j'ai trouvé des résultats non moins variables. A cet égard, on ne peut donner aucun chiffre absolu : tout ce qu'on peut dire, c'est que certaines semences, celles de Lupin blanc, par exemple, donnent des pressions plus fortes que d'autres, telles que celles de Maïs, par exemple.

"La variabilité des résultats obtenus ne doit pas d'ailleurs nous étonner, car cette pression dépend de la position des graines les unes par rapport aux autres et des vides qu'elles laissent entre elles."

Coupin observes that the maximum pressure is not obtained, since the seeds near the orifice of the water-inlet tube swell rapidly, and close the opening, thereby stopping the further supply to the main mass of seeds.

Réguard (12) criticizes these experiments of Gréhant on similar grounds. Macdougal (11) used an apparatus similar to that of Gréhant, but admitted the water through holes in the metal cover. He placed a rubber bag containing water in the centre of the seeds. This bag was connected by a glass tube with an air manometer containing water and mercury.

"The total duration of the experiment was 30 hours, and the final pressure attained was sufficient to compress a column of air from a length of 6·30 to ·78, and the amount is indicated as 630/78 = 8 atmospheres or 120 pounds to the square inch. This pressure was maintained for two days, and then began to decrease slowly, showing but 1·2 atmospheres a week later, or 18 pounds to the square inch."

We have often noticed a slight falling off of pressure after the maximum was reached, but in some cases pressures of over 25 atmospheres were maintained for prolonged periods—once for over six months. Whenever the pressure dropped to any great extent, subsequent examination proved the existence of a leak, which was easily detected, as we used mercury.

Macdougal's lower results may have been also due to leaks; but as the rubber bag in his experiment contained water, the leak would not be detected so easily.

Coupin studied especially the quantity of water absorbed by seeds under varying conditions. His results may be briefly summarized as follows :—

The total water absorbed by dead or anaesthetised seeds is the same as that of live seeds. The amount of water absorbed is independent of the temperature, but the rate of absorption is increased by the rise of temperature. The amount of water absorbed by a seed varied even in specimens of the same species, still more so between seeds of a different species.

He was unable to determine any law which *a priori* would indicate the absorptive powers of the seed.

There is no recognizable ratio determinable between the volume, or weight, and the percentage of water absorbed by the seed.

During absorption of water, the increase in volume of the seed is less than the volume of water absorbed. (To this there is an exception; seeds which become rugose show a relative dilatation during the early stages—Coupin.) Owing to this phenomenon, it is impossible to estimate the pressure of swelling seeds by the simple expedient of placing them in a closed vessel of water attached to a manometer, since there would be a volume reduction and not an increase. In another paper Coupin (2) states, in reference to the variability of the absorptive power among seeds—"La seule chose générale que l'on puisse dire à cet égard est que chez certaines graines, telles que les Fèves et les Haricots, le pouvoir absorbant est plus fort chez les échantillons de petite taille, tandis que chez d'autres, telles que les graines du Lupin et les caryopses de Maïs, le pouvoir absorbant est plus fort chez les echantillons de grande taille."

Gain (7) carried out some investigations on the same subject; he studied especially the absorptive power of seeds grown under conditions of varying humidity. His conclusions are summarized in the following quotations:—"Le poids des graines étant seulement la résultante d'un grand nombre d'influences, il n'y a pas de relation directe entre ce poids et le pouvoir absorbant. Il y a au contraire une relation que paraît être très important entre la concentration des liquides internes au moment de l'emmagasinement des principes de réserve; plus l'eau a été abondante à ce moment, moins le pouvoir absorbant sera élevé à l'époque du gonflement . . . If faut en outre remarquer que les graines qui se forment très tardivement, lorsque la plante est déjà dans un stade de dessication avancée, sont aussi dans des conditions très différentes de celles dont disposaient les premières graines formées."

The first apparatus which we used to estimate the force exerted by swelling seeds was set up for class demonstration.

Although the instrument is simple in design, and consists of materials easily obtainable and readily put together, it provides a striking demonstration of the force generated by swelling seeds.

We believe a brief account of it may be of some pedagogical interest.

The apparatus (fig. 1) is made up of a piece of galvanized gun-barrel steam tubing *F*, three inches in diameter and seven and a-half inches long. The lower end is closed by an end plug-cap *K* screwed on to it. Above the cap is a disc of thick iron *K′*, which gives a good hold for the bolt attached to the channel iron *L*. To the upper end an elbow-piece *D* is screwed to give attachment to a cross-pin acting as a fulcrum *C* of the lever *A*. The corner is cut off obliquely to allow free movement to the lever. A piston *E* is pivoted to the lever by a shackle and bolt *B* two inches from the fulcrum. The piston rests on the mass of swelling seeds *P*, and actuates the lever. Just above the plug the gun-barrel cylinder is pierced by the tube *H* admitting water. A second similarly placed tube facilitates cleaning. A strong metal grating *G*, supported by three stout legs (one of which is figured at *M*), rests on the plug and serves to allow the free passage of water upwards to the seeds. On the grating rests a piece of wire gauze, on top of which is placed a layer *N* (about half an inch deep) of fine gravel. Sand is placed over the gravel, and on this the seeds, mixed with a certain portion of fine sand, are carefully packed.

The sand serves a double purpose: it fills up the spaces between the seeds, and so limits their power of free-swelling movement; and, secondly, it permits the easy passage of water throughout the mass, so that all seeds are uniformly and continuously wetted. Even when registering the maximum pressure, a head of a few inches causes the water to pass through the mass of seeds and sand. A piece of channel steel *L* is bolted on to the bottom plug *K* and strengthened by an angular strut bolted to the lower part of the elbow-piece (*D*). It carries at the distal end a screw-hook, which serves for the lower attachment of the spring balance, the upper end of which is secured to the lever.

The lever is a steel bar an inch and a-half wide, a quarter inch thick, and six and a-half feet long. The free end of the lever is pierced by a drill-hole six feet from the fulcrum. Through this hole the spring balance can be attached by means of a bolt. The balance used registered up to two hundred pounds, but was not nearly powerful enough, since in order to record the maximum pressure we had to use an inconveniently long leverage (6 feet). If we were making another instrument, we should use smaller bore gun-barrel, as a pressure of fifty atmospheres which we registered from peas in the sensitive instrument would lift the three-inch piston with a force of 5250 pounds, or over two tons. Even the one and a-half inch steel lever was bent on its edge, also an inch H-steel girder, which was originally used as the base or fixed arm, in place of the channel steel.

We found it advantageous to select a balance which gave as little extension as possible in proportion to the reading.

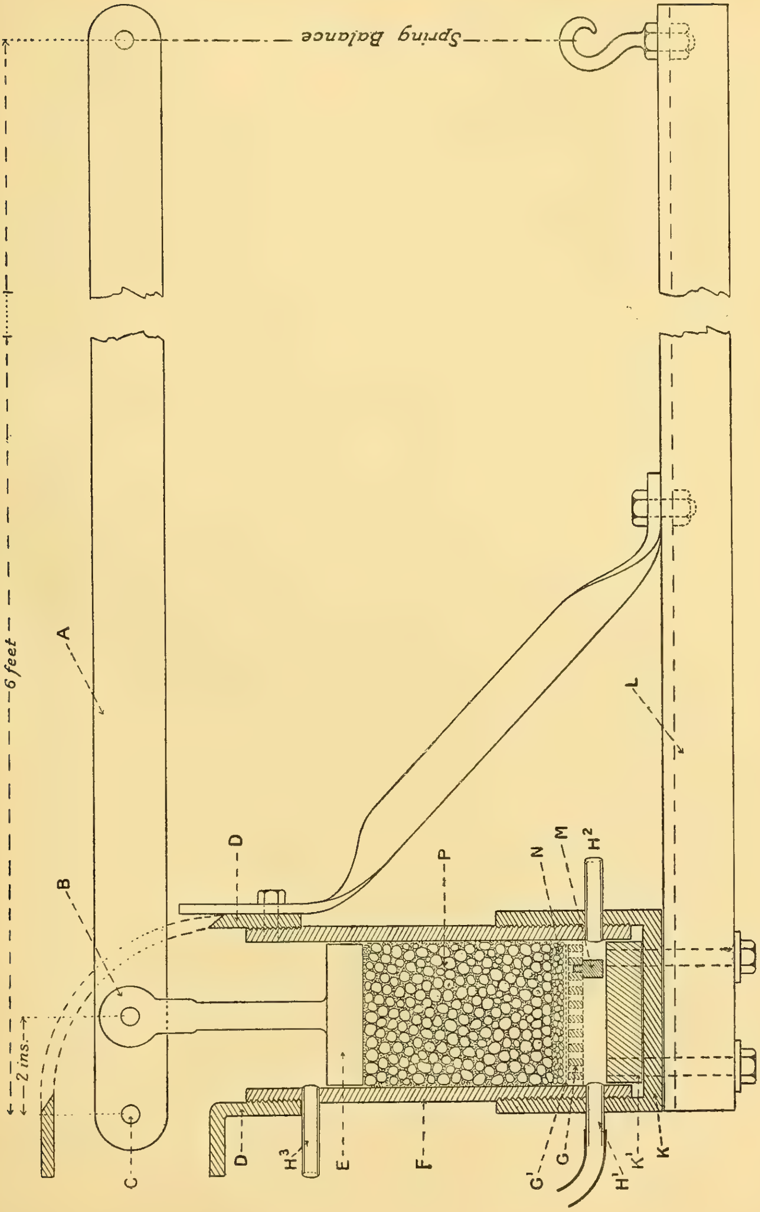

FIG. 1.

The pressure exerted by the peas is calculated as follows:—

$$\frac{\text{Reading of balance} \times \text{leverage}}{\text{Sectional area of piston}} = \left\{ \begin{array}{l} \text{pressure in lbs. per square inch exerted by} \\ \quad \text{peas.} \end{array} \right.$$

To the reading of the balance must be added a correction for the weight of the lever, balance, and piston. When the balance is attached at six feet from the fulcrum, this was found to be equal to ten pounds.

To set up the apparatus the shackle bolt is slipped out and the piston removed; the seeds and sand are then carefully packed up to a level marked on the cylinder. The piston and shackle bolt are replaced, and the spring balance is attached. The water is then admitted gradually to the seeds from below *H*, which is connected with a tubulated supply bottle arranged to secure that the level of the water in the cylinder stands about an inch above the piston.

A considerable advantage is gained in the use of sand and gravel in the above apparatus; this was clearly shown by three experiments set up with—

(A) Beans packed carefully in sand over a large area of gravel.

(B) Beans in sand but no gravel.

(C) Beans alone.

In (A) the pressures were higher and the rise more rapid than in (B). This is due to the more ready access of water in (A); for in (B) the lowest layer of sand becomes washed away, allowing the beans to come in contact with the perforations of the gauze, choke them up, and thus greatly reduce the water-supply. The much lower pressures in (C) demonstrated conclusively the advantage gained by the use of sand. A control experiment was fitted up in which sand but no seeds was put into the apparatus. On admitting water no rise in the pressure took place, or rather could be recorded by the balance, thus demonstrating that the greater rise of pressure in (A) and (B) was not due to a pressure generated in the sand.

In all experiments in which sand is used, it is important to admit the water slowly, and from below, so as to secure the complete removal of air from the spaces between the grains. In an interesting series of papers by Jamin (10) an account is given of the forces generated in porous non-swelling bodies by immersion in liquids:—

"Je prends un bloc d'une matière poreuse solide quelconque bien desséchée de craie, d'argile cuite, de pierre lithographique, de bois, etc., ou un vase poreux de pile rempli d'une poudre fortement tassée, par exemple de blanc d'Espagne, d'oxyde de zinc, d'amidon et même de terre desséchée. Je creuse dans la masse un trou cylindrique et j'y mastique un tube de manométrique droit fermé par le haut, rempli d'air et contenant à sa base, un index de mercure. Il est clair que si la pression vient à augmenter dans la masse poreuse, elle fera monter l'index et pourra se mesurer par la diminution de longueur de la colonne d'air. Cela fait, je plonge l'appareil dans un vase plein d'eau.

" Aussitôt cette eau pénètre dans les pores, refoule à l'intérieur l'air qu'ils contenaient, et la pression augmente progressivement. Au bout de quelques jours elle est devenue égale à 3 ou 4 atmosphères, dans la plupart des cas ; elle en atteint 5 avec l'oxyde de zinc ; elle en dépasse 6 avec l'amidon."

Experiments carried out on peas with this apparatus registered a pressure of 234 pounds per square inch. The levers used at first, however, were badly bent by the pressure, and the balance was stretched beyond its capacity, so that the pressure recorded was not even approaching the maximum. In other experiments on the measurement of the swelling force of seeds—for example, those carried out by Macdougal (11)—no packing material appears to have been used. In this case the seeds can swell considerably by becoming polyhedral without necessarily exercising much pressure on the piston.

If a number of equal spheres are arranged one directly over the other, the pore space is at a maximum and occupies 47·64 per cent. of the total volume; however, when the spheres are arranged as shot is piled, this space is reduced to a minimum of 25·95 per cent. [Hall (9).]

In order to determine experimentally the relative proportion of pore space existing between seeds, we packed a number of peas at random, but as tightly as possible, into a glass-measuring cylinder of three inches diameter until they reached a height of five inches (this gives approximately the size and shape of the reservoir of apparatus described). Keeping them pressed down with a piston, we poured in mercury to the same height, so that all the spaces were filled. The volume of the mercury thus gives the total volume of the pore spaces. In the case of peas, this volume was found to be approximately 34 per cent. of the apparent total volume or one half the actual volume of the seeds. This result is slightly below the mean of the theoretical maximum and minimum quoted above.

The figures given by Hall apply only when the diameter of the sphere is very small compared with the size of the containing vessel. If, however, an allowance is made for the shape and sides of the vessel, and the peas are regarded as spheres of quarter-inch diameter, the calculated minimum pore volume is considerably increased. Further, the smaller the cylinder, or the larger the seeds, the greater the percentage of the minimum pore volume becomes. Hence if a smaller cylinder were used the results obtained would be lower. For this information we are indebted to Prof. H. C. McWeeney. Thus, were it not for the sand, seeds could expand fifty per cent. of their volume without necessarily exerting much pressure on the piston.

The figures are given below :—

Apparent volume of peas = 35 cubic inches.
Volume of mercury poured in = 12 ,,
Actual volume of peas = 23 ,,

$$\frac{\text{Volume of interspaces}}{\text{Apparent volume of peas}} = \frac{12}{35} = \frac{1}{3} \text{ approximately.}$$

$$\frac{\text{Volume of interspaces}}{\text{Actual volume of peas}} = \frac{12}{23} = \frac{1}{2} \text{ approximately.}$$

In some of the experiments after the maximum pressure had been attained, samples of the seeds were set aside to germinate. After a few days the great majority of the seeds were sprouting, showing that their experience had not materially affected their vitality.

A curious point observed was that a dark purple pigment developed in the seed-coat of beans. The pigment is soluble in dilute acid, but is precipitated as a brownish gelatinous substance in alkaline solution. Atkins (1) refers to a purple pigment, probably identical, which develops in the seed-coat of the bean when treated first with acid, then with alkali. The colouring matter observed in our experiments developed in the coat of living seeds, as deeply coloured specimens when set aside germinated freely.

The researches carried out by Coupin (2, 3, 4), Gain (7), and Atkins (1), &c., some of which we have quoted previously, indicate that there is some considerable variation in the amount of water absorbed by individual seeds.

Coupin, and Schull (16) in particular, lay stress on the fact that a large proportion of seeds have injuries or defects in the seed-coat, which are invisible, even on microscopic examination.

For these reasons it appears to us that it would be advantageous to estimate the pressure produced by a single seed. There are certain mechanical difficulties in the way of such an estimation: in the first place, the seeds must be enclosed in a vessel, the sides of which are so rigid that its "give" under pressure would be negligible; secondly, the seed must completely fill the vessel, or else be packed in such a way as to leave it no free swelling space; thirdly, water must be supplied freely to the entire surface of the seeds; fourthly, the pressure exerted on the sides of the vessel must be measured, and yet as little movement as possible be permitted. With these points in view we constructed the following apparatus shown in figure 2. It consists of two sections *E* and *G*, made of cylinder cast-iron, which can be clamped by four five-sixteenth bolts (two of which are shown at *H*).

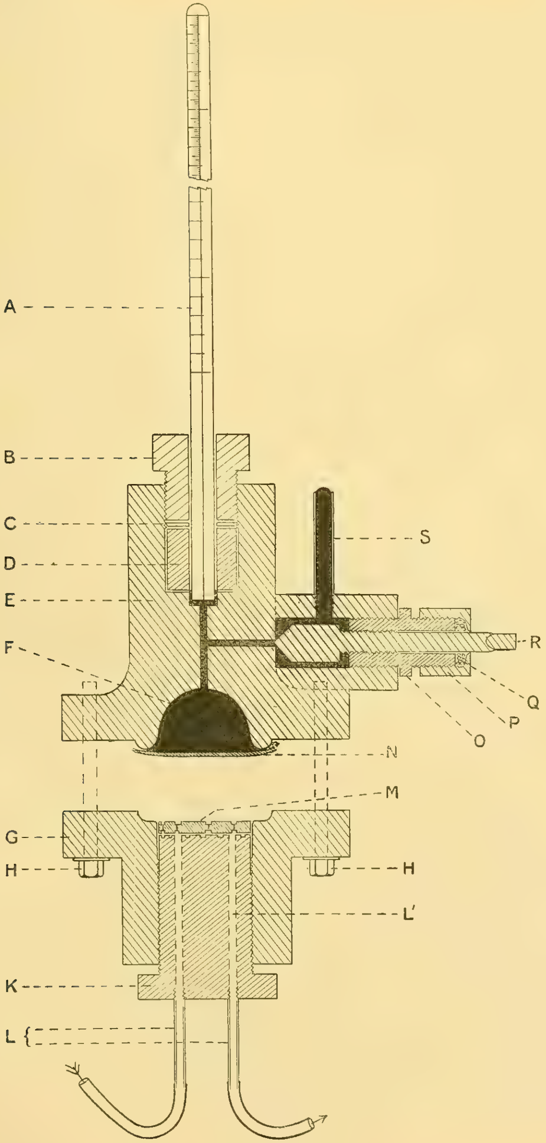

FIG. 2.

The upper section *E* has a central hemispherical cavity *F* one inch in diameter, which is surrounded by a rounded lip. The mouth of this cavity is closed by a rubber diaphragm *N*, held in position by the lower section *G*, when it is bolted on.

The manometer consists of fine bore (approximately half mm. diameter) thermometer tubing, approximately 60 cms. long, accurately graduated volumetrically in the upper 50 cms. The graduations are at distances 5 mm. apart below, but in the upper 20 cms., where the pressure changes more rapidly in proportion to the rise of mercury, the divisions are more closely set (0·5 mm. apart).

The manometer is secured to the casting *E* by the screwing-gland *B*. This has a hexagonal head, by means of which it can be screwed firmly down on the rubber bung *D*, making a firm pressure-tight joint.

A metal washer *C* is placed between the gland and the rubber to diminish friction. The manometer is connected with the space *F* by a narrow hole drilled through the casting. The upper part of this passage is enlarged for about $\frac{1}{8}$ inch to give a good seating to the manometer tubing. In this enlargement a thin washer of fibre, having a central hole, may be placed to avoid chipping the manometer tube against the iron of the seating, though in practice this was not found necessary. To facilitate the adjustments, a by-pass is connected with this narrow passage. The by-pass is closed by a hardened steel needle-valve (*R*), which can be screwed firmly home by a tap wrench. This needle-valve is shown in detail in the figure. A packed gland *P* is fitted to prevent leakage of mercury. The by-pass is continued into the steel tube *S*, through which mercury can be admitted or released. The lower casting *G* has a groove turned in its upper face to fit accurately the lip of the upper casting, allowance being made for the thickness of the rubber diaphragm.

When the bolts *H* are screwed firmly home, the diaphragm *N* is held absolutely pressure-tight. Into a central passage 1 inch in diameter the iron plug *K* screws home; the lower end of the plug carries a hexagonal head to admit of tightening with a spanner. A metal disc *M* $\frac{3}{16}$ inch thick cross-cut on both surfaces, and pierced by numerous fine holes, serves to distribute water. Above this disc a disc of fine wire gauze is placed to prevent the escape of the sand. The steel tubes *L*, which are continued upwards by the drill-holes L^1, serve to allow a current of water or other liquid to be kept up. The flow is controlled by a tap on the right-hand tube while the liquid is admitted through the left-hand tube under a slight pressure. The top of the drill-holes L^1 are counter-sunk, and the end of the screw-plug *K* is cross-cut with fine channels to allow a free water passage under the metal disc *M*. The entire apparatus is set on a circular iron tripod.

To fit up the apparatus, the rubber diaphragm and washer are set in place, and the two castings bolted together, care being taken to ensure an even seating by tightening the screws each half a turn in rotation. With the needle-valve closed, the upper casting *E* is filled with clean mercury, care being taken to expel all air. The valve is then opened to displace the air around the needle. The air in the manometer tube may be dried rapidly by connecting the end of the tube, through a wide glass tube containing calcium chloride, with a vacuum pump, and exhausting the air several times. Again, with the aid of a vacuum pump, a thread of mercury is allowed to enter up to the graduated part of the manometer tubing, which is then dipped into the mercury in the upper part of the casting. The packing rubber *D*, the washer *C*, and gland *B* are adjusted; surplus mercury escapes through tube *S*, by means of a flexible tube and funnel attached to it; the lever of the mercury in the manometer is read off at atmospheric pressure. The needle-valve is then tightly closed, and the tube *S* covered by a cap and the apparatus inverted. The seed is placed on the rubber diaphragm and carefully packed round with sand, the disc *M* is inserted, and water poured in to remove the air and wet the sand.

The fine sand used was sieved, so as to contain only grains of approximately uniform size. It was treated with nitric acid, and washed to remove impurities. The plug *K*, the thread of which is greased to make it watertight, is screwed home with considerable pressure to cause the firm packing of the sand. This causes the mercury to rise in the manometer tube; the needle-valve is subsequently opened to adjust the pressure. It was found advisable to allow a small initial pressure so as to diminish the amount of swelling permitted to the seed. If desired an initial pressure of over 50 atmospheres can be obtained by placing five or six steel balls, similar to those used in motor-bicycle bearings, between the disc and the plug *K*, thus diminishing friction. The tubes *L* are now connected with the water-supply system. The volume of the graduated portion of the bore of our manometers, of which we had six constructed, varies somewhat, but has an average of about 100 cms.

The pressure is calculated from the volume of the gas, according to Boyle's law, and a correction is made for the height of the column of mercury measured from the diaphragm.

Between temperatures of 10° and 100° C. the aberration from Boyle's law, due to the volume occupied by the molecules and the mixed chemical nature of air, &c., for pressures up to 50 atmospheres is negligible, so that a correction on this account is unnecessary.

We have estimated that the bean swelling freely increases on an average about 2 c.cms., hence, in recording the maximum pressure, the bean is allowed to swell less than $\frac{1}{20}$ of the amount it would expand under free conditions. By leaving on an initial pressure of 2 atmospheres the possible expansion is reduced to less than $\frac{1}{40}$.

It is quite possible that the expansion permitted to the seed may be greater than the estimated value, since there may be a certain amount of compression of the sand.[1] It is proposed to carry out experiments to determine the compressibility of sand under the pressures involved.

We give below four examples of records obtained with this instrument; the seeds tested were broad beans, variety Milecross Monarch. The figures are given in Table I. Fig. 3 shows the same results as curves.

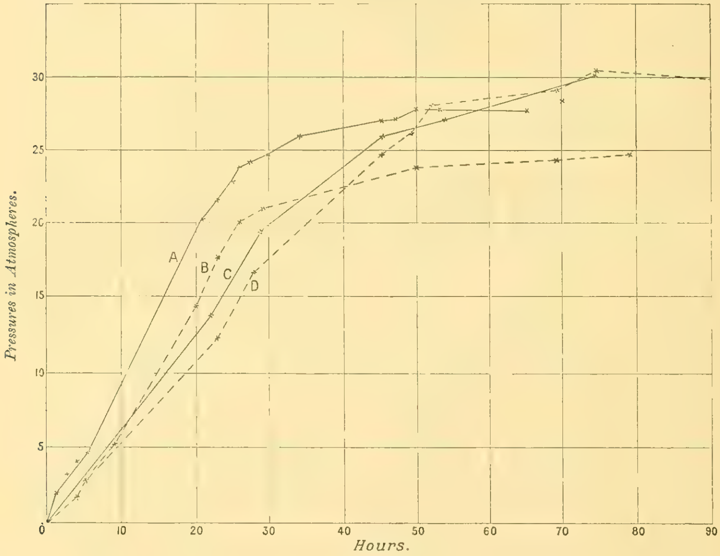

Fig. 3.

These results are given with certain reservations. They are intended to indicate the nature of the records obtainable by the apparatus rather than exact measurements of the pressures of the seeds, as corrections of variations in temperature and barometric pressures were not made.

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[1] Due to fracture of grain and consequent closer packing, rather than to actual compression of the and particles.

TABLE I.

A		B		C		D	
Time in hours	Pressure in all	Time	Pressure	Time	Pressure	Time	Pressure
1	2·4	4	2·0	22	13·8	23	12·4
2½	3·25	5	2·7	28½	19·5	28	16·5
4	4·0	9	5·2	46	26·0	45	24·7
5½	4·6	20	14·4	52½	27·2	49	26·3
21	20·1	23	17·6	70	28·3	52	28·1
23	21·6	26	20·2	74½	30·3	69	29·3
25	22·7	29	21			75	30·4
26	23·8	50	23·7			94	29·9
27½	24·0	69	24·2				
30	24·6	79	24·9				
34	26·0						
44	27·0						
47	27·0						
50	27·9						
53	27·9						
65	27·0						

In A and B the initial pressure was low (about two atmospheres), while in C and D a pressure of about ten atmospheres was applied at the start. In all four cases the beans germinated when set aside in suitable conditions.

Three experiments carried out with peas, six being used at a time, gave maximum pressures of 50·3, 49, and 48·5 atmospheres.

Fig. 4 shows a simple apparatus which we set up to obtain the pressures exerted by a mass of swelling seeds. It is a modification of Macdougal's apparatus to which we have previously referred. It consists of a piece of three-inch gun-barrel tubing *E*, screwed to the lower end of which is the reducing coupling *F*.

To the lower end of this coupling is connected the tube *KL*, and attached to this is a rubber tubing from the water-supply. Three pieces of rod, one of which is shown at *M*, are screwed into the coupling *F*, and act as legs supporting the apparatus. A grating *H* and a wire gauze *G* serve to distribute the water at the inlet. The seeds are packed with sand and gravel as in the first apparatus. The pressure is registered by means of a rubber bag *R* containing mercury (a small football tube acts well) connected with the manometer (A) made of thermometer tubing of 1 mm.

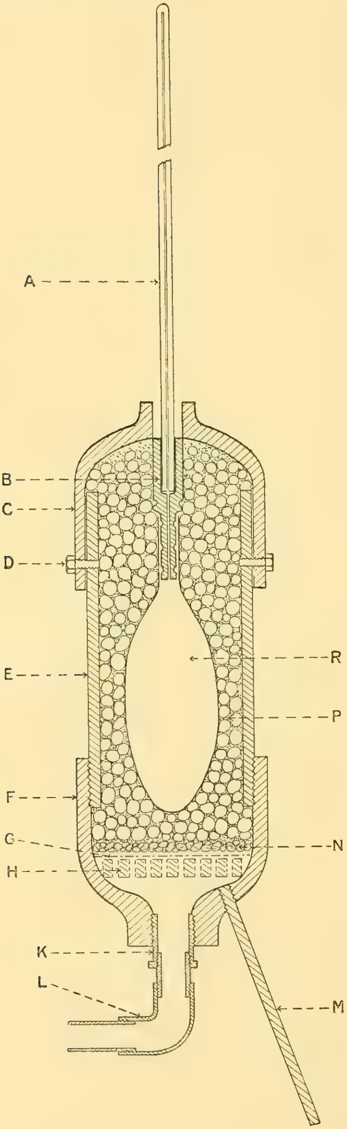

Fig. 4.

bore and 50 cms. long. The upper end of the cylinder is closed by a second reducing coupling, which slips over like a cap instead of being screwed on. (At first we used a screw-on cover, but experienced considerable difficulty in removing it at the end of the experiment owing to the great pressure.) The cover is held in position by six five-sixteenth bolts *D*, screwed into the cylinder. When the cover is attached, any space that remains free is filled up with sand poured in through the neck.

A screw-in adapter (not shown in the figure) is then inserted, and serves to keep the sand in place. The water is now turned on from below, and in the course of a minute or so appears at the neck. The results given by this apparatus are shown in the accompanying Table II.

Table II.

Hours from start of Experiment	Pressures recorded
1	1·9
3	2·8
5	4
14	13·2
16	15·7
18	16·2
19	17·3
20	17·6
23	19·2
25	19·4
29	20·4
40	23·4
42	24·3
48	24·8
64	25·9
69	26·9
72	26·9
75	26·9
85	25·9

Maximum pressure 26·9 atmospheres.

The principal difficulty in using this apparatus was to secure the manometer tube firmly to the rubber bag.

The end of the tube may be drawn out, forming a neck which helps to hold the wire. Even with this arrangement the tube tends to shoot out under high pressure. We are trying an arrangement, shown in the figure at *B*, which so far has proved satisfactory. It consists of a rod of $\frac{5}{8}$ inch iron drilled for $1\frac{1}{2}$ inch from one end to take the end of the manometer tube which is cemented in with shellac. The other end is turned down in the lathe for two inches to fit the tube of the rubber bag. It bears grooves to hold the wire and has a narrow central passage opening into the larger boring and thus communicating with the manometer.

Corrections.

In these preliminary experiments we did not consider it necessary to make any correction for change in barometric pressure. If the original volume of the air in the manometer tube is measured at an atmospheric pressure of say 750, all that would be necessary is to calculate the volume under a standard pressure of 760 mm., and use this volume as a basis for calculations. Any subsequent change of atmospheric pressure would be too small to affect sensibly the volume reading of the compressed air.

In the case of the lower pressures, readings can, of course, be obtained accurately, but with pressures of 50 atmospheres, a rise of about 0·4 of a division is equivalent to an increase of an atmosphere in pressure. In practice it is not easy to read any closer than to 0·2 of a division (i.e. 0·1 of a millimeter), or about 0·5 of an atmosphere at 50 atmospheres pressure. A sliding lens, with cross-lines, is used to facilitate readings.

Heat.

The correction for changes in temperature during the course of experiment has to be considered under two heads:—

(*a*) Increase in volume of air column due to rise in temperature.

(*b*) Expansion of mercury in iron due to rise in temperature.

1. Correction for change in volume of air column.

The source of error is not very serious, as will be seen from the following figures, which are representative of the readings actually obtained.

Length of air column at start, . . = 60.
Temperature, ... = 10° C.
Length of column later (high pressure), . = 2.
Temperature at this time, . . . = 15° C.
If not corrected for temperature, . P = 60/2 = 30 atmospheres.
If corrected for temperature, . . $P = \frac{60 \times \frac{5 \times 60}{273}}{2}$

= 30·5 atmospheres.

The error is therefore 1 in 60 for 5° C,
or 1 in 300 for 1° C.

2. Expansion of mercury in iron due to rise in temperature.

Volume of mercury in apparatus, . . = 4 c.cms.
Coefficient of expansion of mercury in iron, . = ·000143.
Rise in temperature of 5° C.

Increase in volume, = 4 × 5 × ·000143 cms. = ·00286 cms. = 2·8 c.mms.

Now, one division on our manometer represents 1 c.mm. approximately, hence the expansion of mercury would appear to cause a very serious error—one of from 10 to 20 atmospheres. In practice this is, however, not the case. The expansion of the mercury causes a rise in pressure in the manometer; but if the seed is unable to support this increased pressure, it loses water and diminishes in volume, thus automatically correcting for the expansion of the mercury, since the volume change is small.

In support of this view we may point out the uniformity of the readings given in the cases of beans and peas. Beans varied in our latest experiments from 27 to 30 atmospheres pressure when swelling; while peas varied from 48 to 50 atmospheres. These values would vary much more if the mercury expansion effect was appreciable.

Then the following experiments were carried out:—Two instruments were placed in a water bath, and their temperatures rapidly raised through 10° C. One of the instruments contained some peas which were registering about their maximum pressure—in this case the mercury volume rose only ·1 of a division higher. In the second instrument the reading was low at the start—in this case the mercury went up 4 graduations. If the seeds did not reduce in volume, the mercury would have gone up in the first instrument as much as it went up in the second one.

We now carry out the experiments in an incubator, and keep it at a temperature of 20° C. constant to a degree, to avoid this source of error.

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SIMPLIFIED SOLUTIONS OF CERTAIN MENDELIAN PROBLEMS IN WHICH FACTORS HAVE INSEPARABLE EFFECTS.

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XXXVI.

SIMPLIFIED SOLUTIONS OF CERTAIN MENDELIAN PROBLEMS IN WHICH FACTORS HAVE INSEPARABLE EFFECTS.

By JAMES WILSON, M.A., B.Sc.,

Professor of Agriculture in the Royal College of Science, Dublin.

[Read March 23. Published April 12, 1915.]

In a paper entitled "Unsound Mendelian Developments, especially as regards the Presence and Absence Theory," published in the Proceedings of this Society in December, 1912, it was shown that, when the effects of different factors are inseparable, the results obtained from two or more crosses in which the same pair of factors is operating can be combined and the relations between factors which are not operating together determined. For the proof then given a simpler can now be substituted. The following are the necessary observations and deductions from Mendel's work, which hold so long as the characters dealt with are related to each other as dominants and recessives and each pair is distributed independently of the others:—

(1) If the original parents differ in n pairs of characters, then 2^n gives the number of groups into which their hybrids' progeny can be divided, and $(3 + 1)^n$ the ratio in which the groups stand to each other numerically.

(2) Conversely, if their hybrids' progeny consist of 2, 4, 8, 16 . . . 2^n groups, the numbers in which are numerically in the ratio $(3 + 1)^n$, then the original parents differed in n pairs of characters.

(3) If the original parents differ in 1, 2, 3 . . . n pairs of characters, then the characters borne by, and the proportionate numbers in, the groups in their hybrids' progeny are:—

When the parents differ

In one pair.	In two pairs.	In three pairs.
3 X	9 $X\ Y$	27 $X\ Y\ Z$
1 x	3 $X\ y$	9 $X\ Y\ z$
	3 $x\ Y$	9 $X\ y\ Z$
	1 $x\ y$	9 $x\ Y\ Z$
		3 $X\ y\ z$
		3 $x\ Y\ z$
		3 $x\ y\ Z$
		1 $x\ y\ z$

and so on.

(4) The same results follow whether one parent carries all the dominants and the other all the recessives; or the dominants and recessives are carried some by one parent, some by the other. For instance, the same kind of hybrid is produced whether the parents carry the characters XY and xy, or Xy and xY.

(5) The hybrids carry all the dominants of the pairs in which their parents differed.

(6) In the hybrids' progeny the numerically largest group carries all the dominants; the groups next in size carry one dominant less; those next in size again still one less; and so on down to the smallest group which carries all the recessives but no dominants.

(7) In the hybrids' progeny the largest group differs from the smallest in n pairs of characters, each differs from the groups next them in one pair less, and so on. At the same time, the intermediate groups differ among themselves in definite numbers of pairs of characters, which can be ascertained by examining any typical set. For instance, in the two-pair set of four groups

$$\begin{array}{lll} 9 & X & Y \\ 3 & X & y \\ 3 & x & Y \\ 1 & x & y \end{array}$$

the first and last groups differ from each other in two pairs of characters, and each differs from the two middle groups in one pair. At the same time, the middle groups differ from each other in two pairs.

(8) Each character in which the parents differ appears in half the groups of their hybrids' progeny precisely. In the two-pair set of four groups, X appears in two groups, and, in the three-pair set of eight groups, X appears in four groups. Thus, if any character be carried by more than half the groups in a set, it must be carried by all. If, for instance, the third group in the two-pair set

$$\begin{array}{llll} 9 & X & Y & Z \\ 3 & X & y & Z \\ 3 & x & Y & \\ 1 & x & y & Z \end{array}$$

did not carry Z, it would differ from the first and last groups in more pairs than one and from the other middle group in more pairs than two.

(9) If one or more of the groups in a set of hybrids' progeny carry any character outside those producing the set, that character is common to all the groups in the set. If, for instance, the last group in the following set carried Z and the other three groups did not, then the last group would

differ from the first in more than two pairs of characters and from the two middle groups in more than one :—

$$\begin{array}{l} 9\ X\ Y \\ 3\ X\ y \\ 3\ x\ Y \\ 1\ x\ y\ Z. \end{array}$$

The experiments dealt with first in the previous paper were those carried out by Miss Durham on the colours of mice. It will be convenient to consider them first again, and to consider two other important examples after. The details of Miss Durham's experiments are to be found in the fourth "Report to the Evolution Committee of the Royal Society" and in the first volume of the "Journal of Genetics."

In Miss Durham's first experiment, agouti-coloured mice were mated with chocolates, and their hybrids' progeny consisted of agoutis, cinnamon agoutis, blacks, and chocolates in the ratio 9 : 3 : 3 : 1. Since there are 2^2 (i.e. 4) groups numerically in the ratio $(3+1)^2$, there are two pairs of characters concerned; and, since the effects of the factors producing them are inseparable, the four groups can only be set down with "unknown" symbols, thus :—

Agouti,	$9\ X\ Y$
Cinnamon agouti,	$3\ X\ y$
Black,	$3\ x\ Y$
Chocolate,	$1\ x\ y$

In Miss Durham's second experiment, black was mated with a fifth colour, silver fawn, and their hybrids' progeny consisted of blacks, blues, chocolates, and silver fawns in the ratio 9 : 3 : 3 : 1. By being at the top of a set of four groups black is shown to be carrying two dominants. One may be Y, revealed in the first experiment, but both may be new. Assume that both are new. Then the characters carried by the four groups in the second experiment should be, say—

Black,	$9\ Z\ A$
Chocolate,	$3\ Z\ a$
Blue,	$3\ z\ A$
Silver fawn,	$1\ z\ a.$

In that case the characters carried by black and chocolate, as revealed by the two experiments, should be—

Black,	$x\ Y\ Z\ A$
Chocolate,	$x\ y\ Z\ a.$

But this makes black and chocolate differ from each other in two pairs of characters, while the first experiment showed them to differ in one pair only. Thus black carries only one new dominant, say Z, which, since it is outside the characters concerned in the first set, must also be carried by the three remaining groups. The characters carried by the four groups are now therefore—

Agouti,	9	X	Y	Z
Cinnamon agouti,	3	X	y	Z
Black,	3	x	Y	Z
Chocolate,	1	x	y	Z.

Since Y and Z are the characters carried by black at the top of the second set, the two pairs concerned are Y and y, and Z and z, and, since the characters borne by black and chocolate and the positions in the set of blue and silver fawn are already known, we may write down the set as follows :—

Black,	9	x	Y	Z
Blue,	3		Y	z
Chocolate,	3	x	y	Z
Silver fawn,	1		y	z.

But, since it is outside the characters in the set and is carried by both black and chocolate, x must also be carried by the two remaining groups, blue and silver fawn.

It is now obvious that there are three pairs of characters and eight different combinations, all of which may now be set down along with the colours of such as have been identified :—

Agouti,	27	X	Y	Z
	9	X	Y	z
Cinnamon agouti,	9	X	y	Z
Black,	9	x	Y	Z
	3	X	y	z
Blue,	3	x	Y	z
Chocolate,	3	x	y	Z
Silver fawn,	1	x	y	z.

The two unfound colours could have been found by a cross between the first and the last or between any other two differing in three pairs of characters, but Miss Durham found them by two separate crosses between colours differing from each other in two pairs of characters.

Agouti was mated with blue, and their hybrids' progeny consisted of agoutis, dilute agoutis, blacks, and blues in the ratio 9 : 3 : 3 : 1. The characters carried by three of these colours are already known, and, if we set down the set of four with the three known combinations, we shall readily infer the characters carried by the fourth group :—

Agouti,	9	X	Y	Z
Dilute agouti,	3			
Black,	3	x	Y	Z
Blue,	1	x	Y	z.

Since it is carried by more than half the groups in the set, Y must be carried by all. The differentiating characters are therefore X and x and Z and z, and, since three of the combinations they can form, namely XYZ, xYZ, and xYz, are already appropriated, the remaining combination must belong to dilute agouti, whose three characters are therefore XYz.

In Miss Durham's final experiment cinnamon agouti and silver fawn were mated, and their hybrids' progeny consisted of cinnamon agoutis, dilute cinnamon agoutis, chocolates, and silver fawns in the ratio 9 : 3 : 3 : 1. If we again arrange these four groups in the usual order with the characters of the three which are already known set down against them, we shall be able to infer the characters carried by the fourth :—

Cinnamon agouti,	9	X	y	Z
Dilute cinnamon agouti,	3			
Chocolate,	3	x	y	Z
Silver fawn,	1	x	y	z.

Since it is common to three groups, y must also be carried by the fourth. The differentiating characters in the set are therefore X and x and Z and z, and, since three of the four possible combinations are already appropriated, the remaining combination Xyz must belong to dilute cinnamon agouti.

Thus the complete set of eight groups consists of—

Agouti,	27	X	Y	Z
Dilute agouti,	9	X	Y	z
Cinnamon agouti,	9	X	y	Z
Black,	9	x	Y	Z
Dilute cinnamon agouti,	3	X	y	z
Blue,	3	x	Y	z
Chocolate,	3	x	y	Z
Silver fawn,	1	x	y	z.

In parts of the country where tame rabbits have become feral, black young are sometimes found in the nests of wild grey parents. Thus grey seems dominant to black. In the course of his experiments recorded in the "Journal of the Linnean Society" for 1904, Mr. C. C. Hurst bred both black and grey rabbits which bred true, and, when they were mated, their hybrids were grey, while their hybrids' progeny were greys and blacks in the ratio 3 : 1 (actually 38 and 10). Thus the previous inference is confirmed, and black and grey seem each the result of single factors.

But later experiments by Professor Castle of Harvard—a statement of whose work is to be found in "Science" for 1907, new series, vol. xxvi—show this conclusion to be erroneous. He dealt with six colours which he divided in two groups according as they were judged to be produced by or without certain pigments. Two different pigments and a factor which affected their localization were judged to be producing the following six colours (B stands for black pigment, Y for yellow, and A for the localization factor) :—

Series I.			Series II.		
Grey,	B	Y A	Black,	B	Y
Blue-grey,	B (dilute)	Y A	Blue,	B (dilute)	Y
Yellow,[1]	B (traces)	Y A	Tortoiseshell,[2]	B (traces)	Y.

Among the six colours Professor Castle found the following Mendelian relations :—

(1) Grey is dominant to blue-grey, black, and yellow.

(2) Blue-grey is dominant to blue.

(3) Black is dominant to blue and tortoiseshell.

(4) Yellow is dominant to tortoiseshell.

(5) Grey is produced by mating black with either yellow or blue-grey.

Since it is dominant to three different colours, grey must be the result of three different factors at least, and since they are intermatable, so also must the other five colours. There should therefore be three pairs of factors in operation, and eight different colours in all. Since blue-grey, black, and yellow differ each from grey in one pair of characters, the first four groups in the set are determined at once, namely—

Grey, ...	27 X Y Z
Blue-grey, . . .	9 X Y z
Black, ...	9 X y Z
Yellow, ...	9 x Y Z.

---

[1] and [2]; these are the English equivalents of white-bellied yellow and sooty yellow.

But the characters carried by blue and tortoiseshell can also be determined. Blue is recessive to both blue-grey and black, and therefore differs from each in one pair of characters. Thus it carries one dominant less. This dominant cannot be Z, for then blue would differ from blue-grey in three pairs of characters. Nor can it be Y, for then blue would differ from black in three pairs of characters. It can only be X, and the characters carried by blue are therefore Xyz. For similar reasons the characters carried by tortoiseshell must be xyZ. Thus the complete set of eight with the colours so far identified as belonging to six of the possible combinations is

Grey,	27	$X\ Y\ Z$
Blue-grey,	9	$X\ Y\ z$
Black,	9	$X\ y\ Z$
Yellow,	9	$x\ Y\ Z$
Blue,	3	$X\ y\ z$
	3	$x\ Y\ z$
Tortoiseshell,	3	$x\ y\ Z$
	1	$x\ y\ z$.

The two unfound colours could be found by mating blue-grey with tortoiseshell or yellow with blue.[1]

The same conclusion is arrived at by the same line of argument as that previously taken with the mice. The six colours found can be arranged in two sets of four groups each. In a set of four groups the largest is dominant to both the intermediate groups, and each of these is dominant to the smallest. By this method of identification the two sets become, with provisional symbols,

	1			2	
Grey,	9	$X\ Y$	Grey,	9	$A\ B$
Black,	3	$X\ y$	Blue-grey,	3	$A\ b$
Yellow,	3	$x\ Y$	Black,	3	$a\ B$
Tortoiseshell,	1	$x\ y$	Blue,	1	$a\ b$.

By being at the top of two sets, grey must carry three dominants at least, and perhaps four. But, since black appears in both sets, grey can carry only three, for if it carried four the characters of grey and black would be $XYAB$ and $XyaB$, and then the two colours would differ from each other in more

---

[1] It is probable that blue-grey and blue are merely different names for the first two colours in a set of four found by Professor Punnett and called by him cinnamon, chocolate, dilute cinnamon and orange. (Journal of Genatics, Nov. 1912.) In that case dilute cinnamon and orange would bear the characters xYz and xyz.

than one pair of characters. If we call the third dominant Z, then the characters carried by the first set of four groups are

Grey,	9 $X\ Y\ Z$
Black,	3 $X\ y\ Z$
Yellow,	3 $x\ Y\ Z$
Tortoiseshell,	1 $x\ y\ Z$.

Blue-grey differs from grey in one pair of characters. There are only three possible combinations which fulfil this condition, namely, XYz, XyZ, and xYZ, but, as the last two are already appropriated by black and yellow, the remaining one, XYz, must belong to blue-grey.

The characters belonging to three of the groups in the second set are now known, and, if we write down the four groups with the characters of the three already known, we shall be able to infer the characters belonging to the fourth :—

Grey,	9 $X\ Y\ Z$
Blue-grey,	3 $X\ Y\ z$
Black,	3 $X\ y\ Z$
Blue,	1

By being common to three groups, X must be common to all four, and the differentiating characters in the set are therefore Y and y, and Z and z. The only combination left for blue to carry is Xyz. This is precisely the same result as before, and again the two unfound colours belong to the combinations xYz and xyz.

Still another example might be considered—more especially as it has received a different interpretation—in which the distribution of the characters is obscured by some of the factors having inseparable effects and also by the effects of certain factors being suppressed by those of others. This is the well-known example of the fowls' combs. Rose-combed fowl were mated with pea-combed, and, while their hybrids had walnut-shaped combs, their hybrids' progeny had four different kinds : walnuts, roses, peas, and singles in the ratio 9 : 3 : 3 : 1. Since there are four groups in the usual proportion, there are two pairs of characters, but since the effects of the factors are inseparable, the set must be set down with "unknown" symbols, thus :—

Walnut,	9 $X\ Y$
Rose,	3 $X\ y$
Pea,	3 $x\ Y$
Single,	1 $x\ y$.

There is another kind of comb carried by a Dutch breed of fowl, called the Breda, which is described as having "ostensibly no comb. As a matter of fact, in the cocks there are two minute papillæ standing one on each side of the middle line, which are rudiments of a comb structure. As experiment shows, the hens have the duplicity of which these papillæ are the evidence, but in examination of the heads of hens practically no comb-tissue can be seen or felt." Yet these combless fowl carry a factor which suppresses the effects of other factors and another which has the effect of splitting real combs in two.

The Breda comb was mated with both rose and single combs. When mated with the single comb their hybrid had "a large double comb formed as two divaricating singles." From this result four factors can be assigned to the single comb and four to the Breda. The single comb is already known to carry the characters x and y; but, since it produces a real comb when mated with the rudimentary Breda, it must carry a factor for the production of a real comb which is dominant to a factor for the rudiment of a comb in the Breda; and, since it produces a split comb when mated with the Breda, it must also carry a factor for an unsplit comb which is recessive to a factor for producing a split comb in the Breda. If we designate these new pairs of characters R = real comb, r = rudimentary, S = split, and s = unsplit, then the characters carried by the single comb are now, $xyRs$. The Breda comb carries the characters r and S, and it must also carry x and y, for, did it not do so, its hybrid with a single comb would have been something else than a single.

Thus there are now four pairs of characters connected with fowls' combs, and the full set of sixteen groups of hybrids' progeny might be set out with the kind of comb belonging to each combination predicted.

When the Breda comb was mated with the rose comb, "the resulting combs were all duplex roses." Here again the two previously unknown dominants are brought to light, namely, that for a real comb carried by the rose comb, and that for splitting which must have been carried by the Breda comb. When the hybrids were bred from, their progeny were counted into six groups only, but, as there were more than four groups, there must have been at least eight real groups, some of which were inseparable through the action of the suppressing rudimentary factor brought in by the Breda. The rose comb carries the factors $XyRs$, the Breda comb the factors $xyrS$. Thus the two differ in three pairs of characters, and their hybrids' progeny should have consisted of the eight groups which can be formed by combining the three pairs of characters X and x, R and r, and S and s, in all the ways possible consistent with the action of dominance. The eight real groups and the six which were found are as follows:—

	Actual.	Found.
27	Split rose comb,	Split rose comb.
9	Split single comb,	Split single comb.
9	Unsplit rose comb,	Unsplit rose comb.
9	Unsplit single comb,	Unsplit single comb.
3	Split Breda comb with rose suppressed,	Split Breda comb.
3	Split Breda comb with single suppressed,	
3	Unsplit Breda comb with rose suppressed,	Unsplit Breda comb.
1	Unsplit Breda comb with single suppressed,	

The ordinary single comb was mated with still another comb which was brought from Cairo. This comb was itself a single comb, excepting that it was split in two, and, by its progeny with the ordinary unsplit comb, it "proved to be a distinct dominant over single comb." It therefore carried the characters $xyRS$.

Thus there are at least four pairs of characters distributable among fowls' combs; and the complete set of sixteen groups may now be set down with the kinds of combs producible and the ordinary names of such as have been found indicated:—

81	X	Y	R	S	Walnut comb, split
27	X	Y	R	s	Walnut comb, unsplit (*ordinary walnut*)
27	X	Y	r	S	Walnut rudiment, split
27	X	y	R	S	Rose comb, split
27	x	Y	R	S	Pea comb, split
9	X	Y	r	s	Walnut rudiment, unsplit
9	X	y	R	s	Rose comb, unsplit (*ordinary rose*)
9	X	y	r	S	Rose rudiment, split
9	x	Y	R	s	Pea comb, unsplit (*ordinary pea*)
9	x	Y	r	S	Pea rudiment, split
9	x	y	R	S	Single comb, split (*the Cairo comb*)
3	X	y	r	s	Rose rudiment, unsplit
3	x	Y	r	s	Pea rudiment, unsplit
3	x	y	R	s	Single comb, unsplit (*ordinary single*)
3	x	y	r	S	Single rudiment, split (*the Breda comb*)
1	x	y	r	s	Single rudiment, unsplit.

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RADIO-THERAPY: ITS SCIENTIFIC BASIS AND ITS TEACHING.

BY

J. JOLY, Sc.D., F.R.S.

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XXXVII.

RADIO-THERAPY: ITS SCIENTIFIC BASIS AND ITS TEACHING.[1]

By J. JOLY, Sc.D., F.R.S.

[Read March 23. Published April 29, 1915.]

I.

The recent discovery that X rays and γ rays can be defracted into spectra by the natural grating contained in the orderly structure of crystals, sets at rest the question as to the nature of these radiations. They are of the same nature as light-waves, but of very much higher frequency—from 10,000 to 100,000 times as high.[2]

Such rapid vibrations do not evoke the sensations of sight. Our tissues are, indeed, to a considerable degree, transparent to them; the power of penetration increasing with the frequency or as the wave-length diminishes. Whereas light-waves are rapidly absorbed within a few millimetres of the surface of the skin, these rays can penetrate deeply; some of the highest frequency even passing right through the body.

The certitude of the identity of these three classes of radiation leads to issues of much importance to medical science. For medicine had for many years been invoking the aid of the mysterious X and γ rays without in the least knowing what these agents were. It now turns out that they are physically identical with light. This fact secure, medical science is made heir to the discoveries of photo-electric science. It is for the physician now to enter into his inheritance, and to see what it teaches him as to the interpretation of his past results and as to openings for future advance. I shall restate the leading facts of photo-electric science.

In 1887 Hertz noticed that the passage of an electric spark between the terminals of an induction coil was much facilitated by the discharge of another spark in its neighbourhood. He showed that the light from the spark was the agent which promoted the discharge. Ebert, the ensuing year,

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[1] Based on a paper read to the members of the Dublin Clinical Club on March 16, 1915.

[2] The wave-lengths of these various rays are of the following orders:—light, 10^{-5} cms.; X rays, 10^{-8} cms.; γ rays, 10^{-9} cms.

found that the action of the light was on the cathode, and Hallwachs made the important discovery that light could discharge a negatively electrified body, but not a positively electrified one. The discharge was chiefly due to the ultra-violet waves.

Hallwachs' experiment is an easy one to repeat. It is of fundamental importance to photo-electric science. A convenient arrangement is to connect a polished plate of zinc to a gold-leaf electroscope and use an arc light to radiate to it. If we charge the gold-leaf and zinc plate negatively and then turn the light on the zinc, the leaf rapidly collapses. If we charge the system positively, we get no effect from the light. The insulation—best of sulphur—must be carefully attended to, and no bright light allowed to fall upon it.

We can detect the photo-electric effect without first electrifying the illuminated plate. A carefully insulated plate, brightly lit from a source rich in ultra-violet rays, will acquire a positive charge. This charge will rise to a certain potential dependent on the character of the light, and will rise no more.

These results find explanation in the emission of negatively electrified particles from the illuminated plate. If the plate is negatively electrified to start with, the expulsion of the particles is facilitated. If it is positive, they are attracted back and there is no effect. If the plate is neutral to start with, the loss of negative electrons gives it a positive potential. This increases till the attraction is such as to keep back the negative particles. Beyond this it cannot increase.

Since the date of these pioneer experiments much has been ascertained regarding the nature of these negatively electrified particles and the conditions under which they are liberated. In this résumé we cannot, of course, enter into details. The leading facts may be stated as follows :—

(1) The particles expelled carry each a negative charge of the unit amount—in other words, the Faraday unit which appears in electrolysis and in the determination of the valency of the elements.

(2) The particles are alike in mass, no matter from what elemental substance they may be evoked. This mass is very much less than that of a hydrogen atom—about the thousandth part. They are identical with the "electron," with the "corpuscle," with the "β ray," and with the "cathode ray."

(3) The number liberated by the action of light in unit time is directly proportional to the intensity of illumination.

(4) The energy with which they are discharged is independent of the intensity of the light, and is directly proportional to the frequency—i.e.,

the greater the frequency the greater the energy of the photo-electrons. Or, we might say, the shorter the wave-length the greater the velocity of the electron.

(5) The effects are independent of the temperature, taking place freely at the temperature of liquid air.[1] The effect is, therefore, an inter-atomic and not a chemical one. Chemical actions cease at such temperatures.

These are the fundamental laws of photo-electricity. To-day we are in a position to extend these laws to the much shorter and more rapid oscillations of X and γ rays.

It much conduces to a clear understanding of the relations of the several parts of the subject before us if we take into account a comparatively recent generalization which applies to all aethereal radiation—heat, light, X ray, or γ ray. The generalization is due to Planck and Einstein.

According to Planck, the energy of radiation is not sent into the aether as a continuous stream, but in "fits" or discreet rays. The total energy, for instance, which reaches the leaf of a plant from the sun in a second of time is composed of an integral number of definite elementary units of radiation or vibration. The energy associated with each unit of radiation he calls a quantum of energy; and he shows that this is always equal to a certain universal constant multiplied by the frequency. This constant (Planck's constant) has the value $6{\cdot}55 \times 10^{-27}$ erg seconds. If n is the frequency of any aethereal radiation—light, X rays, or γ rays—we can say that the energy of the quantum is $n \times 6{\cdot}55 \times 10^{-27}$ light unit. Such units of energy move in space without spreading or dividing. They keep their discrete and separate character wherever they travel.

The important matter for us to notice here is that the energy increases directly with the frequency. Now, the frequency of an X ray may be 10,000 times that of a light-wave; hence the quantum of energy associated with it may be 10,000 times greater. And as a γ ray of radium C may be of 100,000 times the frequency of a light-ray, the quantum of energy associated with it may be 100,000 times greater, and 10 times as much as accompanies the X ray.

These facts enable us to assign an explanation to some of the laws of photo-electricity which we have recorded above.

We have stated, as matter of observation, that light gives rise to a discharge of electrons, or β rays, from substances in which it is absorbed, and that the velocity and energy of the discharged electrons are proportional to the frequency of the light employed, and are not in any way dependent on

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[1] Joly, "Nature," vol. lxxii, p. 308, 1905.

the intensity or brightness of the light. This at once follows, if we assume that each expelled electron absorbs one quantum of energy, and with this store starts upon its path. The number of quanta falling per second upon unit area of the substance increases, indeed, with the intensity, but each individual quantum remains the same in amount; and thus while the number of electrons liberated from the illuminated surface increases with the intensity, the velocity of the electrons is determined entirely by the value of n.

The fact that γ and X rays give rise to very fast and penetrating β rays when the former are absorbed in matter has, for some years, been known to science, and we are able to state that here, too, and over a much greater range than that of the whole visible spectrum, the law obtains that the velocity of the expelled electron increases with the frequency. What is more, we are safe in stating, from the law of quanta, that such β rays greatly exceed in energy those expelled by light-waves; and we are thereby enabled to give an explanation of their observed much greater power of penetration.

The mechanism by which a light, a γ, or an X ray is transformed into β radiation is unknown. It is most certainly an inter-atomic process; that is, it is consummated within the atom. Whatever the explanation may be, it must involve a reciprocal or reversible mechanism; for it is established that the β ray can give rise to the γ ray, the quantum of energy being handed from one form of activity to the other.

In the case of light a similar transformation explains—according to Einstein, Lenard, and Saeland—the phenomena of fluorescence and phosphorescence. Here the light-wave, imparting its energy to the electron, liberates it from the atom. The electron after a longer or shorter sojourn in the precincts of the parent atom is finally attracted back—a return which may be accelerated by the agitation involved in a rise of temperature. The disturbance attending the return of the electron to the atom sets up vibrations of visible length, and, in fact, gives rise to the phenomena of phosphorescence and fluorescence. These vibrations represent the restoration into aethereal waves of some of the original luminous energy. The fact that there is some loss of energy in the processes of transformation involves smaller quanta in the final luminous vibrations and hence longer wave-lengths. This realizes Stokes's law, which defines the wave-length of the fluorescent light to be longer than that of the exciting light.[1]

In the X ray tube the electric force between cathode and anti-cathode sends a stream of fast moving electrons from the one to the other, and it is where this stream impinges on the atoms of the anti-cathode that X rays

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[1] Einstein, "Ann. d. Physik.," Bd. xvii, 1905. Lenard and Saeland, "Ann. d. Physik.," Bd. xxviii, 1909.

are generated and fresh β rays or electrons are liberated. Each of these X rays carries its quantum of energy depending on its wave-length or frequency, and is in kind identical with the γ ray emitted by radioactive elements; but the quantum is less than is generally associated with the γ ray. It is now known, also, that the X rays from the anti-cathode are evolved in spectra characteristic of the nature of the element forming the material of the anti-cathode.

The increasing gradation of energy from the wave of light to the γ ray introduces certain differences into the phenomena they give rise to. This must be held in mind, for these differences may be important therapeutically. I shall refer here to two important instances.

(1) Photo-electric effects appear in certain cases to be limited by chemical structure. Thus the sulphides of the metals yield free electrons under light stimulus; the corresponding sulphates do not. There is, however, no such limitation when the electrons are evoked by X or γ rays. The limitation imposed in this case by chemical structure almost certainly arises from the small velocity with which the photo-electrons are emitted. There is no reason to believe that light fails to evoke the electron, but the molecular structure is such as to secure a large absorption.

(2) Outside the region of the ultra-violet, but long before the higher frequencies of X rays are reached, there is a spectral region which has been named the Schumann region. This is concerned with wave-lengths of from 2000 to 1500 Ångström units (10^{-8} cms). The absorption of these waves in air, water, etc., is very rapid—far more so than that of visible light. To these waves we will again refer. The point in the present connexion is that the greater frequency has not been attended with increased penetration. This may be due to resonance with some harmonic of the atom. Selective absorption by matter is a conspicuous phenomenon in the case of waves of low frequency. Dealing with such waves, it must not be forgotten that media may be sensitized for particular rays by adding photo-electric substances which absorb those rays. Thus photo-electric effects on bacterial culture can be greatly increased by the addition of eosine or similar dyes.

The β and γ radiations given out by radioactive bodies are those which most concern us. I cannot stop to discuss the views which have been offered as to the source of these radiations in the atom and the kind of properties which must be ascribed to the atom in order to account for the origin and interchange of quanta within it. Suffice it to say that Rutherford has defined in general terms the necessary conditions.[1] Nor is it possible here

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[1] Rutherford, Phil. Mag., vol. xxviii, Sept., 1914.

to do more than mention the facts that the γ radiation given out by radioactive atoms shows continuous as well as line spectra and that the β radiation of such atoms possesses a varied distribution of velocity which, when analyzed by the deflecting influence of a strong magnetic field, gives rise to a linear "spectrum" of velocities, superimposed on a continuous "spectrum" of velocities. Further, it can be shown that the energies characterizing the lines in the "spectra" of the primary β particles escaping from a radioactive atom—for instance, Ra C—are such as would be derived from an integral number of the quanta associated with certain lines of the γ ray spectrum emitted by this same element. Hence the emission of trains of waves (and of quanta) from such atoms is indicated; 10 or even more waves entering into the train according to the nature of the atom and the frequency.[1] Under suitable conditions the train of waves may give all its energy to a single high-speed electron. The high penetrating power of the β particle excited in matter by the γ ray has, according to this view, its source in the peculiar structure of the γ ray emitted by the radioactive atom.

The foregoing statements define in a brief—perhaps too brief—manner the fundamental facts of photo- and radio-electricity. The most important of these facts to carry in mind is the convertible nature of the two sorts of radiation—the electronic and the vibratory. It helps our assimilation of the laws governing this interchange to remember that the activity involved in the γ, X, or light rays is parcelled out into definite quanta which are much greater for the high frequencies than for the low; so that the β rays originating in the interchange possess much more energy in the one case than in the other.

We must now direct our attention to the electron and its influence in bringing about molecular changes. Here, again, we deal in quanta—although differing in kind from the Planck unit. For Faraday long ago showed reasons for assuming that electric currents attending chemical or molecular changes moved in quanta: the Faraday charge on the ion, which—as we have already seen—is the same in amount as that which is associated with the electron. Thus we find Nature on every side ultimately ordered in discontinuous units.

The simplest case in which we can study the effect of the electron is when it is liberated in a gas—say, air. Each electron liberated from an atom of oxygen or nitrogen leaves this atom positively electrified by one unit charge of positive electrification. The atom becomes a positive ion. The electron soon attaches itself to one or more atoms, and in doing so creates a negative ion.

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[1] Rutherford, *loc. cit.*

If now an electric potential is established—say, between two electrified plates contained in the gas—the plus electrons move towards the negative plate and the negative electrons towards the positive plate. Finally, the plates become in this manner neutralized or discharged. The ions, in fact, confer the property of conductivity upon the gas.

Ions can exist in liquids, and can be moved therein by electric forces, as in the case of a gas. The slow motion of the ions in an electrolyte confers on it its conductivity. In recalling these trite facts, we would emphasize the ubiquity of the ion. We might advance yet further. The conductivity of solids is very probably due to the free motion of electrons within them. It is probable that these electrons, the mobility of which is peculiar to conductors, stand in a different relation to the molecules of the substance from electrons concerned with the chemical forces attracting atom to atom. The latter class of electron we shall now consider.

The chemist to-day recognizes that ionization is at the basis of chemical activity, the force of attraction between the positive and the negative ion playing a fundamental part. As we have seen, the loss of an electron converts the neutral atom to the positive ion; the addition of one converts the neutral atom to the negative ion.

It is through the agency of the ion that the light ray, or the γ ray, effects chemical changes. The freeing of the electron is the primary effect. Such electrons—i.e. those concerned with the chemical bonding—are termed valency electrons. How important this action is in Nature will be realized when we consider that the metabolism of green plants the world over depends on the photo-electric activity of the sun's rays.

We might fill much space in merely listing the cases of photo-chemical changes which have been studied within recent years. The chemical changes seem in many cases to be brought about by the reduction of one molecule and the oxidation of another. Such actions can progress in solids, liquids, or gases. They may be produced by visible or ultra-violet light, by X rays, by γ rays, or by β rays. They may be sudden when intense radiation acts upon easily changed substances. Or they may take ages to accomplish, as in the case of mineral changes brought about by the ionising activity of the a rays of radioactive substances acting in the rocks.

On the living cell γ or X rays produce remarkable effects. The study of these effects in plants dates back several years. Schobert, Errera, Molisch, Guilleminot, and others have contributed to it.[1] The rays may retard cell division, and more especially affect the germinating embryo.

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[1] Le Radium, vii, 1910, p. 247.

They may kill such cells. They may also in very feeble doses promote cell division. Gaskell has specially studied the effects of X rays on the embryonic cells of the chick.[1] He found that up to a certain amount of exposure the embryo may make complete recovery from the injurious effects of the rays, but that there is a critical dosage beyond which recovery does not occur and development stops. Certain ultra-violet rays—the Schumann rays—are said to be always very destructive in their action on living protoplasm, giving rise to cytolysis and death in the cases of spirogyro, amoeba, and other unicellular organisms, in less than one minute. These wave-lengths, which are about half the length of visible rays in the violet, are rapidly absorbed even in air. It has been suggested that destructive organic effects are due to untimely oxidation.

The physician possesses in radiation a subtle means of attacking the mechanism of cell-growth, and one of unlimited power. It is a characteristic feature of true scientific advance that new powers, based on newly discovered forces, are placed at our disposal. The conception of physical interference with the atomic linkages of organic structure and its sustaining metabolic processes, is a new one. The older practice recognized one way only of affecting such interference—by the intervention of chemical actions set up by drugs assimilated through the digestive system. Although we are to-day far from a knowledge giving complete control of radiative effects, I venture to think that these will ultimately be found to be more definable and manageable than medicinal treatment.

Let us consider, so far as we can, what we are doing when we insert into a tumour a needle filled with emanation.

Within the tube the radioactive transformations of the atom are attended by three forms of radiations.

(1) α rays, which are positively electrified helium atoms, and which cannot pass the walls of the tube. With these, therefore, we have nothing to do.

(2) Also β rays, or electrons, are sent out. Some of these are so slow as also to be stopped by the thin glass and steel walls surrounding the radioactive substances. But these walls are thin enough, as used in the technique introduced by the Radium Institute of the Royal Dublin Society, to permit a large proportion of them to escape. Their velocity varies over a wide range, some electrons moving at speeds nine-tenths that of light. Their velocities are such as to give, as already stated, both a line and a continuous " spectrum " when sorted out by a magnetic field.

These electrons are known to be the direct agents in effecting ionisation.

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[1] Gaskell: Proc. Roy. Soc., Ser. B, vol. lxxxiii, Feb., 1911, p. 305.

The faster electrons probably penetrate a couple of centimetres in soft tissues, their energy dying out in the creation of ions and secondary γ rays. The latter are again re-converted to β radiations, which again take up the work of ionisation. Thus the whole of their energy, or the greater part of it, is, probably, ultimately spent in the work of ionisation: in other words, on work which is capable of seriously modifying the chemical and molecular processes progressing in the medium.

(3) There emerge, also, from the tube the γ rays of Ra B and Ra C. The latter enormously predominate, most of the rays of Ra B being sufficiently soft to be absorbed in the walls of the tubes. These rays, as we have seen, move with certain definite quanta of energy, or in integrals of a certain quantum in each case: in short, in trains of rays. Wherever they traverse atoms they give rise to β rays. Some of these, taking up the whole energy of a wave-train, move with velocities similar to the most penetrating primary β rays given out by the parent radioactive atoms. This is the inner history of the events leading to the ionisation of the medium according to recent views.

The number of ions which these rays can generate in air has been computed.[1] In reckoning the number of ions two count for one, as each electron separated involves the formation of both a + and a − ion. The numbers given refer, therefore, to pairs of ions.

The quantities of the substances Ra B and Ra C used in the estimates are those which will be in equilibrium with one gram of elemental radium or with one curie of emanation. These substances alone concern the surgeon when he applies radioactive treatment by usage of the emanation. The rays are supposed to act for one second of time.

From β rays of Ra B . .	$0{\cdot}325 \times 10^{15}$
„ β „ Ra C . .	$0{\cdot}64 \times 10^{15}$
„ γ „ Ra B . .	$0{\cdot}084 \times 10^{15}$
„ γ „ Ra C . .	$1{\cdot}134 \times 10^{15}$
Total,	$2{\cdot}183 \times 10^{15}$

Now it is of interest to estimate what these numbers would represent in the therapeutic use of these radiations in body tissues, on the assumption that the energy is in a similar degree expended on ionisation—an assumption which may approximate to the facts, seeing that the mere state of aggregation—solid, liquid, or gaseous—should not much affect the results. I take

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[1] Moseley and Robinson: Phil. Mag., vol. xxviii, Sept., 1914.

the volume of an average cell as 125×10^{-9} cubic centimetres, which is the volume of a cube 1/500 of an inch on the edge.

I shall assume the surgeon inserts the radioactive needles containing the emanation one centimetre apart, and that he has only one millicurie in each needle, the radioactive length of the needle being one centimetre. I shall also assume, as a first approximation, that the radiations are completely absorbed within the boundaries of the tumour being treated.

The number of pairs of ions generated per second by one millicurie will be $2{\cdot}18 \times 10^{12}$. And as the needles are one centimetre apart, we have this number generated per cubic centimetre. In a single cell the number is 272×10^{3} pairs of ions.

In actual practice there may be about five millicuries in each needle. We have then, theoretically, over 1·3 million pairs of ions generated per cell per second. The assumption that all the rays are absorbed in the tumour is not accurate, and again the numbers given apply to quite unscreened radiation only. The softer β and γ rays suffer absorption in the glass and steel envelopes. This loss applies chiefly to the rays from Ra B. In order to make a safe allowance for these sources of error, as well as for the loss of the most penetrating of the γ rays of Ra C which escape from the tumour, I take 50 per cent. of the calculated number of ions, that is 136,000 pairs of ions per cell per millicurie per second; or, in the working conditions, 680,000 per cell per second. In exposures measured by hours the numbers rise to thousands of millions. In ten hours to twenty-four thousand millions of pairs of ions per cell.

These figures are instructive, whether they represent entirely effective and useful ionisation or not. Even if only a small fraction is usefully expended, they reveal the power of radio-therapeutic methods in controlling or initiating chemical changes within the cell.

The effects of this powerful ionisation on the cells of the body have been demonstrated repeatedly by microscopic examination. I would refer more especially to the fine series of photographs obtained by A. Clifford Morson on carcinoma and sarcoma before and after exposure to radiation.[1] After treatment for twenty-four hours with 90 mgrms. of radium the obliteration of structure is far advanced or even, to all appearance, complete. In the case of healthy cells of the rat, Lazarus-Barlow has shown that considerable exposures may produce no more than temporary disturbance of growth, and that even while treatment is proceeding the cells may become again apparently normal.[2] The

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[1] Morson: "Archives of the Middlesex Hospital," xxxiii, p. 110.

[2] Lazarus-Barlow, *loc. cit.*, p. 34.

important point has frequently been brought out that the healthy cell behaves as a less sensitive system. This, of course, is at the basis of radioactive treatment. It is not improbable that a dosage which will do no more than stimulate mitosis in a healthy cell will suffice to destroy the less stable cancer cell. The latter is, indeed, so unstable towards the ionising effects of the rays that a very small dose will arrest development, and even cause the destruction of the cell. A tube guaranteed to contain five milligrams of pure radium bromide was several times applied to cases of cancer in this city, the tube being screened with thin sheet-lead and applied externally. It was subsequently found that the tube contained but 0·8 mgrms. of radium element. This was, therefore, a very small dosage. All the results obtained were, however, beneficial. The whole subject is probably in its first stages of investigation in spite of the work which has been done.

II.

Failing the guidance which investigation will assuredly one day give us, it is interesting and, possibly, important to discuss the cell as a photo-sensitive molecular system, and in so far comparable with another photo-sensitive molecular system, the study of which is less difficult to pursue.[1]

Of all photo-sensitive systems with which we are acquainted the photographic film is at once the most accessible to observation and the best understood; although in this, no more than in any other case, is our knowledge complete, or our views always capable of actual demonstration. We know it to consist of haloginised molecules emulsified in an organic colloid, the relations of salt and colloid being probably complex, and such that they react one upon the other in responding to the photo-electric effect. Certain features in common with the cell will be recognized in this statement. It is, indeed, possible that we might apply it word for word as a general description of the activity of the cell as a photo-sensitive system.

What is the photograph? It is an effect of photo-electric activity. This activity, which operates during exposure, generates the latent image. This is subsequently acted on by the developer, and the negative produced.

In this process we start with a halide of silver, loosely combined with the complex molecule of the gelatine, or in a state of solid solution, the instability of the silver halide being increased by its immersion in the gelatine according to principles which have been pointed out by Sir J. J. Thomson, in the case of ordinary solutions in water.[1] We end up with separated metallic

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[1] Joly: Proc. Roy. Soc., Ser. B, vol. lxxxviii, 1914.

[2] J. J. Thomson: Phil. Mag., vol. xxxvi, 1893, p. 320.

silver. The process is, then, one of reduction on the whole. But it is effected in two stages. First the photo-electric action; then the chemical action of the developer or reducing agent on the latent image.

The view that the process of formation of the latent image is founded in the expulsion of the electron under the photo-electric force is supported by many circumstances.[1] The halides of silver are independently known to be strongly photo-electric. The special sensitizers (certain aniline dyes) which are used to increase the sensitiveness of the film for particular spectral regions are also energetically photo-electric, and, moreover, they are specially active towards those rays which they absorb, and for which they sensitize the film. Again, the latent image formation by radiation is taken out of the category of chemical actions by the fact that it takes place at the lowest extremes of temperature, at which all chemical activity ceases.[2]

The fact last referred to supports the view that translatory molecular movements are not involved in the initial formation of the latent image. What may be described as a state of static ionisation is set up, the discharged electrons creating negative ions by attachment to the surrounding gelatine. The electrons, emanating chiefly from the electro-negative chlorine of the photo-electric molecule, collect around the gelatine-silver-bromide systems, forming an electro-negative region which may be regarded chemically as comparable to an increase in negative ionic concentration. This view is supported by the chemistry of development so far as this is known.

The developer acting at this stage—i.e. when the latent image has been formed—finds the process of reduction facilitated by these conditions. For the developer is essentially a reducing agent possessing generally a concentration of negative hydroxyl ions; that is, it is alkaline in character: and the latent image represents a temporary release of the positive silver ion from attachment to the chlorine ion, the latter having lost its charge.

The latent image is, in short, one stage in the reduction of the complex silver bromide molecule. But it is a stage reached by physical means, and owes its stable character to the solid nature of the medium in which it is immersed. Nevertheless, it runs down in course of time and disappears; the negative electron gradually being attracted back to the central positive system, and recombining with the chlorine whence it came. The process of destruction of the latent image may be accelerated by over-exposure to light, X or γ rays. This is the phenomenon of reversal or solarisation. The probable explanation is that by continued exposure the electrostatic

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[1] Joly: "Nature," vol. lxxii, July 27, 1905, p. 308.

[2] Joly: Proc. Roy. Dublin Soc., vol. viii, 1894, p. 222.

stress set up by electronic segregation accumulates to the point of rupture, and there is a hurried return of the electron to its starting-point under the electric stress. It is noteworthy, however, that the recovery of the plate is not complete. Successive reversals give less and less distinctive results. This would be explained if a certain amount of ionisation in the virgin emulsion was neutralized by the electronic discharge. Describing the phenomenon of reversal in general terms, we may regard the change as referable to an excessive segregation of negative ions, leading to the overbalancing and destruction of the ionic system formed by the moderate exposure of the film.

The latent image may also be induced by friction, pressure, or, generally, by mechanical irritation of the film. The action of the mechanical stimulus is probably to induce directly the separation of electrons; i.e. to promote the negative ionisation. A latent image so formed can be reversed by radiation.

The reversal of the latent image may, in some cases at least, be effected by the infra-red and longer heat waves. This might have been anticipated from the theory of the latent image given above, for we would expect a feeble displacement of the electrons by the long waves, under which effect they would recombine.

We have now to consider the formation of the latent image by chemical means.

If the latent image is essentially the result of a partial reduction of the silver halide due to a concentration of negative ions, we should expect that the introduction of negative hydroxyl ions should assist in its creation. The developers and sensitizers, in point of fact, generally create the latent image, and act most effectively when alkaline, e.g., pyrogallol, gallic acid, tannin, aqueous solutions of nicotine, and—it is stated[1]—alkaline solutions of lactose and glucose. These substances, acting as developers, must, whatever stages may intervene, ultimately neutralize the charge upon the positive silver ion in the emulsion, setting it free as metallic silver. The effects are, in short, probably continuous with those of radiation in creating the latent image. Dilute solution of ammonium hydrate alone will give the latent image. Some substances act as sensitizers, or owe their special efficiency as developers to their active absorption of the halogen. It appears certain that this action occurs in the cases of all strong developers and sensitizers; the halogen being sometimes, however, taken up by the gelatine.

If a solution of gallic acid, especially if rendered alkaline, is poured on

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[1] Meldola: "Chemistry of Photography," Macmillan, 1891, p. 190.

a gelatine dry plate in the dark and, after a few minutes, is again washed off, it will be found that a developable latent image has been formed. Strong pyrogallic acid solution powerfully affects the plate in the same manner, even without the addition of alkali. Alkaline tannin gives the same result, but as it renders the gelatine almost impermeable, the effect is superficial and feeble, sometimes inappreciable. It is an active absorbent of the halogen. Tobacco smoke bubbled through water gives a solution which is intensely active in forming the invisible image.

As opposed to the concentration of the negative hydroxyl ion in promoting development, the action of the positive hydrogen ion upon the latent image is to inhibit its growth, or reverse it if already formed by light or otherwise. This result is made apparent by introducing a very weak solution of a mineral acid. The oxidation of the reduction product, or the rehalogenisation of the partially reduced silver, may be involved, according to the nature of the acid used. The feature common to all acid intervention is the increased concentration of the positive hydrogen ion.

The photo-sensitive molecular system of the film can exist in different states of sensitiveness, ranging from a highly sensitive to a relatively insensitive state. The behaviour of the "ripened" emulsion of the fast plate (i.e. of an emulsion which has been subjected to a process of prolonged heating) is similar in kind to that of the "unripened" emulsion of the slow plate, but in the former all the phenomena are relatively advanced. The latent image is sooner formed under a given exposure, and much more readily reversed. Chemical effects are correspondingly accelerated. The grain of the sensitive or ripened film is much coarser than that of the slow or unripened film.

We now turn to the living cell.

We find that radiation may, if carefully modulated, stimulate, and, if too intense, retard its growth and ultimately destroy the molecular structure required for mitosis. In so far the effects on the growth of the cell—superficially, at least—resemble those on the formation of the latent image.

It is also found—and as already stated the whole efficacy of radioactive treatment turns upon this—that in the case of the pathological cell these phenomena appear all in advance of the like effect in the normal cell. There exist then states of the cell differing in sensitiveness towards radiation just as there exist differing states of the film.

The accelerated mitosis and growth of the pathological cell appear in some cases to be traceable to repeated mechanical stimuli. This is parallel with the formation of the latent image by similar stimuli.

Finally, the destruction of the pathological cell is said to be brought about by thermal radiation of a certain intensity. A method of treatment has even been founded on this. The parallel with the latent image also appears here.

There is, then, a very complete parallel between the effects of radiative and mechanical stimuli in both cases, the latent image and the cell. The formation of both may be promoted by radiation, and by the same radiation in excess may be finally destroyed. It seems permissible to ask if the same parallel does not extend to more definitely chemical effects. The point is important not only on the score of the convenience and accessibility of the plate as a means of investigation, but because of certain conclusions which can be drawn from already known data, and which possibly possess a bearing on what is termed the cancer problem.

We may state the argument thus:—We find certain chemicals producing in the film what are to all appearance identical effects with those generated in it by radiation. And reasoning from the fact that radiation produces parallel results in the case of the cell and the film, we ask if those chemicals which affect the film in the direction of acceleration or retardation may not also in like manner affect the cell. The view that this question is legitimate is supported by some observational facts, as will presently appear.

But first it is necessary to look more closely at what may be really involved in comparing the formation of the latent image with the growth of the cell. If, at any stage of its metabolism, a partial (or complete) reducing action takes place in the cell in which the halogen and the colloid present take a part, the similarity between the two results may be more than a parallel. It may be based on actions chemically or physically identical, or practically so. There may, in fact, exist, as a stage in the life of the cell, relations between the negative halogen ion, a positive ion united with this, and the protoplasm, similar to that prevailing among the elements of the film. If such exists, the explanation of the resemblance in the response of the two systems towards different agents, physical and chemical, is at once forthcoming. We are not in this case involved in the statement that the growth of the cell and of the latent image are parallel actions beyond the inference that a certain molecular rearrangement necessary for the growth of the cell is similar in character to what is presented to our study in the formation of the latent image. In a sense the formation of the latent image is katabolic, that of the cell is anabolic. We are not, however, forcing a complete comparison between them, nor do the observational facts call upon us to do more than recognise *some* photosensitive molecular process involved in cell-growth similar to one involved in the formation of the latent image.

There seems to be no doubt that the growth of the cell is highly sensitive towards ionic concentration. Confirming and extending the results obtained by Loeb in 1898, and using similar methods, Moore, Roaf, and Whitley have found by direct observations on the embryonic cells of Echinus that even small increase in the concentration of the negative ion $(\overline{HO})$ will accelerate growth.[1] But if the alkalinity be increased yet a little, pathological mitoses make their appearance, and at a slightly greater alkalinity the chemical actions necessary for the life and metabolism of the cell are inhibited. On the other-hand, an increased concentration of the positive ion $(\overset{+}{H})$ from the first retards, and, if pushed further, inhibits growth. The parallel with the action of alkaline sensitizers and acid retarders upon the film is obvious.

In the light of the observations of Moore, Roaf, and Whitley we can even offer a probable explanation of the effects of radiation on the cell which is not, fundamentally, unlike that which we have advanced for the formation of the latent image. The electronic discharge in the cell is—as we have seen above—very great. Part of this is directly imported from without (the radioactive supply of β rays), and part is derived from atoms within the cell. The first all goes to the increase of the concentration of negative ions within the cell. The latter may also do so in effect, in so far as it is derived from the less chemically important elements present. The already existing positive ion, as in the case of the silver ion of the film, will not readily part with an electron, but the tendency is for it to be freed from its valency ties or actually neutralized. Thus the negative ion which accelerates mitosis is increased in concentration, and the positive ion which acts to retard it is eliminated. Chemically, the effect is to increase the alkalinity, and all the sequence of events observed by Messrs. Moore, Roaf, and Whitley takes place as the dosage is increased.

Observations showing an abnormal lowering of acidity in the digestive secretions of cancerous patients have been made by several investigators. Messrs. Moore, Roaf, and Whitley in 1905 found that the diminution of hydrochloric acid in the stomach was independent of the location of the disease.[2] Copeman and Hake, in 1908, published results which failed to confirm those of Moore.[3] The question as regards the secretion of HCl cannot be regarded as finally settled. But a lowering of acidity as a frequent feature in cases of malignant disease seems to be accepted as proved. With old age a similar phenomenon is observed, and with advancing years the liability to cancer increases.

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[1] Moore, Roaf, and Whitley: Proc. Roy. Soc., Ser. B, vol. lxxvii, Oct., 1905.

[2] Moore, Roaf, and Whitley: Proc. Roy. Soc., Ser. B, vol. lxxvii, May, 1905.

[3] Copeman and Hake: Proc. Roy. Soc., Ser. B, vol. lxxx, June, 1908.

These facts suggest that the antagonistic action of the acid and the alkali in the cell is parallel with the antagonistic action of restrainer and sensitizer upon the film. The latter may be illustrated by a simple experiment on the film. A latent image is formed on a dry plate, either by brief exposure to light or by application of a sensitizer—such as nicotine, gallic acid, or pyrogallic acid. The application of a wash of very dilute HCl left on the plate for a couple of minutes will then obliterate the latent image, as will be found upon applying a developer. The effect is best obtained with highly dilute acid. Such a strength as is said to prevail normally in the secretion of the stomach—0·2 to 0·4 per cent.—works effectively.

The possibility that substances which act as sensitizers or restrainers on the film may act to promote or retard mitosis in the cell must be admitted from these results, some of which are obtained by direct experiment on the living cell.

The fact that cancer of tongue, lip, and throat, and generally of parts around the mouth, is chiefly confined to the male sex has before now been regarded as raising a suspicion as to the injurious effects of tobacco smoking.[1]

The effects of a solution of the volatile substances evolved from burnt tobacco, upon the film, support this inference. If tobacco smoke is bubbled through water, and a little of this water is poured on the photographic plate in the dark, and again washed off, a vigorous latent image will be obtained, as development will demonstrate.

At the time of communicating the subject-matter of this paper to the Dublin Clinical Club I treated this matter as a purely extrapolatory suggestion. I was not then aware of the fact that C. and R. Hertwig and Galleotti[2] mention nicotine as one of a few substances which they found, by direct observation on animal cells, produced pathological mitosis

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[1] Statistics of cases treated in the Middlesex Hospital in 1913 show that the numbers of cases of the kind in the male and female sexes stand as 8·3 : 1. Thus:—

Tongue,	37	:	3
Lips,	13	:	0
Larynx,	13	:	2
Pharynx,	1	:	3
Palate,	8	:	0
Floor of Mouth,	6	:	1
Tonsils,	5	:	1
	83		10

Archives xxxiii, p. 2.

[2] Referred to by Moore, Roaf, and Whitley: Proc. Roy. Soc., Ser. B, vol. lxxvii, Oct., 1905.

and derangement of cell-division closely similar to those which are observed in cancer growth.

It is now evident that we may find, in this indication of the film, support for our line of reasoning. The substances present in tobacco—probably the intensely alkaline substance nicotine in chief—set up in the cell those same electro-negative conditions which cause or assist it to promote the formation of the latent image, and in this way locally precipitate a state of mitotic instability which from other causes—to be presently discussed—may prevail as a tendency throughout the body cells of the patient. Local mechanical stimuli may contribute. It is, of course, not impossible that in many cases of the kind the effect is so far due to the local causes that but for these the cancer would not anywhere have invaded the body.

An increase in the number of deaths from malignant disease within recent years is admitted by high authorities to be the only conclusion we can draw from the statistics, after every allowance for error has been made.[1] Modern advances in surgical and medical science undoubtedly enable life to be prolonged in many cases, or even cure to be effected where formerly speedy death alone must ensue. This ought to be a set-off against improved diagnosis as a source of error in the statistics. If this increase is a fact, we have to look around for the cause. It is, assuredly, not founded in anything of an evolutionary nature. I say this because if its origin be in the cell itself, a very profound change—profound because seated in the primary organic structure—must be supposed to have taken place within a few decades. Indeed, if the increase is what we judge it to be from the figures, it has taken place within a single generation, or at most two generations. That quite precludes evolutionary change acting through Mendelian factors. The view that some general body-change is involved appears to be supported by the fact that local causes will initiate the disease in some subjects and not in others. Consequently we must look to some article of diet or some custom of life which can reach and affect the stability of the cell. There are obvious difficulties in laying the blame for a change so deep-seated on a custom. Moreover, we look in vain for any custom at all likely to be responsible. When, however, we come to the possibilities of diet, we see much less difficulty.

In view of what has already been stated, it is legitimate to pursue the matter yet further, and to ask if within recent years we are not taking into our bodies more abundantly than formerly some substance or substances

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[1] See "Encyclopædia Britannica," last ed., Art. *Cancer*. I find it stated that the recently issued statistics for cancer in England and Wales (1912) show the highest mortality as yet recorded.—*Daily Mail Year Book*, 1915.

which might be held responsible for the increase of cancer. Many accustomed articles of diet may, doubtless, contribute in some degree towards increased cell mitosis and yet be quite harmless under the conditions of consumption. As already stated, it is known that alkaline solutions of lactose and glucose possess the parallel qualities required for affecting the photo-sensitive films. I have not obtained this action on the ordinary dry plate, nor got any latent image with ordinary sugar in alkaline solution. Milk, however, gives a faint effect, and this may be due to lactose. Sugar is an article of diet, the consumption of which has increased in modern times, and the evidence for its sensitizing activity—either directly or indirectly—should not be lost sight of as possibly concerned in the cancer problem.

But a far more suspicious substance is found in tannin. This substance enters, as all know, largely into the composition of teas of all varieties to the extent of, usually, 11 to 26 per cent. ; and 60 to 80 per cent. of this is obtained in the normal extract. Tea, as an article of diet, has replaced all other beverages in the light meals of the day. This especially applies to the better-off classes. In former years this beverage was only taken at "tea-time." The cancer statistics when compared with the statistics for the consumption of tea in this country show features in common. Both curves rapidly rise for several decades preceding the last, and within recent years show a somewhat less rate of increase.[1]

As regards the consumption of tannin in other countries, it is to be remarked that this substance enters into coffee to the extent of about 22 per cent., and is present in red wines. Obviously without statistics both as to the consumption of tannin-containing beverages and of the prevalence of cancer we cannot discuss the geographical evidence. It is stated that in China cancer has long been a prevalent disease.

Tannin or gallotannic acid is itself a photographic sensitizer, and has long been known as such. It absorbs the halogens.[2] It is the parent substance of a complicated and only partially studied group of substances in which the reducing properties required for development and sensitization seem to prevail. Thus gallic acid, a derivative which does not coagulate albumen, and is said to be absorbed in the body by administration of tannic acid, is a developer and sensitizer. Another derivative is the powerful developer pyrogallic acid, which along with gallic acid is stated by some writers to be excreted by the kidneys. As transported in the circulatory system these substances must, of

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[1] Compare figures given for tea-consumption in Thorpe's "Dict. of Applied Chemistry," article "Tea," with the Cancer Tables in Burns's "Vital Statistics Explained," Constable, London, 1914.

[2] Meldola, *loc. cit.*, p. 98.

course, acquire neutral or faintly alkaline characters. Other substances which possess the requisite reducing properties, and are constituents of the complex tannins, are pyrocatechol and hydroquinone.

Thein (or caffein) does not appear to exert more than a very feeble effect on the film, even when in a state of saturated solution, either neutral or distinctly alkaline.

If the increased prevalence of cancer is wholly or in part traceable to the increased consumption of tannin, we must regard the derivatives of this substance as predisposing the cells throughout the body to the incidence of the disease. The appearance of the disease at any particular point in the body is probably determined by local stimulus of cancerous mitosis. The view suggested is that a general instability or irritability is promoted throughout the body cells by this substance tannin; or rather, by the derivative or derivatives of it which are absorbed in the body; the effects being mainly due to the reducing or halogen-absorbing properties. A state is at length reached after long and excessive absorption of the injurious substance in which local causes are competent to precipitate the pathological mitosis and cell proliferation. These causes are various. It may be a local chemical stimulus, as by the application of a powerful sensitizer such as nicotine, or possibly, "nut-gall ointment." Other local causes, as has often been suggested, may be the increased mitotic activity prevailing in the organs of generation. Here there is already a local approximation to the conditions induced by increased electro-negative ionisation. Mechanical stimuli are probably responsible for the sweep cancer, etc.

The frequent recurrence of cancer after its local extirpation or destruction follows as a matter of course according to the present views. For, even apart from metastatic spread of the disease, the local cure is likely to be only temporary if the patient continues to absorb the sensitizing agent into his system, or, possibly, has already permanently affected his tissues by its use. Where so much is involved, should not the physician consider the advisability of the denial to the patient of tannin-containing beverages?

The effect of tannin as an influence on mitosis is probably responsible for the phenomenon of "vegetable cancers" or galls on trees and shrubs. Galls may contain up to 75 per cent. of tannin. These growths originate under the stimulus of irritation by some insect. Pfeffer, Sachs, and others have recognized that tannin in plants is abundant in places where growth is specially active; such as growing points, pathological growths, and places where the protoplasm is specially irritable.[1] We must remember that when

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[1] Haas and Hill: "Chemistry of Plant Products." (London: Longmans, Green, & Co., 1913.)

we come to the cell there is not so much to differentiate the vegetable from the animal.

We see, then, that not only the parallel with the photo-sensitive film, but also the experiments which have been made on embryonic cells, support the view that the initiation of cancer involves two factors: the presence of a sensitizer which acts to increase mitosis and cell proliferation, and the absence of a substance (the positive ion) which normally acts as a restrainer towards the growth processes of the cell. The presence of the former may be harmless so long as the latter exists in sufficient quantity to neutralize its effects. This view has led us to seek for the cause of the increase of cancer in the increased consumption of such articles of diet, notably tannin, as may introduce sensitizing substances into the system. Reverting to the theory of radio-therapy, we are led to the inference that in radio-active treatment we effect ionic changes in the cell which are equivalent to increasing the amount of sensitizer present. But the cancer cell has already within it almost the limiting amount of the sensitizer which its continued metabolism permits. The result of radioactive treatment is to overstep this limit and bring the amount of the sensitizer up to the lethal concentration. The surgeon must notice that on this view he is increasing the alkalinity of the cell, and, as it is impossible to confine this action entirely to the tumour, excessive radioactive treatment is to be avoided. The use of small multiple centres of radiation, the effects of which are more easily localized and rendered uniform, is preferable to strong centralized radiation where lethal effects are long surpassed in one region before the destructive dosage is reached in another.[1]

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NOTE ADDED IN THE PRESS.

According to Dr. W. S. Bainbridge ("The Cancer Problem," p. 67, New York, The Macmillan Co.), "cancer of lip, tongue, cheek, and buccal mucous membrane is of relatively frequent occurrence in both sexes in India, in

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[1] Stevenson, Brit. Med. Journ., July 4, 1914, and March 20, 1915; Joly, Proc. Roy. Dublin Soc., vol. xiv, May 8, 1914, p. 290.

consequence of the chemical irritation produced by the chewing, or holding in the mouth, of a mixture of betel leaves, areca nut, tobacco, and slaked lime."

The seeds of *Areca Catechu* contain tannin and also arecolin, an alkaloid closely related to nicotine; being, in common with it, a derivative of pyridine. Choline, a strong base answering the general reactions of alkaloids, is also present. The betel leaf is the leaf of *Piper Betle*, and contains yet another alkaloid of the pyridine group—piperine. (Haas and Hill, "Chemistry of Plant Products," London: Longmans, Green, & Co., 1913.)

It is worth noting that in this case both sexes suffer the increased liability to mouth cancer, and both sexes indulge in the habit. Compare the facts respecting mouth cancer and tobacco-smoking.

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CHANGES IN SOILS BROUGHT ABOUT BY HEATING.

BY

MISS A. WILSON, B.A.

[COMMUNICATED BY PROFESSOR H. H. DIXON, F.R.S.]

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Correction referring to p. 513, first paragraph:—

Dr. Russell has pointed out to the author that he only regards the factor therein mentioned as one out of many which may influence bacterial growth in the soil.

XXXVIII.

CHANGES IN SOILS BROUGHT ABOUT BY HEATING.

By MISS A. WILSON, B.A.

[COMMUNICATED BY PROF. H. H. DIXON, F.R.S.]

[Read March 23. Published May 10, 1915.]

In his work on the partial sterilization of soil, Russell (5) has shown that increased production of ammonia in heated soil, or in soil treated with toluene, is due to increased numbers of bacteria, rather than to any other cause. He ascribes this increase in the number of bacteria to the absence from the treated soil of larger organisms, such as infusoria, amoebae, and other protozoa.

In 1888 Frank (1) had shown that the effect of heat on soil was to increase the soluble mineral and organic matter, and also the productiveness of the soil. Pfeffer and Franke (4) and Krüger and Schneidewind (2) showed that plants actually take more food from a heated than from an unheated soil.

Seaver and Clark (6) showed that in soil heated during a period of two hours at various temperatures up to 120°, the growth of plants was greater in soils exposed to the higher temperatures. In samples heated above this temperature the number and growth of fungi increased, while higher plants remained stunted. By chemical analysis of extracts from heated soils Seaver and Clark (6) showed that this result was due to increase in the amount of soluble materials, and to the acid reaction of the extract. They ascribed both these factors to decomposition of some of the soil constituents. G. W. Wilson (7) has also shown that the growth of all plants is accelerated in soil heated up to 95°. Retardation of growth occurred in soil heated between 135° and 175°, and there was greater susceptibility to parasitic fungi, as well as a larger number of soil fungi.

In considering the effects of heated soil on plant growth, both the amount of soluble material in the soil and the absence of larger organisms seem therefore to be important. I am indebted to Dr. Atkins, at whose suggestion the research was undertaken, for the methods of studying the changes in solubilities and for advice during the course of the work.

The change in solubility of soil constituents brought about by heat was studied by two methods:—

I. The depression of freezing-point of a series of extracts from heated soil samples was determined.

II. The electrical conductivities of the extracts were determined. From these results an approximation to the depression of freezing-point due to electrolytes was calculated.

The soil with which the experiments were made was taken from a depth of not more than six inches from the surface, and was freed as far as possible from organic matter, plant-stems, leaves, worms, etc. The soil was then passed through a sieve of quarter-inch mesh to remove large pebbles and hard lumps.

Small flower-pots, each holding about 350 gms. of soil were used for the heating. Each sample was heated separately in an air-oven during a period of not less than two hours; the temperature was noted by a thermometer placed in the soil, and was as far as possible kept constant during the period of heating. Seaver and Clark (6) have shown that the duration of heating after two hours has but little effect on the amount of soluble material.

Samples of soil were heated in this way at temperatures between 60° and 150°. Each sample was allowed to cool during twenty-four hours. An extract was obtained in each case by placing the flower-pot on a retort stand over a filter funnel with paper, which dipped into a clean flask. The same volume of distilled water was poured slowly over each sample. When the soil was saturated, the water dripped through into the filter and appeared as a brownish liquid in the flask. To prevent bacterial growth a few drops of toluene were added to each solution, and the flask closed with a cork.

Reaction to litmus.—The reaction of the solutions to litmus was tested in each case, and found to be neutral.

Colour of the solutions.—The range of coloration in the solutions was marked. An extract from unheated soil was practically colourless; the others varied from a faint brownish yellow colour in that heated at 60° to a very deep brown in that heated at 150°.

Determination of depression of freezing-point.—The depression of freezing-point of each solution was then determined by means of Beckmann's apparatus. The freezing mixture was kept at a temperature of $-2°$, and allowance was made for the depression due to dissolved toluene.

The results are shown in Table I, and are plotted in fig. 1 (Δ).

TABLE I.

Temp.	Electrical Conductivity. $C \times 10^5$	Depression of Freezing-Point.	
		Δ	Δ_e
Unheated	35	0·08°	0·20°
60°	36	0·06°	0·02°
70°	47	0·05°	0·02°
80°	66	0·06°	0·03°
90°	72	0·07°	0·04°
100°	131	0·14°	0·06°
110°	103	0·13°	0·05°
120°	163	0·16°	0·08°
130°	130	0·15°	0·06°
140°	113	0·14°	0·05°
150°	198	0·19°	0·10°

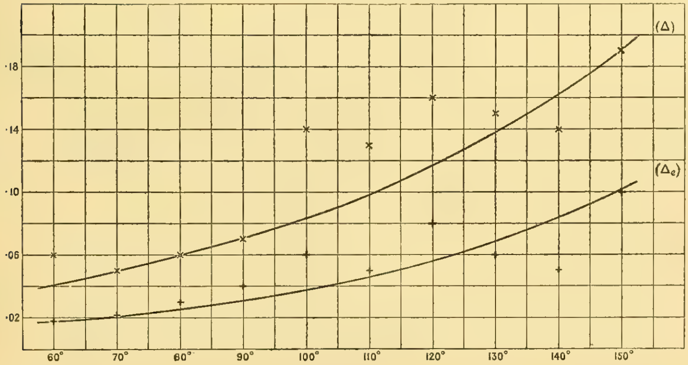

FIG. 1.

Determination of Electrical Conductivities.—The electrical conductivities of the solutions were determined by means of the apparatus devised by Kohlrausch, and described by Ostwald (3). The liquid is placed in a Hamburger tube, and the instrument calibrated by means of an $\frac{N}{25}$ solution of potassium chloride.

The depression of freezing-point due to electrolytes was calculated, and plotted in fig. 1 (Δ_e).

To account for the neutral reaction to litmus, the percentage of combined carbon dioxide in the soil was determined. This was then calculated as calcium carbonate; the latter would neutralize any free acids produced by heating.

These results, with the percentage of moisture in the unheated soil, are given as follows in Table II.

TABLE II.

	Percentages calculated on air-dry weight.
Moisture in soil sample, ...	1·75
Combined carbon dioxide, ...	5·5
Calcium carbonate, ...	12·5

In a preliminary set of experiments it had been noticed that the soil heated to higher temperatures absorbed a larger amount of water. In a second set of experiments the amount of water absorbed in each case was noted. The results are given in Table III.

TABLE III.

Temperature	Volume of water added	Volume of water retained per 100 gms. of soil
15° (Unheated)	200 c.c.	19
60°	,,	13
70°	,,	17
80°	,,	21
90°	,,	28
100°	,,	40
110°	,,	31
120°	300 c.c.	58
130°	,,	60
140°	,,	48
150°	,,	68

(These results are plotted in fig. 2.)

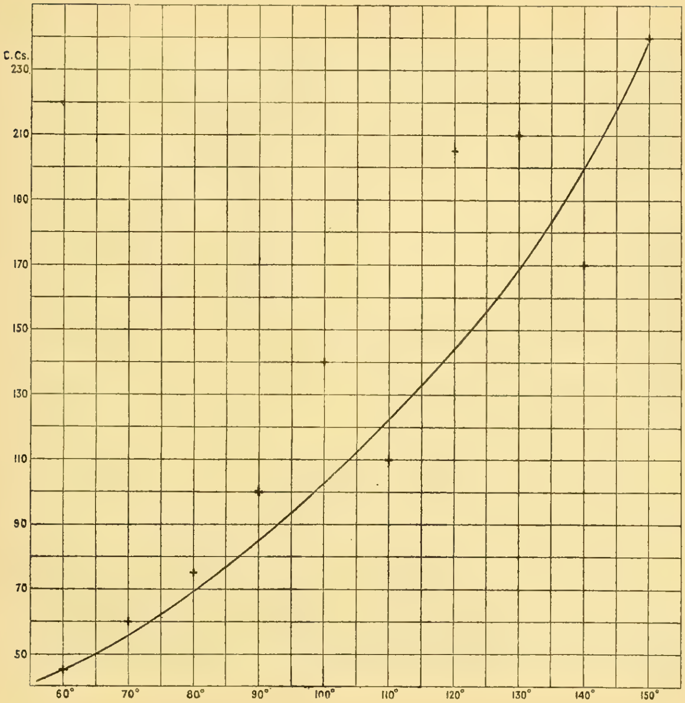

Fig. 2.

From these experiments it is clear that the amount of soluble matter in soil is increased by heating. This is shown by :—

(*a*) Depression of freezing-points of soil extracts, which increases from 0·06° in the case of soil heated at 60° to 0·19° in the soil heated at 150°.

(*b*) The electrical conductivities of the extracts, which show an increase from $36{\cdot}0 \times 10^{-5}$ in the case of soil heated at 60° to 198×10^{-5} in soil heated at 150°.

In each of the extracts about half the depression of freezing-point was due to electrolytes.

The increase in the amount of water absorbed by the heated soil indicates a change in the texture of the soil brought about by heating.

In a further set of experiments soil was prepared in the manner previously described, and allowed to dry at ordinary air temperature during a few days.

In the previous set of experiments it seemed possible that the extract in each case did not contain a fair sample of the soluble soil constituents, owing to the fact that the water had passed through definite channels in the soil, leaving a large portion of the sample untouched.

The soil was placed in beakers, containing about 115 gms., and heated at a constant temperature for not less than two hours. The extract was obtained by pouring 100 c.c. of distilled water on the sample, which had been allowed to cool during twenty-four hours. The soil was then stirred up and set aside for a further period of twenty-four hours, during which it was frequently stirred. The mixture of soil and water was then filtered, and the brownish extract obtained as before. A few drops of toluene were added to each extract.

The depression of freezing-point and conductivity of each extract were determined as before, and the depression due to electrolytes calculated. The results are shown in Table IV and Fig. 3. Several experiments were made at the lower temperatures.

TABLE IV.

Temp.	Electrical Conductivity. × 10^5	Depression of Freezing-Point. Δ	Δ_e
Unheated	26	0·03°	0·01°
60°	38	0·04°	0·02°
70°	42	0·03°	0·02°
80°	41	0·04°	0·02°
90°	42	0·035°	0·02°
100°	37	0·025°	0·02°
110°	48	0·035°	0·02°
120°	53	0·05°	0·03°
130°	72	0·06°	0·04°
140°	90	0·075°	0·04°
58° *a*	44	0·03°	—
b	47	0·03°	—
c	51	0·03°	—
70° *a*	42	0·02°	—
b	42	0·03°	—
c	43	0·03°	—
95° *a*	49	0·03°	—
b	49	0·03°	—
c	52	0·03°	—

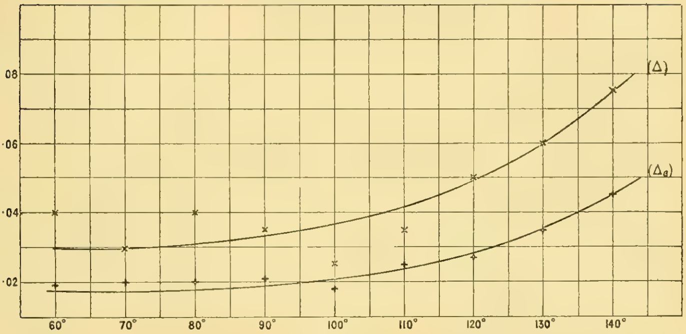

FIG. 3.

In this, as in the previous set of experiments, the solutions showed a marked range in coloration. The electrical conductivity and depression of freezing-point showed a considerable increase in the solutions obtained from soil heated to the higher temperatures. The greater regularity of increase in these solutions was probably due to the method of extraction, which enabled soluble matter to be taken from all parts of the soil sample. Several samples were heated carefully at the lower temperatures, and the electrical conductivities and depressions of freezing-point of the extracts were determined. These measurements show the fluctuations that may be expected in extracts from soil subjected to identical treatment. It may be remarked that the electrical method is the more sensitive for detecting small changes.

SUMMARY.

The results of these experiments show that at any rate part of the increased productivity of heated soil may be due to the increase in soluble matter induced by heating, and to the change in soil-texture, which has a remarkable effect on the retention of water by the soil.

The increase in the amount of soluble matter is seen in :—

(*a*) the range of coloration of the solutions extracted from soil heated at different temperatures;

(*b*) the increase in electrical conductivity of the solutions;

(*c*) the increased depression of freezing-point found in extracts from soil heated at the higher temperatures. About half the total depression throughout the experiments is due to electrolytes.

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THE SUBSIDENCE OF TORSIONAL OSCILLATIONS AND THE FATIGUE OF NICKEL WIRES WHEN SUBJECTED TO THE INFLUENCE OF ALTERNATING MAGNETIC FIELDS OF FREQUENCIES UP TO 250 PER SECOND.

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XXXIX.

THE SUBSIDENCE OF TORSIONAL OSCILLATIONS AND THE FATIGUE OF NICKEL WIRES WHEN SUBJECTED TO THE INFLUENCE OF ALTERNATING MAGNETIC FIELDS OF FREQUENCIES UP TO 250 PER SECOND.

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[Read May 18. Published June 28, 1915.]

In January and in November of last year the present writer brought before this Society the results of some experiments on the subsidence of torsional oscillations, and on the fatigue of nickel wires when they were subjected to the influence of alternating magnetic fields of low frequency.[1]

The present communication gives some results obtained with nickel wires, when alternating magnetic fields of higher frequency were applied, these magnetic fields being produced by means of alternating currents whose graphs—as obtained with an oscillograph—are practically pure sine curves. For a description of the apparatus employed, and of the general methods of experiment, reference should be made to the papers above mentioned. It will be found that in the cases already studied, the nickel wires when under test were subjected to three different loads, and the results showed the effect due to load. In the present investigation the wire was in every case subject to the same load, viz., $1{\cdot}5 \times 10^5$ grammes per sq. cm., which corresponded to the middle value of the loads used in the previous work. With this constant particular load there was used in each case the corresponding magnetic field of 20 c.g.s. units, for which it had been shown that the effects would be a maximum both for the subsidence of torsional oscillations and for the fatigue.[2]

The nickel wires employed were each 226 cms. long, and 0·1675 cms. in diameter; and the millimetre scale for reading off the amplitude of oscillation

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[1] Scient. Proc. Roy. Dub. Soc., vol. xiv, Nos. 14 and 26.

[2] *Ibid.*, vol. xiii, No. 3, pp. 31 and 37, and vol. xiv, No. 26, p. 337.

in the *subsidence* experiments or the steady deflection in the *fatigue* experiments was placed at a distance of 167 cms. from the plane mirror on the vibrator or load on the end of the wire. The maximum deflection of the light-spot which was used in the *subsidence* experiments was at the distance marked 300 on the scale, which corresponded to a torsion or twist of the lower end of the wire equal to an angle of about 5° 10′ on each side of the zero.

In the course of the experiments on "fatigue," the direct current *through* the wire was in each case equal to *one* ampere.

The wire first tested was in the physical state in which it was received from the manufacturer, and when measured it had a simple rigidity of about 810×10^6 grammes per sq. centimetre. When it was placed in the solenoid with the load at its lower end, a deflection or twist was produced of only one millimetre on the scale when the proper currents were sent *round* the solenoid and *through* the wire; the wire therefore in its original state of rigidity could not be tested for fatigue.

It was, however, tested for the subsidence of torsional oscillations, and, for comparison, observations were made when the wire was in two longitudinal magnetic fields, viz. the vertical component of the Earth's field and a field of 20 c.g.s. units, and then when it was in an alternating magnetic field of 20 c.g.s, units, at three different values of the frequency.

The more significant of the results are set out in Table I, and are sufficient to show what the trend of the curves would be if the results were plotted with the number of vibrations as abscissæ, and the corresponding values of the amplitude of oscillation as ordinates.

TABLE I.

Number of Vibrations	D. C.		A. C.		
	$H = 0{\cdot}45$	$H = 20$	$n = 50$	$n = 150$	$n = 250$
0	300	300	300	300	300
15	291	290	274	281	281
30	283	281	252	263	263
50	273	270	225	243	242
70	263	260	203	225	224

The numbers in the tables under the letters D. C. were obtained when direct longitudinal magnetic fields were round the wire, and those under the letters A. C. when alternating magnetic fields were employed at the different frequencies. The wire in this hard state shows very little damping of the torsional oscillations. There is a decrease in the amplitude of only 40 m.m. in the D. C. fields, and the greatest damping with the A. C. fields is obtained with a frequency of 50. There is practically no difference for frequencies 150 and 250 per second.

The wire was now removed from the solenoid, and was suspended vertically under its own weight. It was heated *twice* to a *dull red* heat from the top downwards by means of a broad Bunsen flame, and when it was cold and cleaned up, its rigidity was again measured and found to be about 790×10^6 grammes per sq. cm. It was then replaced inside the solenoid under the same conditions as before, and tested first for fatigue, and secondly for subsidence of torsional oscillations, for each value of the frequency of alternating magnetic field employed. To explain shortly the method of observing the fatigue:—With the direct current round the solenoid to give a longitudinal magnetic field of 20 c.g.s. units, and with *one* ampere through the wire, the lower free end of the wire twisted through a small angle which was read off as a deflection of the light-spot on the scale = D. The direct currents were then switched off both the wire and the solenoid circuits, and an equivalent alternating current sent round the solenoid so as to give an alternating magnetic field of 20 c.g.s. units for, say, *one* minute. This alternating current was then switched off, and separate direct currents of the same values as before were sent round the solenoid, and through the wire, and the deflection again read off on the scale = d, and so on until by the repetition of these processes the deflection d was no longer diminished, then—

F = the fatigue of the wire.
D = the unfatigued deflection.
d = the fatigued deflection.

$$F = \frac{D - d}{D}.$$

The observations for the fatigue for an alternating magnetic field of given frequency having been taken, the subsidence of torsional oscillations was then taken for that magnetic field, and it was found by experiment that the damping curve or curves of torsional subsidence were practically the same whether they were obtained *before* or *after* the fatigue tests. In every case the observations for the fatigue were made first. Now, having obtained the fatigue of the wire and the torsional damping for a magnetic field of a certain frequency, the wire has to be quite recovered from its fatigue before a magnetic field of another frequency may be tried. If the wire is left alone for a long time, it recovers slowly, but it can be restored rapidly as follows:—Ease the wire under test of its load, and put round the solenoid a direct current to give a magnetic field of 20 c.g.s. units for a few seconds, and then a low frequency alternating magnetic field for a few seconds more; the wire will then be quite recovered and in its original unfatigued state. The observations were taken with alternating magnetic fields of five different frequencies. The results for the fatigue are given in Table II, and are shown as curves in Fig. 1. In the Table, *d* means the steady deflection of the light-spot on the scale, and *F* the fatigue, and the frequency of the magnetic field is indicated by $n = 50$, &c.

TABLE II.

Rigidity $\doteqdot 790 \times 10^6$ grammes per sq. cm.

Time Mins.	$n = 50$		$n = 100$		$n = 150$		$n = 200$		$n = 250$	
	d	*F*	*d*	*F*	*d*	*F*	*d*	*F*	*d*	*F*
0	11	0	11	0	11	0	11	0	11	0
·25					10	0·090	9·6	0·125	9·3	0·150
·50	10·5	0·050	9·9	0·080	9·1	0·175	8·7	0·210	8·0	0·275
·75					8·3	0·250	7·0	0·280	6·9	0·375
1·0	10·0	0·090	9·3	0·155	7·4	0·320	6·5	0·350	6·2	0·410
1·5			8·7	0·215	6·2	0·440	6·0	0·460	5·2	0·532
2·0	9·4	0·145	8·1	0·265	5·3	0·520	5·1	0·540	4·6	0·580
2·5									4·4	0·600
3			7·2	0·350	4·6	0·580	4·4	0·600	4·4	0·600
4	8·6	0·215	6·6	0·400	4·4	0·600	4·4	0 600		
5			6·3	0·430	4·4	0·600				
6			6 +	0·450						
7			6 +	0·450						
8	8·0	0·270								
10	8·0	0·270								

From the figures in Table II, and the curves in Fig 1, it will be seen that when the frequency of the applied alternating magnetic field is 50, the maximum fatigue of the wire for that rigidity is 0·27, and it takes eight minutes to attain it; when the frequency is 100, the maximum fatigue is 0·45, and takes place in six minutes, and for the frequency 150 the fatigue is 0·6, and occurs in four minutes, whilst still higher frequencies fail to fatigue the wire more than 0·6, though the actions take place in shorter periods of time.

From previous work one would have thought that the higher the frequency of the applied alternating magnetic field the greater would have been the fatigue of the wire; but, as the above figures show, a nickel wire of a given rigidity can only be fatigued to a certain maximum amount by a magnetic field of a certain frequency; and the application of a higher frequency magnetic field does not fatigue the wire more, but fatigues it to a given maximum in a shorter period of time.

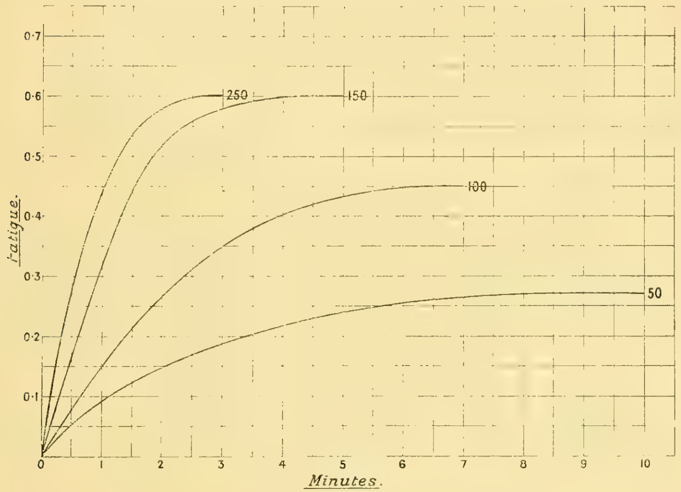

FIG. 1.

In fact, it would appear that *the time taken to effect the maximum fatigue varies inversely as the frequency of the applied alternating magnetic field.* The figures given in Table II for the higher frequency 250 vary slightly from this law; but in this case the maximum fatigue might have happened in 2·4 instead of 2·5 minutes, which would make it fall in with the rule. As for the figures given for the lower frequency 50, on account of the small quantities observed, a slight error might creep in, and the maximum might easily have been 0·271 taking place in 12 instead of 8 minutes; in that case this would also fall in with the law above stated.

Some of the results obtained for the subsidence of torsional oscillations in the same wire are given in Table III; for the sake of comparison, observations were taken in two longitudinal magnetic fields, D. C., as well as in the five alternating magnetic fields, A. C.

TABLE III.

Rigidity $\doteqdot 790 \times 10^6$ grammes per sq. cm.

Number of Vibrations.	D. C.		A. C.				
	$H = 0{\cdot}45$	$H = 20$	$n = 50$	$n = 100$	$n = 150$	$n = 200$	$n = 250$
0	300	300	300	300	300	300	300
15	288	285	280	283	282	281	281
30	277	271	261	267	266	263	262
50	263	253	236	247	247	243	241
70	250	237	213	226	230	225	220

These figures in Table III, as well as those in Table I, show that for the wire in these comparatively hard states, the application of alternating magnetic fields of frequencies higher than 150 per second have very little effect in increasing the subsidence or damping of the oscillations; for softer wires, as shown below, the damping decreases as the frequency of the magnetic field is increased, though at a slow rate.

The wire was again removed, and heated *three times* to a *cherry red* heat in the same manner as before, and when cold its rigidity was measured and found to be about 730×10^6 grammes per sq. cm. It was again put into the solenoid and tested under exactly the same conditions as formerly for both fatigue and the subsidence of torsional oscillations. The wire being now much softer, the fatigue was less than when in the hard state; the maximum was found to be 0·147 for the alternating magnetic fields at the two frequencies tried, and took place in *six* minutes when the frequency was 50, and in *four* minutes when the frequency was 100, per second.

The observations for the subsidence of torsional oscillations were taken as before for both D. C. and A. C. magnetic fields at five different frequencies, and some of them are given in Table IV.

TABLE IV.

Rigidity $\doteqdot 730 \times 10^6$ grammes per sq. cm.

Number of Vibrations.	D. C.		A. C.				
	$H = 0{\cdot}45$	$H = 20$	$n = 50$	$n = 100$	$n = 150$	$n = 200$	$n = 250$
0	300	300	300	300	300	300	300
15	238	160	285	289	290	290	291
30	194	105	271	279	280	281	283
50	153	71	255	266	267	269	271
70	125	52	237	253	255	258	261

In a final test the wire was once more taken down and heated *twice* to a *very bright cherry red,* so as to make it as soft as possible, and when cold its rigidity was measured and found to be about 715 × 10^6 grammes per sq. cm. It was again put into the solenoid, and tested in the same way as before for the subsidence of torsional oscillations. The wire in this soft state was not tested for fatigue, because the fatigue is extremely small even for a prolonged application of the alternating magnetic field. The results for the subsidence of torsional oscillations when magnetic fields of three different frequencies were applied are given in Table V, and some of them are shown as curves in Fig. 2.

TABLE V.

Rigidity ≑ 715 × 10^6 grammes per sq. cm.

Number of Vibrations.	D. C.		A. C.		
	$H = 0{\cdot}45$	$H = 20$	$n = 50$	$n = 150$	$n = 250$
0	300	300	300	300	300
5	271	206	295	296	297
10	245	159	290	293	294
15	223	130	284	290	292
20	204	110	279	286	289
30	173	81	269	280	284
40	151	63	260	273	279
50	133	52	250	267	274
60	118	45	241	261	269
70	107	41	232	255	264

Tables IV and V show the great change in the damping of torsional oscillations in the magnetic field due to a small change in the rigidity of the wire. Taking a mean of the two values of the amplitudes of the 70th oscillation in the last two tables, and comparing it with the corresponding value in Table III, we find that for a decrease of about 8 per cent. in the rigidity the amplitude of the 70th oscillation is *decreased* about 80 per cent. in the D. C. magnetic field, and for an A. C. magnetic field of the same value at a frequency of 50 per second the 70th amplitude has *increased* only about 10 per cent., and for the A. C. field at frequency 250 the *increase* is about 20 per cent.

Three of the sets of observations from Table V are plotted as curves in Fig. 2, which show very clearly the great difference in the subsidence of torsional oscillations in nickel wires in a D. C. magnetic field, and in A. C. fields, and also the small change in the final amplitude in the A. C. field when the frequency of the magnetic field is increased *five* times.

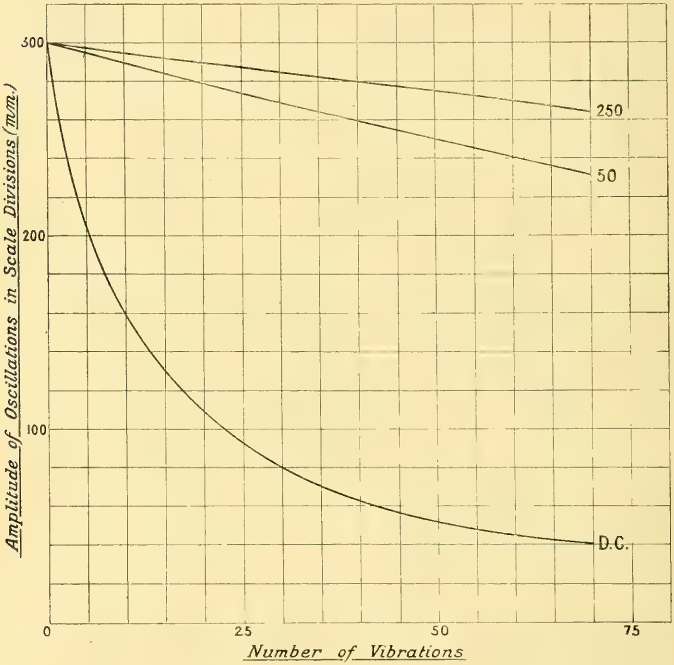

Fig. 2.

For assistance in making some of the observations I am indebted to Mr. J. J. Murphy, a Fourth-year Experimental Science Teacher, in training in this College.

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A METHOD FOR THE ESTIMATION OF HYGROSCOPIC MOISTURE IN SOILS.

BY

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[COMMUNICATED BY PROFESSOR G. A. J. COLE, F.G.S.]

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XL.

A METHOD FOR THE ESTIMATION OF HYGROSCOPIC MOISTURE IN SOILS.

By W. D. HAIGH, B.Sc., A.R.C.Sc.I.,

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[COMMUNICATED BY PROFESSOR G. A. J. COLE, F.G.S.]

[Read June 22. Published July 6, 1915.]

When an ordinary moist soil is spread out and allowed to lose water by evaporation, a point is at last reached when the soil becomes "air-dry" and dusty. It still contains some moisture, however, the amount of which will depend, to a large extent, on the temperature and amount of water-vapour present in the atmosphere. This is known as the hygroscopic moisture, and is held very closely by the soil particles. The amount of this moisture retained by soils will vary greatly with their nature. Broadly speaking, sandy soils retain the least; while clayey soils and those containing much humus material will retain the largest proportion.

It was considered by the earlier experimenters on soils that the hygroscopic moisture was very important for the welfare of plants; but within the last few years it has been shown that this is not the case. The pot experiments by Sach, and F. J. Alway's studies of soil moisture conditions in the "Great Plains" region of north-western America, have shown that it is only the moisture present in excess of the hygroscopic moisture that can be of direct service to crops. Plants will begin to wilt in a clay soil containing as much as 8 per cent. of water, but will flourish in a sandy soil containing only 5 per cent. It has also been shown that wilting begins even before the hygroscopic moisture limit has been reached. Yet it plays an important part, though indirectly, in the life of a crop, since soils of high hygroscopic power can absorb from moist air sufficient moisture to sustain the life of a crop in time of drought, although it cannot maintain normal growth. It is of enormous importance in some regions where hot, dry winds are a feature of the growing season. In such districts the soil containing the greater amount of moisture requires a much longer time to be dried and heated up to the point of injury to the crops than in the case of soils of low hygroscopicity, whose moisture is exhausted by such winds in a few hours, and the surface heats up rapidly to the point of injury. That such occurs in sandy soils much sooner than in clay soils is a matter of common knowledge.

The accurate estimation of the hygroscopic moisture in soils thus becomes important, since it is the amount in excess of this that is directly available for the plant to draw on for its nourishment. The ordinary method of determining the hygroscopic moisture, by heating in the water-oven—that is, at a temperature approximately equal to 100° C., for an arbitrary period of from twelve to twenty-four hours, apart from the length of time the operation takes—does not give very satisfactory results. A soil which contains much organic matter will slowly lose weight for weeks, and it is questionable how much of this loss is due to water and to volatile matter other than water. Even at as low a temperature as 100° C., under the prolonged heating, some of the combined water of the hydrated silicates in the soil may be lost.

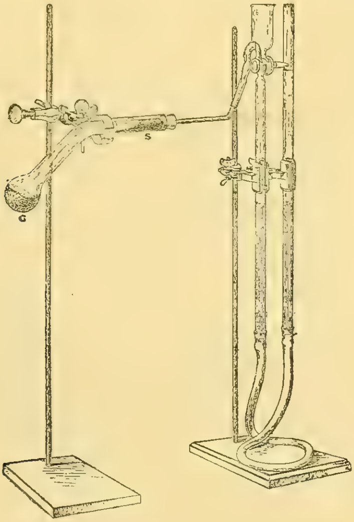
The power of calcium carbide to act as a desiccating agent has of recent years been put to practical use. It was first employed by H. A. Danne, who, in a paper read before the Society of Chemical Industry of Victoria (Australia) in 1900, described its use in determining moisture in organic substances. In 1906, P. V. Duprés (Analyst, 31, 213) described a method for estimating by means of carbide the moisture in cordite and other substances which give off volatile matter other than water-vapour when heated. A modification of Danne's method is now used in Australia to determine the moisture in wool. J. Masson has used it to determine the water of crystallization in salts (Jour. Chem. Soc. Trans. 97, 851). It has also been found of value in determining the water in petroleum.

Consequently it was thought that calcium carbide might be successfully employed to determine the hygroscopic moisture in soils, and a series of experiments were carried out, the results of which are given below.

The apparatus set up was similar, with slight modifications, to that used by Masson.

It consisted of a thick-walled glass tube about one and a half centimetres in diameter, with a bulb on one end, the tube being bent to an obtuse angle just above the bulb. This tube was connected by a short flexible joint to a nitrometer containing mercury; a small sample tube or test-tube which will just fit inside the larger tube, but which will not pass the bend in the latter when the tube is tilted, is slipped inside the larger tube.

When an estimation is to be made, excess of powdered carbide (about three times the weight of the soil used) is put into the bent tube, and the tube is tilted until the carbide falls into the bulb. A quantity of soil is weighed into the small tube which is slipped inside the larger, the upper portion of the latter being kept horizontal. The cork is then replaced tightly, the tube is connected with the nitrometer, and the pressure is equalized. The tube is tilted until the inner tube slips down to the bend, and the soil empties itself on to the carbide; it is then shaken to mix the carbide and soil, the former rapidly taking up moisture from the soil and evolving acetylene, which is collected in the nitrometer. The gas is given off rapidly, and after a few minutes the reaction ceases. If much moisture is present, some heat is generated, and it is necessary to allow the gas to regain the normal temperature of the room before measuring.



C. Powdered Calcium Carbide.
S. Small sample-tube containing a weighed quantity of soil.

It has been found by several observers that the volume of acetylene given off from commercial carbide when allowed to react with water is slightly less than that required theoretically. For every 18 milligrammes of water the theoretical volume of acetylene is 11·2 c.c., while in practice it has been found that only 10·5 c.c. are obtained.

To standardize the apparatus the following experiment was carried out:—Some sand was taken and thoroughly ignited, and allowed to cool in a desiccator. Afterwards the sand was weighed out and a known weight of water added. This was mixed with finely powdered carbide, and the volume of acetylene was measured and reduced to normal temperature and pressure.

Weight of water added.	Volume of gas at N. T. P.	Volume of gas equivalent to ·018 grms. of moisture.
·046 grms.	27·0 c.c.	10·56 c.c.
·038 ,,	22·2 ,,	10·51 ,,
·090 ,,	52·5 ,,	10·50 ,,

These results are in accordance with those obtained by other observers from different methods; and consequently 10·5 c.c. of gas, equivalent to ·018 grms. of water, have been used in the calculations following.

The hygroscopic moisture in a number of soils was determined both by the carbide method and by the ordinary method of heating in the oven. The limit of time for heating in the oven was fixed at sixteen hours, since it was found that all normal soils had reached practically a constant weight in that time. The samples used were a series of good arable soils, with the exception of one peat soil and one sand containing no organic matter. The "fine earths" (material less than 2 mm. in diameter) of the soils were used.

Number of sample.	Hygroscopic moisture.	
	Oven at 100° C.	Carbide.
1. Co. Tipperary, ...	2· 10 °/。	1· 96 °/。
2. King's County, ...	1· 77 ,,	1· 54 ,,
3. Co. Wexford, ...	2· 18 ,,	2· 02 ,,
4. ,, ...	3· 26 ,,	3· 06 ,,
5. ,, ...	2· 16 ,,	2· 05 ,,
6. Co. Dublin, ...	2· 68 ,,	2· 47 ,,
7. Peat, Co. Wicklow, ...	16· 52 ,,	15· 02 ,,
8. Sand (no organic matter), . .	0·262 ,,	0·261 ,,

From the above table it will be seen that, while the results by both methods agree fairly closely, the amount obtained in the case of the carbide method is always slightly lower than that obtained by heating in the oven. The difference increases in a rough proportion to the hygroscopic moisture present. In these soils, which were of a similar type, with the exception of 7 and 8, the increased hygroscopic moisture present was due almost entirely to an increase in organic material or humus. In the case of 7, which is a pure peat, the difference is as much as 1·5 per cent., while in 8, a sand with no organic matter, the results agree. It would appear from this that when a soil contains much organic material, the loss of weight on heating to 100° C. represents more than the hygroscopic moisture present in the soil, part of the loss being made up of other volatile constituents which are driven off on heating. This being so, it should be possible to drive off this volatile material by heating to a moderate temperature, then allowing the soil to cool and absorb moisture from the atmosphere, and the hygroscopic moisture then estimated by both methods should agree very closely. Accordingly the following experiment was carried out. A sample of 6, above, was taken, and the hygroscopic moisture determined by both methods. The result gave ·20 per cent. more in the oven than with the carbide. Another sample of the same soil was heated in an air-oven to 180° C. for several hours. It was then exposed to the air for several days, and the moisture again estimated by both methods, and the results were found to agree to within ·04 per cent.

In carrying out these experiments it was found that in the case of any particular sample of soil the results obtained by the carbide method were more consistent and agreed more closely between themselves than did the results obtained from the same sample in the oven.

The question arises as to how far the carbide affects the combined water in the hydrated silicates in the soil. H. A. Danne[1] has shown that, in applying the method to the estimation of hygroscopic moisture in organic substances, a marked interval exists between the evolution of acetylene from hygroscopic and that from combined water. No such interval has been observed in the case of soils. The gas is evolved steadily and rapidly. Masson, when determining the water of crystallization of salts, found that only the simple salts, such as sodium carbonate and sulphate, were completely desiccated by the carbide at ordinary temperatures, while others react, some completely, some partially, only when heated. Another class is shown to be stable to carbide even when heated to 170° C. From this we may conclude that the effect on hydrated silicates, if any, which in themselves

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[1] *Op. cit.*

form only a small proportion of the soil, is small, and will not vitiate the results.

In the experiments described above it has been demonstrated that the carbide method is a rapid and reliable means of estimating the hygroscopic moisture in soils. Not the least of its merits is the ease with which it can be manipulated.

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ON THE FAUNAL ZONES OF THE RUSH-SKERRIES CARBONIFEROUS SECTION, CO. DUBLIN.

BY

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LECTURER IN PALÆONTOLOGY, UNIVERSITY OF DUBLIN.

(PLATES XXXV–XXXVII.)

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XLI.

ON THE FAUNAL ZONES OF THE RUSH-SKERRIES CARBONIFEROUS SECTION, CO. DUBLIN.

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(Plates XXXV–XXXVII.)

[Read May 18. Published August 13, 1915.]

Introduction.

This section has been excellently described by Dr. C. A. Matley in two papers published in 1906 and 1908 respectively.[1] At the same time an account of the faunal succession and correlation was given by Dr. Arthur Vaughan. The material with which Dr. Vaughan had to work was, for the most part, scanty and fragmentary, and his conclusions are in many cases put forward only tentatively, pending further evidence. This is especially the case with the parts of the section dealt with in this communication.

The present work was undertaken at the suggestion of Dr. Vaughan. Its principal object has been to accumulate more and better fossils, and thus to settle the outstanding questions of correlation.

Several things make this a difficult locality to deal with. The deposits were formed near a shore line, so that the fossils are usually very imperfect. Many of the rocks can only be reached for a short time at low tide, and are covered with seaweed. The section is broken up by gaps and faults, and the rocks are much folded, and, in places, cleaved.

The section occurs at the western extremity of the land barrier which, in Lower Avonian time, separated the Bristol-Dinant Gulf from the waters which deposited the northern and midland rocks.

The same horizons occur in the northern and southern parts of the section in different phase, the northern rocks containing scarcely any fine-grained detritus; whilst slates and shales are characteristic of the southern outcrop.

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[1] Quart. Journ. Geol. Soc., vol. lxii, 1906, p. 275, and vol. lxiv, 1908, p. 413.

This probably indicates, as Matley points out,[1] the proximity of the latter locality to a river mouth. It would explain the considerably greater thickness of the zones in the south.

The present paper deals with the following parts of the section (see map, Pl. XXXV):—

(1) From the southernmost exposure to the bathing-place north of Rush, including the Rush Slates, Rush Conglomerate, with its overlying Limestones, Carlyan Rocks and Kate Rocks;

(2) the Lane Limestone and Conglomerate, and the Holmpatrick Limestone at the northern end of the section, near Skerries.

The map is modified from Matley's maps of the outcrops.[2] The lower portion shows (1) the Rush Slates to the south and east of Rush. They dip first east, then north. Prominent fossiliferous limestone bands are marked (after Matley) R 1 b, R 4 a, R 6 b, and R 8 a. The faults are of insignificant throw. Next in order, northwards, comes (2) the Rush Conglomerate Group (more deeply coloured). This includes the beds to which Matley attached the symbols R 9, R 10, and R 11. It consists of frequently recurring beds of calcareous conglomerate, sandstone, shale, and limestone, dipping steeply to the north. Upon these lie (3) the Supra-Conglomerate Limestones forming the southern boundary of the entrance to Rush Harbour. These and the upper conglomerates are much faulted, but the displacement is never more than a few feet. The succession is broken here by sand and water. North of Rush Harbour are (4) the Carlyan Limestones. These dip north at a low angle, are often pebbly and sometimes oolitic. After a stretch of sand occur (5) the Kate Limestones, dipping mostly northwards, but much folded. Chert layers are common here, and pebbles rare. Just north of these rocks a mile and a half of the coast-line has been omitted from the map. The next beds which concern us occur half a mile north of Loughshinny (Lane Limestone). They are bounded on the south by a branched fault of considerable throw. The dip is nearly north and at low angles. A coarse uniform conglomerate (Lane Conglomerate, coloured more deeply) succeeds this conformably, and is overlain conformably by the Holmpatrick Limestone, both dipping at low angles nearly northwards. The Holmpatrick Limestone is much dolomitized in the lower half, and contains oolitic layers near the base.

Before giving details of the present work, it will be useful to review the state of the problem, in order to exhibit the difficulties which require clearing up.

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[1] Quart. Journ. Geol. Soc., vol. lxiv, 1908, p. 434.

[2] loc. cit.

In 1906 Dr. Vaughan proposed the following correlation for the rocks at the southern end of the section[1] :—

Kate Limestones,		S (? S_2)
gap.		
Carlyan Limestones,		S (? S_1)
gap.		
Supra-Conglomerate Limestones,	70 ft.	S_1
	30 ft.	C_2—S_1
Rush Conglomerate Group,	330 ft.	
	170 ft.	C_1
Passage Beds,	40 ft.	
Rush Slates.	300 ft.	
	1080 ft.	Z_2

In 1908, when dealing with the northern end of the section, doubt was thrown on the foregoing, and the following suggestions were made[2] :—

Holmpatrick Limestone,	180 ft.	? D of unknown position.	? Carlyan Limestone.
Lane Conglomerate,	200 ft.		? Rush Conglomerate Group.
Lane Limestones,	60 ft.		? Rush Slates (top part only).

The position of the Rush Slates and Conglomerates is reconsidered in this paper, and the position is summed up as follows[3] :—"The broad resemblance to a $Z_2 - C - S_1$ sequence is not confirmed by identity of detail. On the other hand, the evidence for a D horizon is far from conclusive, and the failure to find an unquestionably Viséan form in the whole thickness of the *Zaphrentis* slates is without parallel in fossiliferous D beds. The final solution of the question of level must await fresh evidence."

From a consideration of the Clitheroe district in Lancashire Dr. Vaughan got some confirmation of his first correlation, and in August, 1911, referred to a clearing up of difficulties with regard to the position of the Rush Slates and Conglomerates and the Carlyan Limestone.[4]

This, however, he now considers was premature, and in December, 1912,

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[1] Quart. Journ. Geol. Soc., vol. lxii, 1906, p. 279.

[2] Quart. Journ. Geol. Soc., vol. lxiv, 1908, p. 434.

[3] loc. cit., p. 442.

[4] Quart. Journ. Geol. Soc., vol. lxvii, 1911, p. 381, foot-note.

he suggested to the author that, living within easy reach of the locality, he should make an attempt to settle the matter. As a result of studying the material accumulated since then, the following correlation is now suggested (the measurements are after Matley):—

SOUTH-WESTERN PROVINCE.	RUSH-SKERRIES SECTION.		NORTH-WESTERN PROVINCE.
	Southern part.	Northern part.	
S_1	Kate Rocks. 90 ft.	top not exposed.	*Productus corrugato-hemisphericus* zone.
	Gap.	Holmpatrick Limestone, 180 ft.	
C_2	Carlyan Limestone. 180 ft.		*Michelinia grandis* zone.
	Gap.		
	Supra-Conglomerate Limestone 100 ft.		
	360 ft. Rush Conglomerate Series. 540 ft.	Lane Conglomerate, 200 ft.	*Athyris glabristria* zone.
C_1	180 ft.		
	780 ft. Rush Slates 1380 ft.	Lane Limestone, 60 ft. fault	
Z_2	600 ft. base not exposed.		

It will be seen that, in the case of the southern beds, Vaughan's first correlation, and the one to which he reverted, is confirmed in the main. The base of C, however, has been shifted from R 8 a[1] down to R 6 b (480 feet), and the base of S is located in or about the Carlyan Limestone.

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[1] See map, Pl. XXXV.

The chief change, necessitated by the new evidence, is the shifting of the Lane and Holmpatrick beds bodily from D to C and S_1. This settles the long-debated question of the equivalence of the two conglomerate groups. They *are* equivalent, though, perhaps, not exactly coincident. Possibly the Lane Limestone, which contains frequent, inconstant pebbly and shaly layers, may be represented, at least in part, by the lowest beds of the Rush Conglomerate Group. The evidence is not sufficient to decide this.

The fresh material has enabled a close correlation to be made with the exposures round the English Lake country (north-western province), the Holmpatrick fauna containing a great many species in common with the Arnside and other exposures of Garwood's *Chonetes-carinata* sub-zone and "Gastropod Beds."[1]

Acknowledgments.

To Dr. Arthur Vaughan the author is indebted for initiating him into the subject of Carboniferous Zoning, for suggesting the present research, and for frequent help during its progress; to Professor J. Joly, F.R.S., for giving him every facility and encouragement in carrying out the work, and for valuable advice in photographing the coral slices; to Dr. C. A. Matley for the generous loan of his collection from the locality. At least one half the fossils were collected by Mrs. L. B. Smyth.

Faunal Lists and Correlation.

(It has been thought unnecessary to indicate by any mark the species already recorded. They can be ascertained by a glance at the original papers, to which the reader is also referred for particulars of stratigraphy and lithology.)

Holmpatrick Limestone.

Corals:—

Cyathaxonia rushiana, Vau.
,, *cornu*, Mich.
Zaphrentis cf. *enniskilleni*, Edw. & H.*
,, *ambigua*, Car.
,, cf. *densa*, Car.
,, sp.
Densiphyllum aff. *rushianum*, Vau.
Caninia sp.
Campophylloid *Caninia*.
Diphyphyllum subibicinum, M'Coy.*

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[1] Quart. Journ. Geol. Soc., vol. lxviii, 1912, pp. 468 and 470.

Amplexus sp.
Cyathophyllum ϕ, Vau.
Lithostrotion Martini, Edw. & H.*
Clisiophyllid, gen. nov.
Koninckophyllum ashfellense, Gar.*
,, aff. θ, Vau.
,, *densum*, sp. nov.
Carcinophyllum aff. θ, Vau.
Syringopora cf. *ramulosa*, Goldf.
,, cf. *geniculata*, Phil.*
Michelinia sp.

Brachiopods :—

Productus cf. *concinnus*, Sow.
,, *concinno-longispinus*.
,, cf. *plicatilis*, Sow.
,, *youngianus*, Dav.
,, *spinulosus*, Sow.
,, *aculeato-fimbriatus*.
,, *punctato-fimbriatus*.
,, *punctatus*, Mart.* (?)
,, cf. *ovalis*, Phil.
,, *margaritaceus*, Phil.
,, *corrugato-hemisphericus*.*
Chonetes cf. *papilionacea*, Phil. vars.*
Leptaena sp.*
Schellwienella cf. *crenistria*, Phil.*
Schizophoria resupinata (very large).
Rhipidomella Michelini, L'Eveillé.*
Spirifer aff. *bisulcatus*.
,, sp.
Syringothyris cuspidata, Mart.*
Martinia cf. *ovalis*, Phil.*
,, cf. *glabra*, Mart.*
Athyris cf. *glabristria*, Phil.
,, cf. *planosulcata*, Phil.*
,, cf. *globularis*, Phil.
,, *expansa*, Phil.* (?)
,, *ovalis*, Vau.
,, sp. (cf. Dav. Mon. Brit. Carb. Brach. Pl. lv, fig. 4.)
Seminula cf. *ambigua*, Sow.

Camarotoechia cf. *flexistria*, Phil.
,, cf. *pleurodon*, Phil.*
Dielasma cf. *hastatum*, Sow.*
,, cf. *gillingensis*, Dav.

Faunal characters:—

Chonetes cf. *papilionacea*, vars. and *Diphyphyllum subibicinum* are common throughout.

In the middle parts the following are common at a few levels:—

Large *Schizophoria*.
Bisulcate Spirifers.
Concinnoid Producti.
Schellwienella cf. *crenistria*.
Productus margaritaceus.
Cyathaxonia rushiana.
Zaphrentis enniskilleni.
Lithostrotion Martini.

The upper parts are characterized by the abundance of *Productus corrugato-hemisphericus*. The specimens of *Michelinia* obtained are fragmentary. They resemble *M. tenuisepta*, but have larger tubes and thicker walls. Possibly they belong to *M. grandis*. Similar fragments were obtained from the Carlyan Limestone.

In 1908 Vaughan[1] placed these beds, together with the Lane Limestone and Lane Conglomerate, in D, but with some hesitation, owing to the "imperfect and scanty material." The evidence relied upon was the presence of the following:—

(1) Planicostate Spirifers.
(2) "A single, poor specimen" of *Productus humerosus*, Sow. "found loose" in the Lane beds.
(3) *Productus longispinus*.
(4) *Martinia glabra*.
(5) *Michelinia* cf. *tenuisepta*.
(6) A new genus of Clisiophyllid, of which only two other specimens were recorded, one from the top of the Midland Massif, near Wetton, the other from the *Cyathaxonia*-beds of Rush.
(7) A new species of Densiphylloid *Zaphrentis* also occurring in the neighbouring Dibunophyllum beds which have an undoubted D fauna.

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[1] Quart. Journ. Geol. Soc., vol. lxiv, 1908, p. 439.

(8) The *Zaphrentes* of the Lane Limestone are identical with those of the Rush Slates, and agree better with the (?) D forms of the Colne area than with the Tournasian Zaphrentes of the South-Western Province. *Z. Omaliusi* var. *ambigua* . . . is, as yet, not certainly known below D_1.

In view of subsequent work, including the above faunal list, we may remark on these points as follows:—

(1) Planicostate Spirifers have not been found in the course of the present work.

(2) Several specimens of this shell, also poor, but *in situ*, have been found in the Lane beds. It is evidently *P. sub-lævis*.

(3) This is not a typical *P. longispinus*, but, as recorded in Vaughan's faunal list, *P. concinno-longispinus*.

(4) Several fragmentary pedicle valves of a small glabrous Spiriferid without dental plates were found. It would be unsafe to base any conclusion upon them.

(5) The *Michelinia* was only obtained in fragments embedded in matrix. It is now suggested that it may be *M. grandis*.

(6) and (7) These points could only be regarded as corroborative evidence, and have little weight considered alone.

(8) The Rush Slates are undoubtedly of Z-C age (vide infra). *Z. ambigua* is common in the lowest exposure of the Rush Slates, below a typical γ fauna.

HOLMPATRICK LIMESTONE = C S = PARTS OF THE *CHONETES CARINATA* SUB-ZONE AND "GASTROPOD BEDS" OF THE NORTH-WESTERN PROVINCE.

Correlation with the North-Western Province is based upon the following association:—*Diphyphyllum subibicinum,* varieties of *Zaphrentis Enniskilleni, Lithostrotion Martini, Koninckophyllum ashfellense,* large *Schellwienella* cf. *crenistria* and *Productus corrugato-hemisphericus.* All the names, too, marked with an asterisk in the above faunal list occur in Garwood's lists for the above-mentioned zones.[1]

This correlation involves the equivalence of the Holmpatrick beds to C S of the South-Western Province. The following points support this conclusion:—

Lithostrotion Martini is first found in S_1 of the S. W. P.

Cyathophyllum ϕ is a δ species.

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[1] Quart. Journ. Geol. Soc., vol. lxviii, pp. 468 and 470.

The Campophylloid *Caninia* is a typical C S form.

Koninckophyllum aff. θ and *Carcinophyllum* aff. θ (Pl. XXXVI, fig. 5) are both less "advanced" than the typical forms from S_2 and D_1.

Productus corrugato-hemisphericus becomes common near the top.

LANE CONGLOMERATE.

Unfossiliferous.

LANE LIMESTONE.

Corals :—

Cyathaxonia cornu, Mich.
Zaphrentis ambigua, Car.
   ,,   ,,   ,, var. *a*, common.
   ,,   *omaliusi*, Edw. & H.
Amplexus coralloides, Sow.
Syringopora cf. *reticulata*, Goldf.
   ,,   cf. *geniculata*, Phil.

Brachiopods :—

Productus cf. *sub-lævis*, de Kon. (two spp.).
   ,,   sp. (fimbriate).
   ,,   sp. (semireticulate).
Chonetes cf. *comoides*, Sow. common.
Orthotetids (? two spp.) common.
Reticularia lineata, Dav.
Spirifer attenuatus, Sow. ?
Schizophoria resupinata.
Leptaena analoga.

$$\left.\begin{array}{ll}\text{LANE CONGLOMERATE} & = C_2 \\ \text{LANE LIMESTONE} & = C_1\end{array}\right\}\text{APPROXIMATELY.}$$

Assuming that the Holmpatrick Limestone is of C S age (*vide supra*), the Lane Conglomerate and Limestone must lie in C. The former is unfossiliferous. The latter agrees with C_1 of the S.W.P. in the abundance of *Chonetes* cf. *comoides*, and large Euomphali.

Productus cf. *sub-lævis* suggests C_2.

Abundance of *Zaphrentis omaliusi* and *Z. ambigua* suggests C_1 or lower.

Syringopora cf. *reticulata* appears to be the same as the form occurring in the Rush Conglomerate group = C_2 (vide infra).

It seems probable, then, that the Lane Limestone is high in C_1. (See also under Rush Slates and Conglomerates, p. 553.)

Kate Rocks.

Corals :—

Cyathaxonia cornu, Mich. common.
Zaphrentis densa, Car.
,, cf. *constricta*, Car.
,, cf. *enniskilleni*, Edw. & H.
Densiphyllum nodosum, sp. nov.
Caninia sp.
Diphyphyllum subibicinum, M'Coy.
Cyathophyllum φ, Vau.
Koninckophyllum sp.
Clisiophyllum sp.
Carcinophyllum sp. (? aff. θ).
Syringopora cf. *ramulosa*, Goldf.
Michelinia sp.

Brachiopods :—

Chonetes (convex papilionacean).
Schizophoria.
Productus fragments (cf. *P. corrugato-hemisphericus*).
Reticularia sp. ?

Kate Rocks = some part of Holmpatrick Limestone = C S.

This correlation is based on the following association, common to the Kate and Holmpatrick beds, and not found elsewhere in the sequence :—

Diphyphyllum subibicinum, common.
Cyathophyllum φ.
Caninia sp.
Syringopora cf. *ramulosa*.

The commonest brachiopod species in the Holmpatrick beds are *Chonetes* cf. *papilionacea*, *Productus corrugato-hemisphericus* and *Schizophoria resupinata*. The Kate Rocks are full of very fragmentary remains of these three genera, which agree very well with the Holmpatrick species.

The *Zaphrentis* cf. *enniskilleni*, *Carcinophyllum*, and *Michelinia* sp. may be the same.

CARLYAN LIMESTONE.

Corals :—

Cyathaxonia cornu, Mich.
Zaphrentis aff. *omaliusi*, Edw. & H.
,, cf. *enniskilleni*, Edw. & H.
Campophyllum caninoides, Sibly. ?
Amplexus sp.
Diphyphylloid *Lithostrotion*.
Koninckophyllum carlyanense, sp. nov.
Clisiophyllum cf. *oblongum*, Thoms. (Pl. XXXVII, fig. 5).
,, *dublinense*, sp. nov.
,, *spissum*, sp. nov.
Arachniophyllum simplex, gen. et sp. nov.
Michelinia sp.

Brachiopods :—

Productus cf. *concinnus*, Sow.
,, *concinno-longispinus*.
,, cf. *plicatilis*, Sow.
,, cf. *pyxidiformis*, de Kon.
,, *aculeato-fimbriatus*.
,, *fimbriato-pustulosus*.
,, spp. (incl. fragments of a corrugate form).
Chonetes cf. *papilionacea*, Phil.
Leptaena sp.
Schizophoria resupinata.
Orthotetid.
Syringothyris cuspidata, Mart. ? (fragment).

These beds are separated from the Supra-Conglomerate Limestones by the entrance to Rush Harbour. Matley says "it seems probable that there is a strike fault here, though of no great throw."[1]

The evidence now obtained shows that the brachiopod faunas north and south of the gap are nearly identical. We may take it then that the horizons cannot be far apart.

CARLYAN LIMESTONE = HOLMPATRICK LIMESTONE (PART) = C S.

The following species occur in the Carlyan and Holmpatrick Limestones in indistinguishable forms :—

Productus cf. *concinnus*.
,, *concinno-longispinus*.

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[1] Quart. Journ. Geol. Soc., vol. lxii, 1906, p. 289.

Productus cf. *plicatilis.*
,, *fimbriato-pustulosus.*
,, *aculeato-fimbriatus.*
Chonetes cf. *papilionacea.*
Leptaena sp.
Michelinia sp.

The suggested equivalence is also supported by the following considerations:—

(1) To the north of the Carlyan Rocks, and only separated from them by a short stretch of sand, are the Kate Rocks, which have been shown to be probably equivalent to a part of the Holmpatrick Limestone.

(2) The Holmpatrick Limestone is succeeded downwards by a conglomerate. The Carlyan Rocks are separated on the south by the entrance to Rush Harbour from 100 feet of limestone (Supra-Conglomerate Limestones) containing an almost identical brachiopod fauna. This is immediately underlain by a conglomerate.

Very little direct evidence can be adduced for correlation with C S. A Diphyphylloid *Lithostrotion* occurs, and the Clisiophyllids (*C. dublinense* and *spissum*) are of C S type (cf. *C. ingletonense*).

SUPRA-CONGLOMERATE LIMESTONES.

Productus cf. *concinnus*, Sow., vars., abundant.
,, *aculeato-fimbriatus.*[1]
,, *punctato-fimbriatus.*
,, *fimbriato-pustulosus.*
,, cf. *pyxidiformis*, De Kon.
,, sp.
Chonetes sp. (large, convex; fragments abundant).
,, *squamata*, sp. nov.
Leptaena "*analoga*" abundant.
Spirifer sp. abundant.
Dielasma cf. *hastatum*, Sow.
Camarophoria pleurodon, Phil.

These beds may be taken along with the Carlyan Limestone (C S), as the brachiopod faunas are almost identical.

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[1] Quart. Journ. Geol. Soc., vol. lxii, 1906, Pl. xxx, fig. 6.

RUSH SLATES AND RUSH CONGLOMERATE.

Before presenting the new evidence from these beds we must glance at a discussion of their position in the Loughshinny paper of Matley and Vaughan.[1] Here Vaughan says that the correlation suggested in their Rush paper,[2] namely—

Rush Slates—Z_2 ; Rush Conglomerate and Carlyan Limestone—C to S_1, depends upon the similar succession of similar coral-faunas, as set out in the following table :—

	Z_2	C–S_1
In the South-Western Province.	Abundance of *Z. Omaliusi.* Absence of (1), (2), (3), (4).	Entrance and abundance of (1) *Michelinia* cf. *megastoma.* (2) Cyathophyllids. (3) *Lithostrotion.* Entrance (and rarity) of (4) Clisiophyllids of *Lithostrotion*-type.
At Rush.	Abundance of *Z. densa* and *Z. ambigua.* Absence of (*a*), (*b*), (*c*) (notwithstanding the great thickness of the series).	(*a*) *Michelinia megastoma* (common). (*b*) *Lithostrotion cyathophylloides* (abundant). (*c*) Clisiophyllids of *Lithostrotion*-type (rare).
	Rush Slates.	Rush Conglomerate.

He then gives five points which seem to throw doubt upon this correlation :—

"(*a*) *Zaphrentes*, identical with those of the Rush Slates, occur in equal abundance near Colne and in the Lane Limestone, and in both cases the horizon is probably D. . . .

"(*b*) A specimen of *Michelinia tenuisepta,* found loose near the top of the Rush Conglomerate, is identical with the form common in the *Cyathaxonia* beds here assigned to D_{3b}, and is not known from the C–S_1 level.

"(*c*) *Clisiophyllum* cf. *oblongum* bears a much closer resemblance to the highly developed Clisiophyllids of D than to the *Lithostrotion*-like Clisiophyllids of S_1.

"(*d*) *Michelinia megastoma* (Phill.) is probably a D species ; . . .

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[1] Quart. Journ. Geol. Soc., vol. lxiv, 1908, pp. 441–2.

Quart. Journ. Geol. Soc., vol. lxii, 1906, p. 300

"(*e*) . . . *Productus* cf. *fimbriatus* . . , from the top of the *megastoma* beds, occurs commonly in P."

Some of these points may now be discarded :—

(*a*) The Lane Limestone has been shown to be of C_1 age.

(*d*) *Michelinia megastoma* has its maximum in C S. (see Quart. Journ. Geol. Soc., vol. lxvii, 1911, p. 371).

(*e*) *Productus* cf. *fimbriatus* (here called *P. aculeato-fimbriatus*) occurs also in the Holmpatrick Limestone (C S) and the Curkeen Hill Quarry (D), so that it has probably a very long range.

(*b*) is based on a loose specimen.

(*c*) remains for what it is worth.

Vaughan next adds two points against the correlation with D suggested in the arguments just enumerated.

"(*a*) The dominant coral of the *megastoma*-beds and of the Carlyan Limestone—*Lithostrotion cyathophylloides*—is markedly more Cyathophylloid than *Lithostrotion* cf. *affine* of the D P phase, and approaches nearest to a form rare in C S_1 of the South-Western Province and of Ravenstonedale.

"(*b*) The *Lithostrotion*-like Clisiophyllids of the *megastoma*-beds and of the Carlyan Limestone agree more closely with S_1 forms from the South-Western Province, the Ingleborough area, and Arnside than with any D form yet known."

There follows a "faunal comparison of the Rush and Lane Conglomerates" as follows :—

"Resemblances :—

"(1) An identical *Zaphrentis*-facies precedes each.

"(2) *Michelinia* succeeds *Zaphrentis* as a common fossil.

"Differences :—

"(1) The Rush Conglomerate is fossiliferous; the Lane Conglomerate barren.

"(2) *Michelinia megastoma* is common in the Rush, but rare above the Lane Conglomerate. In the case of *Michelinia* cf. *tenuisepta* the reverse is true.

"(3) *Campophyllum* aff. *Murchisoni* and *Diphyphyllum subibicinum* abound, above the Lane, and are absent from the Rush Conglomerate.

"(4) *Lithostrotion cyathophylloides* abounds in the Rush; no *Lithostrotion* is met with above or below the Lane Conglomerate.

"(5) *Chonetes* cf. *comoides* abounds at the top of the Lane Limestone, but is absent from the Rush Slates: papilionaceous *Chonetes* are, however, common in the Carlyan Limestone."

It will be seen that in this paper the conglomerates are taken as of the same age. It is therefore necessary to examine the comparison just quoted. With the resemblances given the author is in agreement. The differences will be considered in turn :—

(1) This is so, but is easily explained by difference in conditions of deposit, e.g. distance from shore or from river-mouth.

(2) The two species were found to be equally common in the Rush Conglomerate. *M. megastoma* was not found *above* either conglomerate, but above both occurs a form resembling *M. tenuisepta.*

(3) *Campophyllum* aff. *Murchisoni* was not found. *Diphyphyllum subibicinum* occurs in the Kate Rocks, which apparently belong not far above the Rush Conglomerate, and which resemble the beds above the Lane Conglomerate in other ways (*vide supra*).

(4) The second part of this statement does not now hold (see faunal list for Holmpatrick Limestone, above).

(5) *Chonetes* cf. *comoides* occurs in the upper part of the Rush Slates (see faunal list).

RUSH CONGLOMERATE (UPPER PART).

R 10 & 11 (except R 10 b & c).

Corals :—

Cyathaxonia cornu, Mich.
Zaphrentis densa, Car.
,, cf. *enniskilleni*, Ed. & H.
,, cf. *disjuncta*, Car.
,, *amplexoides*, Wilmore.
Densiphyllum aff. *rushianum*, Vau.
,, *nodosum*, sp. nov.
Caninia sp. (large).
Campophyllum caninoides, Sibly.
,, *ciliatum*, Gar.
Lithostrotion cyathophylloides, Vau.
Lophophyllum costatum, M'Coy.
Arachniophyllum simplex, gen. et sp. nov.
Carruthersella compacta, Gar.

Carcinophyllum sp. ?
Clisiophyllid[1].
Syringopora cf. *reticulata*, Goldf.
Michelinia megastoma, Phil.
  ,, *tenuisepta*, Phil.
Fistulipora sp.

Brachiopods:—

Productus cf. *concinnus*, Sow.
  ,, cf. *semireticulatus*, Mart.
  ,, *margaritaceus*, Phil.
  ,, *aculeatus*, Mart.
  ,, *spinulosus*, Sow.
  ,, *aculeato-fimbriatus*.
  ,, *fimbriato-pustulosus*.
  ,, cf. *rugatus*, Phil.
  ,, cf. *pyxidiformis*, de Kon.
  ,, *punctato-fimbriatus*.
  ,, cf. *punctatus*, Mart.
Chonetes cf. *papilionacea*, Phil.
  ,, *squamata*, sp. nov.
Spirifer cf. *bisulcatus*, Sow.
Syringothyris subconica, Mart. ?
Athyris ingens, de Kon.
Schizophoria resupinata.

RUSH CONGLOMERATE (UPPER PART) = *Seminula gregaria* SUB-ZONE (UPPER PART), AND THE *Camarophoria isorhyncha* SUB-ZONE (N.W.P.) = C_2.

In addition to the evidence noticed in the course of the discussion given above, the following points may be mentioned:—

The Rush Conglomerate is immediately subjacent to the Supra-Conglomerate Limestones. These have been correlated with CS, therefore the Conglomerate should be of C_2 age.

Campophyllum caninoides is characteristic of C_2 in the Mendip area.

Carruthersella compacta and *Campophyllum ciliatum* are found in the '*Spirifer furcatus* Band' of the North-Western Province, assigned to the middle of C_2.

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[1] Cf. Quart. Journ. Geol. Soc., vol. lxiv, 1908, p. 464.

Rush Slates (upper part) and Rush Conglomerate (lower part).
R 7, 8, 9, 10 b, & 10 c.

Corals :—

- *Cyathaxonia cornu*, Mich.
- *Zaphrentis omaliusi*, Edw. & H.
  - ,, *ambigua*, Car.
  - ,, ,, var. *a*.
  - ,, *densa*, Car.
- *Caninia cornucopiae*, Mich.
  - ,, *patula*, Mich. ? fragmentary.
- Campophyllid, fragmentary.
- *Amplexus* sp.

Brachiopods :—

- *Productus margaritaceus*, Phil.
  - ,, cf. *rugatus*, Phil.
  - ,, sp. (pustulose).
- *Chonetes* cf. *comoides*, Sow.
  - ,, cf. *hardrensis*, Phil., common.
- Orthotetid, common, fragmentary.
- *Leptaena* sp.
- *Athyris expansa*, Phil.
  - ,, *glabristria*, Phil.
  - ,, *lamellosa*, L'Ev.
- *Spirifer* cf. *bisulcatus*, Sow.
- *Spiriferina laminosa*, M'Coy.
- *Schizophoria resupinata*, common.

Rush Slates, R 6 b.

Corals :—

- *Cyathaxonia cornu*, Mich., common.
- *Zaphrentis ambigua*, Car., common.
  - ,, *delanoui*, Edw. & H.
  - ,, *amplexoides*, Wilmore ?
  - ,, spp.
- *Caninia cornucopiae*, Mich., common.
- *Caninia patula*, Mich. ? fragment.
- *Amplexus* cf. *Sowerbyi*, Phil.
- *Michelinia* sp.

Brachiopods :—

Productus aculeato-fimbriatus.
,, *margaritaceus*, Phil.
,, cf. *mesolobus*, Phil.
,, *doulaghensis,* Vau. (St. Doulagh's form).
,, cf. *concinnus,* Sow. (cf. St. Doulagh's form).
Chonetes cf. *hardrensis,* Phil.
,, sp. (? early papilionacean).
Orthotetid (ornament as in St. Doulagh's form).
Leptaena sp.
Athyris glabristria, Phil. (near type).
Spiriferina cf. *octoplicata,* Sow.
Martinia pinguis, Sow.
,, *rotundata,* Sow.
Reticularia cf. *reticulata,* M'Coy.
Spirifer cf. *attenuatus,* Sow.
,, aff. *clathratus,* M'Coy.
,, cf. *convolutus,* Phil.
Syringothyris cuspidata, Mart.
Schizophoria resupinata, Mart.
Dielasma cf. *hastatum,* Sow.
Pugnax cf. *pugnus,* Mart. (St. Doulagh's form).
'*Rhynchonella*' cf. *platyloba,* Sow.
Camarophoria pleurodon, Phil.
Orbiculoidea sp.

RUSH SLATES, UPPER (R 6, 7, 8), AND RUSH CONGLOMERATE SERIES, LOWER (R 9, 10 b, 10 c) = C_1.

The chief elements of the fauna of the Slates continue into the lower Conglomerates. The most marked faunal change seems to occur above R 10 c. Here, for instance, *Zaphrentis ambigua* and *Caninia cornucopiae,* so plentiful below, cease to be found. Here, too, *Michelinia megastoma* first appears. This level has therefore been taken as the base of C_2. R 6 has been fixed as the lower limit (γ) of C_1 for the following reasons :—

1. The earliest trace of a vesicular Caninid occurs here.
2. *Syringothyris cuspidata, Martinia pinguis, Martinia rotundata, Productus doulaghensis, Pugnax* cf. *pugnus* (St. Doulagh's form), &c., are C_1 shells.
3. *Zaphrentis delanoui* (typical form) is not known above γ.
4. There are no traces of large *Chonetes.*

Other C_1 species occurring between R 6 and R 10 c are *Chonetes* cf. *comoides* and *Spiriferina laminosa.*

COMPARISON OF LANE LIMESTONE WITH RUSH SLATES, UPPER + RUSH CONGLOMERATES, LOWER.

Both have been assigned above to C_1. Although their faunas differ considerably, as do their lithological characters, the following points of similarity may be noted :—

Chonetes cf. *comoides* occurs, and the same Orthotetid is common in both.

Zaphrentis ambigua is common in the lower beds, and dies out upwards in both cases.

Zaphrentis ambigua. var. *a*, which is the characteristic coral of the Lane Limestone, is also found in the upper Rush Slates.

Some of the shaly beds in the Lane Limestone have a very strong resemblance to certain beds about R 8 and 9, being full of black, lustrous Orthotetid fragments, Ostracods, Mollusca, and Phillipsids.

RUSH SLATES
lowest exposure to R 4 a (incl.).

Corals :—

Cyathaxonia cornu, Mich. common.
Zaphrentis ambigua, Car. common.
,, *delanoui*, Edw. & H.
,, cf. *disjuncta*, Car.
,, *amplexoides*, Wilmore.
Densiphyllum nodosum, sp. nov.
Caninia cornucopiae, Mich. common.
Amplexus cf. *Sowerbyi*, Phil.
,, cf. *coralloides*, Sow.

Brachiopods :—

Productus Wrightii, Dav.
,, cf. *concinnus*, Sow.
,, *burlingtonensis*, Hall. ?
,, cf. *rugatus*, Phil.
,, cf. *youngianus*, Dav.
,, *laciniatus*, M'Coy. ?
Chonetes cf. *laguessiana*, de Kon.
Orthotetid (fragmentary).

'*Lingula*' *mytiloides*, Sow.
Athyris glabristria, Phil., incl. mut. Z_2.
Spiriferina laminosa, M'Coy.
Spirifer clathratus, M'Coy.
,, *römerianus*, de Kon.
Martinia pinguis, Sow.
Reticularia cf. *reticulata*, M'Coy.
Ambocoelia Urii, Flem. ?
Schizophoria resupinata, Mart.
Rhipidomella Michelini, L'Ev.
Dielasma aff. *Kingi*, de Kon.
,, cf. sacculus, Mart.
Pugnax cf. *pugnus*, Mart.
Camarophoria pleurodon, Phil.

RUSH SLATES, LOWER = Z_2.

These beds should be at the top of Z_2 if the previous correlations are correct. The fauna seems to agree with this position. We may note especially *Athyris glabristria*, mut. Z_2, *Zaphrentis delanoui*, *Chonetes* cf. *laguessiana*, *Spirifer clathratus*, and *Productus burlingtonensis* as characteristic Z forms. *Spirifer römerianus* is known from C_1.

NOTES ON SOME OF THE SPECIES.

Zaphrentis cf. *densa*, Car. (Pl. XXXVI, fig. 3.)

HOLMPATRICK LIMESTONE.

Only one specimen obtained. Younger parts missing. A transverse section near the top is almost identical with *Z. densa*, Car. (type),[1] except that the wall is thicker. A section a little above this (where the structure is probably mature) shows the following differences from *Z. densa*, Car.

(1) The wall thicker still (diam. of sect. 8 mm., thickness of wall 1·5 mm.).
(2) The septa (28 in number) more slender.
(3) The fossula more U-shaped.
(4) The cardinal septum extremely shortened.

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[1] Geol. Mag., 1908, Pl. iv, fig. 7.

Zaphrentis ambigua, var. *a*. (Pl. XXXVI, fig. 7.)

Lane Limestone, common.

Rush Conglomerate Series, R 10 b & c.

This variety seems sufficiently distinct to merit notice. It differs from the typical *Z. ambigua*, Car.,[1] in the following particulars:—Smaller size; thicker septa; counter fossula less exaggerated; counter septum almost or quite reaching central mass; inner ends of septa more or less club-shaped (this is particularly noticeable in the counter septum); cardinal fossula with sides parallel or, more commonly, slightly convergent inwards, and, at the inner end, rapidly converging to form V-shaped termination; cardinal septum reduced to a mere tooth; intermediates scarcely distinguishable.

Zaphrentis cf. *constricta*, Car. (Pl. XXXVI, fig. 8).

Kate Rocks.

A transverse section immediately below the calyx shows 23 septa whose radial disposition is striking. In the counter quadrants there is a slight curvature of the septa concave to the cardinal region. The cardinal fossula is constricted as in *Z. constricta*, Car. The cardinal septum is very short. Minor septa rudimentary, but distinct. A counter fossula is present.

In a lower section the grouping of septa into four groups is noticeable, but their radial arrangement and straightness are still the striking characteristics. A section of the young part has the typical "*Omaliusi*" structure.

This coral seems to be closely related to *Z. densa*, Car., but at no stage are the septa merged in a central mass, the interseptal loculi reaching far in towards the centre.

Zaphrentis cf. *disjuncta*, Car. (Pl. XXXVI, fig. 12.)

Rush Conglomerate Series, R 10.

This coral shows a very considerable resemblance to *Z. disjuncta*, Car. early mut.[2] The only difference appears to be in the neanic stage, which is exactly as in *Z. delanoui*, s.s. No "*constricta*" stage has been observed. The section figured was more than 13 mm. below floor of calyx.

Rush Slates (lowest exposed beds). A single small specimen found at this level may be an early form of the above.

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[1] Geol. Mag., 1908, Pl. iv, fig. 5. The specimen figured by Carruthers is from Horrocksford Quarry, near Clitheroe, which is now abundantly proved to be not higher than C_1.

[2] Quart. Journ. Geol. Soc., vol. lxvi (1910), p. 534, and Pl. xxxvii, fig. 6.

Zaphrentis cf. *enniskilleni*, Edw. & H.

Several forms are included in this term. Two distinct forms are figured.

1. KATE ROCKS. (Pl. XXXVI, fig. 9.)

The young stage agrees very well with that of *Z. oystermouthensis*.

A transverse section of the adult stage has the following characters:—Diam. 9½ mm. Wall thick (·8 mm.). Septa slender, twenty-four in number. Cardinal fossula reaching beyond the middle of the section; inner half parallel-sided, outer half expanding to the wall, bounded by single septum on each side. Cardinal septum short. Alar fossulæ well marked, very oblique to plane of symmetry. Septa convex to cardinal fossula.

A similar form occurs at R 11 g in the Rush Conglomerate.

2. RUSH CONGLOMERATE, R 11 a. (Pl. XXXVI, fig. 14.)

A transverse section of the adult stage has the following characters:—Diam. 9 mm. Septa twenty-three in number. Septal plan as in *Z. enniskilleni.* Fossula wedge-shaped, tapering towards the centre, bounded by two septa only. Cardinal septum thin, and reaching the centre. Septa in cardinal quadrants strongly thickened, especially at their inner ends. Antifossular group rather short and less strongly thickened, their ends meeting. Counter septum shortened.

Densiphyllum nodosum, sp. nov. (Pl. XXXVI, fig. 13.)

The specimen described is from the RUSH CONGLOMERATE (R 11 d), but the species was also found in the KATE ROCKS, and in the lowest exposed beds of the RUSH SLATES.

Transverse section (diam. 8 mm.):—There are twenty-two major septa, curved throughout concave to the cardinal fossula, and very evenly spaced. Each major septum is strongly thickened, the stereoplasm being thickest a short distance from the wall of the coral, and from that point thinning gradually towards the centre and rapidly towards the wall. Thus each septum bears a node-like swelling. Minor septa are present as short blunt teeth. The interseptal spaces have the form of a Y with a long shank and short prongs. The cardinal septum is straight and somewhat thinner than its neighbours. Otherwise no septal break is observable.

At a diameter of 65 mm. the transverse section closely resembles the mature *Zaphrentis densa*, Car., except that some of the septa exhibit beginnings of nodal thickening. A very young stage (diam. 3 mm.) is the same as the young *Z. densa*.

In the immature stages of some specimens the counter septum and its immediate neighbours are grouped, so that their inner ends are parallel. This often produces the appearance of a counter fossula.

The exterior of the specimen described above is not known, but a specimen from a slightly lower level in the Rush Conglomerate (R 11 a) is cornute, costate, about 17 mm. long, and with a calyx 10 mm. in diameter.

Dr. Vaughan has shown the author several slices made from specimens of this coral collected some years ago at Malahide (Co. Dublin) in beds which he correlates with the Rush Slates.

Densiphyllum aff. *rushianum*, Vau. (Pl. XXXVI, fig. 4.)

HOLMPATRICK LIMESTONE.

RUSH CONGLOMERATE, top of R 10.

This form agrees with the type in the remarkable regularity of the septa, thickening of their extremities, absence of alar breaks, constriction of the inner end of the fossula, and presence of rudimentary minor septa.

It differs in the following respects:—It attains a greater size, the septa are much stouter, the wall is not so thick in proportion, and the epitheca bears well-marked costae.

Several specimens were found near the middle of the Holmpatrick Limestone and a single small specimen in the Rush Conglomerate, at the top of R 10. These two horizons are (according to the correlation suggested in this paper) at the top and bottom of C_2, respectively.

Koninckophyllum densum, sp. nov. (Pl. XXXVI, fig. 1.)

HOLMPATRICK LIMESTONE.

Form:—Conical. Length about 30 mm. Diameter of calyx about 30 mm.

Tranverse section (diam. 25 mm.):—External area composed of about ten rows of vesicles regularly radiated by the unthickened major and minor septa. Vesicles somewhat crowded near inner wall. Inner wall very distinct owing to stereoplasmic thickening. Medial area radiated only by major septa which are here strongly thickened. The thickening is less near the inner wall, reaches a maximum further in, and the septa taper to a point at their inner ends. The thickening affects all the major septa, but is greater in the cardinal half. In the medial area there are about six rather crowded tabular intersections showing that the tabulae were widely domed. The cardinal fossula is very distinct, and lateral breaks are marked by short septa. The central area presents a loose, irregular figure, in which one may pick out an unthickened mesial plate, from which radiate a few lamellae.

An early transverse section is *Lithostrotion*-like. The septa unite in groups, and thus reach the central plate. The central plate and all the major septa inside the inner wall are strongly and equally thickened.

Only a very poor longitudinal section was obtained, but it shows the mesial plate and the doming of the tabulae.

Koninckophyllum carlyanense, sp. nov. (Pl. XXXVII, fig 3.)

CARLYAN LIMESTONE.

Transverse section:—External area composed of about four rows of vesicles regularly radiated by both series of septa. Medial area—major septa, thickened at base, tapering to a point, not reaching central area; minor septa projecting as strong, blunt teeth; tabular intersections rare. Central area composed of two or three tabular intersections surrounding a distinct mesial plate which is continuous with the counter septum. A fossula is easily made out.

Longitudinal section:—The tabulae are well spaced. They slope very gradually inwards and upwards until very close to the mesial plate, when they are "tented" up sharply.

Koninckophyllum, aff. θ, Vau.

HOLMPATRICK LIMESTONE.

This appears to differ from the type in two respects—

(1) The continuity of the minor septa is sometimes interrupted.

(2) Crowding of the vesicles at the inner border of the external area is only noticeable in places. This agrees well with the lower horizon of the present form.

Arachniophyllum simplex, gen. et sp. nov. (Pl. XXXVII, fig. 1.)

CARLYAN LIMESTONE.

Rapidly tapering. Diameter of calyx 25 mm.

Transverse section:—At a diameter of 10 mm. there are no minor septa; the major septa, thirty-four in number, all reach the centre. There is a distinct columellar plate continuous with the counter septum. A well-marked fossula is present. Four or five tabular intersections can be seen, but there are no vesicles.

A section at the bottom of the calyx has the following characters:—Diameter 20 mm. An external area of one to two rows of vesicles, radiated by major and minor septa. A medial area radiated by major septa, forty-two in number, and into which the minor septa project as teeth. The major septa reach the central area. The medial area contains two or three tabular intersections. A central area containing about six tabular intersections, radiated by about twelve lamellae, and bisected by a strong mesial plate.

The only longitudinal section obtained was in the calyx. The tabulae are seen to form a very high, steep-sided boss, corresponding to the central area of the horizontal section.

The points characterizing the new genus are (1) the extreme steepness of the tabulae in the central area, forming a high, sharp boss in the calyx; (2) the strong plate bisecting the central area; (3) the simplicity of the external area.

This form is fairly common in the Rush Conglomerate and Carlyan Limestone. The coral figured by Vaughan[1] from the Rush Conglomerate probably belongs here. If so, it cannot be regarded as an early form of *Clisiophyllum curkeenense,* in which the columellar plate is inconstant and confined to the middle of the central area, and the tabulae are not steep.

Clisiophyllum dublinense, sp. nov. (Pl. XXXVII, fig. 2.)

CARLYAN LIMESTONE.

This form appears to be related to *Clisiophyllum ingletonense,* Vau.[2] It differs from the latter chiefly in the central area. This contains far fewer tabular intersections. A "nucleus" is present in which the lamellae are numerous, and tabular intersections almost completely absent. The "aureole" contains only five or six tabular intersections, and lamellae are few. In a longitudinal section the tabulae appear steeply vaulted in the central area, but the division into "nucleus" and "aureole" cannot be traced.

The Cyathophylloid Clisiophyllid from S_1 of the Mendip area, figured by Sibly,[3] seems to have a considerable resemblance to this form.

Clisiophyllum spissum, nom. nov. (Pl. XXXVII, fig. 4.)

CARLYAN LIMESTONE.

Lithostrotion-like Clisiophyllid, Vaughan. Quart. Journ. Geol. Soc., vol. lxiv, 1908, p. 463, and Pl. xlix, fig. 1.

As a number of these "*Lithostrotion*-like Clisiophyllids" are now known, it will be convenient to give this form a name.

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[1] Quart. Journ. Geol. Soc., vol. lxii, 1906, Pl. xxx, fig. 2a.

[2] Proc. Yorkshire Geol. Soc., vol. xvii, Pt. iii, 1911, p. 251, and Pl. xxxviii, fig. 1.

[3] Quart. Journ. Geol. Soc., vol. lxii, 1906, Pl. xxxi, fig. 5a.

The figures are from sections of a new specimen from the same beds as the one figured by Vaughan. The following points, not appearing in the latter figure, may be noted:—The central plate may form a prominent cusp on the central area, opposite the fossula. Many of the minor septa are not continuous to the outer wall. The tabulae in the central area are exceedingly steep, as in the "nucleus" of *C. ingletonense* Vau.[1] In the medial area the tabulae are rather steep and vesicular, as in the "aureole" of *C. ingletonense.* There is no region where the tabulae approach the horizontal position, such as occurs in *C. ingletonense* and in *C. dublinense* (*vide supra*).

Clisiophyllum, sp. (Pl. XXXVI, fig. 10.)

KATE ROCKS.

A fragment only found, much mineralized, from which a single transverse section was obtained.

The septate area is Cyathophylloid. The central area is divisible into nucleus and aureole. The aureole contains about five or six tabular intersections, and is radiated by about ten prominent lamellae. In the nucleus the lamellae are more numerous, but it is too mineralized to make out the structure any further.

Clisiophyllid, sp. nov. (Pl. XXXVI, fig. 11.)

RUSH CONGLOMERATE, R 11 b.

Two fragmentary specimens only were found. In a transverse section the most remarkable features are the highly developed central area, and the entire absence of an external vesicular zone. In these respects it bears some resemblance to "a new genus of Clisiophyllid," described by Vaughan.[2] The present form, however, differs from this in the following points:—Smaller size (diam. ·11 mm.); fewer septa (c. 30 major); fewer (c. 10) tabular intersections in the central area, which is not so distinctly bounded; the presence of a thin mesial plate included in the central area; the more tent-like shape of the tabulae. The septa are blunt-ended, and do not reach the central area. The minor septa are nearly half as long as the major. The central area projects in the calyx as a fairly high dome, on which the lamellae run spirally.

Clisiophyllid, sp. nov. (Pl. XXXVI, fig. 2.)

HOLMPATRICK LIMESTONE.

One imperfect specimen, embedded in matrix. Diameter of calyx 24 mm. Length about 30 mm. Exterior costate.

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[1] Loc. supra cit.

[2] Quart. Journ. Geol. Soc., vol. lxiv, 1908, p. 464.

Transverse section (with diam. 21 mm.):—Central area lemon-shaped, bounded by a strong wall, and containing a mesial plate. The plate is bordered by close-set, short lamellae, giving it a centipede-like appearance. These short lamellae are embedded in stereoplasm. Only some of them are produced beyond the innermost tabular intersection. The produced ones are continued across one or more of the few (5 or 6) tabular intersections, several reaching the outer wall of the area. This wall is composed of two tabular intersections. The inner of these is thickened on its outer side, and from it arise numerous lamellae, only some of which reach the outer tabular intersection. The remainder of the section is extremely simple, consisting of a thick outer wall, from which arise two series of septa. The minor septa are very short. The major septa reach the central area, near which they are spirally deflected. In the septate area there are only two or three tabular intersections, and no vesicles. (One row of vesicles is developed near the edge of the calyx.)

In a younger transverse section the wall of the central area is composed of a single thickened tabular intersection, on which lamellae cannot be detected. In a still younger section (fig. 2*b*), this wall has disappeared, and the ends of the major septa become merged in the thickening of the mesial plate.

A longitudinal section in the calyx shows that the tabulae are extremely steep in the central area, and form a high boss in the calyx.

Productus cf. *sub-laevis*, de Kon.

LANE LIMESTONE.

Two species are included under this designation. All the specimens are extremely fragmentary.

(1) Several specimens of this form were obtained in the upper parts of the Lane beds. They agree very well, as far as they go, with *P. sub-laevis* from C_2 of Belgium. This is the form, a water-worn specimen of which was recorded by Vaughan as *P. humerosus*.[1]

(2) A single specimen, resembling *P. sub-laevis* in form and ornament, but with a quite thin shell (cf. *P. Christiani*, de Kon.).

Two similar forms occur at Clitheroe (Lancashire) above a C_1 fauna.

Chonetes squamata, sp. nov. (Pl. XXXVI, fig. 15.)

RUSH CONGLOMERATE, R 11 & 12.

Length c. 18 mm. Width c. 28 mm.

Ribbing moderately coarse (c. 17 ribs in 1 cm. near anterior margin), ribs

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[1] Quart. Journ. Geol. Soc., vol. lxiv, 1908, p. 438.

forking occasionally. Concentric ornament distinct, making the ribs look as though set with imbricated scales directed backwards. Pedicle valve very convex.

A form resembling, if not identical with, this occurs in the lower part of Worsaw Knoll, near Pendle Hill (probably C_2).

Spirifer, sp. (Pl. XXXVI, fig. 6.)

HOLMPATRICK LIMESTONE.

Pedicle valve:—Ribs on flanks tall, angular, forked, or occasionally split into three. Forking usually occurs near the umbo, but may be delayed until close to the anterior margin. Sinus deep, angular. Ribs in sinus indistinct; quite absent towards the anterior end in some specimens. Concentric lineation very marked towards the anterior margin. Area broadly triangular, extending to the greatest width of the shell.

Brachial valve not known with certainty.

Spirifer cf. *convolutus*, Phil. (Pl. XXXVII, fig. 6.)

RUSH SLATES, R 6 b.

An imperfect pedicle valve. Width at least 70 mm.; length about 30 mm. Resembles *Sp. convolutus* in the nature of its ribbing. The ribs near the sinus are wide, often splitting into two or three; those nearer the cardinal angles are much narrower. Two sharp, prominent ribs bound the sinus, which is V-shaped in section. The anterior part of the sinus is missing. Posteriorly it has smooth sides, the bottom being occupied by a single, narrow rib.

EXPLANATION OF PLATE XXXVI.

PLATE XXXVI.

Fig.

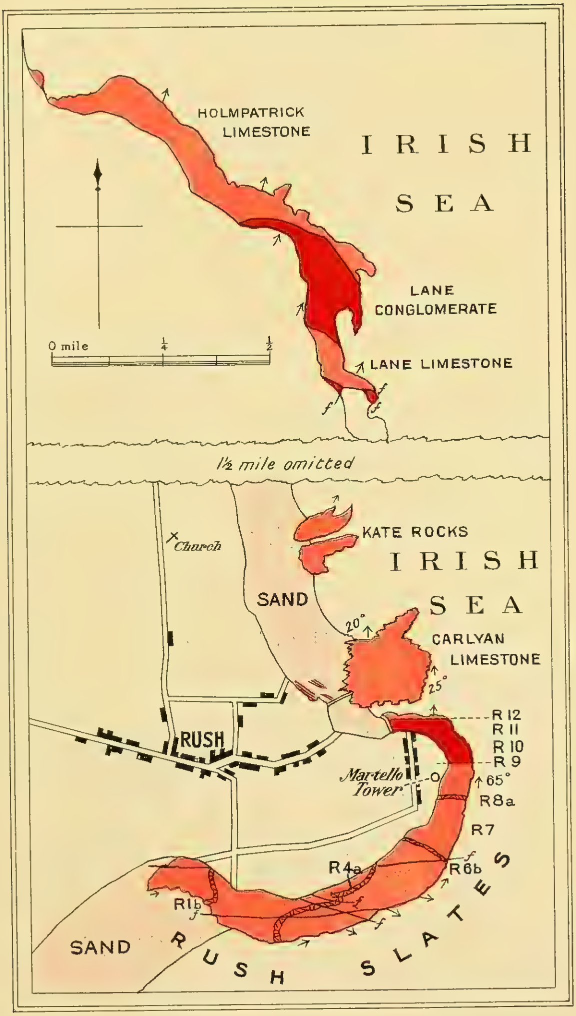
1. *Koninckophyllum densum*, sp. nov. Transverse section. × 1·4. Holmpatrick Limestone (p. 557).
2. Clisiophyllid, sp. nov. Holmpatrick Limestone (p. 560).
    a. Transverse section just below calyx. × 1·8.
    b. Transverse section (same specimen) lower down. × 1·8.
3. *Zaphrentis* cf. *densa*, Car. Transverse section. × 1·7. Holmpatrick Limestone (p. 554).
4. *Densiphyllum* aff. *rushianum*, Vau. Transverse section. × 1·7. Holmpatrick Limestone (p. 557).
5. *Carcinophyllum* aff. *θ*, Vau. Transverse section. × 1·5. Holmpatrick Limestone (p. 543).
6. *Spirifer* sp. Pedicle valve. × 1·1. Holmpatrick Limestone (p. 562).
7. *Zaphrentis ambigua*, var. *α*, var nov. Transverse section. × 1·7. Lane Limestone (p. 555).
8. *Zaphrentis* cf. *constricta*, Car. Kate Rocks (p. 555).
    a. Transverse section just below the calyx, much mineralized. × 1·8.
    b. Transverse section lower down. × 2.
9. *Zaphrentis* cf. *enniskilleni*, Edw. and H. (1). Transverse section. × 1·4. Kate Rocks (p. 556).
10. *Clisiophyllum* sp. Transverse section. × 1·8. Kate Rocks (p. 560).
11. Clisiophyllid, sp. nov. Transverse section. × 1·7. Rush Conglomerate, R 11 b. (p. 560).
12. *Zaphrentis* cf. *disjuncta*, Car. Transverse section taken more than 13 mm. below floor of calyx. × 1·7. Rush Conglomerate, R 10 m. (p. 555).
13. *Densiphyllum nodosum*, sp. nov. Transverse section, much mineralized. × 1·8. Rush Conglomerate, R 11 d. (p. 556).
14. *Zaphrentis* cf. *enniskilleni*, Edw. and H. (2). Transverse section. × 1·4. Rush Conglomerate, R 11 a. (p. 556).
15. *Chonetes squamata*, sp. nov. Pedicle valve. × 1. Rush Conglomerate, R 11 a. (p. 561).

EXPLANATION OF PLATE XXXVII.

PLATE XXXVII.

Fig.

1. *Arachniophyllum simplex*, gen. et. sp. nov. Carlyan Limestone (p. 558).
    - *a.* Transverse section just below calyx. × 1·8.
    - *b* and *c.* Transverse sections (same specimen) lower down. × 1·5.
    - *d.* Longitudinal section (same specimen) in the calyx. × 1·8.
2. *Clisiophyllum dublinense*, sp. nov. (p. 559).
    - *a.* Transverse section. × 1·9. Carlyan Limestone.
    - *b.* Longitudinal section (same specimen). × 1·8.
    - *c.* Transverse section (central part) of another specimen. × 1·5. Carlyan Limestone.
3. *Koninckophyllum carlyanense*, sp. nov. Carlyan Limestone (p. 558).
    - *a.* Transverse section. × 2·2.
    - *b.* Longitudinal section (same specimen). × 2·2.
4. *Clisiophyllum spissum*, nom. nov. Carlyan Limestone (p. 559).
    - *a.* Transverse section. × 1·8.
    - *b.* Longitudinal section (same specimen). × 1·8.
5. *Clisiophyllum* cf. *oblongum*, Thom. Transverse section. × 1·5. Carlyan Limestone (p. 545).
6. *Spirifer* cf. *convolutus*, Phil. Pedicle valve. × 0·8. Rush Slates, R 6 b (p. 562).



Map of the Rush and Skerries Outcrops (Modified after Matley).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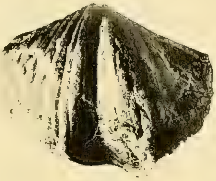
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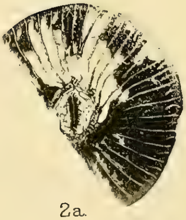
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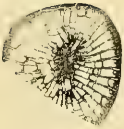
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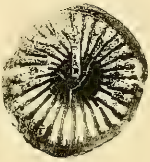
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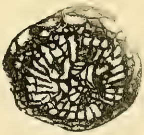
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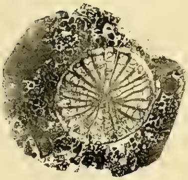
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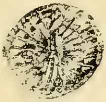
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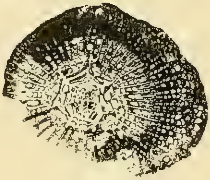
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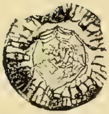
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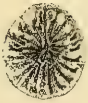
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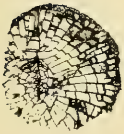
14.


15.

Bemrose, Collo., Derby.

Plate XXXVII.

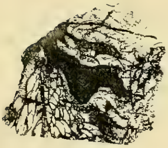


1b.

1a.

1c.

1d.



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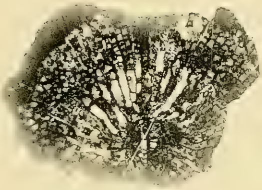

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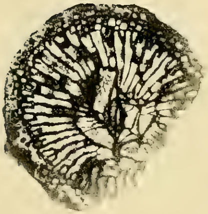

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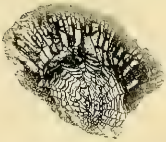

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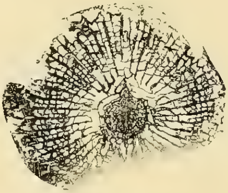
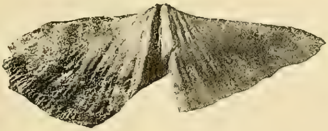


4a.

3b.



6.

4b.

Bemrose, Collo., Derby.

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