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J. W. EYRE, M.D., M.S.Lond., President.

PAPERS ON ANAPHYLAXIS.

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IT has long been recognized that from time to time the injection of a substance of a protein nature, such as blood serum, into animals or man may cause poisonous symptoms, though such an injection is quite without effect in the vast majority. This susceptibility was said to be due to individual idiosyncrasy—a word which explains nothing; it is now known to be a condition of protein sensitization. This condition was familiar to us in other ways: The incubation period of a second vaccination is shorter than that of the first; tuberculin and mallein are without effect when injected into normal animals, but the same injection causes a marked poisonous reaction if made into tuberculous calves or horses suffering from glanders respectively.

Charles Richet in 1902, whilst investigating the action of certain poisons from the sea anemone (congestin and thalassin), noted that after a dog had received a first injection of one of these substances, not sufficient in amount to produce any observable effect, a second injection some three or four weeks later rendered the animal extremely ill, and often caused its death. Therefore the first injection had produced a condition which was the opposite of protection, and to this hypersusceptibility or supersensitiveness Richet gave the name "anaphylaxis." It was soon shown that this reaction was not peculiar to *congestin*, but was general for all substances of a protein nature, just as the formation of antibodies and the precipitin reaction are general for all proteins; in other words, the injection of proteins, toxins, serums, and the like can all give rise to this condition. After an animal has received an injection of some protein it is hypersusceptible to that protein and to no other, the reaction being specific for each protein. The tissues do not react in this manner to drugs and chemical substances of known constitution. Certainly it has been stated that if blood serum from patients who are supersensitive to antipyrin or iodoform is injected into the guinea-pig, this animal also will exhibit an increased sensitiveness to these drugs; this condition, however, bears no true relation to anaphylaxis.

One remarkable fact is the extremely small dose of the sensitizing protein (antigen) necessary to produce the anaphylactic state. A guinea-pig which has received one hundred-thousandth part of a cubic centimetre of such an inoffensive serum as that of the horse is never again quite the same animal. If, let us say in a month's time, it again receives an injection, which would be quite without effect on a normal animal, it may die in a few minutes. Even such a minute dose as 0.000,000,05 gram of egg-white has been stated to be enough to induce the anaphylactic state in the guinea-pig; large doses act quite well, but the latent period before the condition is observed is longer. The protein molecule is the essential reacting agent, since pure crystalline egg-albumin will produce anaphylactic shock in a guinea-pig which has been sensitized to egg-white. The conditions, then, essential for anaphylaxis are the introduction of a substance of a protein-like nature into the body, and of which it is not a normal constituent, followed after a suitable interval by a second dose. The interval between the first and second injections should be not less than a week; in man the full effect is not reached till a much later period, whilst in the dog the maximum effect is about the fifteenth day. This condition of supersensitiveness when once present lasts for a variable time in different animals—from some weeks in rabbits to some years in guinea-pigs. Within certain broad limits the symptoms of anaphylactic shock are the same for each animal no matter what the nature of the sensitizing protein employed. Hence it has been argued that these identical symptoms probably require a common cause, and this is one reason why the hypothetical poison, anaphylatoxin, has been requisitioned. All tissues do not necessarily respond to antigen in the same degree. The liver in the dog is affected by antigen to a greater extent than other organs, and in this instance it certainly looks as if some movable poison is formed, as the other tissues are affected secondari'y from the liver.

The *symptoms and signs* of anaphylactic shock can be divided into three groups—circulatory, muscular, and local.

Circulatory symptoms are perhaps the most characteristic: the arterioles are paralysed, the vessels become widely dilated, and as a direct result blood pressure falls. The heart-beat is weaker, but not sufficiently weak to cause the fall of blood pressure. The liver is mainly responsible for this condition, since anaphylactic shock is ill-defined or absent if the liver is excluded from the circulation. These circulatory effects are especially characteristic in the dog and cat. It is not known how the liver induces shock; its vessels are dilated and its vascular volume is much increased, but of course this is not enough to account for the changed blood pressure, and it seems not unlikely that toxic products are liberated in relatively large quantities here which cause a secondary intoxication. No doubt some of the nervous symptoms which have been described are secondary effects of the low blood pressure.

Plain muscle tissues in certain situations undergo tonic contraction. This effect is especially well seen in the bronchioles and occurs in all animals, guinea-pig, rabbit, cat and dog, but it is peculiarly intense in the guinea-pig because in this animal the bronchioles are lined with a thick and folded mucous membrane which effectively blocks the lumen when constriction occurs; it is well marked in rabbits, but perhaps less defined in cats and dogs. After an intravenous injection of the antigen the bronchioles close up immediately and atropine, which paralyses the motor nerves to the bronchiolar muscles, has no influence either in preventing or relieving the spasm. Some of the nervous symptoms, convulsions, tonic and clonic spasms, may be regarded as secondary results of asphyxia. Other plain muscle which may be thrown into contraction is that of the stomach and intestines, and vomiting and diarrhoea is a not uncommon effect of anaphylactic shock in the dog. The uterus also enters into tonic contraction. In all these instances the only essential factors, so far as we know for certain, are the sensitized plain muscle and the antigen.

Local effects generally take some hours to manifest themselves. The experiments of Dunbar and Weichardt are instructive; each was injected under the skin with a suspension of pollen grains. Dunbar was a martyr to hay fever, and almost immediately he experienced an acute attack, with the coughing, sneezing and congestion of the mucous membranes, congestion and swelling of the face, and general urticaria; the attack passed off in twenty-four hours. Weichardt being normal was not influenced by the injection. Skin symptoms such as itching and oedema are not uncommon in cattle, and form one of the milder group of symptoms in man. A second injection of antigen subcutaneously in the rabbit sometimes causes local oedema and sloughing at the seat of the first injection. Bloch has shown that the reaction between antigen and sensitized tissue is strictly local; he transplanted the skin from a subject known to be highly susceptible to trichophyton upon a normal person. After the skin had grown healthily again it was found that the transplanted portion was still supersensitive to trichophyton, whilst the rest of the skin was normal. Tuberculin, which acts like anaphylatoxin in the tuberculous, can be used also to obtain a local reaction—Calmette's ophthalmo-reaction, and von Pirquet's skin reaction. The anaphylactic symptoms last only a few hours, and the animal either recovers rapidly or dies.

The explanations which have been offered to account for

anaphylaxis fall naturally into two groups—the side-chain view and the ferment view.

The supporters of the side-chain view point out that the production of anaphylaxis and also of antibodies runs a parallel course, and the incubation period is roughly the same, and they suggest that the symptoms of anaphylactic shock are due to a direct combination of the antigen with the cells of the sensitive animal. They believe that the sessile receptors which are the precursors of antibodies are the basis on which supersensitiveness depends. A cell which carries an increased number of these sessile receptors free to combine with a foreign protein is hypersusceptible to that protein.

The second or ferment view depends upon three facts. First, that all protein molecules contain a poisonous group which can be liberated by an appropriate ferment. Secondly, that the introduction of a foreign protein into the animal body determines a new ferment which splits up that protein and no other. And thirdly, that the symptoms of anaphylactic shock and those resulting from the injection of these products of partial protein hydrolysis (peptones) are identical. Supposing that some serum from an anaphylactic guinea pig is examined for the presence of such peptones, they will not be found present until after the addition of a little antigen, when proteolysis commences immediately, though the results as shown by peptone formation are not very manifest until after fifteen minutes.

These are the facts; the theory consists in assuming that these products of proteolysis are the cause of anaphylaxis. It may be assumed that the sensitizing injection leads to the formation of a specific ferment, and that the protein of this injection is so slowly digested that the poison set free at any one time is not enough to produce any obvious effect. This view affords an explanation why such minute sensitizing doses can produce such large effects after the reacting dose. It is quite easy to test the effect of antigen directly upon isolated sensitized plain muscle of the guinea-pig. Schultz tried the effect on the intestine and Dale on the uterus. The organ was washed free from blood and suspended in Locke's fluid, so that the automatic contractions could be recorded, and it was found that the addition of traces of the sensitizing protein of the fluid caused marked contraction, whilst all other proteins were without effect. The contraction is immediate, and therefore Dale argues that it is unlikely to be caused by a ferment liberating some toxic cleavage product, because this would take time. A gradual onset of anaphylactic symptoms is perhaps the rule in the dog, but in the guinea-pig and rabbit there is no delay. Although this criticism is certainly valid and cogent, especially as Abderhalden has shown that fifteen minutes' incubation are required between sensitive tissue and antigen before peptones can be detected, yet it is not completely destructive, and especially should it be remembered that such a strip of plain muscle probably carries a large amount of ferment in proportion to the amount of antigen necessary to cause the effect, and further, that the peptones would be liberated under ideal conditions for producing an effect. Any fat-free protein when heated to 78° C., with 2 per cent. caustic soda in alcohol is split up into two fractions, one of which, a poisonous fraction, remains in solution in the alcohol, and when injected into animals causes the symptoms of anaphylaxis. The addition of antigen to sensitive serum induces proteolysis, and whatever the nature of the antigen the poisonous substance has identical physiological properties. To this substance, which is sometimes regarded as precipitin, the name of "anaphylatoxin" has been given. It is easy to prepare a similar toxic substance from the normal guinea-pig's serum by digesting it with china clay, agar, or even an emulsion of nerve-tissue, and in each case after standing the supernatant liquid contains an anaphylatoxin.

Here, then, is a third view as to the cause of anaphylaxis, that the poison is produced by physical means due to the adsorptive properties of a colloid. It is, then, at least possible that the formation of anaphylatoxin from mixing antigen and serum may be the result of some physical change in the state of aggregation of the colloids inducing, as Traube believes, a change of surface tension. Other evidence is collecting which supports this view; the injection into the circulation of animals of colloidal solutions of inorganic substances produces some of the characteristic effects of anaphylaxis; a solution of common salt given by the mouth to sensitive guinea-pigs for some days inhibits the action of antigen; the administration of the drugs belonging to the group of indifferent hypnotics and anaesthetics, the narcotic power of which run parallel with their effect on surface tension, will prevent anaphylactic shock at least for a time. These facts certainly suggest strongly that the reaction between antigen and sensitized serum is not chemical. It may be noted before proceeding that if the serum from a supersensitive animal be injected into a normal animal, that property is transferred, and the supersensitive state is also transmitted by the female to her progeny.

Clinical Significance.

Anaphylaxis is not merely a question of academical interest, but is also one of considerable practical importance; a person may be rendered hypersusceptible to a disease at will. It is true that few people show any decided supersensitiveness to an injection of ox serum even though they may be meat eaters; but then we do not absorb our protein as such, but as the relatively simple amino-acids.

Attention has been drawn already to hay fever, and spasmodic asthma may be cited as a condition easily brought about in the supersensitive. In this connexion cat asthma, which is generally regarded as reflex constriction of the bronchioles from the irritating odour of the cat, assumes a new significance. Horse asthma is also not unknown, and there are those who suffer from asthmatic attacks merely from riding behind horses. Expired air has been shown to contain a protein which is able to sensitize animals, and this, when inhaled, may account both for the local anaphylaxis in asthma and for the condition in those cases in which there is hypersusceptibility to horse serum. In the latter instances all the symptoms of true anaphylaxis may, within a few minutes, follow a first injection of horse serum. This condition is, however, one of great rarity. These cases of true anaphylaxis must not be confounded with serum disease, which is generally characterized by fever, a rash, and some oedema; here the larger the dose of serum administered the greater the chance of an attack, but the trouble rarely arises until a week or ten days after injection. Calcium salts, even when given by the mouth, are said to check this condition.

A relatively large number of people exhibit some supersensitiveness to foods; some cannot eat eggs, especially if they are lightly boiled, because cutaneous rashes, migraine, and alimentary disturbances occur. This idiosyncrasy is often seen in members of the same family, and it may be hereditary. It is worth noting that such an alimentary anaphylaxis to eggs has been obtained experimentally. Mussels, plaice, crayfish, oatmeal, strawberries, and other foods occasionally cause similar effects. In the supersensitive these symptoms are rapidly and certainly produced; minute doses only are required, and the symptoms are identical with those which are seen in the lower animals produced by experimental anaphylaxis. Sometimes, though not often, an apparent cause for these conditions can be found; thus Billard mentions a child nourished on horseflesh, which showed typical anaphylactic shock after an injection of antidiphtheritic serum.

The means by which such shock can be eliminated deserves some consideration. Sometimes it is urgent that a patient who has received a dose of serum at some earlier period should now receive a second dose. For example, during an epidemic of diphtheria at a school some of the scholars may receive a prophylactic injection of antitoxin and at a subsequent period may contract the disease. It may be argued that the principle of prophylactic injections under these circumstances is wrong, as we may be masking a carrier, but still the practice is adopted and must be considered. In such cases an antitoxin prepared from some animal other than the horse can be substituted for the ordinary antitoxin, and some of the manufacturers prepare an ox serum for this purpose. If a patient receives a series of injections at short intervals, not longer than five or six days apart, anaphylaxis does not occur. If it is doubtful whether a patient is supersensitive to horse serum, several methods exist for determining this factor. Perhaps the simplest is to obtain 2 c.cm. of the patient's serum and add this to 1 c.cm. of

horse serum. If this mixture is incubated for fifteen or twenty minutes anaphylatoxin is formed provided the patient is sensitive, and in this case its injection into a guinea-pig will result in anaphylactic shock. In man the period of maximum danger extends from one to six months after an injection of serum, and the reaction may not disappear for a year. A principle of treatment which is frequently adopted in cases in which anaphylactic shock is feared consists in a preliminary injection of 0.1 c.cm. If after three hours no untoward symptoms appear a full dose may be given.

II.—G. SIMS WOODHEAD, M.A., M.D.,
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THOSE who have gained some practical knowledge of the methods of production and manifestations of anaphylaxis realize what an important share this phenomenon is destined to play in discussions on certain pathological conditions, and in moulding opinions as to the nature of the processes involved in the cure of infective disease. Although this question has come to the front, especially in connexion with treatment consisting of the injection of single or repeated doses of antitoxic serums, this is by no means the most pressing of the questions associated with the anaphylactic condition. Indeed, we may take it that although phenomena closely allied to those produced by the true anaphylactic condition (supersensitiveness) are observed in serum treatment, this condition is merely a somewhat atypical example of supersensitiveness. As Dr. Dixon has pointed out, the anaphylactic reaction is the result of the introduction of small doses of a protein into the "exposed" tissue of a living animal organism. This primary injection, spoken of by the French school as the preparatory, or sensitizing, dose, if followed at an interval of from ten days to several weeks by a "stimulating" or "exciting" dose of an even smaller quantity of the same serum, gives rise to phenomena so marked that there is little difficulty in distinguishing the condition; moreover, the specificity of the process is such that several workers have claimed this anaphylactic process as being useful in the demonstration of the specific character of the blood derived from various animals, and have thus ascribed to it considerable value in forensic medical investigations. It is certainly interesting to note that by the application of the anaphylactic method, it is possible to obtain a specific reaction with quite as small quantities of blood as are necessary for the precipitin test; though I think it would appear that more blood is required for the carrying out of this test than for the deviation of complement test. Further experience and elaboration of the process, however, may enable the medical jurist to obtain still more delicate results.

The next condition which requires further elucidation is that associated with disorders such as nettle-rash in persons who are said to have definite "idiosyncrasies." Many different forms of food are *anathema* to certain individuals. I myself, for instance, have on several occasions after eating crab-pie or wild strawberries suffered more than intense discomfort from severe nettle-rash, with the result that, in self-defence, I now avoid both. Moreover, drugs such as salicylates, certain salts of quinine, iodoform, the bromide salts, and even the iodides, may sometimes prove themselves very objectionable through their sensitizing action.

In connexion with the absorption of foreign proteins from the alimentary tract, it is interesting to note that when albuminoids are introduced into the upper part of the tract, where they are submitted to prolonged and active chemical treatment, anaphylaxis is very rarely developed unless there be some lesion in the walls of the canal. When, however, the injection is made into the lower part of the tract—the large intestine, whence, as we know, absorption of material in a comparatively unaltered condition may take place—an anaphylactic condition may result. May this fact not give us some indication as to the lines to be adopted in using tuberculin? Intrarectal administration of tuberculin has, of course, been both recommended and tried; but the recorded results are at present too few and too inconclusive to allow of any very definite teaching being founded upon them. One scarcely associates the supersensitive condition with the ingestion of milk, though it is well known that certain individuals exhibit a great intolerance to this aliment. In the adult this intolerance has been attributed to imperfect digestion, especially when the milk is taken in bulk and coagulated in large masses, and is therefore carried in an undigested condition into the large intestine, where, as has been observed in certain experiments on guinea-pigs, a protein may first act as a preparatory injection, and at a later stage as a stimulating injection. In breast-fed children suffering from lesions of the alimentary tract (and sometimes when no definite lesions can be demonstrated) characteristic anaphylactic symptoms, even followed by death, have been recorded when the children were suddenly transferred to a diet of cow's milk—the homogeneous protein of the mother inducing no supersensitive condition, but the heterogeneous protein of the cow acting violently as soon as there has been time for the induction of the supersensitive condition.

It is evident that the diagnostic value of the anaphylactic condition depends very largely on the specificity of the protein. Jenner foreshadowed this in his extremely careful observations on the local supersensitive condition; this observation is alluded to by Hektoen [1] in an article on "Allergy or Anaphylaxis in Experimental Disease," from which I quote verbatim:

> In his *Inquiry into the Causes and Effects of Variolae Vaccinae, a Disease discovered in Some of the Western Counties of England, particularly Gloucestershire, and known by the Name of Cow-pox*, 1798, he (Jenner) records (page 13, Case 4) that a woman who had had cow-pox thirty-one years before was inoculated with variolous matter, and that "an efflorescence of a palish red colour soon appeared about the parts where the matter was inserted, and spread itself rather extensively, but died away in a few days without producing any variolous symptoms." In a footnote he comments on this phenomenon as follows: "It is remarkable that variolous matter, when the system is disposed to reject it, should excite inflammation on the part to which it is applied more speedily than when it produces the small-pox. Indeed, it becomes almost a criterion by which we can determine whether the infection has been received or not. It seems as if a change which endures through life had been produced in the action, or disposition to action, in the vessels of the skin; and it is remarkable, too, that whether this change has been effected by the small-pox or the cow-pox, the disposition to sudden cuticular inflammation is the same on the application of variolous matter." This remarkably clear statement probably records the first observation of allergy in an infectious disease.

This rapid manifestation of a local sensitiveness may be looked upon as the outcome of a general anaphylactic condition, and the early appearance of the local manifestation, as recognized by Jenner, affords a constant and characteristic diagnostic reaction, which we now know to be almost as characteristic and constant as is the Pirquet cutaneous diagnostic reaction obtained with tuberculin in cases of tuberculosis. In this connexion we should note a further remarkable manifestation in cases of haemorrhagic small-pox. Although vaccination appears to afford very complete protection where the dose of the small-pox virus is not excessive, it would seem that a vaccine virus exerts little or no protective influence against those "massive" doses that occasionally gain access to the individual. Haemorrhagic small-pox has been described, though comparatively rarely, in vaccinated individuals, and Hektoen notes that

> it has been suggested that haemorrhagic small-pox may be an expression of a specially intense reaction between antigen, the preparatory substance, and antibody, the substance produced.

Lytic changes are set up, not in the floating cells alone, but also in the epithelial cells of the capillaries; the result is severe damage to the walls of the capillaries, owing to the action of the lytic antibodies set free by the exciting virus.

An interesting example of the untoward results of the setting up of anaphylaxis is one that affords some explanation of a most mysterious condition associated with operations for the removal of hydatid cysts. There is, of course, but a small percentage of albumin in the fluid of a hydatid cyst—only some 0.5 per cent. It appears, however, that this albuminous fluid may, under certain conditions, act as a "preparatory substance," either through general absorption, or by some slight lesion; for in certain cases where, during operation, there has been an escape of the fluid from a hydatid cyst into the peritoneal cavity, and consequent rapid absorption, death with distinct anaphylactic symptoms has supervened. This phenomenon, of course, was inexplicable before the thread of events involved in anaphylaxis had been somewhat unravelled.

Experiments confirmatory of those carried out by Professor Chauffard may add no new crude facts to our knowledge of this special form of supersensitiveness; but work carried on with a less complex form of albumin than many of those hitherto experimented with may enable us to elucidate further secondary features, and to learn something more as to the specificity of the process.

It has been stated that the proteins of the placenta and even amniotic fluid may act as preparatory and exciting substances in the production of a hypersensitive condition in an individual of the same species as that from which placenta and fluid are taken; but it has never yet been proved that there is any specific poison contained in either of these substances. Wolff-Eisner[2] maintains that the alterations produced by the infection of these substances into animals have not been characteristic of eclampsia, though he agrees that the eclampsia, with its convulsions, nephritis, haemorrhages, etc., resembles closely the "complex" set up by the repeated reabsorption of heterogeneous albumin. He seems to think that the placenta partakes as much of the male element as of the female, and that to that extent the proteins are heterogeneous. We know that under normal conditions the placental villi are actually absorbed towards the end of the period of gestation, and it is quite possible that this process of absorption may be considerably accelerated under conditions in which there is some lesion of the uterine surface, and especially when there is breaking down of the villous tissue; and it is quite possible, and even probable, that the lytic processes that occur towards the end of the period of gestation may, under certain conditions, be greatly exaggerated, and thus give rise to the eclamptic or hypersensitive condition above referred to. Rosenau is distinctly of the opinion that the repeated absorption of villous elements plays this important part in eclampsia: and Wolff-Eisner[3] writes:

> We now know positively that the heterogeneous albumin from the syncytium finds its way into the circulation of the pregnant woman, and under certain conditions eclampsia ensues as the sign of the real absorption of the albumin and the resulting hypersensitiveness.

This author holds, indeed, that the eclamptic condition is only the severest of a group of phenomena, of which urticaria, albuminuria, and the vomiting of pregnancy are the commoner members, resulting from the absorption of heterogeneous albumin.

It is obvious that we can know little of the causes of this series of phenomena until we have a much wider knowledge of what takes place in the absorption of these heterogeneous albumins. Here is a very suggestive line of inquiry for us. Is not contraction of the uterus, even under normal conditions, in part, at any rate, brought about by sensitization of the muscle fibre by an anaphylactic process in which the intermittent absorption of the villous processes of the placenta or their proteins play a part? Gräfenberg,[4] quoted by Wolff-Eisner, states in this connexion that:

> In the first three months the placental villus shows heterolysis—that is, it acts digestively on the serum plate. The purpose of this process is perhaps to facilitate the embedding of the ovum. The fact that during pregnancy, probably as a reaction from the reabsorption of tryptic ferments from the chorion, the amount of antitrypsin in the maternal serum increases to double the normal content, is to be regarded as a sign of the reabsorption of parts of the chorion by the maternal organism.

Whatever we may think of the explanations given, the facts are of extreme interest in connexion with the production of the supersensitive condition in its relation to puerperal eclampsia.

It was in Arloing's laboratory that we first had observations leading up to the inference that in the tubercle bacillus are substances which, introduced into the animal body, diminish the resistance of the tissues, and render them more sensitive to a second infection; but it was left to Koch to describe in detail a series of changes which we now recognize as being associated with a condition of supersensitiveness to these products. He showed that when a normal or healthy guinea-pig receives an injection of the living tubercle bacillus into the subcutaneous tissue there appears to be a latent period, during which comparatively little change goes on at the site of the injection. After this, however, in from ten days to a fortnight, a firm nodule appears. The skin ulcerates, and the tuberculous process progresses until the animal succumbs, the ulcer remaining open until the last. When the guinea-pig has already been inoculated with the tubercle bacillus, and a tuberculous process set up, a secondary inoculation with the tubercle bacillus gives rise to much earlier, though not always more extensive, local changes. Swelling appears on the second or third day; in the centre of the swelling the tissues seem to die *en masse*, the skin ulcerates, and the necrosed mass is thrown off, after which healing of the ulcer may take place, and there is little or no extension of the tuberculous process beyond the point of introduction. In effect, there appears to be a local supersensitiveness, accompanied by a surrounding tissue immunity, which interferes with the extension of the tuberculous process. Tuberculin and dead tubercle bacilli respectively appear to cause corresponding reactions in the healthy and in the tuberculous guinea-pig; and there can be little doubt that this condition of anaphylaxis was associated, in Koch's mind, with the local protection against the invasion of the living tubercle bacillus.

Whilst, on the one hand, we must accept this evidence, it must also be noted, as pointed out by Austrian,[5] working at the Phipps Tuberculosis Dispensary in Baltimore, that the pounded bodies of washed tubercle bacilli give up to water a substance which may be precipitated by absolute alcohol. This substance—a protein—when injected into the peritoneal cavity of a guinea-pig or a rabbit, renders it supersensitive; but in the doses used (15 mg. for the guinea-pig and 50 mg. per kilogram weight of rabbit) it conferred absolutely no immunity against even small doses of tubercle bacilli. In fact, it appeared to render a certain proportion of the animals considerably more susceptible than were the unsensitized animals. So marked, indeed, was this in the rabbit that doses of human bacilli incapable of setting up tuberculosis in the normal rabbit induced a progressive tuberculosis which in some cases proved fatal—apparently bringing the human type of tubercle bacillus almost into line, as regards virulence, with the bovine.

The marked differences in the anaphylactic or supersensitive condition obtained when a heterogeneous protein is introduced into the alimentary canal, as compared with its tremendous sensitizing or anaphylactic power when introduced parenterally, has always attracted attention. Gastric and intestinal splitting up of the proteins certainly runs on different lines from the dissociation that goes on in the other tissues of the body. Moreover, the mucous membrane appears to limit very sharply the passage of any but digested albumin beyond the alimentary tract. In spite of this, a number of those interested in the treatment of tuberculosis have persisted—and not unnaturally, in view of the unfortunate consequences that have arisen as the result of the subcutaneous injection of "large" doses of tuberculin—in giving tuberculin by the mouth, and some appear to have convinced themselves that they have obtained satisfactory results. Under present conditions, I should be inclined to encourage this method of administration, if the substance is to be given indiscriminately by all and sundry, but I should encourage it because I should expect tuberculin so introduced to exert little, if any, profound or constant effect upon tuberculous tissues. Tuberculin, like any other protein-containing body, is digested in the alimentary canal, and not until this has taken place can it be passed on to the tissues. In the digestive tract it appears to be non-toxic, though when introduced parenterally it is so tremendously active. To those who have doubts on this point I would call attention to the results recorded by Dr. L. Cobbett and Dr. A. Stanley Griffith in their report on tuberculin to the Royal Commission on Tuberculosis. They found that large quantities of "old" tuberculin—in one instance as much as a pint—fed to thirteen tuberculous calves had not sufficient stimulating effect upon their tissues to give rise to a diagnostic tuberculin temperature reaction. In 10 of the 13 experiments, there was no rise of temperature at all, whilst in the other 3 there was a rise in one case of 0.2° C., in another of 0.4° C., and in a third of 0.7° C. Other kinds of tuberculin *may* act more energetically when given by this channel. According to Sahli, all tuberculins differ in degree and not in kind, and depend for their activity upon one set of substances—the proteins; but these experiments certainly afford little

promise that the administration of tuberculin by the mouth will help in treatment.

Richet[6] insists that up to the present we have obtained no evidence that tuberculin sensitizes to tuberculin, although the anaphylactic reaction of tuberculous subjects to tuberculin is so pronounced. Although in tuberculin what he calls the "exciting" or "stimulating" substance is present in large quantities, the "preparatory" substance is entirely absent. These observations may be set alongside similar observations made whilst he was studying the action of actino-congestines, the substance with which he first produced the anaphylactic condition. He found later that exciting substances could be dissociated from the preparatory substances, the latter, or a combination of the two, sensitizing the animal, the exciting substance inducing the anaphylactic phenomena. Again, he demonstrated that in an animal infected by the tubercle bacillus preparatory substances are present "which are not present in the tuberculin we use, either because they are not developed in the culture media with the same intensity and with the same ease as in the infected organs," or perhaps because precipitating by alcohol or heating to 200° may so alter the tuberculin that it loses its preparatory power whilst retaining its exciting power. In all probability the immunization set up by the use of small doses of tuberculin is associated with this preparatory process in the first instance, and although the stimulating or exciting process may be essential, a state of unstable equilibrium may be very easily induced during which reaction useless or harmful to the patient, rather than beneficent, may be set up. At present our knowledge of this process is comparatively sketchy. It is maintained by Yamanouchi,[7] Bauer,[8] Edward Lesne and Dreyfus, and Helmholz that a passive anaphylaxis to tuberculin may be transferred from guinea-pig to guinea-pig along with the serum of the prepared animal. The results obtained by these observers, however, are somewhat fragmentary, and Marelli and Josef, and Simon, repeating these or similar experiments, have been unable to corroborate their results. The question is so important, however, and has such a very grave bearing on the treatment of tuberculosis by tuberculin, in view of the many suggested methods of immunization, that more knowledge on this subject must be sought.

This anaphylactic condition promises, I am afraid, to be the *bête noire* of those who are investigating the serum and cell products of cancer. It is obvious that in an animal capable of being supersensitized the periodical injections of emulsions of cancer cells, of growing cells of cutaneous or mucous tissue or of serums from cancerous animals must involve great danger of producing an anaphylactic condition, and just before I came down to this meeting I was interested to receive a letter from Professor Grünbaum, of Leeds, who hoped that light might be thrown on this subject by some of those taking part in the discussion, as he has lost such a very large number of his experimental animals from a supersensitiveness so highly developed that it has become a difficult matter for him to carry on his experiments owing to the high death-rate amongst them. The occurrence of such a condition during the course of treatment must limit our field of operations as regards experiments on the cure of cancer, but, on the other hand, it opens up to us a line of research in connexion with the diagnosis of disease that affords distinct promise of a fruitful issue, and I have great confidence in recommending its study to those interested, especially in view of the fact that cases of sudden death from unexplained causes are of by no means rare occurrence in cancerous patients.

It is evident, from what has already been said, that in certain specific infective diseases sensitized animals may be killed by the rapid action of the very powers that help to immunize. Through the changes that are set up in the fluids and tissues the sensitized animal is in a position to disintegrate by some lytic process the proteins of specific bacteria. During the process of lysis the specific poison, whatever may be its character, is set free. This disintegration may take place so slowly that any poison elaborated may be summarily dealt with by the tissue cells and fluids, and the micro-organism associated with the disease is reduced to the position of a saprophyte and is rapidly killed off. If, however, the lytic power be too highly developed, so that when a large amount of vaccine—or, alternatively, too great an amount of infective virus—is introduced, such an enormous amount of poison may be set free that the very magnitude and perfection of the processes usually helping to protect the patient may result in such profound poisoning of important tissues and organs that the patient succumbs, although practically every micro-organism may have been destroyed.

Victor C. Vaughan,[9] writing on the relation of anaphylaxis to immunity and disease, gives an excellent example of the fact that "there is no constant and fixed relation between the toxogenic and the pathogenic properties of bacilli." The non-pathogenic *Bacillus prodigiosus* contains in its bacillary protein over fifty times as much intracellular poison as does the highly pathogenic anthrax bacillus, and he offers, as an explanation of the difference between the pathogenicity of the two organisms, the fact that the secretions of the *Bacillus prodigiosus* are incapable of digesting the proteins of the animal body, or perhaps—and he considers that this is the more probable and satisfactory explanation—the secretions of the body cells destroy the bacillus, and thus prevent the production of toxins by the bacillus. The anthrax bacillus, however, although it actually contains less poison in its protein, is able to resist the action of the body cells and to elaborate ferments which have the power of digesting the proteins of the animal body. The one micro-organism, *Bacillus prodigiosus*—a mere saprophyte, and unable to attack living tissues—is incapable of multiplying, whilst the other, the anthrax bacillus, able to digest the proteins of the animal body, obtaining from them its nutriment, can grow, multiply, and form toxic substances in large quantities. He offers this also as an explanation why "a given bacillus may be pathogenic to one species or one race, and wholly devoid of effect on other animals." It is this power of lysis apparently that plays perhaps the most important part of all in the production of the anaphylactic condition.

Sahli[10] maintains that the recurrent attacks of such conditions as articular rheumatism, pneumonia, and erysipelas are the result of acquired hypersensitiveness, and that this is accompanied by a degree of protection which renders the later attacks milder and of shorter duration than the initial attack. He puts the matter very clearly:

> By virtue of a previous recovery from these diseases the organism acquires a hypersensitiveness, and therefore, when again exposed to the infective agents, relapses more quickly and more frequently than the healthy organism. On the other hand, just because of this hypersensitiveness and the resulting production both of the non-specific inflammatory and the specific antibody, it recovers more quickly and more easily from the infection than the normal organism.

I know that some are not prepared to accept the suggestion that the crisis in pneumonia has anything to do with the anaphylactic condition, but Wolff-Eisner[11] points out that the bacteriolysins present in the body ensure a continuous liberation of endotoxins from the lysed pneumococci. As a result of the liberation of toxins and antigens a larger amount of lytic antibody is produced, and at a certain stage these ensure the disintegration of the whole of the pneumococci that have made their way into the tissues. The tremendous amount of toxin thus set free gives rise to the symptoms of crisis, but as soon as these toxins have done their work, there being no more pneumococci present, the condition of the patient improves rapidly—the crisis is passed. In certain cases, however, where the destruction of the pneumococci has not been complete, further lytic antibodies are formed under the action of the remaining antigens, the protein of the pneumococci, and a secondary or pseudo crisis may result. On this point a very suggestive and interesting note is added to his text by Wolff Eisner. He says:

> It would be wrong to assume that antibodies accumulate before they begin the attack. They begin the attack as soon as they are formed. The formation, however, is stimulated when, owing to the irritation of the endotoxins liberated by the bacteriolysins spontaneously present, they are cast off from the cells, particularly, it would seem, in the haematogenic organs.

Many individuals who are supposed to be exceedingly susceptible to colds and catarrh may be individuals highly supersensitized to some special substance; and one cannot help thinking that many of those who suffer from so-called nasal catarrh and coryza have been supersensitized, and kept in a highly sensitized condition during

certain periods of the year, by the waste proteins accumulating in rooms where there are large congregations of people, the stimulating or exciting dose of protein being applied at intervals. We know that the exhalations, or at any rate matter, conveyed from the horse may induce severe asthma in the human subject. That is now recognized as being an anaphylactic condition. May we not have a similar but modified series of phenomena resulting from the inhalation of the organic exhalations of crowds? Of course, it may be that a supersensitive condition is set up to the "lysed" micro-organisms, staphylococci, streptococci, etc., present in the mucous membrane—micro-organisms which under ordinary conditions kept outside the tissues, may on congested surfaces gain more ready access to the deeper naked tissues, and so set up a supersensitive condition. These are all points of interest.

Before I close, may I be allowed to introduce a side issue, but one that involves some of the most important practical outcomes of recent studies in anaphylaxis? it bears on the suggestion that in certain cases antidiphtheria and antitetanic serum should be prepared in goats and in oxen. Surely it is "practical politics" that all prophylactic doses, especially of antidiphtheria serum, should be taken from some animal other than the horse, so that should it be necessary at a later stage to inject large quantities of antiserum, say, in the treatment of cases of diphtheria, the patient would not be already sensitized to horse serum. May I suggest that those engaged in the preparation of antitoxic serums should keep a separate series for prophylactic treatment, and that these should always be obtained from the ox or the goat?

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ANAPHYLAXIS IN ITS BACTERIOLOGICAL ASPECTS.

BEFORE considering the special bacteriological aspects of anaphylaxis it is necessary to review briefly the nature of the reaction, and to eliminate other causes producing sudden death in animals.

I. THE NATURE OF THE ANAPHYLACTIC REACTION.

The first point to be discussed is the nature of the anaphylactic antibody. It is still considered by some observers that the antibody which produces anaphylaxis is a special one, and distinct from the amboceptors or precipitins, etc.

We have performed a series of experiments in which we have endeavoured to show that the anaphylactic antibody is identical with the amboceptor, and that, given a sufficient amount of amboceptor, anaphylaxis can always be produced. We inoculated intravenously a series of guinea-pigs of even weight with graded quantities of an anti-sheep haemolytic serum from a rabbit of high titre. After thus passively sensitizing the guinea-pigs they were injected intravenously with washed sheep's red corpuscles (twenty-four hours later). It was found that acute anaphylaxis could be always produced in animals which had been sensitized with a sufficient quantity of amboceptor, the quantity necessary for a guinea-pig of 250 to 300 grams being 1,680 units and above, a unit being that quantity of haemolytic serum which will, in the presence of complement, just produce complete haemolysis of 100 c.mm. of a 10 per cent. red corpuscle suspension. Below 1,680 units acute anaphylactic death never occurred, but only some of the minor manifestations. On the other hand, however much amboceptor is used anaphylaxis can always be produced if a sufficient quantity of antigen is subsequently inoculated intravenously. That is to say, that excess of antibody does not protect against anaphylaxis In the guinea-pig it is very difficult to produce immunity to a foreign protein by repeated inoculations in the ordinary way, the animals continually dying after the second or third injection. Further, if a highly immune animal be obtained, intravenous injection of a sufficient quantity of antigen always kills from acute anaphylaxis. For example, a guinea-pig of 300 grams was immunized to sheep's red cells and had a lytic titre as high as 4 c.mm., which represents over 56,000 units in the animal's body. The intravenous inoculation of 0.2 c.cm. of red cells caused acute anaphylactic death. There thus seems to be a direct parallel between the sensitizing power of an amboceptor containing serum and its amboceptor content. We can also say that in guinea-pigs an excessive amount of amboceptor does not protect against anaphylaxis. The above quantitative relationships also hold when the guinea-pigs are passively sensitized with homologous haemolytic serums.

II. THE ACTION OF THE ANTIBODY.

The question arises as to whether acute anaphylactic death in the guinea-pig is due to the action on the bronchial musculature of a toxic substance produced by the interaction of the antibody and the antigen, or whether it is caused by mechanical irritation produced by an alteration in surface tension due to beginning precipitation from the interaction of the antigen and antibody. With regard to the latter, Dale has suggested that the reaction in excised involuntary muscle of a sensitized animal is so rapid that it cannot be assumed to be due to ferment action, but is probably due to the irritation produced by a sudden alteration in surface tension from beginning precipitation. The points against this view are:

1. That removal of its whole protein content does not appreciably alter the surface tension of a fluid. The surface tension depends on the lower molecular bodies.
2. That when the homologous antigen is mixed with the sensitive serum the alteration in surface tension for the first two hours at room temperature is the same as when the antigen is added to normal serum. These observations on the surface tension were kindly performed for us by Dr. Lovell.
3. That substances such as bile salts, which produce a rapid alteration in surface tension, do not cause anaphylactic death.

Again, in some of our experiments egg-yolk was used as the sensitizing antigen, and on comparing the effect of the egg-yolk on sensitive and normal serums there was an alteration in surface tension, which was equally rapid in the two cases and equal in degree, and was wholly due to surface tension altering substances in the egg-yolk. Egg-yolk inoculated intravenously into a normal animal produces no obvious results, although it contains surface tension altering substances. Thus we should say that the anaphylactic reaction is not due to a sudden alteration in surface tension.

Now, with regard to the extreme rapidity of the reaction, this is used as an argument against it being due to toxic substances developed as the result of ferment action on the antigen. If this be so it equally holds against the idea that the condition is due to an alteration in surface tension produced by the interaction of the two substances. Time is also a factor here; time is also required for the combination of precipitin and precipitinogen. So if the one can produce instantaneous effects, why not the other?

III. DOES THE ANAPHYLACTIC ANTIBODY ACT AS A PROTEOLYTIC FERMENT?

The only real objection to the antibody being a ferment is the fact that it is supposed to act quantitatively, that is, that it cannot do an indefinite amount of work like an ordinary ferment, that it does not become free when the reaction is completed. That this is erroneous is shown in the following way:

If a complement fixation experiment be carried out in a test tube the complement once bound never becomes free again. If, however, the experiment be performed in a diffusion membrane through which protein cleavage bodies can pass against normal saline, the complement becomes free again after a few hours. This was demonstrated by taking out samples from time to time and adding to them haemolytic serum and red corpuscles. The samples were not in themselves directly haemolytic. The first samples were unable to activate a haemolytic system, but those removed some hours later were able to do so. The diffusate after

concentration was shown to contain protein cleavage bodies. This shows that complement which has once become bound can, if the products of its action are removed, become free again. That the antibody can also become free can be shown in the same experiment. If a sample be taken at the time when the complement is free again and fresh antigen be added to it, it will be seen that on further incubation in a test tube the complement now becomes fixed. Thus both complement and antibody must have become free, and can attack a further quantity of antigen.

Muir has also shown that if fully sensitized isogenous red corpuscles be inoculated into an animal, the amount of corpuscular destruction is by far greater than can be accounted for by the lysis of the inoculated sensitized corpuscles, showing that the lysin attached to the red cells has become free and has attacked fresh ones.

Further, it can be shown that complement does not act quantitatively except in minimal doses. Thus we have shown that if the unit of ambceptor be added to a unit of red cells a certain quantity of complement is necessary for the haemolysis. If, now, one hundred times the quantity of red cells and one hundred times the quantity of amboceptors be mixed together, to cause complete haemolysis one hundred times the amount of complement is not necessary; even as little as twenty-five times the quantity is sufficient—that is, one quarter of the calculated amount.

Thus we have shown that the antibodies fulfil some of the most important laws of ferment action. That the antibody has a proteolytic action is seen by the examination of the diffusate of its action on the homologous antigen.

The rapidity of the anaphylactic reaction remains to be discussed: (1) The amount of toxic substance necessary to produce acute death is exceedingly small, since acute anaphylactic death in a guinea-pig can be produced with the toxic substance obtained by the action of normal guinea-pig's serum from half a drop of horse serum. (2) The action of a ferment on a fermentable substance is at the commencement of the reaction infinitely rapid, as degradation substances accumulate the reaction is slowed.

Thus, in the animal body, the ferments acting under their optimum condition can very rapidly produce the necessary amount of toxic substance to cause death. In the sensitized involuntary muscle the ferment is bound to the muscle; there are no end bodies to slow ferment action, so that the ferment action will be infinitely rapid; the necessary small amount of toxic substances will be liberated in immediate contact with the muscle. It is thus unnecessary for there to be any appreciable latent period.

There still remains for discussion the action of colloidal substances when inoculated intravenously into guinea-pigs. The substance most frequently used in connexion with experiments of this description is colloidal silica. Kaolin and kieselguhr have also been injected intravenously and, like colloidal silica, produced rapid death, and this death has by all previous observers been thought to be identical with or very like acute anaphylactic death. This, however, is quite erroneous, since, on careful observation, it is seen that the real cause of death is intravenous clotting. After the injection of a few cubic centimetres of a suspension of colloidal silica the animal dies—convulsions are not a marked feature; on *post-mortem* examination, carried out immediately after death, it is seen that the blood in all the veins is clotted. The lungs may show a certain degree of distension, the distended lungs are very hyperaemic and somewhat oedematous, and there are in addition many areas of collapse. In the case of kaolin and kieselguhr the injection of a suspension in normal saline also produces similar sudden death. Here, also, there is rapid intravascular clotting and the lungs are only slightly distended. It will thus be seen that the mode of death is quite different from and is not comparable to the mode of death in acute anaphylaxis. It can thus be said that acute anaphylactic death, with its typical features, is only produced when the toxic substances are produced in the animal's body by the interaction of the antibody with its homologous antigen, or when this toxic substance is inoculated preformed into the animal.

In examining a large number of guinea-pigs, dying from various forms of bacterial toxaemia or septicaemia, it was noticed that the mode of death was identical in all cases. The animal was never febrile, the temperature fell to below 94° F., there was paresis of the posterior extremities, gradually extending to the anterior, ruffling of the coat, shivering and varying degrees of respiratory spasm. At the autopsy there was always right-sided congestion and venous engorgement. The lungs, in quite 90 per cent. of the cases, showed a fairly marked degree of emphysema, and the gall and urinary bladders were distended. This condition is exactly parallel, both in its symptoms and *post-mortem* appearances, to that seen in delayed anaphylaxis, and is comparable to what happens when a normal guinea-pig is inoculated intravenously with a sufficient amount of fully sensitized sheep's red corpuscles. Thus if a guinea-pig of 300 grams is inoculated intravenously with 5 c.cm. of a 10 per cent. suspension of fully sensitized sheep's red corpuscles, the animal dies in about eight to twelve hours, with symptoms exactly like those described above. The *post-mortem* findings are the same. Here the cause of death can only be due to toxic substances formed from the corpuscles by the action of the guinea-pig's complement on the sensitized red cells, the rate of liberation of toxic material being just rapid enough to produce delayed toxic death.

The next point for consideration is the result of the inoculation intravenously into healthy guinea-pigs of the serum, blood, and effusions of guinea-pigs or rabbits dying from bacterial toxaemia or septicaemia. In our first experiments the serum or effusion was filtered through a porcelain filter, but it was found that, although the material so treated produced intense toxic symptoms in the inoculated animal, acute death rarely occurred; whereas with the unfiltered material acute death did occur, so that it appeared that the toxic substance underwent some change as the result of this treatment, and in our further experiments this method was not adopted. With the unfiltered serum, or whipped blood, or citrated blood, or effusion, on inoculation intravenously into a healthy guinea-pig, acute anaphylactic death could always be produced if a sufficient quantity were inoculated, the quantity varying from 2 to 5 c.cm. of the material. The mode of death and the *post-mortem* appearances were absolutely typical. This result was obtained by inoculating the fluids from animals dying from infection with the bacillus of chicken cholera, *B. phlei*, *B. danysz*, *B. coli*, *B. tuberculosis*, *B. proteus vulgaris*, *B. hoffmanii*, *Staphylococcus aureus*, *B. prodigiosus*, etc. Sublethal quantities produced either a great fall of temperature or fever, according to the amount inoculated. It might of course be argued that the toxic substance was not preformed in the blood used for the inoculation, but produced by the ferments of the normal animal into which it was inoculated, either directly from the bacteria, or macerated bacterial protoplasm, or from the sensitized macerated bacterial protoplasm present in that blood. In order to eliminate this possibility of error, healthy animals were inoculated either with large quantities of finely disintegrated bacteria or with sensitized disintegrated bacteria, but with the only exception of the inoculation with tubercle bacillary emulsion, acute death never occurred. In the case of the tubercle bacillary emulsion it was demonstrated that hydrolytic cleavage bodies were present, and that if other bacteria were allowed to undergo autolytic degradation they also become directly toxic. Now, the blood and effusions of the animals examined were never allowed to stand for any length of time, so that degradation of the bacteria could not occur subsequent to the removal of the material from the infected animal. It therefore follows that the toxic substance in these fluids was preformed, and was not formed subsequent to removal from the animal infected, either by autolytic degradation of the bacteria present or by the subsequent action on them of the antibodies of the normal animal inoculated. We must come to the conclusion that there is in the blood and effusions of animals dying from bacterial toxaemia or septicaemia a substance which is present in sufficient quantity to cause acute anaphylactic death when inoculated into healthy animals, and that this substance is always present, no matter what the organism is which has given rise to the infection. By diffusing the effusions of animals dying from bacterial toxaemia or septicaemia, hydrolytic cleavage bodies can be demonstrated in the diffusate, so

that the toxic substance is produced from the bacteria during the process of hydrolytic cleavage of the protein by the ferments in the animal body.

From these results it will be seen that guinea-pigs dying from bacterial toxaemia or septicaemia all die in exactly the same way. The *post-mortem* findings are identical and the toxic substance found in the blood is identical, and this toxic substance is present in relatively large amounts, as can be easily seen when it is considered that a few cubic centimetres of blood or effusion are sufficient to kill a perfectly normal guinea-pig. Such being the case, it appears to be superfluous to suppose that there is, in addition to this, a special toxic substance, characteristic of the bacterium—namely, a specific endotoxin. No matter how large a quantity of finely divided bacterial protoplasm is inoculated into a normal animal, acute death can very rarely be produced, and when it does occur it always has the character of acute anaphylactic death and is then probably due to the autolytic degradation bodies present. In all other cases, no matter what the bacterium is, death occurs in exactly the same way. The animal develops fall of temperature, ruffling of the coat, rigors, paresis, urination, and the *post-mortem* appearances are always the same, the only exceptions being that suprarenal haemorrhages or intestinal hyperaemia may be marked features. These, however, are by no means constant for special bacteria, but are frequently noticed with very widely different varieties of bacteria.

This leads, then, to the conclusion that bacteria have no specific toxic substance, and that the bacterial protoplasm is primarily non-toxic, and only becomes toxic when it has been acted upon by the antibodies present in the infected animal, and the degree of toxicity of a bacterium depends upon the rate of accumulation of these toxic degradation bodies, and this will depend upon the relation between the activity of the antibody present in the animal at the time and the quantity of bacterial protoplasm present—that is, upon the primary dose inoculated and the rate of multiplication of the bacteria. Two examples may be given supporting this view on the above idea. Animals are immune to certain bacteria either because the antibody activity of the animal against the bacteria is so high that the bacteria are rapidly degraded beyond the toxic stages, or because the antibody activity is so low that the toxic substances do not accumulate to any degree.

Now, dealing with an example of the latter—there is the *B. mycoides*—this is an organism which naturally occurs in the soil, and will not grow at body temperature. By a process of gradually educating it to an increasingly higher temperature it is possible to obtain a strain from the original which will grow at the body temperature. This organism is not pathogenic to guinea-pigs in doses of three agar slopes and more. If, however, a guinea-pig be inoculated with 20 mg. of dead *mycoides* a week previously, one agar slope of the organism will now in that animal produce septicaemia, and the blood and effusions of the infected animal will give rise to acute anaphylactic death when injected intravenously into a normal animal. There is no difference in the bacteria inoculated; the difference is in the animals. In the normal animal even 20 mg. only produce a transient temperature variation.

The possibilities are:

1. That the animal is immune because there is so little antibody that the toxic substances can only be formed slowly—so slowly that they do not accumulate in sufficient amount to injure the animal; but that after the injection of the preliminary dose the ferment activity is raised so that the ferments are now in such amount that sufficient toxic substances can be formed to kill the animal. That in one week antibody formation can be great enough is seen from the injection of 20 mg. dry weight of red corpuscles, when the lytic titre of the serum to the homologous antigen becomes such that a unit is contained in about 40 c.mm. of the serum.

2. That a negative phase is produced so that the toxic material of the second dose cannot be dealt with.

Dealing with the latter first, the following experiment negatives the idea of the result being due to the production of a negative phase. During our experiments on the *B. mycoides* several strains of closely allied and similar organisms were isolated from various soils. These grew easily at the body temperature. They were found to be directly pathogenic to guinea-pigs in doses of half an agar slope and less. Guinea-pigs were also inoculated with 20 mg. dry weight of these bacteria, and after intervals of respectively two, three, four, and five days, they were reinoculated with a dose certainly fatal to a normal animal—namely, half an agar slope, which usually produced death in less than eight hours—the animals all recovered, and even from larger doses. So that if in the one case the septicaemia and toxaemia are due to a negative phase, in the other a similar negative phase protects the animal. Experiments with other organisms bring out the same fact.

A possible third theory would be the state of anti-anaphylaxis—that is, that the 20 mg. of the first inoculation bound all the antibodies, and so allowed the second dose to be unattacked, to multiply, and so produce death. This is really the previous idea over again in other words. The only possible conclusion is that the whole matter rests on relative ferment activity. In the case of *B. mycoides* there is very little antibody activity, consequently toxic substances cannot be split off from the bacteria in sufficient amount to do damage and inhibit phagocytosis; with the other closely allied strains the antibody activity is just great enough to allow of a sufficient accumulation of toxic material so as to inhibit phagocytosis, allow the bacteria to multiply and cause death.

With the former a previous inoculation of dead bacteria causes an increase in the antibody activity, and at a certain period it is sufficiently great to bring about the conditions necessary for the death of the animal and the causation of septicaemia. With the others the antibody activity increases so that the toxic substances first formed are rapidly rendered non-toxic by the further rapid action of the antibody, so that the animal can now recover from what, under ordinary conditions, would have been a fatal dose. Then there are animals, which are immune because of the great antibody activity against certain bacteria. By diminishing the activity of the antibody against these bacteria a fatal result can always be produced. Here the immunity is due to the fact that the antibodies can so rapidly deal with the bacteria that the early-formed toxic substances do not accumulate, but are rapidly further converted into non-toxic ones.

Thus the toxicity of a bacterium depends on the animal—that is, on the relative antibody activity to the bacterium and not on the bacterium *per se*; the same bacterium producing different results according to the antibody activity in the different animals. The bacterium does not secrete a toxin or have a preformed toxin; the toxin is produced by the antibodies from the bacterial protoplasm, and is the same for all bacteria—that is, a toxic hydrolytic cleavage body of the bacterial protoplasm, producing death in the same way in all bacterial infections, and present in the infected dying animal in large amounts, so that a small amount of that animal's blood can produce in a healthy animal acute anaphylactic death. Hence we must not regard this state of so-called anaphylaxis or hypersensitiveness as a freak state nothing to do with immunity proper, but as a state that can always be made to occur and is relative, depending on the power of the antibody and amount of bacterial protoplasm present; according to the relations between them, acute toxic deaths, delayed toxic death or recovery may occur, the toxicity of a bacterium depending upon these factors.

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CLINICAL ASPECTS OF ANAPHYLAXIS.

I have been asked to say a few words on the clinical aspect of this subject, and I do not think I can introduce what I have to say better than by relating the histories of two cases. The illness in each case was due to a certain cause, but for the present I shall not say what it was. The first case was published by Dr. Blain.[1] The patient was a woman, aged 24. On January 24th, 1907, at 6 p.m. a certain event happened to her.

> Within twenty minutes . . . a profuse urticaria appeared over the entire body. The oedema of the periorbital tissue completely closed the eyes. The patient was greatly prostrated, respiration sighing, absence of radial pulse, complained of feeling faint and chilly, also of fullness in the throat. The bowels moved freely at 8.45 p.m., the patient complaining of

cramps in the bowels and soreness in the stomach. At 9 p.m. the radial pulse became perceptible, but was thready and intermittent; 10.30 p.m., pulse low in tension and irregular; 11 p.m., pulse somewhat improved, respiration still sighing, patient very restless; 12 midnight, bowels again moved freely. . . . After a restless night, at 7 a.m., the pulse was 88; the patient coughing a good deal from bronchial irritation; at 6 o'clock was able to retain a small amount of nourishment. Temperature rose to 100° F. at the beginning of the attack, but otherwise did not go above 99°. After a few days in bed the patient gradually recovered.

The second case was related by Dr. Atkinson.[2] The patient was a married woman, aged 40, who had always enjoyed good health except for periodical attacks of migraine. At about 7.45 a.m. on September 9th, 1907, a certain event happened to her.

> Within five minutes she began to itch all over, and felt herself swelling from head to foot. This was followed by abdominal pain and a desire to defaecate. . . . She then collapsed, and her husband and a maid carried her . . . to bed. She was then unconscious. I (Dr. Atkinson) arrived about 9 a.m. . . . I found her in a semiconscious condition, and the whole body irregularly convulsed. She rallied when spoken to, and then relapsed into apparent unconsciousness again. . . . The whole of her body was covered with a dark, measly rash. . . . The skin felt hard and tense and brawny. The hands, and especially the finger tips, were livid. The face was congested and the eyes suffused. She tells me there was a feeling of nausea all the time. The skin was cold, the pulse was feeble, and the heart sounds faint. A distinct wheeze could be heard over the chest. . . . At 10 a.m. she felt better, but complained of the tight feeling all over the skin and the difficulty of breathing. At 2 p.m. the rash had almost gone. She felt much better, but there was still some dyspnoea and feeling of constriction. . . . At 9 p.m. I found her very hot and flushed, and the skin now covered again from head to foot with a very bright scarlatiniform rash, and on the back and hips were many extensive wheals. On the morning of September 10th all rash had practically gone, but there was some oedema of both eyelids. All the other symptoms had disappeared.

There are several points of similarity between these two cases. It is true that the latter was more severe than the former, but it is to be observed that in each case the onset of the illness arose very quickly after the event which was believed to have occasioned the attack; that there was a rash, urticarial or erythematous; that there was enfeeblement of the heart's action; that there was some respiratory embarrassment; and that there was some gastro-intestinal disturbance. The first of these two cases was undoubtedly an example of anaphylaxis. The attack came on after a subcutaneous injection of diphtheria antitoxic serum in a patient who had been treated with serum about a year previously. In the other case the symptoms came on almost immediately after the sting of a wasp on the left foot.

Hitherto anaphylaxis in the human subject has been observed chiefly in connexion with antitoxic and antibacterial serum in diphtheria and other diseases. The serum almost invariably employed has been that of the horse. Now in the vast majority of cases an injection of horse serum is perfectly harmless at the time it is given. No symptoms arise then; but in about one-third of the cases treated with serum, after an interval or latent period of a week to a fortnight, the patient undergoes an attack of what is now known as serum sickness, of which the most common symptoms are an urticarial or erythematous rash, pyrexia, and in a few cases slight multiple arthritis. But serum sickness is not anaphylaxis, nor can it be taken as evidence that the affected person is necessarily in the anaphylactic condition. In order to demonstrate the existence of this condition, it is necessary to give another injection of horse serum, and this fresh injection must not be given before the end of the average latent period of serum sickness—that is, before the lapse of nine or ten days from the first injection. If the first injection has rendered the person anaphylactic, this second injection, given after the latent period, may be anything but harmless. An attack such as that which occurred in the first of the two cases related above, or even a more severe one, may follow either within a few minutes or hours, or, at any rate, well within the ordinary latent period. The latent period is, in fact, much curtailed. The primary injection has rendered the person much more sensitive to the action of the serum. Other evidence of over-sensitiveness is to be found in the occurrence, in unusual severity, of the symptoms of ordinary serum sickness (a rash and pyrexia), and also in the additional occurrence of symptoms which are not met with in ordinary serum sickness (cardiac depression, respiratory embarrassment, rigors, convulsions, coma).

The anaphylaxis which is brought about by an injection of horse serum is an acquired one, which it takes ten days or a fortnight to produce. But some persons are apparently naturally anaphylactic, for the symptoms of anaphylaxis may be evoked in them by the first injection of serum. This form of supersensitiveness may be called congenital, and, curiously enough, it is in this form of the condition that the most severe examples have been met with. Nearly all the fatal cases have belonged to this variety. There is also evidence to suggest, if not actually to prove, that many asthmatics are by nature in an anaphylactic state as regards horse serum.

I need hardly do more than point out that horse serum anaphylaxis is but one instance of the anaphylaxis which can be produced in animals by any protein. But in looking for instances of anaphylaxis in clinical medicine we must remember three important facts which have emerged from the experience of the laboratory. The first is that the protein must be foreign to the animal; the second is that to bring out the symptoms of an acquired anaphylaxis it is necessary to employ for the reacting dose the same protein as was used to establish the anaphylaxis; the third, and most important from the clinical standpoint, is that, whatever the protein, the anaphylactic symptoms are the same in the same species of animal.

If, therefore, we wish to find anaphylactic diseases in the human being, we must look for the symptoms of serum anaphylaxis. I have given one example in the case of wasp sting reported by Dr. Atkinson. The doctor, at the end of his report on the case, asks the question, "Is it possible that all this trouble could arise from the sting of a wasp?" To which I should have no hesitation in replying, "Certainly it is possible." One of the remarkable facts about anaphylaxis is that a very minute quantity of protein will suffice for the reacting and a by no means large one for the sensitizing dose. Amongst the agents that will produce in man symptoms which are extremely like those of serum anaphylaxis, certain articles of diet and certain insects pre-eminently figure. The ingestion of the one and the stings or bites of the other are the methods by which the reacting dose of the protein is introduced to the system. Certain fish, especially shellfish (mussels, crabs), a few fruits (strawberries, raspberries), and the stings or bites of some insects (bugs, wasps), are well known to be capable of evoking the anaphylactic syndrome. But while I have little, if any, hesitation in ascribing the symptoms set up by these agents to the anaphylactic state, I am not so sure that we know of any other agents which act similarly. From time to time cases are met with, I have seen them very occasionally in the course of some of the acute infectious diseases, which suggest anaphylaxis. But when we search for the cause which has produced and elicited the supersensitiveness, we have to confess ourselves baffled. I refer especially to odd febrile attacks with rashes of the same kind as those seen in serum sickness.

The general reaction set up by tuberculin would appear to be of an anaphylactic nature; but it is extremely doubtful when Wolff-Eisner's, Calmette's, and von Pirquet's local reactions are. Arthus's local anaphylactic reaction—as seen in rabbits—is never, or at any rate extremely rarely, produced by horse serum in man.

During the last two or three years more than one writer, clinical as well as bacteriological, has suggested, nay, has even boldly asserted, that several morbid conditions besides those I have mentioned should be included in the anaphylactic group; for example, puerperal eclampsia, the shock produced by burns and scalds, the idiosyncrasy to certain drugs, such as quinine, the rashes of some of the acute infectious diseases, specially of those of which the incubation period is seven to fourteen days. To me, however, the evidence brought forward in support of these suggestions is far from being convincing. From the clinical and from the experimental evidence I hold that inasmuch as anaphylactic symptoms are of the same kind whatever may be the protein concerned, when anaphylaxis is made manifest, it will be revealed by much the same symptoms as are seen in horse-serum anaphylaxis.

In respect of that form of anaphylaxis in man which is best known—namely, horse-serum anaphylaxis—there are

several points concerning which further clinical observations are required. I have mentioned above that apparently some persons are naturally oversensitive to the serum. But I think it is quite a question whether there really is a congenital anaphylaxis. May not those persons who react so strongly to a first dose of serum have been sensitized before? Some of the asthmatics have been peculiarly sensitive to the emanations from the horse. Possibly in them the anaphylaxis has been induced in this way. So also one may argue in the case of shellfish and insect anaphylaxis. The lady who suffered so severely from the effects of the sting of a wasp had been stung more than once previously by those insects, and she may have been sensitized by one of the earlier stings. Another question which awaits an answer is: How long will a person who has been rendered anaphylactic remain in that condition? The longest period I have known has been just over seven years. Not every person who is treated with serum becomes anaphylactic. I have had under my observation during the sixteen years ending December 31st last 203 persons who have been injected with serum twice at intervals varying from a fortnight to upwards of thirteen years, and 114 of them, or 56 per cent., were anaphylactic. There are a few cases on record which go to show that a person may not be sensitized by the first, but may be by the second, injection. In the first of the two cases I related at the beginning of my remarks the young woman had been treated with serum three times, the second occasion was six weeks after the first, and then no anaphylactic symptoms appeared. The third time was a year after the second, and then there was a most serious attack. Another question which arises is whether the anaphylactic condition varies in its intensity from time to time in any sensitized person. It has been stated by more than one observer that there is an optimum period for anaphylaxis in persons who have been treated with horse serum. Thus Gruyez and Bernard have concluded from certain experiments that in man there is little development of the condition within five weeks of the injection, that the symptoms are most likely to be brought out from the fifth to the twenty-seventh week, and that between the twenty-eighth and the forty-ninth week the supersensitiveness disappears. But these conclusions are not in agreement with what I have noticed in my cases. Of 45 patients reinjected within five weeks 17, or 37.7 per cent., were anaphylactic; and of 26 injected later than the twenty-seventh week 19 were so, or 73 per cent. Twenty-one were injected after the forty-ninth week, and of these 17, or 80.9 per cent., gave signs of anaphylaxis. My cases, therefore, afford no evidence of the existence of an optimum period, nor of the disappearance of the increased sensibility after a particular length of time. Another question which requires working out is whether the anaphylactic state, acquired or congenital, can be transmitted to the children of the affected person. It has been shown that the condition can be transmitted by the female guinea-pig, but not by the male, to its offspring. But I have not yet had occasion to inject with serum the child of any woman who had been injected before the child's birth. In the ordinary course of events there should be some, if only a few, such cases in the not very distant future.

Lastly, can the anaphylactic state be avoided or abolished? Certain experiments on animals go to show that it can be, at any rate for a time. Some of the methods used are quite inapplicable to the human subject, and the results of those which might be used are very uncertain. It is stated that if you inject a person, who may have been rendered anaphylactic by a previous injection, with a very small quantity of serum no general reaction will take place; and, further, that this small dose abolishes anaphylaxis for a time at any rate, so that a large dose may be injected with impunity within a few hours or perhaps a day or two. More especially is this the case if the small injection has evoked no local reaction. I have not had an opportunity of trying this method.

It has been stated that if an animal survives one anaphylactic storm it will not experience another—that it is, as it were, immunized against anaphylaxis. This is certainly not invariably the case in human beings. I have twice known anaphylactic symptoms to arise in patients after a third injection of serum, in whom the second injection had aroused those symptoms; and one of the cases I related at the beginning of my remarks shows that a person may be anaphylactic to a third injection who was not so to the second. It has been stated that if you give a second injection within a fortnight of the first—that is, within the latent period of ordinary serum sickness—you will protect the person so treated from anaphylaxis should a third injection have to be given subsequently. But I have had more than one case under my observation which has shown that that statement is not correct.

Fortunately, severe anaphylactic symptoms are not common in reinjected persons. But I should be very chary of giving serum to a patient whom I knew to be asthmatic. I should not give it unless he was suffering from a dangerous attack of the disease for which the serum was indicated. The suggestion that for a second injection the serum of a different animal from that whose serum was used for the first injection should be employed, is certainly to the point and quite worth a trial. I understand that such serums can be obtained. But more clinical evidence is required before we shall be able to speak with any certainty on this part of the subject, and the scantiness of the evidence must be my excuse for having so little that is of value to say about it.

It is a curious circumstance, for which I can offer no valid explanation, that while I still continue to witness undoubted anaphylactic reactions in persons reinjected with horse serum, yet these reactions are not so severe as those I saw when I first became acquainted with this phenomenon some sixteen years ago. This serum, made for the Metropolitan Asylums Board by Dr. Cartwright Wood, is less noxious than it used to be—a fact which is shown by the lessened severity not only of the anaphylactic but also of the ordinary serum reactions.

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THE ACTION OF THE ASBESTOS MINERALS AND ALLIED MATERIALS ON BACTERIAL AND OTHER SUBSTANCES.*

By MYER COPLANS, M.D.
(From the Department of Pathology, University of Leeds.)

THIS series of experiments arose out of an attempt to isolate the toxin of tetanus from a bacilli-free broth culture of *B. tetani* by gravity filtration through a recently prepared hydrate of alumina formed as a filtering layer upon filter paper.

As a preliminary it is necessary to wash the hydrate free from admixed electrolyte by means of a gentle and continuous stream of distilled water through the undisturbed layer of the hydrate; and, after six hours of such treatment, the filtrate contains greatly diminished yet appreciable quantities of the electrolyte in solution. About this period, however, the constantly shrinking gelatinous hydrate commences to show deep fissures, and the process is rendered abortive.

To overcome the difficulty finely carded Tyrolese asbestos was incorporated with the hydrate, and after prolonged washing the toxin-containing broth was allowed to percolate the prepared compound layer of hydrate and fibre. The filtrate proved to be toxin-free, and on treatment with alcohol the compound of hydrate and fibre yielded a considerable proportion of the tetanus toxin.

As a control test a sample of the toxin-containing broth was shaken with the asbestos fibre without the intervention of hydrate of alumina, and, after an interval of five minutes, the expressed liquid was found to be toxin-free. It failed, in addition, to give a positive biuret reaction, in strong contrast to that yielded by the untreated broth.

The investigation of this phenomenon has been extended so as to compare the activities of various kinds of finely divided "asbestos" minerals, including the Russian, Italian, and blue varieties of asbestos, chrysotile-asbestos of Canada, and, in addition, the steatites, as exhibited towards toxins (including those derived from *B. diphtheriae*, *B. tetani*, *B. tuberculosis* and *B. mallei*), certain antitoxic

* A portion of the expenditure in connexion with this research has been met by a grant from the Science Committee of the British Medical Association.

serums, cobra venom, complement and amboceptor of a haemolytic system, specific agglutinins, certain proteins, pigments and colloids, while as a convenient example of a ferment diastase was included, and submitted to similar tests.

All these substances apparently readily disappear from watery solutions, or are rapidly changed in character when brought into intimate contact with finely divided minerals of the asbestos group, while watery agar, watery gelatine, and watery isinglass, after similar treatment lose the power of setting.

The action of finely powdered and fibrous substances such as kaolinite, spun glass, the vegetable fibres (jute, flax, cotton and ramie), silk and wool, and, in addition, animal charcoal, have been under investigation in a parallel series of experiments. As an outcome of the results following the interaction between certain radio-active substances in solution and members of the asbestos group of minerals, the investigation is being extended to include the felspathic and plagioclasic granites, which, on weathering, give rise to complex hydrated silicates known as zeolites.

Arising out of the action on toxins in particular by members of the asbestos group, I have carried out a series of observations on the effect of introducing asbestos fibre into fluid nutrient media inoculated with various pathogenic organisms.

Experiments relating to the phenomena of super-sensitiveness have been carried out. In no instance, up to the present, have toxic properties been conferred upon a normal serum by means of treatment with asbestos.

Certain Chemical and Physical Characters.

Many substances are classified as belonging to the group of "asbestos" minerals, and their chemical and mineralogical properties are shown in the subjoined table. With regard to the fibrous varieties, the fibres vary greatly in diameter and length. Their thickness depends upon the degree of mechanical disruption to which they have been subjected. Some of the finest fibres are but 0.6 μ in diameter (33,000 to the inch) in the carded Canadian varieties, while with others the thickness varies from 1.0 μ (20,000 to the inch) and upwards.

CHEMICAL RELATIONSHIPS OF "ASBESTOS" MINERALS.

"Asbestos" may be divided into the following three groups:

1. *Anthrophyllite Group* [(Mg Fe)O Si O_2].
2. *Amphibole or Hornblende* [RO.Si O_2], where RO may represent CaO; NgO; FeO, etc.
   - (*a*) Tremolite [(3MgO.CaO.2SiO_2)].
   - (*b*) Actinolite [3(MgFe)O.CaO.2SiO_2].
   - (*c*) Hornblende asbestos, hydrated (Italian asbestos).
   - (*d*) Mountain leather, mountain wood, mountain cork.
   - (*e*) Crocidolite (Blue or African, West Griqualand) [NaFe (SiO_3)3. Fe Si O_3].
3. *The Serpentine Group* (3MgO.2 SiO_2. 2H_2O).
   - (*a*) Picrolite (Canada).
   - (*b*) Chrysotile-asbestos (Canada-Quebec).
   - (*c*) Talc.

N.B.—*Kaolinite* = ($Al_2O3.2$ $SiO_2.2H_2O$).

Proximate Composition of "Asbestos" Minerals (Dana).

	Tremolite.	Actinolite.	Crocidolite.	Chrysotile.	Talc.	Picrolite.
SiO_2 ...	57.7	61.82	49 6	40.87	59 59	37.88
Al_2O_3 ...	—	1.12	—	0.9	1.76	1.10
FeO ...	—	6.55	19.8	2.81	0.79	0.36
Fe_2O_3 ...	—	—	22.0		—	2.7
MgO ...	28.9	23.98	—	41.5	32.92	43.29
CaO ...	13.4	1.63	—	—	0.59	0.82
Na_2O ...	—	—	8.6	—	0.56	—
H_2O ...	—	5.45	—	13.55	3.79	14 52

Fibrous varieties of asbestos differ materially in their behaviour towards atmospheric moisture, as is testified by the following table:

Humidity Tests for Relative Absorption, as Compared with Condition at 103° C.

Process.	Temp	Time.	Change, Per Cent	Chrysotile.		Crocidolite (African).
				Canadian.	Russian.	
	C.	hrs.				
1. Drying ...	120	3	Loss	0.49	0.48	0 19
2. Exposure to saturated atmosphere*	40	3	Gain	17.13	28.86	12 12
3. Drying ..	40	3	Water retained	0.33	0.34	Nil.
4. Exposure to saturated atmosphere†	13	12	Water absorbed	2.47	19 23	0.24

* Moisture = 50 grains per cubic metre.
† Moisture = 10 grains per cubic metre.

General Method.

1. For effect of treatment of liquids asbestos fibre equal to half the weight of the liquid has been added. The result is a moistened ball of asbestos which must be pressed to obtain any exudate.

2. For the effect on growth of pathogenic organisms in nutrient media 7½ per cent. by weight of finely-carded asbestos fibre has been added.

3. The fibre as a preliminary is dried at a temperature of 120° C., and must be kept in a moisture-free vessel. In the case of treatment with diastase the fibre was subjected as a preliminary to firing at a cherry-red heat. Heating above 120° C. degrades the mineral and alters its physical qualities, the fibres tending to disintegrate, while crocidolite is ruined for all test work if the temperature be raised to red heat.

4. When powdered mineral substances have been added to liquids the vessels have been placed in suitable rocking apparatus.

With regard to the method of preparation, I have come to the conclusion that it is better to use the finest carded varieties available, free of all coarse impurities, and that no further treatment is necessary. On the contrary, additional treatment, whether it be the process of firing or simple boiling with distilled water, causes degradation of the fibre both physically and chemically. Indeed, simple friction between the palms of both hands will reduce fine fibre to powder.

The action of distilled water on the asbestos minerals, on kaolin, and on animal charcoal, is of importance. Asbestos fibre is said to be "practically insoluble," or rather unacted upon by water. This is untrue, for in the early stages of this investigation prolonged experimental tests, some of them extending to four months, were made especially to prepare a purified form of asbestos fibre by means of the process of boiling in distilled water, or in dilute mineral acids followed by distilled water. By using electro-conductivity tests with direct reading apparatus, it is easily shown that asbestos is constantly acted upon and degraded by distilled water alone. In short there appears to be no useful purpose served by any attempt at purification by such methods.

The following table shows the results of such tests with distilled water, and for the purpose of comparison, animal charcoal and glass wool have been similarly treated. (The

Expressed Liquid under Test derived from	Conductivity of Expressed Liquid after			
	24 hrs.	42 hrs.	90 hrs.	280 hrs.
Control: Distilled water	3.2	3.2	3.2	3.2
Distilled water + Russian asbestos ...	321.28	551	756	1,472
Distilled water + Canadian asbestos...	1,152	1,696	2,204	2,272
Distilled water + African asbestos ...	448	630	702	1,056
Distilled water + Tyrol asbestos ...	988	1,152		
Distilled water + Austrian talc ...	102.4	121	130	186
Distilled water + animal charcoal ...	800.0	886.0	930	960
Distilled water + glass wool	2,560	2,793	3,444	4,216

units of conductance throughout are in terms of reciprocal megohms, cm^{-1}, at 20° C.) In each case to the distilled water there is added 50 per cent. by weight of the material, the degradation (or solution) of which is under test.

The constant and increasing degradation of all these substances in the presence of distilled water assumes importance if their rate of degradation or solution is materially altered—either hastened, stayed, or diminished—by the presence of substances in the liquid which apparently are inert. For instance, peptone water—0.2 per cent. solution of Witte's peptone in distilled water—when brought into contact with any of the foregoing varieties of fibrous asbestos, rapidly loses all trace of the proteose and peptone originally present. The conductivity of the expressed liquid, however, rises in an extraordinary manner, as shown in the following table:

Liquid.	Conductivity of Liquid (after 24 hrs.).	Biuret Test for Purpose of Comparison.
Distilled water...	3.2	0
Distilled water +0.2 % Witte's peptone	102.4	100
Distilled water +50 % Russian asbestos	321.28	0
Distilled water +0.2 % Witte's peptone +50 % Russian asbestos	620.8	0

Here the phenomenon of the disappearance of the diffusible proteoses and peptone from the solution is accompanied by an increased rate of degradation of the mineral substance, which ultimately is evidenced in the solution in the form of electrolyte.

In the case of non-diffusible colloids, such as suspensions of dialysed iron and methylene blue, the colouring matter of the suspension is quickly yielded to the asbestos fibre, the exudate becomes colourless, and its conductivity is markedly diminished. In short, the disappearance of the non-diffusible colloid, and the staining of the asbestos fibres, is accompanied by a deviation of electrolyte originally present in the solution.

In the case of colloidal gold in water there is in the presence of asbestos fibre immediate deposition of the gold on the fibre, giving rise to a stain which varies in intensity from puce to crimson. The conductivity of the liquid is that obtained from a simple experiment using asbestos fibre steeped in plain distilled water.

The results yielded by animal charcoal, when used in place of asbestos, are somewhat comparable. Both with animal charcoal and asbestos fibre the subsequent history of the liquids under test appears to be that the rate of disintegration in every case with non-diffusible colloids is somewhat lessened, due possibly to the formation of a protective coating around the particles either of the animal charcoal or asbestos fibre used. In addition the action of finely divided asbestos fibre on the following substances may be briefly mentioned:

Blood.—5 per cent. serum in normal saline yields negative tests for protein after two hours' contact. 1 per cent. haemoglobin in normal saline: colouring matter rapidly disappears.

Haemolytic System.—In the course of twelve hours' contact complement wholly disappears, and over 50 per cent. of the haemolytic amboceptor.

Agglutinin.—Serum diluted in normal saline 1 in 25: agglutinin (typhoid) entirely disappears in two hours.

Cobra Venom.—A solution made up so that 0.1 c.cm. contains a lethal dose for a large rat, the solution giving a well-marked biuret reaction, after twelve hours' contact yields a non-toxic liquid, and, in addition, the biuret reaction is entirely negative.

Diastase.—After four days' contact the expressed liquid shows no signs of the presence of the ferment when suitably tested with starch.

Boiled Starch (0.5 per cent. in water). — After four days' contact no trace of starch in solution as tested by iodine. The expressed liquid has lost its opalescence.

Mixture of Dye-stuffs.—Methylene blue in the presence of eosin rapidly yields all the blue to the fibre which is stained blue. The expressed liquid appears to contain eosin only.

Radio-active Substances.—Radio-active water from St. Austell, Cornwall, was placed in contact for one week; the radio-activity of the liquid diminished from 7 to 3. Five per cent. thorium nitrate solution placed in contact for three weeks; the emanations of the expressed liquid had diminished from 22 to 2. There was no appreciable diminution of the nitric acid in the liquid computed as nitrate, while conductivity tests gave the folowing results:

Liquid Tested.	Conductivity of Expressed Liquid.	Emanation Activity.
Distilled water	3.20	Nil.
Distilled water + 5 % thorium nitrate	17,472.0	22
Distilled water + Russian asbestos (50 %)	1,728.0	Nil.
Distilled water + 5 % thorium nitrate + Russian asbestos (50 %) —after twenty-one days' contact	16,832.0	2

[NOTE.—Mr. S. A. Edmonds, of the Physics Department of Leeds University, to whom I am indebted for the figures relating to the emanation activities, has kindly undertaken to investigate generally the fate of radio-active substances in the presence of asbestos, and a preliminary note on the outcome of his work is appended.]

Degelling Phenomena.

Watery gelatine (10 per cent.), watery agar (1 per cent.), watery isinglass (3 per cent.), after melting, are placed in contact for twelve hours with asbestos fibre at refrigerator temperature. Thereupon the expressed liquids are found to have lost the power of setting.

Bacterial Poisons.

Solutions of tuberculin, mallein, diphtheria toxin, and tetanus toxins, when diluted 1 in 25 in normal saline and placed in contact with the fibre, appear rapidly to lose the whole of their toxicity, as far as can be judged, within twenty-four hours.

With regard to diphtheria and tetanus toxins, the strength of the solutions taken was such that 1 c.cm. contained 400 × M.L.D. A volume of the exudate equal to 50 × M.L.D. of the untreated diluted liquid proved entirely non-toxic to animals, while the reaction changed likewise from markedly positive biuret to one entirely negative.

In the case of Koch's old tuberculin a reaction occurs during contact which may be divided into two stages: (*a*) The proteoses entirely disappear and the biuret reaction becomes negative; (*b*) later the toxicity diminishes, the latter being tested by biological reactions both in tuberculous man and tuberculous guinea-pigs.

Using the diluted toxins of diphtheria and of tetanus, diluted to such strength that 0.1 c.cm. contains 1 × M.L.D., contact with asbestos leads to an immediate loss of the toxicity of the liquid, and a like loss of potency ensues with diluted antidiphtheritic serum in which 0.1 c.cm. contains unit protective dose.

It is important to note that the extract from a mixture of asbestos fibre and distilled water after being in contact for several weeks does not, when added directly to these toxins or antitoxin serum, materially diminish their potency.

In extremely dilute solutions glass wool appears to have little effect upon the toxins of diphtheria or of tetanus. These, however, appear to be slowly destroyed in the presence of finely divided china clay or of animal charcoal. Cotton-wool, after being cleansed with alcohol and ether, has a somewhat similar effect, but the reaction is better marked with diluted tuberculin and mallein (1 in 25).

The action of asbestos fibre on concentrated toxins, using a toxin the strength of which is such that 1 c.cm. contains 400 × M.L.D. placed in contact with asbestos 50 per cent. by weight, is as follows:

Material Used.	M L.D. Doses Given.	Death.	M.L.D. Doses Given.	Death.
A. Diphtheria toxin—1 c.cm. contains 400 × M.L.D. (control)	1.5	< 30 hours	1	30 hours
B. = A. after 24 hours' contact	1.5	60 hours	1	44 hours
C. = A. after 48 hours' contact	1.5	120 hours	1	Not killed in 17 days
D. Tetanus toxin—1 c.cm. contains 400 × M.L.D. (control)	1	4 days		
E. = D. after 24 hours' contact	250	6 days		
	117	19 days		
	45	Did not kill in 42 days		
	22			

The action of asbestos fibre is obviously greater upon concentrated tetanus toxin solution than upon an equally concentrated diphtheria toxin.

Growth of Virulent Organisms upon Nutrient Broth containing 7½ per cent. by Weight of Russian Asbestos Fibre.

B. tuberculosis (Human and Bovine Types).—After five months there is a fair amount of growth. The liquids are proteose- and peptone-free, and their toxicity is about equal to that of control cultures grown on normal glycerine broth which had to be freed of proteoses by precipitation with alcohol. The

exudate from the asbestos broth culture has been tested by means of the Lignière, Woodcock, and von Pirquet methods of cutaneous reaction and, broadly, the results are comparable. The organisms in one culture flask (*T. bovinus*), however, seem to have lost all their virulence, as tested upon twelve guinea-pigs, none of which, at the end of six weeks following inoculation, have developed signs of tuberculosis on *post-mortem* examination. The organisms derived from the culture flash sown with *T. humanus* appear to have lost none of their virulence in a parallel test now being conducted. The organisms show certain changes both in morphological and staining appearances.

B. anthracis; B. pestis.—One strain of each type has been under observation. The organisms show certain changes in morphological and staining appearances and have lost a great measure of their virulence during two months' growth. Mice inoculated with small quantities of the expressed fluids are apparently unaffected.

B. diphtheriae (American Variety).—No toxin appears in the asbestos broth culture at the end of eight days' growth; 5 c.cm. of the expressed liquid is without harmful effect when inoculated subcutaneously into guinea-pigs, whereas $\frac{1}{20}$ c.cm. of the control broth culture proved fatal within thirty-six hours. There is a great alteration in the staining and morphological appearances in the growth on asbestos broth, while the intensity of the biuret reaction of the expressed fluid is less than 2 per cent. of that of the control.

NOTE ON THE ACTION OF ASBESTOS UPON RADIUM AND THORIUM IN SOLUTIONS OF THEIR SALTS.

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THIS investigation was suggested by Dr. Coplans, and it was thought that a short account of the results achieved might be of interest in conjunction with his work on asbestos.

Preliminary experiments showed that asbestos had the power of diminishing the activity of a solution of a radio-active body. Thus, 25 grams of Russian asbestos were left in contact for one week with 50 c.cm. of radio-active water from the Cornish Radium Mine, and the solution extracted from this asbestos by pressure possessed only three-sevenths of the activity of the original solution while another 25 grams of the same sample of asbestos left in contact for three weeks with 50 c.cm. of a 5 per cent. solution of thorium nitrate yielded 12.5 c.cm. of liquid with an activity per cubic centimetre diminished to one-eleventh of the original solution, the asbestos retaining the remainder of the active thorium and being correspondingly radio-active, the total activity of asbestos and liquid being the same as that of the thorium solution before treatment with the asbestos fibre.

The active product in the asbestos could not be washed out with distilled water, but on boiling with 300 c.cm. of water for four hours, about 100 c.cm. of cloudy solution were eventually obtained with an activity per cubic centimetre of one-fifteenth of that of the original solution. The deposit from this cloudy solution was inactive, and the total activity thus recovered in the extracted liquids was one-sixth of that of the original liquid, while the asbestos retained an activity equal to five-sixths of that of the original thorium nitrate solution.

A solution of thorium nitrate was then made up, 1 gram in 200 c.cm., and different weights of chrysotile-asbestos from Canada were sealed in contact with 50 c.cm. of this solution for four days, after which they were compressed.

The volume of liquid thus extracted was measured, the remaining compressed solid was weighed, and the activities of each obtained by the "emanation method," as devised by Boltwood (*Phil. Mag.*, 1905).

The solution under test was boiled *in vacuo*, and the dissolved gases driven through a drying tube into an ionization chamber connected to an electrometer, the deflections of which were proportional to the ionization currents produced in the gas in the chamber by the α particles shot out from the emanation on disintegrating. While devised primarily for radium emanation, the method of estimation can be used for thorium, provided the gases are admitted quickly into the chamber—thorium emanation decaying to half its strength in one minute—and may be calibrated by repeating the test with standardized radium or thorium solutions.

The activity thus measured is proportional to the amount of thorium or radium emanation present, and consequently to the amounts of thorium or radium from which these emanations were produced.

The results of testing Canadian asbestos (chrysotile) were as follow:

Quality of Fibre.	Weight (Grams).	Liquid Removed (c.cm.).	Emanation Activity		
			Of Liquid.	Of Asbestos.	Per Gram Asbestos.
	A.	B.	C.	D.	E.
1. Canadian, fine ...	2.17	46.5	4.51	0.79	0.363
2. ,, ,,	3.85	46.0	3.95	1.35	0.351
3. ,, ,,	5.10	43.5	3.44	1.86	0.365
4. ,, coarse	3.34	47.0	4.19	1.11	0.333

Time of contact: 4 days. Emanation activity of original liquid: 5.30.

After eight days' contact the figures showing the loss of emanation activity by the asbestos used in experiments 1, 2, and 3 are respectively 0.63, 0.65, and 0.64 per gram.

The numbers in columns C and D represent the thorium contents of the extractate and the asbestos respectively, while in column E it is seen that, using different weights of fine Canadian fibre, equal amounts of thorium are removed per gram, so suggesting a chemical replacement of the magnesium in the chrysotile (usually a magnesium silicate combined with various oxides) by the thorium.

The amount of nitric acid present in the liquid reckoned as nitrate appears to be unchanged throughout the process.

It is hoped to elucidate these phenomena by further research.

HYPERTHYROIDISM: ITS EXPERIMENTAL PRODUCTION IN ANIMALS.

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EXOPHTHALMIC goitre in man is generally held to be due to thyroid changes producing an increased secretion, which is the immediate cause of the symptoms and the pathological changes of the disease. These suppositions appear to be borne out by the results obtained in the treatment of the disease by partial thyroidectomy. Observers mostly insert a saving clause that the symptoms may be due to an altered secretion, that is, a dysthyroidism rather than a hyperthyroidism.

The essential change in the thyroid is a cell hyperplasia with absence of colloid, the cells are also altered in shape and approach the columnar type. The hyperplasia is commonly associated with a macroscopic enlargement of the gland, sometimes with an adenoma, though cases are recorded where the size remained normal. Walter Edmunds,[1] Halsted and Horsley,[2] found that after partial thyroidectomy in a normal animal the remaining portion enlarged and underwent a histological change indistinguishable from the hyperplasia seen in exophthalmic goitre. Marine and Lenhart[3] hold that thyroid hyperplasia is accompanied with deficiency of thyroid secretion.

Exophthalmic goitre is apparently rare in animals, but undoubted cases of a similar condition have been recorded by Albrecht, Cadiot, Marek, Ries, and Rider, in cows, dogs, and horses. So the symptoms and pathological changes that are due to thyroid excess should be capable of reproduction in animals by thyroid feeding. The results that have been obtained by thyroid feeding are extremely contradictory, as the following short account of the principal published work shows.

Nearly all the observers have obtained in animals increased appetite, loss of weight, gastro-enteritis, and diarrhoea. They appear to agree that the loss of weight is due to an increased oxidation of fat and increased nitrogenous metabolism in cretins, myxoedema, normal man, and in animals (Schoendorff,[4] Underhill and Saiki,[5] Dutto, Roos,[6] and Magnus Levy[7]).

Loss of hair, glycosuria, nervousness, and tremors are among the symptoms that have been described by some observers and not by others. Any observations made as to nervousness are open to error, as animals tend to become more tolerant of man when ill, and estimations made as to their behaviour to some sudden noise are most unsatisfactory. No definite opinion can be given on tremor, as the animals are in a wasted condition and weak; any tremor present may arise from the tachycardia.

Most observers have failed to obtain exophthalmos, but it has been described by Edmunds[8] in monkeys, by Hoennicke[9] in rabbits, by Baruch[10] in dogs, rabbits, and rats; also by Ballet and Enriquez[11] and Gagnevin. It is quite impossible to compare the eyes of thyroid-fed animals to the exophthalmos in man, owing to the anatomical differences and the wasted condition of the animals.

The great question is tachycardia; it has been obtained by the experiments of Ballet and Enriquez,[11] Canter,[12] Georgiewski,[13] Hellin,[14] Lanz,[15] and by von Fürth and Schwarz[16] in dogs and rabbits; in guinea-pigs by Angiolella[17] and Ludke,[18] in goats by Lepine,[19] in rats by Peiser,[20] in monkeys by Edmunds,[8] and in cats by von Fürth and Schwarz. These papers were summarized by von Fürth and Schwarz[16] in 1908, and they concluded that tachycardia could be produced, but not invariably. The more recent workers, especially the American, deny the production of tachycardia; Marine and Lenhart[3] have never produced it in dogs, Cunningham[21] did not produce it in man, monkeys, cats, dogs, rabbits, and chickens by feeding with fresh thyroids, but obtained it by feeding with desiccated thyroid and by subcutaneous injections. Carlson, Rooks, and McKie[22] did not observe it in pigeons, chickens, rabbits, ducks, guinea-pigs, rats, foxes, cats, dogs, and monkeys.

In all these experiments the heart-rate was taken by the finger, and this has often proved unsatisfactory—for example, Carlson found that no observations could be made on monkeys, foxes, or rats, as they were too excitable, while dogs' hearts were too irregular, and those of rabbits and guinea-pigs too rapid, and he appears to have made no attempt to count the rate in ducks or chickens. So his categorical statement is based on observations on two cats, one of which was pregnant. His observations would carry greater weight if they were controlled by the use of some instrument such as a polygraph. The contradictory results obtained thus make it uncertain whether the tachycardia of exophthalmic goitre can be reproduced by thyroid feeding, though most authors appear to hold that it results from it. (See Biedl and Swale Vincent.)

Some observers have used exophthalmic goitre thyroid, and have obtained contradictory results. Soupault[23] fed guinea-pigs with it, and found it less active than normal thyroid. Schoenborn[24] used it on cats and rabbits, and found no essential difference from the normal. Klose and Lampe,[25] Klose and Liesegang[26] produced tachycardia in nervous fox terriers by using exophthalmic goitre thyroids within half an hour of their removal, and concluded that the results were due to some dysthyroidism. Fonio[27] and Marine[28] found that exophthalmic goitre thyroid was no more active than thyroid of a similar iodine value. The previous observers had not estimated the iodine value.

The conflicting results may be due in part to the different preparations that have been used and to the different methods of administering them. The preparations have varied from fresh glands, normal or goitrous, thyroid powder, iodothyrin, thyroglobulin, glycerine extracts, colloid solutions, to iodized protein. For the most part the iodine has not been estimated, and when this has been done it was found to be low. The preparations were introduced intraperitoneally, subcutaneously, in the food or in gelatine capsules. The iodine content is of the utmost importance. Marine and Lenhart[29] found that the compensatory hypertrophy after partial thyroidectomy could be prevented by the administration of iodine. Marine and Williams[30] found that the physiological value of the thyroid was dependent on its iodine content. Stoland,[31] a pupil of Carlson, by feeding experiments, found that thyroids of a low iodine value were less toxic than those of a high value.

Technique.

The selected animals—cats, rabbits, guinea-pigs, and dormice—were taken as being typical, carnivorous, herbivorous, and hibernating. The last were investigated in order that the effect might be noted on the large thymus these animals possess in the winter months. The material used was Duncan and Flockart's thyroidea sicca. A sample of this was found to contain 0.3289 per cent. of iodine. Marine and Lenhart found that normal to colloid glands contained 0.4–0.95 per cent. The material used in my experiments thus contained a high percentage of iodine, and, according to Marine, would have a high physiological value. It is about three times as high as that used by Carlson, Rooks, and McKie, which contained only 0.08–0.13 per cent.

The powder was given in the whole state mixed with either milk or meat, and was taken quite readily; if any was left over it was added to the next meal.

In one experiment subcutaneous injections of thyroid purified according to Hutchison's method were given. In three cats thyroid that had been previously subjected to gastric digestion for 12 hours was employed. An objection was open, that any of these might contain some toxic agent other than thyroid, so three controls were taken by previously boiling the thyroid for some minutes. As the same effects were produced by all these methods in cats, it was not thought necessary to repeat the controls for the other animals. Controls were kept of all the animals, to note the effect of confinement in the laboratory; these remained normal and put on weight, except the dormice.

Accurate records were made of the pulse rates, by means of a polygraph, and these rates were compared with estimations made directly; the direct observations invariably proved inaccurate. There was no difficulty in using the polygraph, the animals were placed in the observer's lap, and petted until they became quite quiet, when the tambour was placed over their heart and records taken. All tracings were discarded, where there was any question of any increased rate from either movement or excitement.

No attempt was made to record either the temperature or the respirations. Owing to the difficulty in collecting the urine free from contamination only occasional tests were made for sugar in cats. The morbid changes were examined both macroscopically and microscopically; other observers have not examined the organs microscopically. The nervous system was not examined histologically, as a very large series would have been necessary to be of any value, owing to the irregularity of the appearance of changes in the nervous system in exophthalmic goitre.

Effects Produced.

1. *Cats.*

Thirteen cats were divided into five groups.

A. Four cats fed on thyroid powder.
B. One cat injected every day with a 2½ per cent. solution of purified thyroid (subcutaneously).
C. Three cats fed with previously digested thyroid.
D. Three cats fed on previously boiled thyroid.
E. Two cats kept without thyroid feeding.

The doses given were from 1 to 3 grams per day.

The results obtained in the first four groups were similar, and so may be taken together.

General Account.—The effect of the thyroid feeding produced but little change during the first three days. On about the fourth day the animals became suddenly weak and went off their legs; this stage of weakness passed off in about forty-eight hours, but then the toxic symptoms mentioned below set in and slowly progressed until the animals became moribund. Some of the cats appeared for a time to come to a standstill, but they eventually succumbed. In four the feeding was stopped when they became moribund, as they were required for other purposes.

Fur Changes.—The hairs came out and the fur became unkempt; when the animals died the fur was very thin.

Body Weight.—There was a gradual loss of body weight from 28 to 53 per cent. This was accentuated at those times when food was refused and occurred most rapidly two to three days before death. Muscular weakness corresponded to the loss of weight. In the two cats in which the thyroid feeding was stopped and which were allowed to recover, a steady increase of weight followed.

Alimentary Tract.—Salivation took place during the later stages of the experiments, its intensity varied with different cats, at the last it was represented by a thick ropy mass hanging from the mouth. The appetite was much increased though occasionally they would refuse all food for two or three days at a time. Diarrhœa constantly occurred even in the cat subjected to subcutaneous injections.

Glycosuria.—The urine reduced Fehling in some of the cats, but not in others.

Circulatory.—The daily records made with the polygraph showed that the pulse-rate steadily increased. The highest was 384. The heart's impulse appeared to be diffused over a larger area of the thorax than previously. The rates steadily diminished in the two cats in which the thyroid feeding was stopped.

Exophthalmos.—There was no prominence of the eye that could be compared to that seen in exophthalmic goitre.

Tremors.—The cats became tremulous, and the hair

could be seen to shake, but it was quite impossible to differentiate this from the effects of the general weakness and rapidity of the heart's action. It cannot be compared to the tremor of exophthalmic goitre.

Nervousness and Excitability.—The cats during the experiments became quite tame, and purred on being handled; in the later stages they appeared to start at a sudden noise.

Macroscopic Post-mortem Appearances.—There was almost entire absence of fat, the muscles were extremely wasted. The intestinal mucous membrane showed haemorrhagic changes, but not invariably. The intestinal contents were conspicuous by the absence of worms, which are generally present in large numbers in normal cats. The mesenteric lymph glands were sometimes enlarged, but not more so than is commonly seen in cats, so that no comparison can be made with the lymphoid changes seen in exophthalmic goitre.

Morbid Histology.—This will be described with that occurring in rabbits.

The following is an account of each individual cat:

GROUP A, No. 1.

Date.	Thyroid per Day.	Body Weight.	Average Pulse.
	Grams.	Grams.	
November 23, 1910	1	3,560	153
December 3, 1910	1	2,650	232
December 5, 1910	1	2,410	Dead

Feeding days 11. The cat died, having lost 32.5 per cent. of its total body weight.

GROUP A, No. 2.

Date.	Thyroid per Day.	Body Weight.	Average Pulse.
	Grams.	Grams.	
December 9, 1910	1	1,850	132
December 15, 1910	1	1,750	160
December 22, 1910	1	1,630	214
January 7, 1911	Stopped	1,440	272
January 21, 1911	Nil.	1,520	210
January 30, 1911	Nil.	1,550	176

Feeding days 33. When the feeding was stopped the cat was extremely weak, and had lost 28.7 per cent. of its body weight; it was obvious that it would have died in the next few days had the feeding been continued. On January 30th, twenty-two days after the feeding had been stopped, the cat had regained 180 grams in weight and its heart-beat had fallen from 272 to 186.

GROUP A, No. 3.

Date.	Thyroid per Day.	Meat.	Weight.	Average Pulse.
	Grams.	Grams.	Grams.	
March 31, 1911	3	100	3,070	160
April 7, 1911	3	100	2,690	263
April 19, 1911	3	50	1,760	320

Feeding days 19. The cat died, having lost 42.9 per cent. of its body weight. Its pulse-rate rose from 120 to 320.

GROUP A, No. 4.

This cat was fed on 1.5 gram of thyroid daily from November 23rd to December 1st, 1910. It was pregnant, and aborted on the fifth day after the feeding commenced; the kittens were not full term, and they died in about half an hour. The cat died on the eighth day. Carlson fed a cat when pregnant on thyroid, and it carried its kittens to full term on the seventy-second day, but his cat was given increasing doses, starting with 0.5 gram, and the iodine value was low.

GROUP B.

This cat received fifty-eight subcutaneous injections of 5 c.cm. thyroid solution. The 5 c.cm. corresponded to 6 grams of thyroid powder.

Date.	Weight.	Average Pulse.
	Grams.	
December 9, 1910	2,610	160
December 16, 1910	2,440	192
January 2, 1911	2,360	144
January 30, 1911	2,000	240
February 12, 1911	1,700	384
February 26, 1911	1,670	300
March 9, 1911	1,780	225

From December 23rd to January 2nd the cat received no injections. They were continued from January 2nd to February 12th, when they were finally stopped owing to the development of an abscess in the neck. It had lost 37.1 per cent. of its body weight. On March 9th—twenty-five days after the injections were stopped—it had regained 50 grams in weight, and its pulse dropped from 384 to 225—a fall of 159 per minute.

GROUP C, No. 1.

Date.	Thyroid per Day.	Weight.	Average Pulse.
	Grams.	Grams.	
January 30, 1911	1	3,400	182
February 6, 1911	1	3,400	192
February 13, 1911	1	3,360	217
February 27, 1911	1	2,780	270
March 8, 1911	1	2,250	293

The cat died on the thirty-seventh day, having lost 33.8 per cent. of its total body weight.

GROUP C, No. 2.—*Digested Thyroid.*

Date.	Thyroid per Day.	Meat.	Weight.	Average Pulse.
	Grams.	Grams.	Grams.	
April 1, 1911	3	150	4,190	150
April 15, 1911	3	150	3,590	246
April 29, 1911	3	150	3,580	294
May 13, 1911	3	150	3,070	320
June 4, 1911	3	150	2,720	312

Feeding days 65. It was killed when moribund, having lost 35.1 per cent. of its body weight.

GROUP C, No. 3.

Date.	Thyroid per Day.	Meat.	Weight.	Average Pulse.
	Grams.	Grams.	Grams.	
April 1, 1911	39	100	2,610	170
April 8, 1911	39	100	2,350	251
April 22, 1911	39	100	1,650	293
April 27, 1911	39	100	1,460	

On the last date the pulse was irregular and intermittent. The cat died on the twenty-fourth day, having lost 37.5 per cent. of its total body weight.

GROUP D, No. 1.—*Boiled Thyroid.*

Date.	Thyroid per Day.	Meat.	Weight.	Average Pulse.
	Grams.	Grams.	Grams.	
December 7, 1910 ...	1	—	2,780	127
January 1, 1911	1	—	2,430	131
January 15, 1911	1	—	2,060	169
January 29, 1911	1	—	2,050	190
February 12, 1911 ...	1	—	1,950	255
February 26, 1911 ...	1	—	1,940	283
March 16th, 1911	1	—	1,290	320

The cat died on the ninety-eighth day of thyroid feeding, having lost 53.8 per cent. of its total body weight. Its pulse-rate increased 204 per minute.

GROUP D, No. 2.

Date.	Thyroid per Day.	Meat.	Weight.	Average Pulse.
	Grams.	Grams.	Grams.	
April 1, 1911	3	100	2,010	195
April 14, 1911	3	100	1,670	288
April 24, 1911	?	?	1,160	384

The cat went off its food on the twentieth, and no thyroid was taken after the twenty-second. Number of feeding days twenty. The cat died on the twenty-fifth day, having lost 42.3 per cent. of its total body weight.

GROUP D, No. 3.

Date.	Thyroid per Day.	Meat.	Weight.	Average Pulse.
	Grams.	Grams.	Grams.	
April 1, 1911	3	100	3,020	162
April 14, 1911	3	100	2,480	192
April 28, 1911	3	100	2,390	272
May 8, 1911	3	100	1,930	303

The cat was killed on the thirty-eighth day of the experiment, when moribund. It had lost 36.1 per cent. of its total body weight.

GROUP E.—*Control Cats.*

These two cats steadily increased in weight, and their pulse-rates varied between 120 and 190.

2. *Rabbits.*

Nineteen rabbits were fed on thyroid in milk in doses varying from 0.2 to 1 gram daily. They rapidly lost

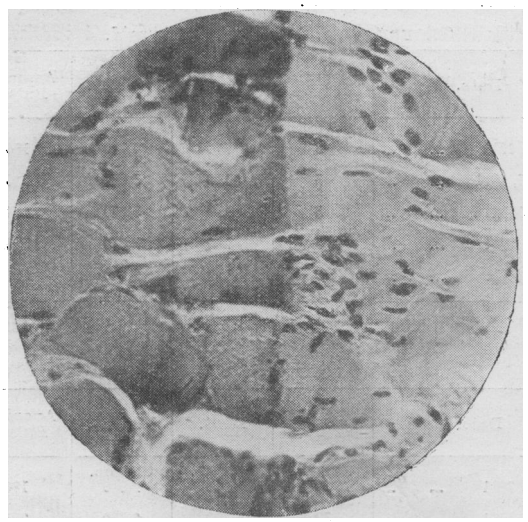

Fig. 1.—Cat's heart showing type of degeneration, cut transversely and vertically.

weight and died, except five in which the experiments were not pushed to their termination. Death occurred from the fifth to the nineteenth day with one exception; this one survived twenty-eight days. The fur became unkempt and fell out. The loss of weight varied from 19 to 42 per cent. of their total body weight. The appetite was much increased, and they ate ravenously right up to the time of their death. Diarrhoea was a constant symptom, but no salivation was noted. The urine was not tested or estimated. The pulse-rate steadily increased, the highest recorded being 420 per minute. No exophthalmos or constant alteration in the nictitating membrane was noticed. The rabbits became weak and tremulous, but no comparison can be made between this and the tremor of exophthalmic goitre.

Three of the five rabbits in which the experiment was not pushed to a termination died suddenly while being given ether as an anaesthetic.

Naked-eye Morbid Anatomy.

There was extreme wasting of the fat and muscles. The stomach invariably was found full of food; the intestinal mucous membrane showed haemorrhagic changes.

No enlargement was seen of the lymphoid group of glands.

The following may be taken as a typical feeding experiment on a rabbit:

Date.	Thyroid.	Weight.	Average Pulse.
	Grams.	Grams.	
December 7, 1910	0.2	2,150	167
December 9, 1910	0.2	2,040	192
December 14, 1910	0.2	1,775	240
December 19, 1910	0.2	1,320	352
December 20, 1910	0.2	1,290	Dead

The Morbid Histology of Thyroid Fed Cats and Rabbits.

Evidence of the extreme degree of fat absorption is seen in the illustration, which represents a cat's omentum spread out on a slide.

Muscles and Heart.—The change seen in the heart varied from a condition of general wasting of the fibres to what may be described as a condition of hyaline degeneration. The fibres appeared swollen with few nuclei and no processes or transverse striations; both the fibres and the nuclei stained badly. Fig. 1 shows this appearance; it corresponds to a myocarditis without evidence of inflammation.

Kidneys.—The majority of sections showed no change, but in some, as in Fig. 2, there was a condition approaching tubular nephritis; the cells were in a peculiar swollen condition, without evidence of inflammation.

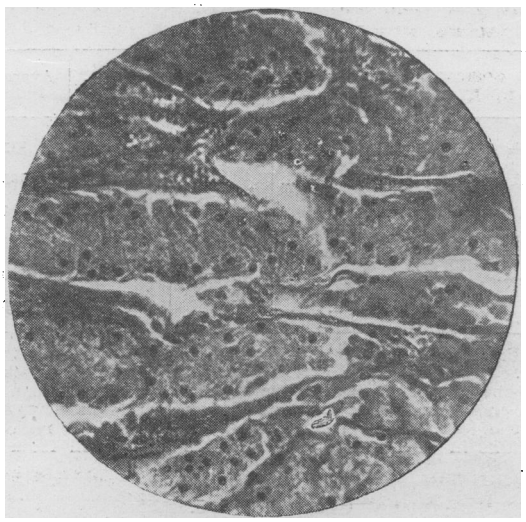

Fig. 2.—Cat's kidney showing degeneration

Liver.—The liver showed a condition of fatty degeneration, most marked around the centre of the lobules.

Pancreas.—Certain of the animals were killed, so that an immediate *post-mortem* examination might be done and the true condition of the pancreas ascertained uncomplicated by *post-mortem* degeneration. Considerable enlargement of the islets of Langerhans was seen. Others showed a degeneration of the islets with fibrosis, but the possibility arises that this fibrosis might have been due to previous inflammation independent of the thyroid feeding.

Ductless Glands.—The thyroid showed a marked increase in the staining capacity of the colloid, otherwise it was quite normal. No change was found in the pituitary or adrenal. The most constant of these changes was the enlargement of the islets of the pancreas and the alteration in the heart. The degrees of the changes in the organs varied in each individual animal; all the changes were not to be seen in any one animal.

Guinea-pigs.

Fifteen were fed on thyroid daily, from 0.2–0.6 gram. The fur changes, loss of weight, diarrhoea, and increased appetite that occurred agreed with the changes seen in

the rabbit; the naked-eye pathology was also similar. No observations were made as to the occurrence of tachycardia owing to the tambour being too big to fit over a guinea-pig's heart.

Dormice.

Twelve dormice were fed on 0.025 gram of thyroid a day, twelve others were used as controls. Beyond falling out of the fur, diarrhoea and loss of weight, no changes were to be noted. They died in four to nine days, having lost 13 to 38 per cent. of their weight. The main object of the experiment was to notice the effect on the thymus. It was found that it had diminished; in some at the time of death it was quite small, and no fat was to be seen in it, whilst others died before all the fat had disappeared.

The control dormice lost some weight, but appeared healthy when the thyroid fed ones died, but they themselves died one night, presumably owing to a hard frost.

Comparison of the Effects in these Animals.

The effects produced by thyroid feeding in cats, rabbits, guinea-pigs, and dormice may be compared as follows:

The cats proved the most resistant, the other three were very susceptible; the rabbits lived longer than the guinea-pigs; this one would expect from their size.

Carlson suggested that the difference in the resistance of carnivorous and herbivorous animals might arise from some toxic effect of animal protein to herbivora, but French,[33] one of Carlson's pupils, found that large quantities of meat could be given to herbivora without producing any toxic effect.

The cardinal symptoms in all were fur changes, loss of weight, weakness, increased appetite and diarrhoea.

Tachycardia was well marked in the cats and rabbits, but no reliable observations could be made in either the guinea-pigs or the dormice, owing to the difficulties involved in their small size. The question arose in cats and rabbits as to the occurrence of nervousness and tremors, both of which, along with exophthalmos, have been observed in higher animals—by Edmunds in monkeys, and by Notthaft[32] in man.

Comparison with Exophthalmic Goitre.

A comparison of the symptoms obtained may be made with those seen in exophthalmic goitre. In making this comparison, it should be borne in mind that the length of time over which thyroid excess is produced in animals is much less than the duration of the average case of exophthalmic goitre; also the amount of thyroid given daily represented a much larger quantity than the whole gland of the animal. An early acute case is the most suitable for comparison.

The change in the fur is analogous to the change in the hair. The loss of weight, from 28 to 53 per cent. in cats, and from 19 to 42 per cent. in rabbits, agrees with the average or extreme loss seen in the disease. The salivation seen in cats corresponds to the moistness of the mouth. The appetite is rather different, as the typical appetite of exophthalmic goitre is capricious whilst that of the thyroid fed animals is increased until they are very ill. Diarrhoea is an almost constant symptom in both, as also is tachycardia. The occasional glycosuria, seen in cats, agrees.

Exophthalmos for the most part can be said not to be present and tremor cannot be distinguished as such, nervousness remains doubtful. Fineness of the skin and sweating cannot be reproduced in these animals for obvious reasons.

In a comparison of the morbid anatomy the changes may be divided into two main groups, other than changes in the nervous system which were not looked for.

The fat absorption, muscular wasting, degeneration of the heart, kidneys and liver, the enlargement of the islets of Langerhans, and the haemorrhagic changes in the intestine are to be found in both. The type of the degeneration, a hyaline without evidence of inflammation, agrees, also the uncertainty of their occurrence. The second group consists of those that are found in exophthalmic goitre, but cannot be reproduced in animals by thyroid feeding. They are the hyperplasia of the thyroid, the alteration in the pituitary, the enlargement of the spleen and certain lymph glands. Of these the hyperplasia of the thyroid is not to be expected in animals fed on thyroid gland, so that the chief differences between exophthalmic goitre and experimental hyperthyroidism consist in the absence of the alterations in the latter in the pituitary, spleen and lymph glands.

SUMMARY.

One may conclude that thyroid feeding is toxic to these animals, but that their resistance varies both as to the species and to the individual of that species.

A complete picture of exophthalmic goitre cannot be reproduced in these animals, but a modification can be produced that resembles the disease in certain features. These are fur changes, loss of weight, bodily weakness, diarrhoea, tachycardia, and occasional glycosuria, combined with a disappearance of fat, muscular wasting, degeneration of the heart, liver, and kidneys, enlargement of the islets of Langerhans, and haemorrhagic changes in the intestine. The deduction can be made that the thyroid hyperplasia of exophthalmic goitre is associated with excess of secretion, and that this is the cause of these symptoms and morbid changes.

It is suggested that in regard to symptoms that cannot be reproduced no deduction can be made either as to their being or not being caused by thyroid excess, owing to the anatomical and other differences between animals and man.

The absence of the morbid changes in the ductless glands and lymphoid system precludes their production by thyroid excess.

The experimental work was done at University College in Professor Cushny's laboratory. I am much indebted to him for his care and supervision, without which the work would have been valueless. I am also indebted to Dr. Marris for the loan of his polygraph.

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DISCUSSION.

Dr. CROFTON asked for information concerning the occurrence of glycosuria and albuminuria in the experimental animals.

Mr. FARRANT replied that the difficulties of collecting urine in animals without contamination were great, but that glycosuria was not infrequent.

ON THE VALUE OF THE BLOOD COUNT AS AN AID TO DIAGNOSIS IN OBSCURE BACTERIAL AND OTHER INFECTIONS.

By H. MILLER GALT, M.B., B.Sc.Glasg.,

Pathologist to the "Stephen Ralli" Memorial Laboratory, Royal Sussex County Hospital, Brighton.

BRIEFLY this is a plea for the much more extended use of the examination of the blood as an aid in diagnosis and a guide to treatment.

Within certain fairly well defined limits there is a very remarkable constancy of the histological elements of the blood. All these elements, however, are liable to alteration in number and character under certain influences, mostly toxic; this is particularly the case with regard to the leucocytes, which show fluctuations under very slight influences.

With regard to the leucocytes, the usual alteration is in the form of increase in number—leucocytosis. This may be in the form of a simple leucocytosis, with a proportionate increase of all the varieties of leucocytes. On the other hand, the increase may entirely or specially affect the polymorphonuclears, the lymphocytes, or the cosinophiles — polymorphonuclear leucocytosis, lymphocytosis, eosinophilia. In some bacterial infections the unusual form of leucopenia is manifested; this, again, may be general or special. Lastly, there may be diurnal, seasonal, digestive and other leucocytic fluctuations which may be considered normal.

The chief alterations of the erythrocytes are with regard to number and haemoglobin content. In this connexion the relation between these two factors—the colour index—is an important one.

The term "homogeneous anaemia" may be applied to cases where all the histological elements of the blood are proportionately diminished.

In general it may be said that there are few pathological conditions which do not show some associated alteration in the blood count, while in other cases a negative result from the examination may be equally valuable.

Of diseases associated with leucocytosis we have examples in the following: abscesses in general, practically all local inflammatory conditions, diffuse suppurative processes, lobar pneumonia, acute rheumatism, endocarditis, the infective fevers generally (with a few remarkable exceptions as noted under), hepatic cirrhosis, acute yellow atrophy of the liver, ptomaine absorption, and often malignant disease in the later stages.

Of the diseases associated with leucopenia the two most striking examples are enteric fever and kala-azar. With regard to the former I personally place greater reliance upon the leucocyte count than upon Widal's reaction; many cases in my own experience have been substantiated thus where the Widal's reaction has been indefinite or even negative. The other striking condition accompanied by marked leucopenia is kala-azar; in one case of this I obtained a leucocyte count of 350.

Myelocytes are always abnormal in healthy blood, though it is stated by some authorities that occasionally a cell indistinguishable from a myelocyte may be found in apparently healthy blood. In spleno-medullary leukaemia myelocytes are abundant and are characteristic of this disease.

Splenic anaemia and the severer anaemias generally are also usually accompanied by a more or less marked leucopenia. Finally, there are a number of conditions where the leucocyte count is affected slightly or not at all. We have examples of this in influenza, malaria, mumps, and uncomplicated tuberculous conditions; also some cases of acute nephritis.

Turning to the erythrocytes we have *oligocythaemia* in the later stages of practically all forms of wasting diseases —for example, tuberculosis, malignant disease, chronic lead poisoning, helminthiasis, malaria, and severe haemorrhages generally.

Apart from congenital conditions polycythaemia is rare, and when it does occur is usually very temporary in nature. Visitors to high altitudes usually have evanescent polycythaemia. Then there is the temporary polycythaemia of the newly-born child.

Extreme variations in shape of the red cells—poikilocytosis—may or may not be associated with extreme variations in size—anisocytosis. These two changes are common in all the severe anaemias, and are most marked in those of pernicious type.

Nucleated red cells are always abnormal in the *circulating* blood. Normoblasts are of less significance than megaloblasts, the latter being absent in normal bone marrow, while normoblasts are always found there. In all the forms of severe *secondary* anaemias normoblasts are usually found, but the presence of megaloblasts usually points to an anaemia of true pernicious type.

Basophile stippling of the red cells is also always abnormal, and may exist by itself or in combination with the diffuse form of polychromatophilia, and the latter may again be present without stippling. Both are evidence of immature red cells, and thus they are present in the severer anaemias in general and in some forms of poisoning, notably chronic lead poisoning.

High colour index, if it is marked and persistent, almost invariably points to an anaemia of pernicious type. Low colour index, on the other hand, is common in chlorotic conditions, splenic anaemia, and general malnutrition.

I have given this brief summary in the hope that examination of the blood may become more of a routine practice than it is at present. It seems strange that, while the examination of the urine has become a routine practice, the state of the blood has not received the attention it deserves. It is quite a mistake to imagine that special skill and costly apparatus are necessary; the general practitioner will find that he can obtain in very many cases information of the utmost value with a minimum of trouble. The apparatus is neither costly nor cumbersome, and it can be employed at the bedside with practically no discomfort to the patient.

The evidence obtained by an examination of the blood in this way forms a link in the chain which should not be neglected even in cases where the diagnosis is clear without it. For example, a definite lobar pneumonia which shows a distinct leucopenia on examination indicates a severe and generally fatal attack, and may suggest treatment with the view of producing an "artificial" leucocytosis. In some cases, indeed, the examination of the blood, as in lymphatic leukaemia, may constitute the entire chain of evidence as to diagnosis; in others it may be a centre link without which the other links are inadequate, and even where it forms an end or additional link its value is great.

In conclusion I may quote a few illustrative cases of the value of the blood examination.

1. A youth sent in to the Royal Sussex County Hospital for immediate operation for ruptured appendix. I saw the case on admission. There was deep cyanosis, acutely tender abdomen, and the patient appeared in a moribund condition. Operation was delayed and a blood examination made. This showed a leucocyte count of 2,400, with 45 per cent. of lymphocytes, and on the strength of this I reported that the case was in all probability enteric fever. No operation was done, and the diagnosis was completely established later, recovery following.
2. A young man sent in to the Royal Sussex County Hospital as a case of acute appendicitis. The patient appeared remarkably well, and the pulse and temperature were only slightly above normal, while the abdomen was soft and not tender. Operation was begun while the blood examination was in progress, and the gangrenous appendix with abscess which was present only bore out the blood findings of 27,000 leucocytes, with 87 per cent. of polymorphonuclears.
3. Severe and long-continued anaemia, with occasional remissions, in a lady. Examination of the blood showed 800,000 red cells, leucocytes normal, but marked poikilocytosis, anisocytosis, and nucleated red cells (some of these megaloblasts), all pointing to an anaemia of pernicious type. This case was treated with salvarsan intramuscularly, with rapid and progressive improvement, the patient finally showing perfectly normal blood, and remaining in this state for a period of eighteen months—up to the present time.
4. A case of severe anaemia occurring in a lady, in which no improvement followed various lines of treatment, and in which a blood examination was finally performed. This showed at once that the case was one of acute lymphatic leukaemia, and the fatal conclusion followed shortly after.

Cases like the foregoing might be multiplied *ad libitum* from my own experience, but sufficient has been said to show the advantage to be derived from this easily applied aid to diagnosis, and it is with the hope that the general practitioner will be induced to carry out this examination in many cases that I have ventured to emphasize what is, of course, an admittedly valuable line of investigation.

DISCUSSION.

Dr. J. T. LEON (Southsea) asked as to the strength of solutions used for leucocyte count. He regarded $\frac{1}{3}$ per cent. acetic acid as too weak. He had seen cases of cancer of the stomach in which a pseudo-pernicious anaemia was present. He desired to support Dr. Galt in his plea for a routine examination of the blood, especially from the point of view of those in a general medical practice.

Dr. BERNSTEIN (London) recognized the valuable aid given by blood counts in diagnosis, but wished to point out that here, as in all laboratory work, the blood count could only be of service if used discriminately by clinicians as an adjunct to other methods of diagnosis.

Dr. HICKS (London) also agreed that one must not be a "blood fanatic," and said that correct diagnosis was essential to correct treatment. He quoted some interesting cases.

Dr. CROFTON (Leeds) agreed that blood counts did not point to the cause, as many different causes produced similar results.

Dr. EYRE (President) discussed the leucocytic variations in anomalous cases of pneumonia.

EMPUSA MUSCAE AS A CARRIER OF BACTERIAL INFECTION FROM THE HOUSE-FLY.

[*With Special Plate.*]

By R. M. BUCHANAN, M.B., F.R.F.P.S.,
Bacteriologist to the Corporation of Glasgow.

CARRIAGE OF BACTERIA BY THE HOUSE-FLY.

IT has been demonstrated within recent years by exact methods of experimental research on the part of several

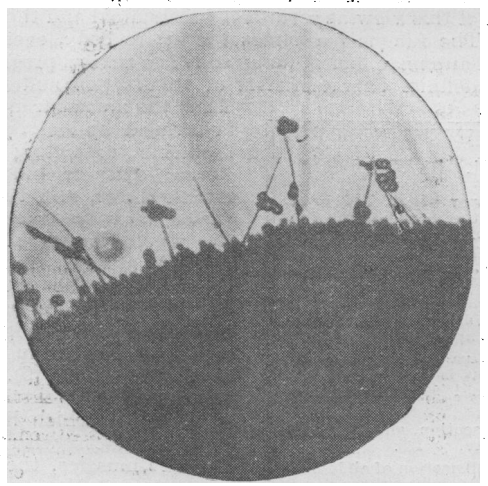


Fig. 3.—Profile view of intersegmental region of *M. domestica* showing the outcrop of conidiophores. Some conidia have been arrested in their outward flight by the setae on which they tend to accumulate in bunches. × 90.

observers that the common house-fly, *Musca domestica*, may carry infection upon the surface of its body and thus act as an agent in the spread of disease. The insect's life-cycle, its habit of life and the conformity of its appendages are even such as to favour the acquisition and distribution alike of pathogenic and non-pathogenic bacteria—the

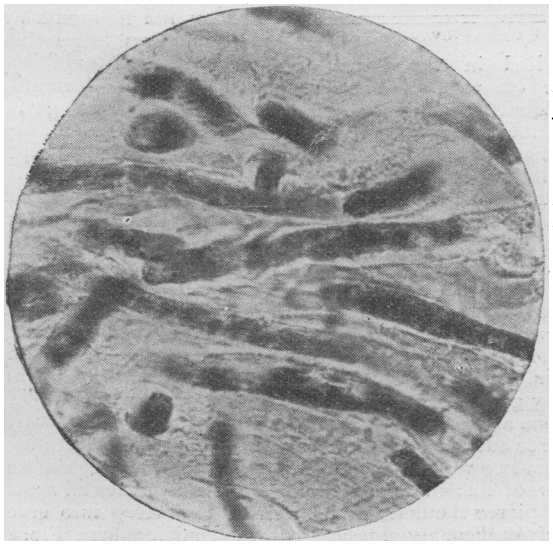


Fig. 5.—*Empusa muscae* invading thoracic muscle. × 500.

species being varied as the substances with which it comes into contact. But while an indefinite variety of bacteria may be carried on the surface of the fly's body valuable work has more recently been in progress showing that a large number of organisms also find natural or temporary lodgement in its alimentary canal.

Thus Graham-Smith (1909), in a series of experiments to ascertain what proportion of flies were infected with organisms of the colon group, isolated thirteen varieties from the intestines, while Nicoll (1911), in studying the natural intestinal flora and following the lines adopted by Graham-Smith, found twenty-seven varieties. Further experiments by Graham-Smith (1910) have shown that while such bacteria only survive a few hours on the surface, others may be retained in the alimentary canal of the fly for seven days under conditions of natural infection (tubercle bacilli in sputum) and for sixteen days under conditions of experimental infection (anthrax spores in emulsion). Again, Bacot (1911), experimenting with *Musca domestica*, has proved the persistence of *Bacillus pyocyaneus* in pupae and imagines raised from larvae infected by pure cultures, and Ledingham (1911), extending

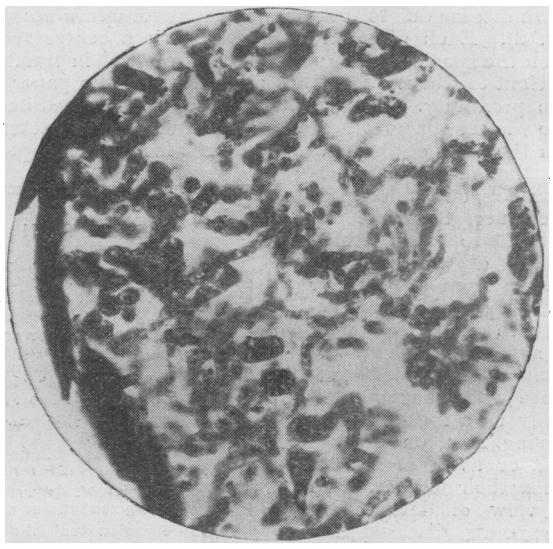


Fig. 10.—Posterior portion of the head of *M. domestica* occupied by *Empusa muscae*. The chitinous plates forming the back of the head capsule are seen on the left. (The formation of gemmae is conspicuous.) Longitudinal section. × 250.

this line of investigation to *Bacillus typhosus*, found that this organism also was able to adapt itself to some extent to the conditions prevailing in the larval and pupal interior (the examination of imagines not having been so far possible).

As regards the presence of infective bacteria in the

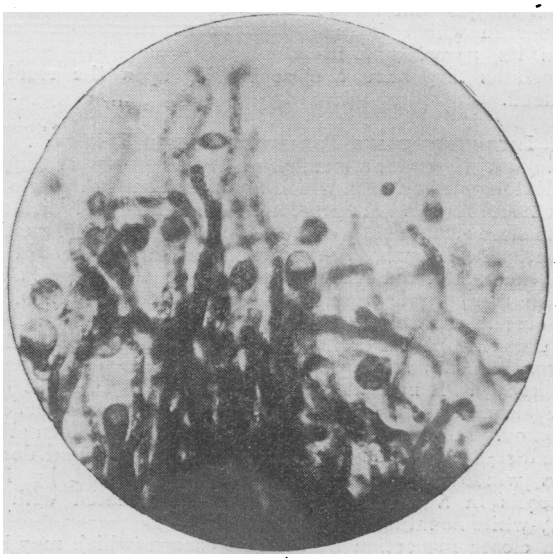


Fig. 11.—A fragment of the felted mass of fungal hyphae from the abdominal cavity of *M. domestica* showing the formation of gemmae. × 250.

intestine of the fly under what may be called conditions of natural infection, this has been demonstrated by Ficker (1903), Hamilton (1903), and Faichnie (1909). Moreover, Odlum (1908) and Faichnie brought forward evidence in support of the view that in the transmission of typhoid

infection by flies, the source from which the specific bacilli were chiefly derived was their breeding ground, and that the infection was carried within rather than upon the surface of their bodies.

The experiments and observations of all these observers thus indicate that the alimentary tract rather than the surface of flies plays the more important part in the transmission of infective organisms.

CARRIAGE OF BACTERIA BY SPORES OF EMPUSA MUSCAE.

Another aspect of the subject has presented itself in the course of some observations on the distribution of house-flies in city dwellings, and one which lends support to the latter view. Among the flies collected from different houses by means of fly-papers, individuals began to appear towards the end of August exhibiting the well-known disease due to the parasitic fungus *Empusa muscae* (Fig. 1). Each diseased fly appeared as a centre from which the spores of the fungus were showered in number sufficient to whiten other flies stuck fast on the paper in close proximity. The question thereupon suggested itself as to whether or not the spores carried with them bacteria from the body of the fly. Beyond the fact that the question has in itself considerable epidemiological importance, there is its relation to the transmission of infective organisms from the interior of the fly, and its significance in connexion with the suggestion which has recently been made to propagate the fungus artificially as a means of destroying flies on a large scale, or at least of controlling their number. With the view of determining these points, diseased flies caught during September and October were subjected to cultural tests and microscopical examination, while attempts were made to grow the fungus on nutrient media.

At this stage some reference may be conveniently made to the life-history of the fungus and to the manner in which it displays its parasitism, inasmuch as they have a close bearing upon the results obtained.

DESCRIPTION OF SPECIAL PLATE.

Fig. 1.—Portion of ribbon-shaped fly paper which had been exposed for twenty-four hours. Four of the flies present the characteristic black and white swollen abdomen due to *Empusa muscae*.

Fig. 2.—White nebular zone formed on glass by conidia projected from the surface of the abdomen of a diseased fly. The abdomen was fixed proximal end downwards in the centre of a Petri capsule. The white wing-like centre is a denser accumulation of conidia on either side of, and close to, the abdomen.

Fig. 4.—The ventriculus of *M. domestica* passing into the abdomen on the right. The wall of the viscus is surrounded and invaded by hyphal filaments. Longitudinal sagittal section. × 125.

Fig. 6.—Leg of *M. domestica* in oblique section, showing the hyphae of *Empusa muscae* among the muscles. × 125.

Fig. 7.—Longitudinal (slightly dorso-lateral) section of abdomen of *M. domestica*, showing *Empusa muscae* developing throughout cavity and erupting through the delicate membrane stretched between the chitinous plates in the form of conidiophores. × 28.

Fig. 8.—Eruption of hyphae through intersegmental membrane with the development of conidiophores and the formation of terminal conidia. × 125.

Fig. 9.—The dome-shaped terminal conidia. Various stages from the commencing bud to the ripe conidium are seen. × 500. (See Fig. 14.)

Fig. 12.—Gemmae of *Empusa muscae* from abdomen of *M. domestica* sprouting and developing into hyphae. Two conidia appear in the field, one in the right and one in the left, affording a contrast between the two kinds of reproductive elements. × 250.

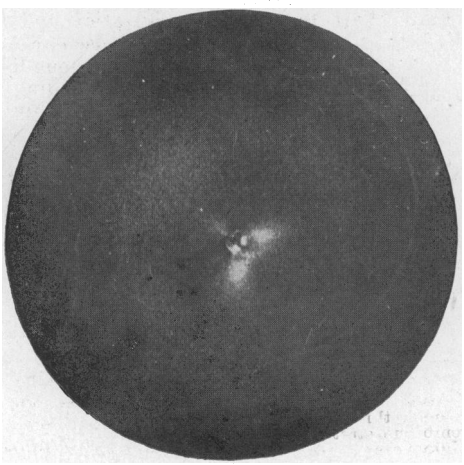
LIFE-HISTORY AND PARASITISM OF THE FUNGUS.

Empusa muscae is a member of a small group of fungi, the Entomophthorineae, which live parasitically in certain insects and larvae. Its position amongst fungi is indicated by its close relationship on the one hand to the parasitic Peronosporaceae, whose mycelium is so destructive to the vine, the potato, and other plants, and on the other hand to the saprophytic Saprolegniaceae, which are mostly aquatic and grow on the surface of diseased or dead fish and decaying plants or insects. A fly affected with the fungus presents an appearance very characteristic and well known. It may attract attention while in flight by sluggish movements and while at rest by the distended abdomen. More commonly the insect, with abdomen still more distended and ringed black and white, is found dead on a window pane, wall, or curtain, attached by its proboscis in a life-like attitude, and associated with a dusty-white zonal marking (Fig. 2).

On closer inspection by the microscope (which may be easily accomplished by placing a diseased fly on a glass slide and leaving it for some hours) it will be seen that the dusty-white zonal marking is due to a deposit of spores or conidia. The fly itself shows a whitish fungal growth distending the abdomen and protruding between the black chitinous segmental plates, thus giving rise to the black and white ringed appearance. The fungal ring takes the form of a serried rank of conidiophores, each forming, or bearing, or having parted with, a terminal conidium or spore (Fig. 3). The conidia are dome-shaped, and on reaching maturity are shot off to varying distances around the fly. This ejectment may be viewed under the microscope by placing an affected fly in a Petri dish, and may be likened to a microscopic bombardment, the conidia being easily seen as they flash across the field or alight upon the glass. They adhere at once to the glass, and do not trundle about; hence the comparative uniformity of the zone on a vertical surface. They may be projected to a distance of an inch and a quarter from the fly.

The manner in which the fungus gains an entrance to the body of the fly—in other words the mode of infection—is not definitely known. It is usually stated as occurring in the manner described by Brefeld (1871): the germination of a spore which has become attached to the surface of the insect and the penetration of the resulting hyphal tube through the body wall. In the flies which I have examined there has been no microscopical evidence in support of this view as regards the abdominal and thoracic walls. The fungus permeates the entire body, even the legs and antennae, but in doing so it has always presented certain definite characteristics of localization and form. Thus the muscular system is invaded by long hyphal filaments extending between and within the muscle fibres, and the invasion may be traced in the whole length of the cephalic muscles from the proboscis to the occiput, in the great mass of thoracic muscles (Fig. 5), and down into the muscles of the legs (Fig. 6). In the case of the ventriculus or stomach, which lies in close contact with the thoracic muscles, the fungus also follows the direction of the muscular fibres, and so envelops and permeates the wall of the viscus in a circular manner (Fig. 4). The abdominal cavity (Fig. 7) and also the space forming the posterior portion of the head are entirely occupied by the fungus. The hyphal filaments are more or less intertwined or felted in the latter two regions, and exhibit a stage of vegetative reproduction in the formation of globular bodies or gemmae, which in turn germinate into ramifying hyphae (Figs. 10, 11, 12). These gemmae, it appears, are formed by certain fungi, where conditions of environment are unfavourable to the production of the more specialized spore bodies. In the abdomen the hyphae assume a close parallel arrangement in contact with portions of the intestinal wall, pointing towards the viscus. This is also noticeable in connexion with segments of the crop, but in a lesser degree, and is frequently absent in the sections. At other segments of the crop and portions of the intestine, the fungus is applied to the walls so as to suggest growth from within outwards, but this received no confirmation from the existence of any growing fungal hyphae within these viscera. The hyphae appear to seek the path of least resistance towards the free surface of the body (or the lumen of the intestine), gradually swelling into thick clublike processes containing a row of nuclei. Approaching the intersegmental spaces they pierce the delicate membrane to develop into erect, closely packed, aërial processes—the conidiophores (Fig. 8). In thin sections stained with Loeffler's methylene blue the conidiophores show all the stages in the formation of the conidium, from a bud-like constriction of the apical region to the complete dome-shaped body (Fig. 9). In several flies the intestine was penetrated by the hyphae, but only in one instance did conidiophores bearing conidia appear within the tube. As a rule, the ends of the hyphae which had invaded the intestine presented a digested appearance.

FIG. 1.



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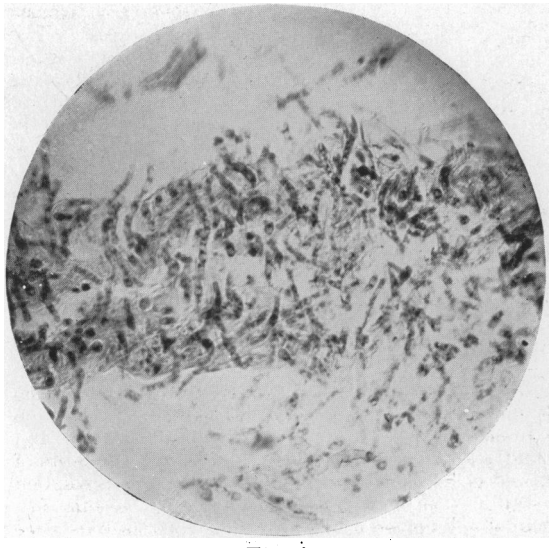

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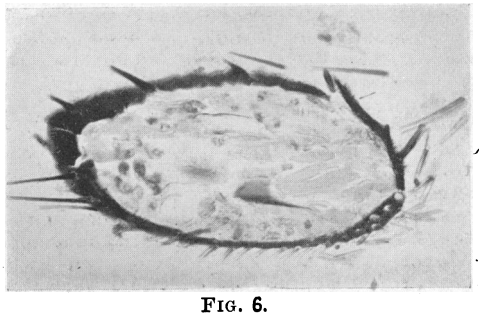
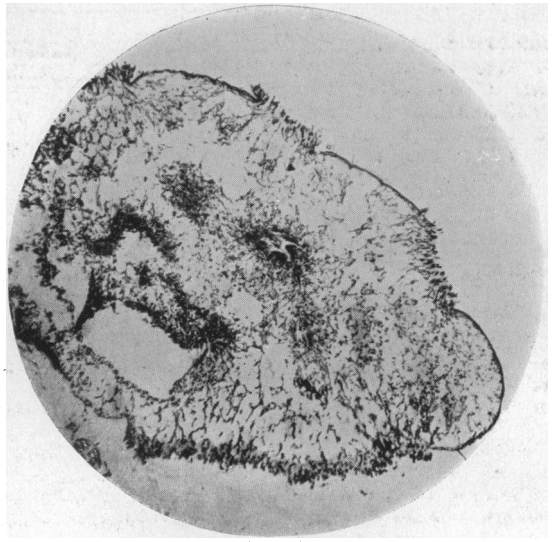


FIG. 6.



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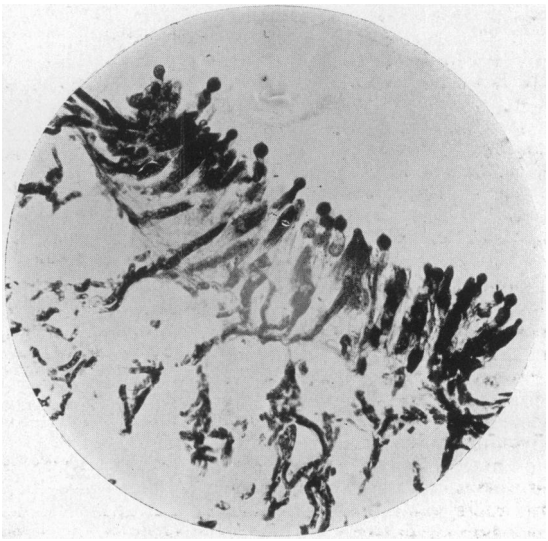

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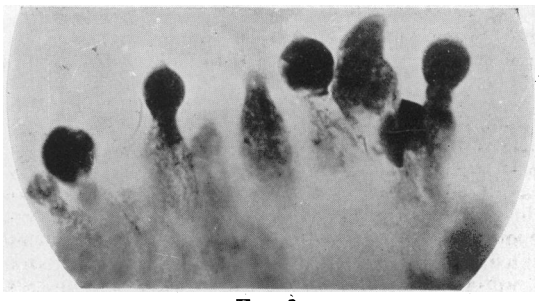

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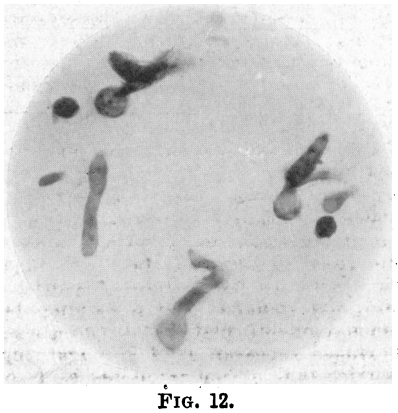

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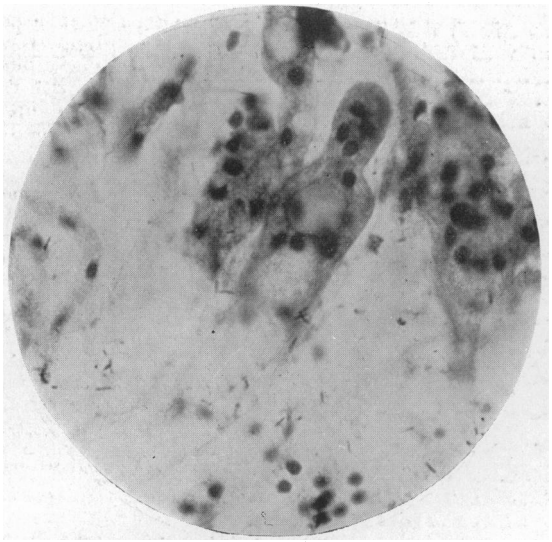

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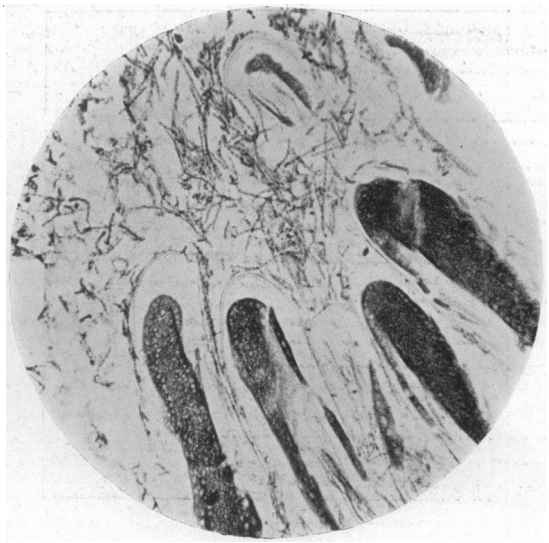

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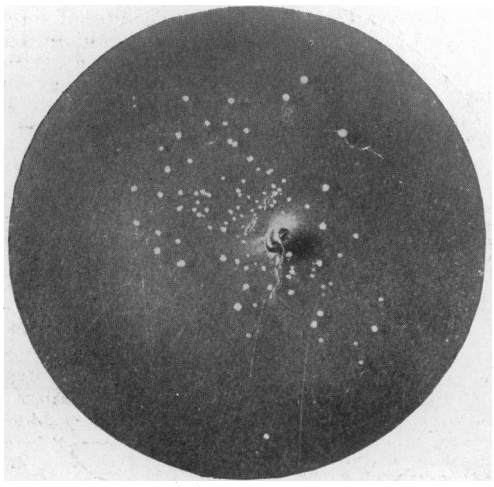

FIG. 15.

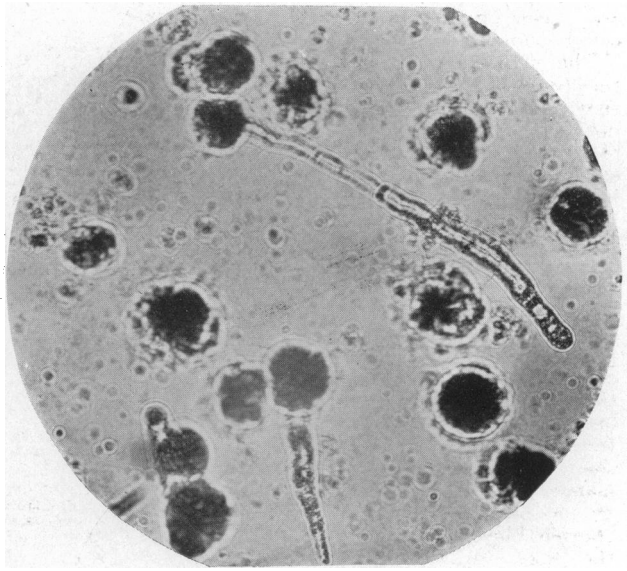

FIG. 20.

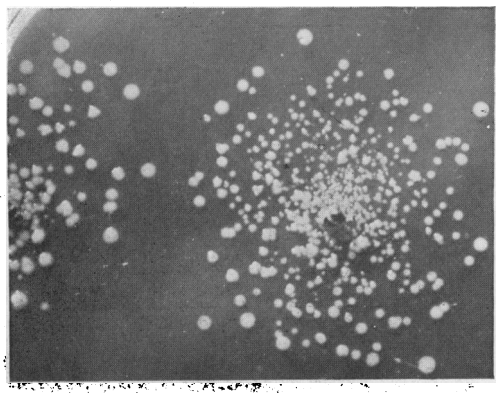

FIG. 16.

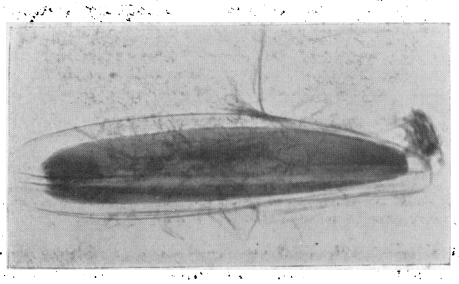

FIG. 17.

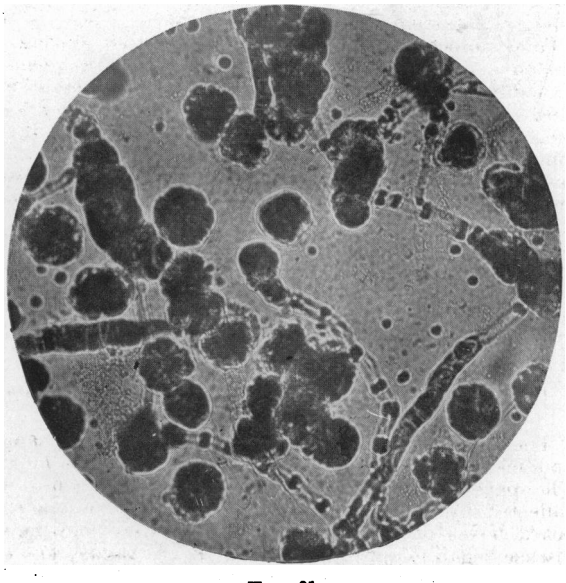

FIG. 21.

The majority of the flies presented great numbers of bacteria in the intestines, and amongst the hyphae of

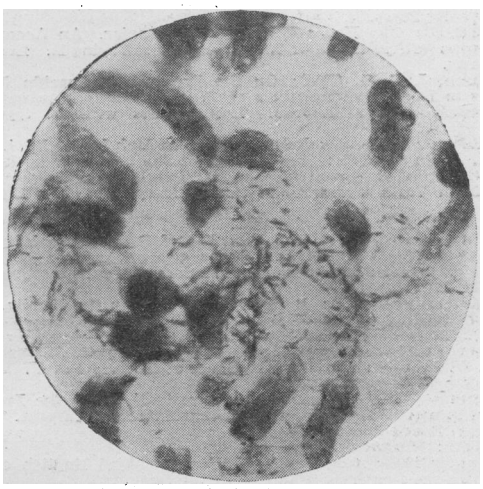

Fig. 13.—Bacteria amongst the hyphal filaments in the abdomen of *M. domestica*. × 1,000.

the abdominal cavity they were widely distributed (Fig. 13), spreading towards the abdominal wall. In Fig. 14 they are seen amongst the conidiophores.

The manner in which the *Empusa muscae* persists from year to year is not known. The formation of resting spores would serve to explain this hiatus in the life-history of the fungus, but the existence of such bodies has not yet been definitely determined. On the other hand, a probable means of persistence from season to season has presented itself in the course of these investigations. It was found in the examination of the eggs from a diseased fly that some of the mycelium remained firmly attached to the outer covering or chorion (Figs. 17, 19). When the eggs are viewed *in situ* in the body of the affected fly they appear in a feltwork of mycelial threads closely applied to the chorion but not penetrating it (Fig. 18). The deposit of eggs by a fly harbouring the fungus thus renders it extremely likely that the larvae would in turn become infected. It is even probable that during the epidemic period of the disease this may be a means of transmitting the parasite from generation to generation of flies. A somewhat analogous method of continuing the species occurs in the life-history of the potato disease fungus, *Phytophthora infestans*. Some of the mycelium passing into the tubers remains in a resting condition, and by this means, without the intervention of spores, the disease can be transmitted from one generation of the plant to another (Massee).

It may be mentioned that the strikingly uniform invasion of the tissues of the diseased imago would be consistent with infection at an earlier stage in its life-history.

DESCRIPTION OF SPECIAL PLATE.

Fig. 14.—Bacteria amongst the hyphae at the surface of the abdomen. (A conidiophore shows the nuclei passing into the terminal bud preparatory to the formation of a conidium.) Longitudinal section of abdomen. × 1,000.

Fig. 15.—Colonies of bacteria (chiefly coliform bacilli) arising from organisms carried by the projected conidia of *Empusa muscae*. A diseased fly was fixed head downwards on agar in a Petri capsule. The colonies are disposed around the fly and coextend with the range of the conidia, which may be seen forming a nebular white zone on the agar surface.

Fig. 16.—A similar experiment to that illustrated by Fig. 15, but showing the type of growth mostly obtained. Two diseased flies were fixed on the plate in this instance, one at the centre and one at the side.

Fig. 17.—Egg of *M. domestica* separated from the felted abdominal mass, and bearing mycelium firmly attached to the outer covering or chorion. × 57.

Fig. 18.—Longitudinal section of *M. domestica* showing eggs *in situ* and their envelopment by the fungus. × 75.

Fig. 20.—Conidia of *Empusa muscae* showered on glucose-agar from a diseased fly. Development of hyphal filaments from one of two conidia which happen to be contiguous. × 150.

Fig. 21.—Active growth of hyphae from conidia projected from fly upon glucose-agar. The thickened septate hyphal development in the left of the field suggests the formation of a third reproductive element. × 250. (See Fig. 12.)

TESTS FOR BACTERIAL DISSEMINATION BY THE FUNGAL SPORES.

The flies were obtained, as already indicated, by hanging up sticky papers in the form of a ribbon about 30 in. long. The papers were exposed for twenty-four hours, and collected by placing them edgeways in slots in long cardboard boxes of just requisite depth. Flies showing the disease began to appear in the middle of August, and continued through September and October; in November they were only in evidence twice. In proportion to apparently sound flies their number was small. The fungus showed itself almost entirely in *Musca domestica*; only two flies of the species *Fannia canicularis* were discovered with it.

In view of the eruption of the fungus from the interior of the fly and the relatively wide range of the conidia, a test was made to determine whether the conidia in their outward flight carried bacteria from the fly to the surface upon which they were projected. For this purpose a diseased fly was fixed head downwards upon nutritive agar in the centre of a Petri plate. In the course of a few days a zone of colonies appeared around the fly within the area bestrewn by the conidia (Figs. 15, 16). It was also observed (under the microscope) that colonies developed along the margins of certain fungal filaments that grew outwards in the medium from the fly's body. Ten flies were dealt with in this way, with the uniform result of producing a zonal crop of colonies. Taking the first five plated for more detailed examination it was found that the colonies numbered from 70 to 400, and that their distribution was well within the furthest range of the conidia. By the usual fermentation tests the colonies were proved to be almost entirely representative of groups in the colon family, as shown in the accompanying table. One fly supplied four types of coliform bacilli; two gave two each, and two one each. These results are representative in some measure of the intestinal flora

Fly.	Date.	Number of Colonies.	Groups of Coliform Bacilli Represented in Colonies.
1	Sept. 5th, 1912	400	*Bacillus lactis aerogenes* (Escherich).
2	Sept. 17th, 1912	107	*Bacillus neapolitanus.*
3	Oct. 2nd, 1912	350	*Bacillus neapolitanus.* *Bacillus proteus.*
4	Sept. 14th, 1912	Overgrown by *B. proteus* (uncountable)	*Bacillus pneumoniae.* *Bacillus coli communis.* *Bacillus proteus.* Bacillus No. 19 of Morgan.
5	Oct. 11th, 1912	70	*Bacillus enteritidis* (Gaertner). *Bacillus proteus.*

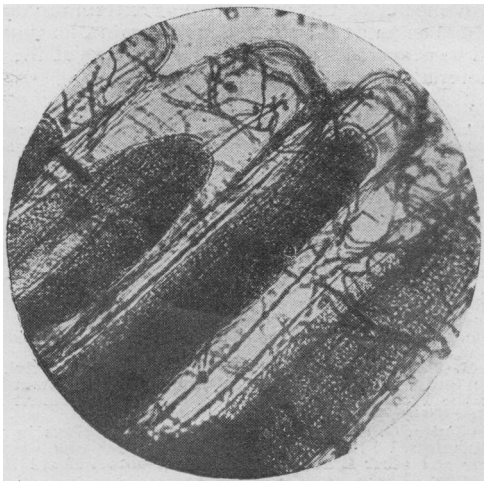

Fig. 19.—Four eggs of *M. domestica* separated by teasing from fungal mass in abdomen and showing network of hyphae firmly attached to the outer covering or chorion. × 100.

of the house-fly as found by Graham-Smith (1909), Orr (1910), Nicoll (1911). It is noteworthy that they are also representative of bacteria which may be found in the human intestine, as was coincidently demonstrated in the laboratory in the course of the examination of the excreta of infants suffering from diarrhoea.

In fly No. 4 there was also a golden yellow coccus in large number, but non-liquefying and non-virulent.

CULTURE OF THE FUNGUS.

Attempts were made on various media to establish artificial growth of the fungus, but while these resulted in an abundant germination of the conidia and the production of characteristic hyphae of considerable length (Fig. 20) development on subcultures was not obtained. The best results were got on glucose agar and on agar to which a small percentage of fat was added, an experiment suggested by the view put forward by some writers that the fungus spreads in the insect from the fat body.

It is perhaps worthy of special note that germination was frequently observed from a conidium which was in contact with another conidium. In the plate having glucose agar a condition suggesting the formation of zygospores was manifested (Fig. 21).

Since these observations were made I learnt a few days ago from an editorial note in the BRITISH MEDICAL JOURNAL of January 4th that Dr. H. de Reimer Morgan, of the Lister Institute, had succeeded in obtaining an artificial cultivation of the fungus. Attention is also directed in the same note to the fact that another observer (Mr. Edgar Hesse of London) had obtained what he believed to be a culture of the organism. This was confirmed by Dr. Julius Bernstein, who has been engaged for some time in the investigation of the subject on behalf of the Local Government Board. Mr. Hesse placed his results on record in the *English Mechanic and World of Science* for July 12th, 1912, and by further experiments it appears that he also succeeded in killing flies by feeding them on food containing spores derived from one of his own artificial cultures. He believes that *Empusa muscae* is polymorphic, and by nature a saprophyte endowed with the capacity of living a parasitic existence. Mr. Hesse's experiments lend support to the view that the fungus gains entrance to the body of the fly by way of the alimentary canal.

SUMMARY.

The part played by the house-fly as a carrier of infective bacteria is not yet fully appreciated and is far from being fully known.

The alimentary canal of the insect rather than the surface of its body appears to play the more important part in the transmission of infection.

Empusa muscae proves to be a potential means of bacterial dissemination not hitherto recognized.

The fungus is probably spread from one generation of flies to another by their larvae.

The suggestion of using this natural enemy of house-flies as a means of their reduction or extermination is to be regarded as an expedient of doubtful value, inasmuch as it would be attended by the risk of spreading at the same time bacterial infection to man.

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DISCUSSION.

DR. S. M. COPEMAN desired to congratulate Dr. Buchanan on the interest and value of his research work on the parasitic fungus of the house-fly, as set out in his paper; and on the excellence of the series of lantern slides, mainly of his microscopical preparations. As one result of the investigation on house-flies as carriers of infection that had been carried out during the past few years by the Local Government Board evidence had been obtained that flies could be infected with *Empusa* by way of the alimentary canal; and indeed it seemed not improbable that this, rather than infection from the exterior, was what usually occurred in nature. On the hypothesis generally accepted it was difficult to understand how any really extensive infection could be brought about. The fact that, as Dr. Buchanan had observed, conidia were formed immediately the fungal hyphae reached the exterior of the fly, whereas they were only found exceptionally in the crop or intestine, merely indicated that the fungus was markedly aërtropic, and did not in any way render it improbable that infection originated by way of the alimentary canal. Moreover, the further observation of Dr. Buchanan that bacteria of the *coli* group accompanied the hyphal growth, and might even be thrown off from the exterior of the fly, along with the conidia, seemed to indicate that the growth must have commenced from within the walls of the intestine. The fact that hyphal filaments had been found on the exterior of eggs within the ovary could hardly be of practical importance in reference to carriage of infection, seeing that it was improbable that these eggs would ever be laid. As regards attempts at cultivation of the fungus in artificial media, these had all failed hitherto. Morgan had indeed claimed, in a letter to the JOURNAL, to have been successful; but Mr. Massee, of Kew Gardens, who examined his preparations, did not find any *Empusa* growth in the subcultures. Under these circumstances, other methods, such as the slow burning of pyrethrum powder, must for the present, at any rate, be depended on for the destruction of such flies as obtained access to dwellings. But the ideal was the removal of all facilities for the breeding of flies.

DR. BERNSTEIN expressed pleasure at being present to see the remarkable series of pictures shown by Dr. Buchanan, and stated that one of his initial difficulties in starting to work with the fungus was the absence of any illustrations. In conjunction with Mr. Hesse he had been working at the *Empusa muscae* on behalf of the Local Government Board in order to verify the statement of Mr. Hesse that the fungus could be given artificially and used for inoculating flies. This work would be published later after presentation of the report to the Board. But he felt inclined to agree with Dr. Buchanan that it would be more satisfactory to prevent the growth of flies by destroying their breeding grounds than to await to kill them in later life; though perhaps in dealing with other pests such as the cinch bug and the locust the knowledge might prove of greater value.

TWO CASES OF PARATYPHOID B INFECTION TREATED WITH VACCINE.

BY

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THE paratyphoid B bacillus appears to produce two sets of symptoms. By far the most common is an illness clinically indistinguishable from ordinary typhoid fever. Much less commonly it causes acute gastro-enteritis.

Both the following cases occurred in the practice of Dr. Garratt, of Kingstown, to whom I am very much indebted for allowing me to report them. I desire also to express my obligations to Professor E. J. McWeeney and to Dr. H. F. Moore (Research Scholar) for the cultural and serological diagnosis of the two organisms isolated.

CASE I.

An elderly gentleman, aged 65, was taken ill on Thursday, January 16th. He had a history of attacks of pain in the abdomen and diarrhoea whenever he got a chill. For six weeks previously he had been suffering from swollen and inflamed gums with mucous patches. When seen by Dr. Garratt his temperature was 101°, pulse 96, respirations 20. He had pain in the region of the umbilicus and was having twelve to fourteen diarrhoeal motions in the twenty-four hours. He was given 10 grains of tannalbin every hour and ½ grain of opium with 6 grains of tannigen twice daily, and starch and opium enemata. The next day (17th) his temperature was 102°, his tongue was dry and heavily coated, and the stomatitis had spread over the soft palate and into the pharynx, making swallowing difficult. His abdomen was distended, and there was exaggerated gurgling through the ileo-caecal valve.

On January 18th his temperature was 102.8°; still twelve to fourteen motions a day. He continued t grow weaker. He was at times delirious and his heart was intermitting. On the 21st I saw him with Dr. Garratt. I made cultures from the pockets round his teeth, and also from his stools. I suggested the use of a germicide pending the making of a vaccine, and 1 c.cm. of iodine-menthol-radium compound was given into the gluteal muscles. That evening his temperature fell to 99.2° and the diarrhoea was less.

The next day (22nd) 1 c.cm. of iodine-menthol-radium compound was given, and the evening temperature was 99°. Blood cultures were made but gave no growth. The cultures from the gums gave chiefly streptococci with some *Micrococcus catarrhalis*. On Drigalski plates the faeces gave two kinds of colonies—(1) pink (acid); (2) blue, both Gram-negative motile bacilli. From the pink colonies the bacilli were feebly motile; from the blue they were more actively motile. I gave the Conradi-Drigalski plate to Professor McWeeney for further investigation, and made a mixed vaccine from an agar plate that I had made at the same time. I told Dr. Garratt that the case was most probably one of either typhoid or paratyphoid B fever. The blue colonies proved to be paratyphoid B. The vaccine was ready on the 23rd, and 5 million of the mixed vaccine were given on the 24th. His temperature was then 102° F., and he had to have 120 minims of chlorodyne, and starch and opium enemata, which kept down the motions to six to seven a day. He was delirious at times. That night he had the best night since the beginning of his illness, and the next day his temperature had fallen to 99° F. He felt better and was not delirious. His temperature was normal from this on, and instead of diarrhoea he became constipated, and enemata were necessary, which were probably the cause of an erythematous rash which came out on February 3rd. He had 7½ million of vaccine on January 28th, and was so much better on February 2nd that he was given solid food. He had 10 million on February 4th, and 15 million on February 11th. On February 22nd he had 20 million of the coli-paratyphoid vaccine, and 2½ million of streptococcal vaccine made from his gums as they were still a little sore. On March 1st he had 50 and 5 million, and on March 8th 70 and 10. His gums were then healed.

CASE II.

Male, aged 18, was seen by Dr. Garratt when he was, from his history, apparently at the beginning of the third week of what was clinically typical typhoid fever. The patient was semiconscious, restless, and sleepless. His abdomen was distended, there were a few rose spots on it, and he was tender in the region of the caecum. He was constipated, and his mouth and tongue were typically typhoid. He was refusing food. His temperature was 103° F. and his pulse was 110. Widal negative. As in the last case, cultures on Conradi-Drigalski from his stools gave red and blue colonies. A mixed vaccine was made. He was given 2½ million on March 9th. That night he was very weak and restless, and was given a saline enema and stimulants. The next morning his temperature was 97° F., his pulse 65. His flatulence was relieved, tongue clean, and sordes cleared up, and delirium had disappeared.

On the following night (March 11th) his temperature went to 100° F., but his pulse was only 65, and his temperature kept entirely normal from that on. He was put on to more solid food in a day or two, and made an uninterrupted recovery, and was given full diet on the 19th. On March 17th he had 5 million of the vaccine, and had no reaction after it.

Comment.

When I first saw Case I I thought it was probably one of acute gastro-enteritis, most likely produced by a mixed infection with streptococci from the gums and coliform organisms in the intestines, which, in my opinion, is the most common cause of inflammations of the gastro-intestinal tract and its adnexa. The iodine-menthol-radium compound seemed to produce a temporary improvement, but the patient relapsed, and his condition on the 24th, when he got the vaccine, was most precarious. The change in a few hours, after the injection, from a temperature of 102°, an intermittent pulse, a distended abdomen, delirium and diarrhoea hardly controlled, to a condition of temperature nearly normal, steady pulse, no distension and feeling of comparative well-being, was most remarkable and dramatic. The same may be said of Case II, whose condition at the time he got the vaccine—namely, temperature 102°, pulse 110, weak, and of small tension, delirious, with distended abdomen. Twenty-four hours after he is quite conscious, his abdomen is no longer distended, his pulse is 68 and of good tension, and his temperature is normal.

I can find little reference in the literature to the treatment of paratyphoid by means of vaccine, but there is a pretty extensive literature of vaccine therapy of typhoid fever, which, according to most authorities, is clinically indistinguishable from paratyphoid. I cannot find anything comparable to the sudden turn from severe illness to convalescence which occurred in these cases, and suggest that it may have been due to the use of the mixed vaccine, for in all these cases the *B. coli* must always be also pathogenic, since it becomes so at the slightest lowering of the resistance of the cells with which it comes in contact.* These cases are in marked contrast to the two fatal cases of paratyphoid referred to below.

Manufacture of Vaccine.

Since both of these cases were in a precarious condition, it was essential to get the vaccine made in as short a time as possible. The faecal material was plated out on an ordinary agar and on Conradi-Drigalski medium, and broth cultures were made. As I have said, on the Conradi-Drigalski medium two kinds of colonies appeared, the larger were red, the smaller blue. The larger were feebly motile, the smaller more actively motile. It was presumed that the microbes belonged to the typhoid-coli group, the red colonies belonging to the coli side, the blue to the typhoid side of the group.

The cultures were made in the afternoon, examined the next morning, and a mixed vaccine made from the agar plates. I prefer, if possible, to sterilize vaccines with as little heat as possible. As a rule, in making typhoid vaccine I do not heat at all, but allow the ½ per cent. phenol to kill the microbes, or, if in a hurry, take some of the suspension and heat it for fifteen minutes with the ½ per cent. phenol saline solution, and use it for the first dose or two. It takes a very long time to kill *coli* organisms this way; even half an hour at 58° C. frequently fails to kill them all, and so in these cases I heated them for forty-five minutes at 58° C. They were then tested, and the agar cultures made being sterile after twenty-four hours, doses were measured out. It is thus possible to have the vaccine ready within forty-eight hours. (The Conradi-Drigalski plates were then handed over to Professor McWeeney and Dr. Moore for exact diagnosis.) The diagnosis that the blue colonies were paratyphoid B organisms was arrived at by their cultural and serological reactions, which were compared with those of other paratyphoid organisms and with those of other members of the typho-coli group. Taking the cultural reactions first, it will appear from the accompanying table (p. 1374) that they behaved as typical paratyphoid B organisms.

* Sanarelli has proved the increased toxicity of the colon bacillus in typhoid fever.

TABLE I.

	Arabinose.	Dulcite.	Glucose.	Inulin.	Lactose.	Maltose.	Mannite.	Raffinose.	Saccharose.	Salicin.	Xylose.
1. Faecal coli	G. A.	G. A. R.	G. A.	A.	G. A.	G. A.	G. A. R.	G. A.	G. A. R.	G. A.	G. A.
2. Typhoid	—	A. R.	A.	— R.	—	A. R.	A. R.	A.	— R.	— R.	— R.
3. Gaertner	G. A.	G. A. R.	G. A.	A. R.	—	G. A. R.	G. A. R.	A. R.	— R.	— R.	G. A. R.
4. P.B., Redmond ...	G. A.	G. A. R.	G. A.	A. R.	—	G. A. R.	G. A. R.	A. R.	— R.	— R.	G. A. R.
5. P.B., R.A.M.College	G. A.	G. A. R.	G. A.	— R.	—	G. A.	G. A.	A. R.	— R.	A. R.	G. A. R.
6. Crofton I	G. A.	G. A. R.	G. A.	— R.	— R.	G. A.	G, A.	A. R.	— R.	— R.	G. A.
7. Crofton II	G. A.	G. A. R.	G. A.	— R.	— R.	G. A,	G. A.	— R.	— R.	— R.	
8. P.B., Malta	G. A.	G. A.	G. A.	— R.	— R.	G. A.	G. A.	— R.	— R.	—	
9. P.A., R.A.M.College	G. A.	G. A.	G. A.	—	—	G. A.	G. A.	—	—	—	
10. Murray	A.	A. R.	G. A.	— R.	—	G. A.	G. A.	—	— R.	A.	
11. Sligo	G. A.	—	G. A.	—	G. A.	G. A.	G. A.	—	— R.	G. A.	

G. = Gas. A. = Acid. R. = Reduction of litmus colour.

Comments.

1. This strain of faecal coli is not classically typical, because it forms gas and acid on saccharose.

2. Typhoid, an old strain (ten years), behaves typically on these sugars.

3. "Gaertner" was isolated by Professor McWeeney from a meat poisoning epidemic in Limerick. It was, and still is, very virulent. It produced nine deaths in the original epidemic.

4. "P.B., Redmond." From a *fatal* case of supposed typhoid fever in the Mater Misericordiae Hospital, Dublin. The organism was isolated from the intestinal contents. The alimentary canal from and including the stomach downwards was found at the autopsy to be swarming with paratyphoid B bacilli. There were ulcers of a typhoid character round the ileo-caecal valve. This strain differs from the other paratyphoids in forming acid in inulin. It agrees with (5) and (6) in forming acid in raffinose, while (7), (8), and (10) do not.

5. A strain obtained from the Royal Army Medical College. It agrees with Redmond and Crofton I in forming acid in raffinose. It also forms acid in salicin.

6. Was isolated from the acute diarrhoea case described above. Its only cultural difference from the strain obtained from Case II is in its forming acid in raffinose. This latter strain may be termed "Crofton II." It is No. 7 on this list.

8. "P.B., Malta" was obtained from the Royal Army Medical College, and agrees with Crofton II in not forming acid in raffinose.

9. "P.A." was also obtained from the Royal Army Medical College. It differs from the other paratyphoids in not discharging the litmus colour in the media containing inulin, lactose, raffinose, saccharose, and salicin. Its main difference, of course, is its inability to alter neutral red and its behaviour on litmus whey.

10. "P.B., Murray" was isolated from the faeces of a patient whose illness was complicated by peritonitis from which a staphylococcus was isolated *post mortem*. Its sugar reactions are atypical, since it forms no gas in arabinose and dulcite and forms acid in salicin like (5) "P.B., R.A.M.College."

11. "Sligo" was isolated from the faeces of patients from an epidemic of illness, attributed to the ingestion of abnormal milk, in Sligo Workhouse. The blood of the patients did not give sufficient agglutination to connect the microbe with the disease. From its cultural reactions it appears to be an atypical *coli*.

By Erhlich's method none of these microbes gave indol except *Bacillus coli*. Loeffler's new plate medium and green solutions, while it distinguished typhoid from coli, paratyphoid A, paratyphoid B, and the other food poisoners, did not distinguish the food poisoners from each other. The same may be said of Buchholtz neutral red medium.

TABLE II.—*Agglutination Experiment.*

Serum.	Crofton I.	Crofton II.	Murray.	Redmond.	Malta.	P.A.	Aertryck.	Gaertner.	Coli.	Typhoid.
Anti-Crofton I	50,000	50,000	40,000	40,000	5,000	20	50	10	10	20
Anti-Crofton II	60,000	70,000	50,000	30,000	30,000	100	50	100	20	50
Anti-Gaertner	100	50	100	100	100	100	—	10,000	0	100
Anti-P.A....	500	500	500	1,000	500	30,000	—	50	0	100
Anti-typhoid	500	500	1,000	500	500	100	—	1,000	500	30,000
Anti-Aertryck	10	16	20	16	20	50	10,000	20 (?)	100	20

Comments.

1. Anti-Crofton I serum was obtained from a rabbit by intravenous injections of a killed suspension. Five injections at six days' intervals were given, when the serum was drawn off to test. Another injection was given fourteen days later. The animal rapidly became ill and died in about two days after this injection. Cultures from heart's blood and spleen gave no growth, the vaccine was proved sterile, and the serum had *post mortem* lost all its agglutinating power. (For these reasons we are inclined to think it died from anaphylaxis.) On account of this its agglutinating effect on Crofton II and Malta could not be verified.

2. Anti-Crofton II serum was prepared in the same way.

3. Anti-Gaertner serum. This was obtained from the Lister Institute, our own efforts to obtain an antiserum having failed, owing to the extreme toxicity of the killed cultures, which were derived from the Limerick epidemic.

4. Anti-P.A. serum was also obtained from this source.

5. Antityphoid serum was obtained from Messrs. Burroughs, Wellcome and Co.

6. Anti-Aertryck was made by giving four injections of killed suspensions subcutaneously. This immunization was carried out by the subcutaneous instead of the intravenous method, owing to the extreme toxicity of the killed microbes—two rabbits having died after the first *intravenous* injection.

Controls were done in every series of tests by using normal rabbit serum instead of the specific agglutinating serum. The microscopic method was used, and was controlled in many instances by Wright's macroscopic technique. It is interesting to note that in none of these experiments was a zone of inhibition experienced.

Anti-Crofton I and II did not agglutinate Aertryck in high dilution.

The only further comment necessary is that the figures as set forth in Table II clear up the diagnosis of the group to which the microbe isolated from Murray belongs, since the sugar reactions left this in doubt. The figures make it abundantly

evident that it is a paratyphoid B. organism, and yet it does not ferment dulcite.

The identity of these microbes—namely, Crofton I and II—was further confirmed by a few absorption experiments.

TABLE III.—*Absorption Experiment.*

Anti-*paratyphosus* A serum saturated with Murray (lowest dilution examined 1 in 50).

	Crofton I.	Crofton II.	Murray.	Redmond.	Typhosus.	P.A.
Titre before absorption...	500	500	500	1,000	100	30,000
Titre after absorption ...	0	0	0	0	50	10,000

This further tends to confirm the identity of Murray with Crofton I, Crofton II, and Redmond.

The anti-P.A. serum used in this case was obtained by us in similar manner to the antiserums to Crofton I and II.

TABLE IV.—*Absorption Experiment.*

Anti-Crofton II serum saturated with Redmond (lowest dilution examined 1 in 20).

	Crofton I.	Crofton II.	Redmond.	P.A.
Titre before absorption...	60,000	70,000	30,000	100
Titre after absorption ...	0	0	0	100

The typical P.B. microbe (Redmond) has taken all the agglutinins for Crofton I and II from Crofton II serum, again confirming the identity of these microbes.

DISCUSSION.

Dr. C. W. HUTT (Brighton) said that the case of acute gastro-enteritis recorded was of interest to those who were concerned with "diarrhœa," especially from the standpoint of the public health official. The connexion of diarrhœa among infants with *Bacillus paratyphosus* B had been proved, among others, by Drs. Clements and Thomas Orr. The case mentioned by Dr. Braxton Hicks was also of interest in this connexion. If the association of *Bacillus typhosus* and *Bacillus paratyphosus* B was more common than was usually held, it was possible that the cases of diarrhœa among children, shown by Dr. Niven ,by means of Widal reactions to be overlooked cases of typhoid fever, might also harbour in their intestines the *Bacillus paratyphosus* B. It would be useful to hear of the results of the use of vaccines in cases of diarrhœa among infants and children.

Dr. EYRE pointed out that Conradi-Drigalski medium was most valuable because in a few hours characteristic colonies could be picked out. He mentioned the probability of a mixed vaccine being more satisfactory than a pure vaccine, owing to the exaltation of the *B. coli*. He deprecated the haphazard use of sugar media, and thought it would be better to classify these according to their chemical structure—namely, monosaccharides, disaccharides, etc.—in order to arrive at a better understanding of what these organisms could do. He pointed out that, though some organisms might form acid litmus media might not be discoloured. With regard to vaccines in treatment of diarrhœa in infants, he said that some years ago Minett obtained promising results with Morgan's bacillus No. 1.

Dr. HICKS (London) related two interesting cases in which agglutination reactions had proved of value and in which vaccines had been of great assistance.

Dr. BERNSTEIN (London) pointed out that for the purposes of treatment it was not absolutely essential to exactly place the offending coliform organism, provided that its association with the diseased state was evident. He also suggested the possibility of a spontaneous cure of these infections, which would possibly ultimately wear themselves out, though there could be no doubt of the value of vaccines in such cases.

Professor MCWEENEY, in reply, said that the prognosis in these cases was so bad that it appeared unlikely that their recovery could have been due to spontaneous recovery. He agreed that for purposes of treatment it was sufficient to allocate the isolated microbes to their groups, this giving sufficient guide to initial dose. The cases referred to by Dr. Hicks clearly indicated that mixed autogenous vaccines were essential in the treatment of typhoid and paratyphoid infections. He agreed with Dr. Eyre that the sugar reactions should be grouped according to their chemical composition.

ON THE ACTION OF ASBESTOS ON CERTAIN PHYSIOLOGICAL SUBSTANCES.

BY

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WE have found that solutions or suspensions of certain substances, after being in intimate contact with asbestos for varying periods are altered either in quantity or quality.

The substances were classified as follows:

A. *Ferments:*
1. Malt diastase.
2. Pepsin—liquor pepticus (Benger).
3. Rennet—Benger's preparation.
4. Pancreatic ferments—liquor pancreaticus (Benger).

B. *Carbohydrates:*
1. Starch.
2. Dextrin.
3. Dextrose.

C. *Proteins:*
1. Non-diffusible: egg albumin.
2. Diffusible: peptone (Witte).

D. *Alkaloids:*
1. Curare.
2. Strychnine.

E. *Hormone:*
1. Suprarenin; synthetic.
2. Adrenalin (Parke, Davis and Co.).

Method.—To each gram of asbestos fibre 2 c.cm. of the solution or suspension substance were added. This proportion sufficed to enable the asbestos to soak up all the fluid, leaving no excess. The liquid was recovered from the asbestos by means of pressure exerted by a small hydraulic press. We propose to call the exuded liquid the exudate, and the material which cannot be recovered by pressure the residue material.

A. FERMENTS.

1. *Malt Diastase.*—The malt diastase before being placed in contact with asbestos was very active, rapidly converting starch into sugar. After being in contact with asbestos for twenty-four hours the exudate was quite inactive. The asbestos containing the residual material became activated or sensitized, and rapidly hydrolyzed starch.

2. *Pepsin.*—The undiluted liquor pepticus (Benger) was used. It was in contact with asbestos for four weeks. The exudate was added to swollen fibrin in 0.2 per cent. HCl and maintained at 37° C. No proteolytic action could be detected. The asbestos with its contained residual material was steeped in 10 per cent. egg albumin which had been acidulated with HCl and the whole placed in the incubator at 37° C. On examining the fluid which could now be exuded it was found to be entirely free not only from albumin but also from the products of

peptic digestion. One of the following phenomena had taken place:

(*a*) The albumin was absorbed unchanged, and remained unchanged as residual material.

(*b*) The albumin was absorbed unchanged, became residual material; this residual material was hydrolyzed, and the products of hydrolysis remained as residual material.

(*c*) The albumin was first hydrolyzed, the products of hydrolysis absorbed, becoming residual material.

Further research in the form of fractional analysis will be necessary in order to elucidate this point.

3. *Rennet.*—Benger's undiluted preparation was used and squeezed out from the asbestos after the lapse of four weeks. The exudate contained no milk-curdling ferment. The asbestos has not been satisfactorily examined for residual material on account of the difficulty experienced in determining whether a liquid which cannot be expressed from the interstices of a solid is or is not curdled.

4. *Pancreatic Ferments.*—Benger's undiluted liquor pancreaticus. This preparation contains four ferments—namely, trypsin, amylopsin, steapsin, and a milk-curdling ferment. After being in contact with asbestos for four weeks the exudate was examined, with the following results: 97 per cent. of the trypsin, as estimated by Roaf's method, had disappeared, and no trace of the remaining three ferments could be found.

Fibrous asbestos, with its residual material, was steeped in 0.5 per cent. starch paste, and kept at 37° C. After twenty-four hours—it was not examined before—the exudate contained no starch, no erythrodextrin, but a quantity of reducing sugar, and the asbestos itself gave no reaction with iodine.

An attempt was made to examine the residual material for steapsin and milk-curdling ferment, but the result was negative. With improved technique we hope to obtain a positive result in the near future. It is quite within the bounds of possibility that the several pancreatic ferments may be thus removed fractionally from solution.

B. Carbohydrates.

1. *Starch.*—A 0.5 per cent. starch paste was placed in contact with asbestos, and pressure applied after ninety-six hours. The exudate contained no starch, it contained erythrodextrin and reduced Fehling's solution slightly, but so slightly that we could not exclude the possibility of the reduction being due to the dextrin and not to one of the reducing sugars. This experiment was repeated, it being possible that the asbestos contained some organic ferment. The asbestos was therefore boiled in water, dried and kept at a temperature of 300° C. for several hours. Even after this treatment its catalytic action was unabated. On treating the asbestos with iodine, the characteristic starch reaction was obtained.

Some 0.5 per cent. starch paste was left in contact with asbestos in a sealed bottle for three months at room temperature. On squeezing out, the exudate was free from starch and dextrin, but contained a large amount of reducing sugar, which proved to be glucose, a glucosazone being readily formed. The asbestos gave no reaction with starch, showing that all the starch had been converted into glucose, or achroodextrin and glucose.

2. *Dextrin.*—A 0.5 per cent. solution of pure dextrin was placed in contact with asbestos. After the lapse of twenty-four hours the exudate contained 0.25 per cent. dextrin. On testing the asbestos fibre with iodine the reaction for dextrin was obtained. We quite expect to find, after a long period, that the dextrin which exists as residual material will ultimately be converted into glucose.

3. *Dextrose.*—Dextrose is only slowly and imperfectly removed from solution by means of asbestos.

C. Proteins.

1. *Non-diffusible Egg Albumin.*—Weak solutions or suspensions of egg albumin lose all their contained protein in twenty-four hours when placed in contact with asbestos. The exudate is protein-free, while the asbestos containing the residual material gives the xanthoproteic reaction and reaction with Millon's reagent. The more concentrated the albumin solution the more protein will the exudate contain. It is possible that the residual material contains the products of protein hydrolysis; this we have not yet determined.

2. *Diffusible Protein: Peptone.*—A 0.2 per cent. solution of Witte's peptone was employed; this is a mixture of proteose and peptone, and was always removed quickly from solution, so that the exudate was proteose and peptone free. The biuret reaction was used as an indicator, a standard solution of copper being prepared by adding 1 c.cm. of fresh Fehling's solution to 20 c.cm. of 20 per cent. NaOH, 1 c.cm. of this solution was added to 1 c.cm. of the exudate and the depth of colour compared with a 0.2 per cent. solution of Witte's peptone.

Table showing Removal by Asbestos of Protein from Solutions of Different Concentrations.

Experiments.	Egg Albumin per Cent.	Protein per Cent.	
		Exudate 24 Hours.	Exudate 48 Hours.
1	0.09	Nil	Nil
2	0.1	Nil	—
3	0.15	Nil	—
4	0.19	Trace	Trace
5	0.25	Trace	—
6	0.26	Trace	—
7	0 34	0.1	—
8	0.38	0.1	Trace
9	0.46	0.2	—
10	0.5	0.2	—
11	0.54	0.22	—
12	0.58	0.28	0.15
13	0.64	0.38	—
14	0.72	0.38	—
15	0.78	0.5	0.39
16	0.98	0.68	0.62

Apropos of diffusible proteins we should mention that we have observed some interesting phenomena with regard to the behaviour of asbestos towards the products of peptic and tryptic digestion. We are at present making further investigations.

D. Alkaloids.

1. *Curare.*—A 0.1 per cent. solution of curare in 0.6 per cent. saline was used. After twenty-four hours' contact with asbestos 1 c.cm. of the exudate was injected into the dorsal lymph sac of a frog; the frog showed no symptoms of paralysis.

A frog of similar size was given a 1 c.cm. dose of the original solution. It showed marked paralysis in fifteen minutes, and was dead at the end of an hour. The exudate was also much less deeply pigmented than the original solution.

2. *Strychnine.*—The liquor strychninae, *British Pharmacopoeia*, diluted ten times, was employed. On testing the exudate after twenty-four hours' intimate contact with asbestos no trace of strychnine could be found.

E. Hormone.

1. *Synthetic Suprarenin.*—A 0.0025 per cent. solution in normal saline was in contact with asbestos for twenty-four hours. The exudate contained no pressor principle as gauged by its action on the excised eyeball of the frog.

2. *Adrenalin* (Parke, Davis and Co.).—A solution containing one part in 10,000 parts of normal saline was used. After twenty-four hours the exudate contained no pressor principle.

It is proposed to repeat the experiments with suprarenin and adrenalin, using the colorimetric method for estimation as recently described by Folin, Cannon, and Denis.

The following table shows the solubility of various forms of asbestos and other materials in distilled water. The figures refer to the conductivity of the exudate in reciprocal meghoms cm^{-1}. In each case 50 c.cm. of distilled water were added to 25 grams of material.

	24 Hours.	42 Hours.	90 Hours.	230 Hours.
Asbestos, Russian ...	336	551	756	1,472
Asbestos, Canadian, A.	1,205	1,636	2,204	2,272
Asbestos, Canadian, B.	602	722	851	1,088
Asbestos, African ...	468	620	702	1,056
Glass powder	200	229	254	322
Talc, Austrian	107	121	130	185
Talc, French	98	111	117	160
Kaolin	200	177	172	192
Asbestos powder ...	277	295	310	361
Glass wool	2 770	2,793	3,444	4,216
Fuller's earth	236	244	263	326
Animal charcoal ...	941	886	930	960

When in contact with distilled water, asbestos continually undergoes a degradation or solution; electrolyte is being continually added to the water. This degradation is increased during the disappearance of diffusible substances from solution, and diminished during the adsorption of colloidal substances, such as colloidal gold, dialysed iron and methylene blue. These latter apparently form a protective coating on the asbestos fibre.

The following table shows the increased solubility of asbestos fibre and kaolin during the disappearance of a diffusible substance from solution. The asbestos and the kaolin were steeped in a 0.2 per cent. solution of Witte's peptone:

	Conductivity in Reciprocal Megohms, cm^{-1}.			Biuret Reaction.		
	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.
Distilled water ...	3.2	3.2	3.2	100 %	100 %	100 %
Peptone 0.2 per cent..	102 4	115.8	133.4	—	—	—
Kaolin	107.0	177.0	—	—	—	—
Russian asbestos ...	336.0	551.0	756 0	—	—	—
Kaolin and peptone, 0.2 per cent.	224.0	263 8	288 0	20 %	20 %	20 %
Russian asbestos + peptone 0.2 per cent.	620.8	736.0	851.0	10 %	0 %	10 %

The following table shows the diminished solubility of asbestos in distilled water during the adsorption of colloidal substances:

	Controls.	+ ½ Weight Asbestos.		+ ½ Weight Animal Charcoal.	
		After 15 Minutes.	After 22 Hours.	After 15 Minutes.	After 22 Hours.
Distilled water ...	3.2	317.8	342.4	874	896
Colloidal gold ...	258.8	345.2	530.8	760	760
Dialysed iron, 2 per cent.	501.8	403.2	729.2	102	102
Dialysed iron, 0.7 per cent.	239.6	258'8	934	—	—
Methylene blue, 0.01 per cent.	230.4	134.4	486	716	716

The removal of peptone from solution by asbestos and other materials is shown in the following table:

Materials.	Weight in Grams.	Volume of Solution, 0.2 per Cent. Peptone in c.cms.	Per Cent. of Peptone remaining as judged by the Biuret Reaction.	
			Powders Shaken 8 Hours; Fibres Standing 16 Hours.	Powders Shaken 16 Hours; Fibres Standing 55 Hours.
Asbestos, Tyrolese ...	1	5	2	10
Asbestos, Tyrolese, after treatment with alcohol and ether	2.5	5	1	0
Silk	0.85	4.25	60	50
Cotton	1	5	100	60
Scottish wool (long fibre)	1	5	90	0
Merino	1	5	75	Brown
Ramie	1	5	90	10
Flax	0.22	1.2	40	40
Asbestos, Canadian...	1	5	25	0 (?)
,, Russian ...	1	5	27.5	0
,, blue ...	1	5	35	Dirty brown
Glass wool	1	5	100	100
Animal charcoal ...	1	5	0	0
Kaolin	1	5	10	100
Glass powder	1	5	90	90
Talc, Austrian ...	1	5	20	100
,, French	1	5	27.5	95
Asbestos powder ...	1	5	0	0 (?)
Sand (clay present) ...	1	5	50	50
Control (not shaken)			100	100
,, (shaken) ...			100	100

Oscillatory Phenomenon.

We have observed that in a few instances the peptone was first removed from solution and later given up; the figures in the following table refer to the percentage of peptone of original solution remaining. The original solution was 0.2 per cent.

Russian asbestos + 0.2 per cent. peptone	24 hrs. standing, 10 %	48 hrs. standing, 0 %	68 hrs. standing, 10 %
Kaolin powder + 0.2 per cent. peptone	8 hrs. shaking, 10 %	16 hrs. shaking, 100 %	
Tyrolese asbestos + 0.2 per cent. peptone	16 hrs. standing, 2 %	55 hrs. standing, 10 %	
Talc, Austrian, + 0.2 per cent. peptone	8 hrs. shaking, 20 %	16 hrs. shaking, 100 %	
Talc, French, + 0.2 per cent. peptone	8 hrs. shaking, 27.5 %	16 hrs. shaking, 95 %	

The above research is a continuation following on some remarks made by one of us at a meeting of the Pathological Society of Great Britain and Ireland, held at St. Bartholomew's Hospital in January, 1913. The experiments were not performed under conditions of strict sterility.

PROTEOSE-FREE TUBERCULIN.

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In the application of old tuberculin for the diagnosis of tuberculous disease a large number of positive reactions are obtained in persons in whom no clinical signs of tuberculosis are found, and who give no history indicative of a tuberculous infection. At the same time a number of tuberculous patients give a negative or slightly positive reaction, from which it is difficult to decide whether the reaction is due to specific toxins existing in the tuberculin, or to substances which are not specific products of the tubercle bacillus.

In a series of experiments, performed with the view of discovering whether any of the ingredients used in the preparation of old tuberculin possess toxic properties, it was found that the medium itself, when used instead of old tuberculin in the manner described by von Pirquet or Woodcock, produced a reaction very similar to that produced by old tuberculin, alike in tuberculous and non-tuberculous subjects. It was further found that a 1 per cent. solution of commercial peptone, containing peptones and proteoses, gave a similar result.

It was inferred from this fact that the proteoses in old

tuberculin are not specific, and that they are toxic is shown by the inflammatory process they induce when applied as a cutaneous test by any of the cutaneous methods. Their presence, moreover, in old tuberculin tends to mask any reaction which might be produced by specific toxins excreted into the culture medium, or derived from the bodies of the bacilli during the process of growth.

Accordingly, it was thought that if these foreign proteoses were removed from old tuberculin a positive reaction would more definitely indicate the presence of tuberculosis.

Koch evidently thought that the presence of these substances were injurious, since he endeavoured to exclude them from tuberculin by growing bacilli on an albumose-free medium containing asparagin, citrates, and inorganic salts. This modification is now known as T.A.F.

Béraneck excludes foreign proteoses from his tuberculin by using for his culture medium a watery extract of veal, obtained by macerating veal in cold water for two hours.

A third method for excluding proteoses from tuberculin was described in a former paper, and consists in growing tubercle bacilli on peptone veal broth in the manner described by Koch, and then adding absolute alcohol to the filtered product to precipitate the proteoses. The supernatant fluid is decanted, and the alcohol evaporated off at a low temperature. The remaining fluid is proteose-free, and contains in solution a toxin which has been shown to be specific to tuberculosis.

Another method, recently described by Coplans, is that in which finely divided asbestos is added to peptone veal broth; the medium is then inoculated with tubercle bacilli, which are allowed to grow until a thick film of growth has formed. The fluid is removed by squeezing the asbestos dry, filtered through porcelain, and 1 per cent. phenol added. When tested by the "biuret" method this fluid is found to be proteose-free, the proteose having been absorbed by the asbestos. It is, however, toxic to tuberculous subjects.

If the proteose derived from tuberculin be dissolved in water and made up to the original bulk with water, and the solution used instead of tuberculin in the manner described by von Pirquet or Woodcock for cutaneous reaction, a positive reaction is obtained in practically every case, whether tuberculous or non-tuberculous. If, after the removal of the alcohol, the supernatant fluid is employed in a similar manner no reaction is obtained in patients in whom no clinical signs of tuberculous disease are found, whereas in persons who are known to have tuberculosis, a positive reaction is obtained in 98 per cent. of cases. Those tuberculous patients who gave a completely negative reaction were very advanced in the disease, and all died within two weeks of the application of the test.

For diagnostic purposes the method which has been principally used is a modification of that advocated by Lignières. This method was chosen on account of its painlessness and the ease with which it can be performed. The following is briefly the method of procedure:

The skin of the forearm having been cleaned with soap, spirit, or alcohol, sufficient friction being applied to induce a slight hyperaemia, one drop of proteose-free tuberculin is injected from a sterile hypodermic syringe between the epidermis and the Malpighian layers. On withdrawing the needle a little of the injected fluid escapes and is removed on a piece of cotton-wool. As a control, a drop of sterile peptone veal broth, from which the proteose has been removed by precipitation with alcohol, is introduced in the same way a short distance from the site of the first injection, any fluid escaping being removed on cotton-wool. No dressing is required.

At the end of twelve hours the arm is examined. If a positive reaction is produced, an area of hyperaemia is noted around the part into which tuberculin was injected. The hyperaemic area enlarges and becomes more pronounced, until, at the end of twenty-four hours, the complete reaction is produced. The control should be free from hyperaemia.

The reaction, when positive, varies in intensity according to the stage of the disease, but in the inverse ratio; an early case of phthisis, for example, produces a well-marked reaction, while a more advanced case produces a smaller and less intense reaction.

The reaction in an early case is seen to be as follows: Around the site of injection a well defined area of induration and hyperaemia, measuring about 1 in. in diameter appears within twenty-four hours, the edges gradually fading to normal skin; it is somewhat tender to touch, and the patient complains of a feeling of tightness in the arm and a slight burning sensation. In about forty-eight hours the tightness and burning have disappeared; the hyperaemia now begins to fade, and at the end of another twenty-four hours it has practically disappeared, leaving only a brownish discoloration, which may last one or two weeks.

In an advanced case the appearance of the reaction is materially the same; the area of hyperaemia and induration, however, is very much smaller, and may only measure ¼ in. in diameter, and is less marked. All degrees of intensity of the reaction are met with, from the well-marked and tender area of the early case to the small, ill-defined, painless area of the advanced.

Another factor of importance is the time which intervenes between the application of the test and appearance of the reaction, which in an early case is developed in twelve hours, whereas in a more advanced case it is not developed until the lapse of twenty-four hours. It has been noted also that the earlier the case the longer the reaction remains visible—an early case retaining the hyperaemic area for three or four days, while in an advanced case it begins to fade on the second day.

It is possible to tell, therefore, from the appearance, onset, and disappearance of the reaction, how far advanced the disease is.

Children react well to this method of cutaneous diagnosis, but the test should be performed with a diluted tuberculin. It has been customary to use a dilution of 1 in 25 for children under 10 years and of 1 in 10 for those from 10 to 16 years.

The appearance of the reaction has been described as appearing in three types—first, second, and third—corresponding to the first, second, and third stages of the disease. It has been noted that in cases in which the disease has become arrested, the reaction tends to approximate to that of an early case. In a number of cases in whom a reaction of the second type was obtained on admission to a sanatorium, a reaction almost as well marked as that of an early case was obtained after six months' treatment. So by this method it is possible to decide whether a case under treatment is progressing favourably or otherwise.

When performing the intradermal test for the diagnosis of tuberculosis the needle should be held parallel to the skin, and the skin rendered tense by encircling the forearm with the thumb and forefinger, and drawing the skin taut. The point of the needle is then inserted obliquely between the epidermis and the deeper layers. The point should not penetrate deeper than the epidermis. A small bulla is produced by the injection, on the surface of which, a few small dimples are to be seen, with a cutaneous hair in each, if the injection has not been too deep.

Koch's original tuberculin was concentrated, by boiling, to one tenth its volume; this would seem to be unnecessary, since a tuberculin which has been heated to over 60° is less toxic than one which has not been heated, and the proteoses are more easily precipitated by alcohol when the tuberculin has not been exposed to temperature above 60° C. Judging from the analogy of other bacterial toxins, heating would seem to reduce the toxicity of tuberculin; this has been proved in a series of experiments undertaken to determine the toxicity of old tuberculin.

The treatment of tuberculosis was undertaken with proteose-free tuberculin in about 100 cases; of these, 50 completed a full course. The results indicate a more extensive trial, and compare very favourably with those obtained with other forms of tuberculin.

It is easily borne, and can be given in cases where other forms of tuberculin produce a marked general reaction, and for this reason it is very useful in the treatment of ambulant cases. A general reaction is rarely produced, and only in acute cases, or with large doses. When this does occur it is not very severe, and usually is of short duration. Headache and lassitude are the principal symptoms complained of; the temperature may be elevated one degree, but usually falls within a few hours.

It can be given with safety in febrile cases; in fact, it has a beneficial effect on the temperature. In many cases the temperature has been reduced to normal by its

administration. The initial dose is found to be 0.001 c.cm., but in acute cases, if this dose produces a general reaction, the dose should be reduced to 0.0001 c.cm., and rapidly increased. The dose is increased every other day by 0.001 c.cm. until 0.01 c.cm. is reached, when the increase may be more rapid. The maximum dose is 1.0 c.cm., and to reach this about three months is the usual time required. For children the initial dose is 0.0001 c.cm. This should be gradually increased by 0.0001 c.cm. every other day until tolerance is established, when the increase may be more rapid.

Improvement is usually manifested in about a week after the commencement of the administration, and is evidenced by a feeling of improvement expressed by the patient; at first there is a slight increase in the cough and expectoration, followed by a diminution, and soon there is a cessation of night sweats.

During the treatment of the 100 cases only one patient had haemoptysis, and that only of a slight nature. It would seem, therefore, that proteose-free tuberculin does not increase the tendency to that condition.

Of the 50 cases who had a complete course of proteose-free tuberculin, 45 were suffering from pulmonary tuberculosis, 2 from early lupus, 1 from early spinal caries, 1 from tuberculous glands of the neck, and 1 from tuberculous disease of the hip-joint.

Of the 45 cases of phthisis, 17 were in the early stage, with the disease confined to one apex; 12 were in the second stage, with the disease in both apices or in parts of two lobes; 16 were in the third stage, with cavity formation, or having some tuberculous complication, such as phthisis laryngei.

Some of these cases were treated in hospital, some in a sanatorium, and some by the ambulant method. All derived benefit from the treatment, especially the early cases of phthisis and the surgical cases.

The time which has elapsed since the cessation of the treatment is not long enough to decide whether the benefit is permanent or not, but the rapidity with which the physical signs cleared up in many of the cases, and the general improvement which was seen to follow the administration of proteose-free tuberculin, justify the continuance of the treatment on similar lines.

I am indebted to Dr. Margaret Sharp, of Bradford, for the notes of several of the cases.

IT is estimated that there are 100,000 blind persons in the United States. The knowledge of this fact led some time ago to the appointment of a commission by the Medical Society of the State of Pennsylvania, and on the initiative of that commission the Pennsylvania Society for the Conservation of Vision has recently been formed. It includes laymen as well as doctors. An active campaign has been begun against ophthalmia neonatorum, avoidable eye injuries in trades, trachoma, wrong lighting of buildings, and the like causes.

THE week of celebrations organized by the *Evening News* to commemorate the publication of its 10,000th number was opened on Monday, November 17th, when an interesting series of medical and scientific films was exhibited to an audience of over seven hundred doctors and nurses at the West-End Cinema, Coventry Street. The programme included films showing the movements of protoplasm, the germination of pollen grains, the process of obtaining the intestinal juices and the digestion of albuminoids, the blood circulation in the lung of a frog, trypanosomes of sleeping sickness in the blood of a rat, and numerous other items of scientific and biological interest. A demonstration of the progress of radio-cinematography was also given; and Mr. George Cunningham, the organizer of the Cambridge Dental Institute for Children, presented an interesting series of photographs illustrating the formation, growth, and decay of the teeth, and the prophylactic work carried on at the Dental Institute under the direction of Mr. W. H. Jones, the borough dentist of Cambridge. Sir James Crichton-Browne, who presided, called special attention to this section of the programme, and declared that it was high time that the nation was awakened to the ravages of dental decay and the need of taking measures to arrest it. The existing state of affairs was not inevitable, and he was not one of those who believed that dental decay was an essential feature of modern civilization, or that the superman of the future would have a swollen head and toothless gums.

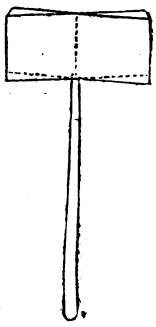
Memoranda:

MEDICAL, SURGICAL, OBSTETRICAL.

NYSTAGMUS.

As reported in the JOURNAL of November 1st, p. 1152, Mr. Harrison Butler stated at the Brighton meeting that miners' nystagmus was not of the same type as ordinary or non-miners' nystagmus; that the movements in miners' nystagmus were often those of divergence of both eyes together, convergence of both eyes together, or one eye rose while the other fell, and that this was not the case in non-miners' nystagmus; in other words, the movements in miners' nystagmus are non-concomitant, and in non-miners' nystagmus concomitant.

This observation, if confirmed, becomes important from a medico-legal point of view; and, further, if miners' and ordinary nystagmus are distinct entities, then a difference in etiology may be expected. In order to investigate this point I have had put together two 12-degree square prisms, edge to edge—that is, bases out—held together by a band of white metal with a handle fixed to it. The observer looks at the patient's eyes from a distance of about 18 in., and then interposes the prisms at about 8 in. from the patient, and by moving them nearer or further from the patient the eyes are seen to approximate and separate, and a distance can be selected at which the corneae are seen edge to edge, when the movements of the eyeballs can be studied. It is necessary to hold the prisms vertically, or one eye will be seen at a different level from the other. It is also convenient to place on the patient's face a trial frame with a *plus* 20 D sphere over each eye to obtain magnification.



During the last three months I have seen 13 cases of nystagmus, and of these 2 were miners. In both these miners the movements were concomitant. One of the miners presented vertical movements; the eyes rose and fell together. Two of the patients were infants, and restless; but all the others, with one exception, presented concomitant movements. The exception was a girl aged 11 years, and here the movements were non-concomitant—that is, the eyes converged together and diverged together.

These observations are not in harmony with Mr. Butler's statement.

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STRUMA AN IMPORTANT FACTOR IN EYE DISEASE.

I WAS much interested in Dr. Harrison Butler's paper on this subject in the JOURNAL of October 18th, and in the main ophthalmic surgeons will agree with his opinions. I wish, however, to draw attention to one or two important points, not sufficiently emphasized, under the heading of phlyctenular ophthalmia.

This disease in children plays havoc with the eyesight, chiefly because of the recurrent attacks, and only by attacking the cause can the recurrences be prevented. Dr. Butler is inclined to attribute the trouble to an infected milk supply, but he cannot account for the etiology in 30 per cent. of cases. Careful examination will, I am sure, convince him that in 100 per cent. of cases adenoids, almost always in a very unhealthy condition, are present.

We know that tubercle bacilli have been, and can be, demonstrated in the adenoid tissue; but to this is usually added a septic infection. Whether the "attenuated tuberculosis" toxin is produced in the adenoid tissue or in another tuberculous focus arising from infected milk is a debatable point, but I am quite certain that complete removal of the former focus allows the child to resist the latter successfully, at any rate until puberty.

I consider that all children suffering from phlyctenulosis are strumous, and consequently agree with Professor