ON THE BEHAVIOR OF THE DISSOCIATED CELLS IN HYDROIDS, ALCYONARIA, AND ASTERIAS[1]

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THIRTY FIGURES

INTRODUCTION

1

It is known that monaxonid sponges may be broken into their constituent cells and these will recombine to form restitution masses which have the power to transform into perfect sponges (Wilson, '07b, '11b; Müller, '11). Several kinds of cells enter into the composition of these masses, not only the indifferent or totipotent amoebocytes, but also differentiated elements. What is the fate of the latter elements? Do they undergo a process of de-specialization, passing into an indifferent or totipotent state, in which they persist as part of the restitution mass when it begins to transform? Or are they incorporated and digested by the amoebocytes? In general terms, in the development of such masses does regressive differentiation of cells into an indifferent condition play an important part? The large number of amoebocytes, at any rate in the monaxonida, makes it difficult to decide this question in the case of sponges.

It seemed that a study of the coelenterates might throw light on the matter. In the hydroids we have two tissue layers, ectoderm and entoderm, and a comparatively small amount of material corresponding to the totipotent amoebocytes of sponges. The question was formulated: if all the anatomical connections

[1] Published with the permission of Hon. Geo. M. Bowers, U. S. Commissioner of Fisheries. The experimental work was carried on at the Beaufort Laboratory of the U. S. Bureau of Fisheries during the summer of 1910. Brief mention of the results has already been made (Wilson, '11a; '11b).

and physiological interrelationships, due to position, between the cells composing the hydroid layers, were broken up, would the cells recombine and form masses of totipotent regenerative tissue?

Experiments were conducted on two hydroids, Pennaria tiarella and Eudendrium carneum. The phenomena were essentially the same in the two forms. The hydroid or, in one experiment, the coenosarc only was cut into small fragments and these were pressed through gauze (fine meshed silk bolting cloth). The flesh is thus broken up into cells and small cell aggregates. Fusion between these begins quickly and large masses are produced which exhibit a slow amoeboid change of shape. Continued fusion goes on with the formation of massive lumps of tissue or in other cases of sheets. The size and shape of such masses are under control. These bodies acquire a smooth surface and secrete a perisarc within about one day. They are solid and of a syncytial structure, although cell bodies are here and there marked out. During the two or three days following their formation, the bodies are subject to great mortality. Many isolated masses or nodules of larger masses, however, remain alive and differentiate ectoderm and entoderm layers, together with a central yolk mass, much as in the case of a coelenterate planula. These now send out cylindrical coenosarcal outgrowths which under favorable conditions continue to grow and produce well formed hydranths. Such hydranths appear to be normal. In the case of Pennaria they developed both the characteristic sets of tentacles and were equal in size to the smaller subapical hydranths of the adult, and were of about the same size as hydranths obtained from metamorphosing planulas. In Eudendrium also hydranths of adult size with the characteristic hypostome and number of tentacles were obtained in this way.

We apparently have here in the hydroids a plain case of the despecialization of tissue elements and their union to form masses of totipotent regenerative tissue. The indifferent elements which give rise to the sex cells or in some forms to buds (Braem, '08) are practically absent in the coenosarc, and yet as experiment shows the coenosarcal tissue will give rise to the restitution masses. After their sudden and violent separation the coenosarcal cells are

spheroidal and without vacuoles, whereas in the hydroid itself they are in some measure vacuolated and provided with outgrowths which probably all interconnect. This change in appearance is perhaps correlated with a change in physiological state whereby they pass as a result of the shock and isolation into an indifferent condition, in which condition they unite to build up the restitution mass. The mass differentiates like a planula, the outer stratum becoming the ectoderm, while the inner mass gives rise to an entoderm and material that is used up as food. After the several kinds of cells unite to form the restitution mass, they undergo changes and it becomes impossible to trace them. It is conceivable that some degree of specification persists and that elements derived originally from the ectoderm migrate in the mass until they reach the region of the surface, while the elements derived from the entoderm shift towards the interior of the mass. However, I find no facts in support of this idea, and it seems to me extremely improbable.

I conceive then that in the formation of the hydroid restitution masses the phenomenon is essentially one of regressive differentiation. The cells of the hydroid layers perhaps as a result of isolation, perhaps in part as a result of the shock, pass quickly into a simplified indifferent state. The restitution masses of sponges are so like those of hydroids that in them too regressive differentiation of tissue elements probably plays a great part.

2

It may be anticipated that there are other animals besides sponges and hydroids in which the somatic cells when forcibly disjoined, will fuse and give rise to totipotent regenerative material. Perhaps where the body cells in general have not this power, it may still be possessed by comparatively undifferentiated amoebocytes in such forms as ascidians. And it is possible that the sex cells in some animals before they have progressed too far in their special differentiation, may possess this power. From these standpoints a few observations were made on the common alcyonarian Leptogorgia, and on the immature gonads of the starfish, Asterias.

For Leptogorgia it was shown that when short pieces of the colony are pressed through cloth as before, or simply squeezed under water with forceps, small lumps of tissue and isolated cells are pressed out. An active fusion goes on between all these, with display of amoeboid phenomena. Masses are thus formed which become more or less spheroidal and acquire a smooth surface. By bringing together the smaller ones, bodies of considerable size up to and over 1 mm. in diameter may be produced. These bodies remained alive in laboratory dishes for many days, but exhibited no metamorphosis.

In the case of Asterias, immature gonads about an inch long were cut up and pressed through gauze. Abundant cells and cell masses were thus obtained, which fused actively forming reticulated plates and massive lumps. These soon acquired a smooth surface. They remained alive in laboratory dishes for a couple of days, but underwent no further change.

Experience in the handling of regenerative sponge masses suggests that possibly in the case of the alcyonarian the fusion masses might metamorphose if transferred to the harbor water. It seems incredible that anything in the shape of an individual could come from the fused germ cells of Asterias, however probable this might be in the case of a coelenterate. On the other hand, where the mass of cells is unable to give activity to the regenerative power when removed from the body fluids to water, it might conceivably display regenerative power, in some degree, if replaced in a proper body. Perhaps in some animals it might give rise to a bud individual, or under other conditions become merged into the tissues of the locality, or die gradually in some process of absorption, or be extruded as something foreign, or finally pass into one of the categories of tumors. From this point of view, an experiment was started to determine what the Leptogorgia balls, above described, would do if replaced in the Leptogorgia body. The experiment which must be regarded as no more than a tentative one is described farther on in the paper.

3

Some facts concerning the regressive differentiation of cells have long been familiar. The choanocytes of fresh water sponges for instance, it is known since the time of Lieberkühn's investigations ('56), give up during the winter their distinctive features and assume the character of mesenchyme elements. Metschnikoff observed the same phenomenon in marine sponges that were kept in foul water ('79). The flagellated chambers in both these cases again developed with the return of proper conditions, but there is no reason to suppose that the same cells that once were choanocytes again became transformed into such elements. It is possible, of course, that this is so, in which case the de-specialization of the choanocytes is partial and temporary, and a complete return to the indifferent (totipotent) state of the typical amoebocyte is not made. On the other hand it is certainly possible that the choanocytes do retrogress into this state, from which they ontogenetically arise in the gemmule development of the spongillidae and at any rate in a percentage of individuals in some cases of larval metamorphosis (Wilson, '94; Evans, '99). A third possibility, that they are used up as food material, must also be considered, until more intensive investigation settles the question.

The idea of a thorough going de-specialization of cells into indifferent elements underlay the older account of the origin of gemmules in the spongillidae, according to which the gemmule is made up of the transformed cells, of all kinds, located in a region of the sponge body. More precise investigations have shown that the spongillid gemmule and some other asexual reproductive bodies in sponges (Tethya buds, e. g., Maas, '01) arise as a congeries of similar mesenchyme cells. The origin of these cells especially in cases where the sponge body degenerates, is a matter of physiological interest, and deserves to be better known. It is not impossible, as I have said ('07b, p. 252) that they are "groups of amoebocytes which are in part recruited from transformed collar cells and other tissue cells, such as pinacocytes (flat cells of canal walls), that have undergone regressive differentiation into an unspecialized amoeboid condition." Maas ('10, p. 124) more recently advances the same idea.

In recent times the question of the de-specialization of cells has become a prominent one. Some pathologists as is well known believe this occurrence to be at the bottom of tumor formation. And in regenerative phenomena of many kinds it has been shown that cells lose their specific features, become less specialized, and re-acquire a capacity for new differentiation. Schultz reviews numerous cases in his essay on reduction ('08). In none of the instances, however, which he records as occurring in animals is there an obvious assumption of the totipotent character. Possibly this occurs in certain tumors and in the case of Moniezia reported by Child ('06). In Moniezia muscle cells lose their fibrillae and become spermatogonia, and it may be that this behavior is to be interpreted as a return to the totipotent character followed by a subsequent differentiation into sex cells. In the well known case first described by Driesch ('02) and again studied by Schultz ('07) where the excised branchial sac of ascidians is remodelled into the condition of a stolon bud, which then transforms into a small ascidian, the cellular metamorphosis stops short in its backward path before the condition of totipotent regenerative tissue is reached. In the reduction of starving hydras studied by Schultz ('06), the hydra body is greatly simplified, becoming a mouthless spheroidal sac. But in this sac the two layers, ectoderm and entoderm, persist, and although some of the cells assume an embryonic appearance, there is no obvious metamorphosis of cells into totipotent regenerative tissue. Still one striking feature of this case of reduction, is the great development of sex cells (male) which increase in number and ripen while the body in general dwindles, and it is conceivable that here, as in Moniezia, we have the transformation of cells into indifferent elements which then differentiate into male germ cells. In the reduction of hydra, the spheroids eventually degenerate and die. They do not undergo a regenerative awakening as in the case of the ascidian branchial sac. And this in turn may be due to the differentiation of the indifferent elements, as fast as they are formed, into germ cells.

In a case of reduction in the coelenterates which is not recorded by Schultz, there would appear to be extensive de-specialization of cells. I refer to the observations of Perkins on the larva

of the medusa, Gonionema ('02). Perkins observed that the hydra-like larvae of Gonionema after they had been for some time in an aquarium retracted their tentacles and became transformed into shapeless masses which had the power to throw out pseudopodia and creep about. In such plasmodial bodies the original differentiation into layers and even cells appeared to be lost, while the nuclei remained visible scattered unevenly through the substance. These bodies continued alive for two months and underwent repeated fission until owing to their diminution in size it became impossible to follow their history. It is possible that under favorable conditions these masses would have behaved like the restitution bodies of Pennaria and Eudendrium, and once more have developed the normal structure of the species.

Regressive differentiation of cells undoubtedly occurs in sponges when owing to confinement or abnormal chemical environment the body gradually breaks down into masses of regenerative material. I have recently ('11b) touched upon the question as to whether in these instances the de-specialized cells persist as part of the regenerative tissue. The latest accounts (Maas, '10; Müller, '11b) indicate that while the choanocytes are absorbed, other de-specialized cells persist.

4

Eugen Schultz in a series of papers ('04, '06, '07, '08) discusses a variety of phenomena which he groups under the head of 'reduction.' Under this head he classifies such simplifications of structure as the normal cyclical loss of gonads and associated organs in Planaria (Curtis, '02), the loss of organs and cellular changes in starving planarians, the changes occurring in starving hydras that have been referred to, and the remodelling of the ascidian branchial sac into the condition of a stolon bud. While it is not clear, as Schultz admits, that all of these simplification processes should be regarded as belonging in one category, a consideration of them and of other cases leads Schultz to conceive of a phase in the life history of organisms which may or may not

occur according to circumstances. To this phase he applies the term, reduction, and he conceives of it as an inverse process to ontogeny. Under unfavorable circumstances a simplification process may set in, in the course of which differentiations that were acquired during ontogeny are given up in inverse order. Such an orderly retrogressive course of events may, like ontogeny, be disturbed by coenogenetic adaptations. In this process, the organism acts as a whole, that is the course of reduction is determined not by a struggle for existence between the different kinds of cells, but rather the needs of the entire organism dictate what structures shall be sacrificed. The behavior of the organism in reduction is thus looked on as an adaptive response. What there is of new and old in these ideas, is sufficiently stated in Schultz's essay ('08).

Schultz's theory of a reduction process may be applied to the behavior of sponges which give up their organization under the influence of confinement (Wilson, '07a; Müller, '11b) or abnormal chemical environment (Maas, '06). In the monaxonid Stylotella, for instance, I have found ('07a) that the oscula and the bulk of the pores close, much of the canal system is suppressed, the skeletal arrangement is simplified, and the flagellated chambers for the most part broken up into their constituent cells which become scattered in the mesenchyme. At any time the sponge may be made to reassume its normal differentiation on transfer to better conditions. The passage of the body into this simplified condition, which is essentially like the winter state of some spongillidae (Weltner, '93), is obviously an adaptive response on the part of the whole sponge, which thus protects itself against the bad water of the aquarium, as the spongillid does against extreme cold. Moreover this simplified state is very similar to a stage in the metamorphosis of such a mass of totipotent regenerative tissue as a sponge gemmule. In them both we have a simple flat epithelial covering layer and an internal mass, which in the case of the gemmule is composed of indifferent amoebocytes and in the case of the 'reduced' sponge is largely so composed. Or again it is much like the 'pupal' stage (just after fixation) of those larvae of silicious sponges in which amoeboid cells split up to

form the choanocytes.[2] The stage then may fairly be looked on as repeating an embryonic stage that actually occurs, whether or no it be a coenogenetic modification of the more typical (palingenetic) course of development.

In the later history of this behavior of sponges in confinement, etc., the sponge ceases to act as an individual. It breaks up either with (Stylotella) or without (Calcarea, Spongillidae) considerable death into numerous masses of totipotent tissue. If we are to apply the reduction theory here we must assume that owing to coenogenetic adaptation, the sponge body does not continue to dwindle and simplify itself until it reaches the condition of an egg, or an individual heap of blastomeres, or a single gemmule-like mass of totipotent cells. This would entail a tremendous loss of substance and altogether is a process which it is foolish to contemplate as a possibility. In the backward development of the sponge, coenogenesis prevents a too strict adherence to the

[2] Maas in his recent study ('10) of the cellular changes involved in the breaking down of a sponge body into masses of regenerative tissue, views the phenomena from a standpoint not strictly that of the reduction-theory. This is not the place for a discussion of Maas' standpoint, but I may mention that he debates the question as to whether a fundamental similarity exists between late stages in the reduction of sponges and the 'pupal' stage in ontogeny and decides there is none, because in the former the interior is chiefly made up of amoebocytes and in the latter of immigrated ectodermal cells destined to become directly transformed into choanocytes. There are still a good many unsolved problems concerning the behavior of the layers in sponge ontogeny, and among these, if we accept the current view that the choanocytes typically arise from the ciliated covering cells of the larva, is the relation between the typical pupae and those in which the interior is chiefly filled with amoebocytes, or as I have called them 'formative cells' ('94), which divide up and form the choanocytes. My account ('94), it seems to me, established the fact that such pupae are exceedingly common in certain species of monaxonida, so common that I regarded them as typical. There is no doubt that in my work of that time the bulk of the sponges reared came from pupae of this sort. Evans in studying spongillas several years later found ('99) that the choanocytes not infrequently arose in this way, as I and writers before me had described in detail. The occurrence of such pupae cannot be passed over as pathological, and if we keep them in view instead of the type which is densely filled with small cells, the similarity between the pupal stage and the reduced sponge is obvious. Maas has some comments on larvae of this type in Sycons ('10, p. 105), and is inclined to believe that they are to be looked on as individuals in which the amoebocytes have actually incorporated and digested the immigrated ectodermal elements.

path over which the organism has come, by setting into activity a process of division. The sponge body in the simplified condition above described may conceivably divide up again and again until very small masses result which then go over the last steps of reduction, transforming themselves each into a single embryo-like heap of totipotent cells. Or the division may stop when the masses resulting therefrom are still of considerable size. Or a division of far-reaching extent may take place which consists in the breaking of all ties, anatomical and physiological, between the component cells. Such a process would result in the virtual dissolution of the sponge body into an enormous number of independent cells, and these, or such as could, might then go through the last phases of reduction becoming totipotent. In the actual behavior of the sponges all these varieties of the division process are associated together in a complex and over-lapping fashion.

When the division process leads to the production of independent cells, these wander about on or through the old skeleton and are attracted to one another or to masses of reduced tissue, and so unite to build up or aid in building up such masses. This is a useful habit to such cells. In the body of an animal, since by hypothesis they are totipotent, they might conceivably through growth and division give rise to a group large enough to transform into an organism of that species. But out of the body or cut off from the possibility of growth, their only hope for life would lie in fusion. Only so could they give rise to organisms. The habit of fusion displayed by these elements being useful may have been acquired. More probably it has been preserved as an inheritance from early ancestors.

In the retrograde differentiation of organisms it is conceivable that there might be a difference in the path followed according as to whether the individual had its origin in an asexual mass or in an egg. Schultz in passing suggests ('07) that possibly some difference might be found between the reduction process of ascidians derived from eggs and buds. Following out the logic of the theory we should expect in the one case to find stages resembling the free larva and egg, in the other case no such stages. But

such a difference seems one too good to be hoped for—coenogenesis would surely not allow the oozooid to go back through the complications of tadpole larva and egg development. Even with lower forms, sponges and coelenterates, it is incredible that the path of reduction would ever lead through the stage of the ciliated larva to an egg, which by virtue of its egg nature, in contrast with that of a simple totipotent cell, would again in its upward differentiation develop into a ciliated larva! It is indeed highly probable that coenogenesis would usually take care, in animals capable of asexual development that reduction should lead to the formation of bud-like or in other cases gemmule-like anlages rather than to the production of eggs and sperm. The idea is that the bud with its quick development or the gemmule-like anlage made up of totipotent regenerative cells, unhampered by a tendency to retrace the path of phylogenesis and free to develop at once into an organism, would present a great advantage over an egg to an animal seeking, so to speak, to restore itself.

It may be seen then that with some use of the coenogenesis idea it is possible to view the whole set of retrogressive changes undergone by sponges in confinement or under abnormal chemical conditions as reduction phenomena. Schultz's theory it seems to me enables us to get a better picture of the facts as a whole than is possible without it. Müller evidently entertains the same opinion, since he classifies ('11b) the changes in the spongillidae under the caption of reduction.

5

In the preceding section, it has been shown that the gradual breaking down of a sponge body into small masses and eventually, in part, into independent and indifferent cells may be thought of as a case of reduction, as a return to an embryonic condition. When we compare with such phenomena the forcible breaking down of a sponge or hydroid into elements which can live independently for a time and which unite to build up new organisms, it would seem that we have in these elements the same end result that is reached in the comparatively slow process of reduction.

The mechanical division of the flesh and the attendant stimuli (shock) apparently, in the case of the pressed out tissue, bring on the final stage at once.

The same facts may be viewed from a somewhat different standpoint if we confine ourselves within the narrower limits of physiology. Child has just published an interesting essay ('11), the kernel of which is the idea that when a part of an organism becomes physically or physiologically isolated, there is a tendency for it to develop into a new whole, provided it has the power of complete regulation or in other words, is made up of totipotent material. (By physiological isolation, Child means the condition in which the part is cut off, through one cause or another, from the exchange of stimuli which maintain that mass of material as an individual organism.) Such a statement well covers the facts of the pressing experiments and the reduction of sponges, provided, (1) we remember that the cells as they exist in the stem of the hydroid, for instance, are not at the time totipotent, but only become so by retracing their development; and provided, (2) we employ the category of tropisms for the union of the dissociated cells to form masses.

Finally it may be of interest to consider the question, does any phylogenetic significance attach to the power of an organism to break up into small bits of indifferent protoplasm which then recombine? Did the early ancestors of existing animals practise such habits, which in modified form have proved useful and so have been retained? It is at least possible that the early organisms were amorphous masses of protoplasm. Assuming this, it is plain how useful the power of easy, quick division might be, from how many dangerous situations an organism might escape which had the power to break up into parts, these retreating from the situation independently, leaving perhaps dead or dying tissue—a "*sauve qui peut*" proceeding in short. But when we consider the difficulties besetting the life of very small plasmodial masses of this kind,[3] it is also plain how useful would be the power to fuse with one another. We may then conceive of these early

[3] I have found that the very small plasmodial masses of sponges are at a disadvantage ('07, p. 257).

ancestors as creatures amorphous in shape and inconstant in size. According to local conditions and needs of the moment, the body divided or fused with a corresponding mass. The behavior of the totipotent cells which we have been considering may, for speculative purposes, be considered as a survival of such primitive habits.

EUDENDRIUM. RESTITUTION FROM DISSOCIATED CELLS

Species used. The common Eudendrium of Beaufort harbor is E. carneum Clarke (Mem. Bost. Soc. Nat. Hist., III, no. 4, p. 137; Nutting, '01, p. 333). The most striking characteristic of the species concerns the arrangement of the male gonophores, which are "four or five chambered, borne in a verticil around the body of aborted hydranths which are themselves joined to pedicels bearing ordinary hydranths, the two being thus borne in pairs symmetrically disposed on the branches" (Nutting).

Experiment July 9. A clean male colony was chopped up with scissors into pieces about 3 mm. long and a mass of such pieces pressed through gauze in the usual way: the mass was laid on a square of gauze, and the latter folded to make a sac, which was immersed in a watch glass of sea water and squeezed repeatedly with fine forceps. The hydroid flesh streams through the pores of the gauze and falls on the bottom as a fine sediment. A little is sucked up with a pipette and examined on a slide. It consists of isolated cells and minute cell masses with some larger fragments. The latter are picked out. Fusion is observed to go on under the microscope. The tissue is now transferred to watch glasses of fresh sea water in which it is shaken towards the center with the purpose of facilitating the fusion process, and the watch glasses are immersed in large bowls of water. Within a few hours time fusion in the watch glasses leads to the formation of irregularly lobed flattened masses, varying from a fraction of a millimeter to about 5 mm. in diameter with a thickness of 1 mm. or less. Such masses exhibit slow changes of shape. All the masses of any considerable size are later in the day carefully picked out with a large pipette and transferred to fresh sea water. On sub-

sequent days they are transferred in this fashion three times a day. An effort is made to keep them clean and free of the bottom. On the day after their formation the surface of all these masses is smooth and a thin perisarc is to be seen round many of them.

On July 13, some of the masses have begun to transform. In fig. 1 is shown one of the smaller from which an outgrowth has sprouted. The perisarc round the outgrowth, *c s.*, is thinner than round the original mass, and in the outgrowth the ectoderm and entoderm are clearly distinguishable in the living object. The base of the body representing the original mass appears in life uniformly opaque. A number of the flattened larger masses are in the condition shown in fig. 2, which represents a part of a mass 6 mm. long by 3 mm. wide, of an irregular shape, more or less lobed, and partially subdivided into spheroidal nodules, *a*, *b*, *c*, each with its own perisarc. A good deal of the mass is dead. One of the spheroidal nodules, *c*, has sprouted, and in the outgrowth, *c s*, the ectoderm and entoderm are distinct. In such a mass the living parts are easily distinguished by their bright color and sharp contour. The perisarc is distinguishable round some parts of the mass that are dead, but in other such places it cannot be made out. In the case of such large masses possibly the perisarc does not form round the whole mass. The nodule or lobe from which an outgrowth has sprouted is often not cut off from the general mass by perisarc. This is true of the masses shown in figs. 2 and 4. In other cases, however, the nodule that has sprouted is completely surrounded by perisarc and is merely imbedded in the general mass (fig. 3).

Some of the more promising masses alive on July 13 were isolated. Among these was the mass shown in fig. 4. The condition of this mass on the next day is shown in fig. 5. During the twenty-four hours that have elapsed since fig. 4 was made, outgrowth *c s 1* has died, and the general mass from which the outgrowths project is dead. Outgrowth *c s 2* has however grown and bifurcated. One subdivision, *a*, adheres to the glass like an ordinary hydrorhiza, the other subdivision, *b*, projecting up in the water and now ending in a hydranth. The latter

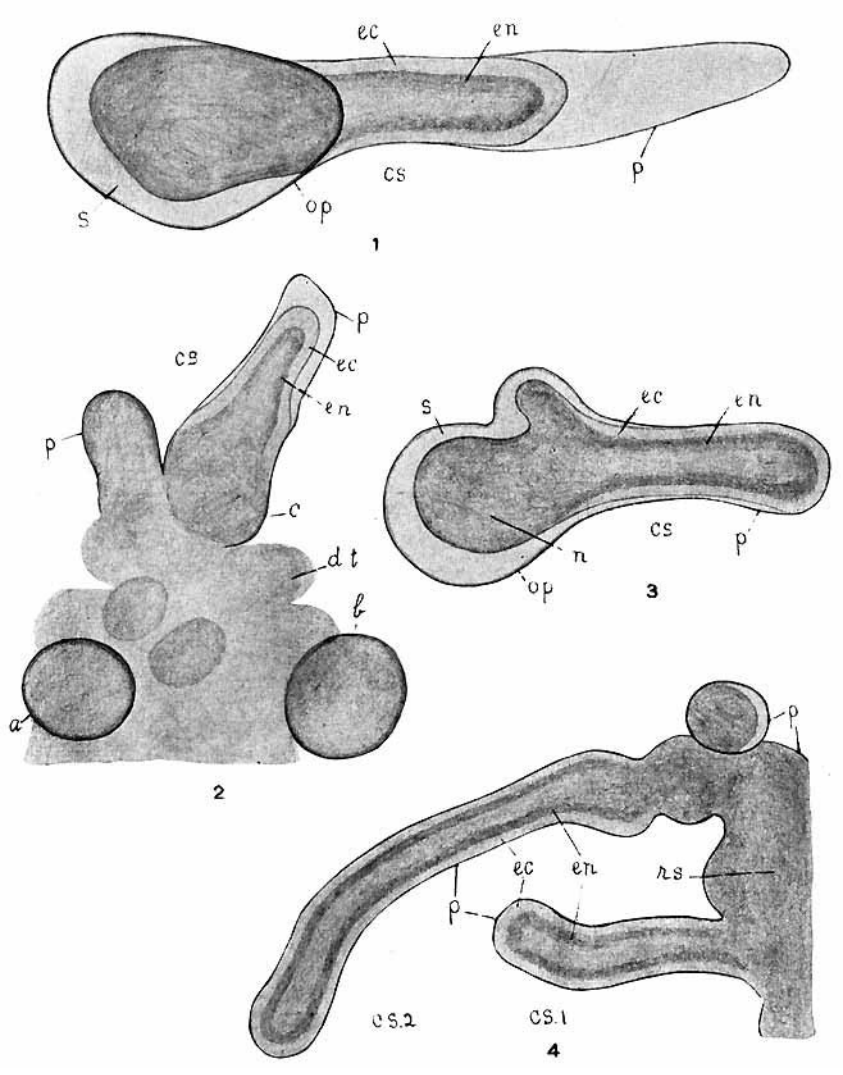


Fig. 1 Eudendrium. Restitution mass four days old. *cs*, coenosarcal outgrowth; *ec*, ectoderm; *en*, entoderm, *op*, perisarc of original mass; *p*, perisarc of outgrowth; *s*, space between original mass and its perisarc. × 150.

Fig. 2 Eudendrium. Part of large restitution mass four days old. *a*, *b*, spheroidal nodules of living tissue with perisarc; *c*, similar nodule with coenosarcal outgrowth, *cs; d. t.*, dead tissue; other lettering as before. × 90.

Fig. 3 Eudendrium. Nodule of living tissue *n*, with coenosarcal outgrowth, *cs*. Nodule was part of a large restitution mass. Other lettering as before. × 150.

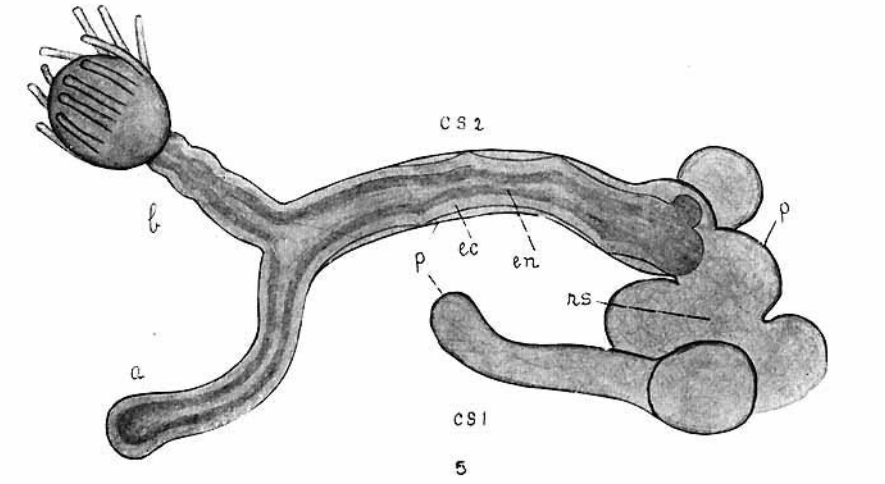
Fig. 4 Eudendrium. Part of large restitution mass, *rs*, with two coenosarcal outgrowths, *cs.1*, *cs.2*. Other lettering as before. × 90.

has not yet acquired the characteristics of the species, although considerable differentiation has gone on. Nettle cells in the enlarged tentacular ends can be made out. The ectoderm of *c s 2* has contracted away from the perisarc, thus assuming the condition common in the adult. In the entodermic cavity of the coenosarc a movement of small spheroidal particles goes on constantly. Such particles are perhaps remains of the interior of the original mass, serving now as food material. The specimen is preserved in the condition drawn (fig. 5).

Two of the masses that were isolated on July 13, at that time in the condition of fig. 4, have developed completely formed hydranths on July 15. One of them is shown in fig. 6. The general mass, *r s*, from which the outgrowth has sprouted, is for the most part dead, although it still includes some spheroidal nodules, *n*, of bright orange color that are obviously alive. The original outgrowth, *c s.*, is a hydrorhiza, and its apex has apparently died, for here there is a regeneration point, *r p*. A branch, *a*, ascends in the water and bears the hydranth. This has the characteristic shape and size, the long slender tentacles, large hypostome, and bright orange color of the normal adult polyp. The other mass is also largely made up of dead tissue, but an outgrowth has survived and become a hydrorhiza bearing two fully developed hydranths like that of fig. 6. Both masses now preserved.

Summing up, it may be said that the larger masses of this experiment, of the general character shown in fig. 2, gave rise to a number of coenosarcal outgrowths like those of figs. 2 and 4. In all, about three dozen such were obtained from July 13 to July 20. A good many were preserved as soon as this state was reached. From the others that were kept for further development, three masses gave four hydranths as already recorded. My experience with sponges suggests that exposure to the natural water of the harbor would give a higher rate of survival and transformation.

Along with the larger plasmodial masses of this experiment, very many small lumps were formed, 200 to 300μ in diameter, which became spheroidal and in about one day after their forma-



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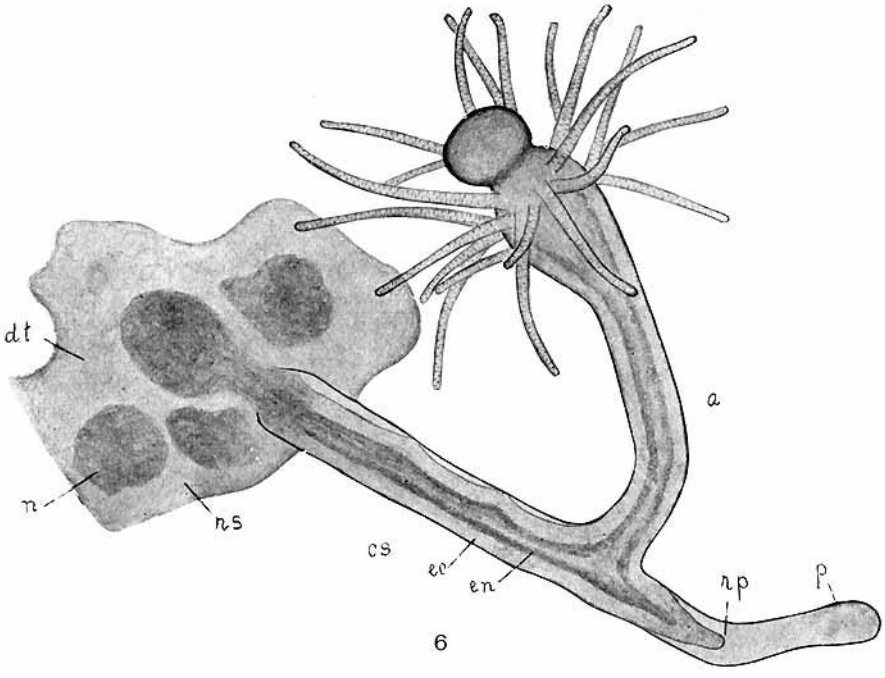

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Fig. 5 Eudendrium. Restitution mass of fig. 4, twenty-four hours later. General mass, *rs*, and outgrowth, *cs.1*, now dead. Outgrowth, *cs.2*, has bifurcated, and one branch, *b*, has developed a hydranth. × 90.

Fig. 6 Eudendrium. Restitution mass, *rs*, with coenosarcal outgrowth, *cs*, the latter bearing a vertical branch, *a*, which ends in a hydranth. *d. t.*, dead tissue; *n*, nodule of living tissue; *rp*, regeneration point. Other lettering as before. × 90.

tion secreted a perisarc. These small masses adhered so firmly to the glass that the water could be drained off when the glass was changed to a fresh bowl. I had expected that many of them would transform. As a matter of fact only half a dozen developed outgrowths, and these were short and in no case gave rise to a hydranth. These masses remained alive for days. Perhaps their failure to produce hydranths was due to the small amount of material making up the mass.

Experiment July 14.[4] Colonies chopped up and pressed through gauze 4 p.m. The tissue was distributed with the pipette in watch glasses, on slides and covers, and all were immersed in large bowls of water. The tissue from the start had a bad color, not orange but a dirty brown. The colonies I used were probably not clean enough, and perhaps too much Perophora and other organisms infesting the hydroid got in the cultures. All of these preparations died before the tissue even reached the state of smooth compact masses.

Experiment July 15. Colonies, some with male, some with female gonophores, others without gonophores, were pressed out at 4 p.m. The tissue is handled in essentially the way described for Exp. July 9, and at 7 p.m. fusion has led to the formation of flattened plasmodial masses and lumps of all sizes. The color of the masses at this time is good, reddish-orange, and the surfaces are not far from smooth. But on the following day much of the tissue is dead. Many of the nodules and lobes composing the larger masses show, however, a thin perisarc. The masses including nodules of live tissue are transferred to fresh sea water. The masses gradually died or when nodules remained alive they exhibited no change, continuing in a dormant condition. In this and most of the subsequent experiments I feel satisfied that the cause of failure lay partly in the fact that too much tissue was pressed out. This made the first steps in the handling slow and the aggregations of tissue too large.

Experiment July 18. A clean colony was pressed out in the usual way in watch glasses, the cells settling on the bottom.

[4] The records of several very unsuccessful experiments are given in the expectation that they may serve to point out features in the method of treatment which are to be avoided.

These are allowed to stand about fifteen minutes, during which time they are shaken to the center a couple of times. The glasses are then transferred to bowls of water. Finer sediment floats off, but the coarser clings to the bottom. After ten minutes the glasses are removed to fresh bowls. Again some sediment is lost, but not much. The peripheral sediment is now gently dislodged with the pipette, and heaped up towards the center of the glass. After an hour the tissue is sucked up with a large pipette, an effort being made not to break it up more than is necessary, and deposited on bottom of fresh bowls in heaps $\frac{1}{2}$ to 2 mm. thick and about 5 mm. in diameter. The tissue was later on again transferred in the same way. The technique of this experiment proved wrong, for on the next day most of the tissue was dead. Probably there was too much handling, and the heaps made were too massive.

Experiment July 19. Colony pressed out 11:30 a.m. Tissue collected in watch glasses and shaken to center after ten minutes. Glasses transferred to bowls 12 m., and again to fresh bowls 1 p.m. Glasses now removed from bowl and with pipette the tissue is transferred to fresh watch glasses of water and there deposited in heaps about 5 mm. in diameter and $\frac{1}{2}$ mm. thick. The material is now coarsely lumpy, showing that fusion has gone on, and the heaps are therefore porous, not dense and compact. Glasses transferred to fresh bowls at 1:20 p.m. gently so that the tissue aggregations are not disturbed. Again so transferred at 3 p.m.

The coherence and slow contraction of the heaps of tissue is shown by the fact that at 5 p.m. they have curled up slightly at the edge and are now free or nearly free from the bottom. In this condition the heap or cake can be pushed with the pipette over the bottom. Only the thinner heaps have this amount of coherence—to behave in this way they should not be over $\frac{1}{2}$ mm. thick. Glasses transferred to fresh sea water at 5 p m. and at 7 p.m.

On the next day the result was disappointing. Most of the material was dead. Some of the smaller cakes were however alive or alive in places, and had developed a perisarc. One of

these is shown in fig. 7. Several such were preserved for sections. Again I attribute the failure, comparatively speaking, of this experiment to too much handling and to the fact that heaps of too large a size were made. It must be borne in mind that when any considerable part of the tissue dies during the first day, the surviving masses having been infected have a poor chance for further growth.

Experiment July 22. Colonies pressed out 3:10 p.m. Only tops of clean colonies were used and female gonophores were mostly excluded. Tissue collected in watch glasses and transferred to bowls, about as before. At 7 p.m. tissue was coarsely lumpy, showing that fusion had gone on. It was transferred to bowls of fresh sea water and deposited in areas 7 mm. in diameter and $\frac{1}{2}$ mm. thick. Tissue nearly all dead the next day.

Experiment July 23. Tops of clean male colonies were pressed out at 3:30 p.m. At 7 p.m. tissue was sucked up with pipette and distributed in fresh bowls to form thin areas about 10 mm. in diameter. These areas soon become transformed into reticular sheets having considerable coherency. They become free or nearly free from the bottom, and some curl up slightly at the edge —indications of contraction. Another set of preparations were made at the same time and treated in same way, from the tops of female colonies. This tissue too went so far as to form reticular sheets of considerable coherency. But on the following day the tissue was practically all dead.

Experiment July 25. Male and female colonies were pressed out at about 2 p.m. The tissue collected from each colony was kept by itself. One-half hour after preparation, the tissue which had been squeezed out in watch glasses, was shaken to the center of the glass, and the glasses were transferred to bowls. The glasses were transferred to fresh sea water at intervals of two hours until 9 a. m. on the next day. Five hours after preparation the tissue had united to form thin sheets, which soon began to crack evidently owing to a process of contraction. These sheets of tissue however went no further in development and on the next day were dead.

In the peripheral region of each watch glass numerous small masses of tissue, a fraction of a millimeter, formed. These are alive on July 26 and show a smooth contour. They are still alive on July 27 and now have an obvious perisarc. Many of them cling to the bottom, but many are free and are held together in loose, thin, flat aggregations by débris and the perisarc of dead tissue. On July 28 and 29 most of these masses are still alive and a dozen have sent out coenosarcal outgrowths. Several are shown in fig. 8. A little group of masses all interconnected is also shown in fig. 9.

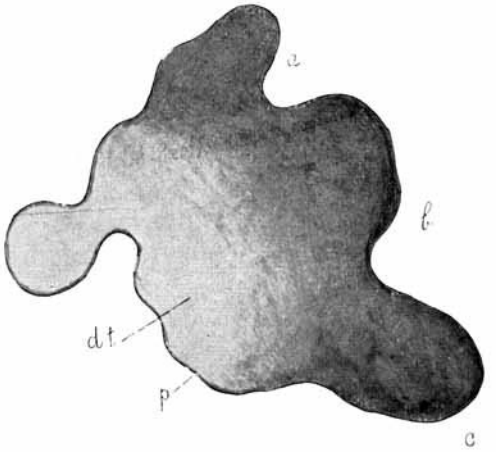


Fig. 7 Eudendrium. Restitution mass twenty-four hours old. *a*, *b*, *c*, lobes of living tissue; *d*, *t*., dead tissue; *p*, perisarc. × 150.

Experiment July 27. A clean male colony pressed out at 1:45 p.m. in a watch glass. Tissue was shaken to center after a few minutes, and the water in watch glass was renewed after the tissue had once more settled on the bottom. The tissue was then distributed with a large pipette over the bottom of two watch glasses and shaken to center. In each watch glass a central collection of tissue is thus formed in the shape of a thin area 10 to 15 mm. in diameter. Outside of this are scattered many small lumps, 1 mm. and less in diameter. The glasses are transferred to bowls 3:30 p.m. Four such cultures were made.

At 5 p.m. coherent cakes of open, reticulated texture have been formed. These are free or nearly free from the bottom. They are transferred with a large pipette to fresh bowls of water. At 7 p. m. they are again transferred, and several are cut in pieces. They have a good color and are obviously alive. On the next day many of the cakes are still alive and have formed a perisarc over much of their surface. Where the perisarc has formed, the included tissue is uniformly opaque and by reflected light appears distinctly orange. The formation of the perisarc cuts out, I think, such masses as pieces of gonophores, tentacles, and hydranths, from the undifferentiated mass, round which it forms. Gonophore and tentacle fragments are to be sure sometimes partially surrounded by the growing (fusing) masses of tissue, from which they may be seen projecting. But probably a considerable part of each such mass dies, and with it the fragments of gonophores, etc.

On July 29 most of the tissue is dead or dying. Bacteria and infusoria are abundant. Nodules of live tissue surrounded by perisarc occur imbedded in the general mass. In some of the disintegrating cakes the arrangement of the perisarc is much plainer than it was when the whole cake was alive. It may now be seen that the perisarc was secreted round compact nodules, lobes, and anastomosing cords. All such are intricately combined with débris and tissue that never secreted perisarc to form the general mass.

All the larger masses soon died. But a good many of the small lumps that lay in the outer part of the watch glass, away from the central cake, were still alive on August 1. Several of them by this time had sprouted coenosarcal outgrowths, and were essentially like fig. 1, although in some instances the mass had given rise to two opposite outgrowths.

The practical problem in handling this tissue seems to be to get masses large enough to provide the necessary amount of material for hydranth development, and yet thin and reticular enough to expose all parts of the mass to the water. Small masses, a fraction of a millimeter in diameter, are much easier to keep alive than large masses.

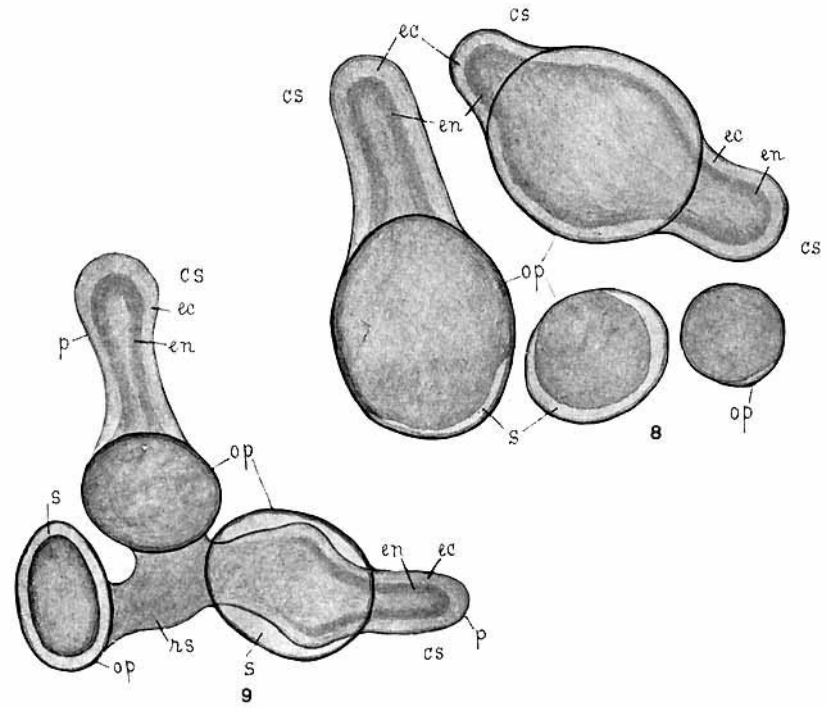


Fig. 8 Eudendrium. Group of restitution masses three days old; two still spheroidal; one with a coenosarcal outgrowth, *cs.;* one with two such outgrowths. *op,* perisarc of original mass; *s,* space between original mass and its perisarc. Other lettering as before. × 150.

Fig. 9 Eudendrium. Three restitution masses interconnected, four days old; two masses with coenosarcal outgrowths, *cs; rs,* central part of original restitution mass. Other lettering as before. × 90.

Experiment August 1. Colony pressed out at 3:30 p.m. Tissue in comparatively small amount is collected in watch glasses and allowed to settle. Water is gently drawn off and fresh added without disturbing the layer of 'sediment' that clings to the bottom. Glasses transferred to bowls after thirty minutes; transferred again 6 p.m. On the following day all the larger masses dead or dying. Many of the very small masses are also dead. Still there are very many small masses, a fraction of a millimeter in diameter, that are alive. These are more or less spheroidal and covered with thin perisarc, some attached to bottom of glass,

some to cover glasses that had been put in the watch glasses. On August 3 many of these masses are still alive, surrounded by perisarc, but they have not thrown out coenosarcal processes.

In this experiment a small amount of tissue was pressed out, and until the details in the method of treatment are more precisely marked out, this is certainly a safe step. It will be noticed that the tissue was left *in situ* where it was first deposited on the bottom of the glass. The results indicate that this is not a good method for the larger masses. And yet it seems desirable for the tissue to establish some connection with the bottom, and this it will not do if disturbed and dislodged too much. The experiment records show clearly what a great influence apparently slight differences in the treatment had on the vitality of the fusion masses, and how much in the dark I remained as to what details were good, and what bad.

Experiment August 2, a. Colony pressed out at 4:15 p.m. Tissue collected in a watch glass, shaken gently to the center, and water changed several times, each time the tissue being stirred up considerably by the pipette current. Glass transferred to bowl 5 p.m.; transferred again 6 p.m. Tissue does not cling to the bottom, as it does when left undisturbed where it first settled. At 7. p.m. the tissue coheres sufficiently for pieces 2 to 3 mm. wide to be sucked up with large pipette. Other smaller pieces about 1 mm. wide are sucked up. All pieces are thin, about $\frac{1}{2}$ mm. thick. These pieces, forty-five in number, are scattered over the bottom of three bowls.

On August 3 at 9 a.m. the masses are alive and of good color. Some are free, some slightly attached to the glass. The latter are freed and all are gently transferred with large pipette to fresh bowls of water. They resemble the Pennaria mass shown in fig. 14. Examination shows that the perisarc has formed over parts of many plates, but in other places while the surface of the plate is smooth, no perisarc can be seen. In still other places the contour is rough, the periphery here consisting of rounded cells. Bacteria are present, here and there in swarms, but not much of the tissue is dead.

On August 7 a good deal of the tissue is now dead. But a large number of the pieces include lobes and nodules of living tissue surrounded by perisarc. Two of the plate-like masses show each a coenosarcal outgrowth of considerable length, about like those of fig. 4. On August 8 two other masses show each a similar outgrowth. On August 9 another mass has developed an outgrowth, which soon becomes sickly, losing its well marked layers and developing in the interior numerous dark masses. On August 11 two other pieces show each a coenosarcal outgrowth. These outgrowths are horizontal and creeping and each bears a vertical branch. Both are sickly as is shown by the fact that the layers are not everywhere uniformly differentiated, but in places appear to be breaking up, while in other places the tissue is densely concentrated.

The method practised in this experiment is evidently good and yet too much of the tissue dies leaving the surviving masses slow to transform. In the hope of stimulating these masses they were given a liberal supply of pure oxygen on August 6, but with no discoverable results.

While an effort was made in this and the other experiments to pick out pieces large enough to be distinguished with the eye, some pieces of gonophores and tentacle fragments remain in the cultures. Some of these are incorporated by the plasmodial masses. Many others undoubtedly die without being incorporated. Another structure too deserves mention as being occasionally present in the cultures. In cutting up the hydroid, many hydranths are snipped off at the very base. Some of these get in the cultures and escape notice. I have found that when such hydranths are isolated a process of reduction takes place analogous to that described by Schultz for hydra ('06), the hydranth gradually becoming in the course of a few days a mouthless spheroidal body. Apparently such a process goes on in the pressed out cultures more rapidly, for occasionally spheroidal bodies are seen quite like the reduced hydranths just referred to. I have moreover several times found such a body embraced by a plasmodial mass. Where bodies like gonophores, tentacle fragments, and reduced hydranths (or the bodies that look like

such) become surrounded by the plasmodial tissue, it is a question what becomes of them. As already said, the formation of the perisarc, I am inclined to think, cuts out such bodies from the comparatively homogeneous material round which it forms. Pictures are sometimes had which indicate that possibly such bodies are attacked by the plasmodial tissue, the latter invading and absorbing them. Again a stray fragment of stem perisarc from the colony used may get into the cultures, and if some of the coenosarc has been left inside, it may form a regeneration knob at one end of the piece. I have seen a piece or two of this kind. Such a fragment might very well regenerate a hydranth in the midst of the plasmodial masses. But fragments of this sort are easily distinguished from the plasmodial masses or nodules of the latter.

Experiment August 2, b. A colony was pressed out and the tissue allowed to settle on a cover glass immersed in a watch glass. In transferring, the cover glass was always kept in the watch glass, and thus the tissue was not directly exposed to the air. The material settling on the cover soon transformed itself into a multitude of small, more or less spheroidal masses, a fraction of a millimeter in diameter. They went so far as to form a perisarc. A large number of them died about one day after preparation, but many remained alive for days; were still alive on August 9. They had not sent out coenosarcal outgrowths, but as was learned later from sections the originally solid mass in several cases, perhaps in all, had developed into a sac, the wall of which was made up of ectoderm and entoderm layers.

In the same way on August 2 tissue was allowed to settle over the bottom of a watch glass, forming a very thin deposit. Great numbers of small lumps formed which had the same history as the above. It is quite possible of course that a few of these lumps sent out coenosarcal outgrowths, but that such escaped notice.

Summary. Eleven experiments with Eudendrium were made. In all experiments fusion led to the formation of plasmodial masses. In eight experiments an extensive formation of perisarc took place. In five experiments numerous small masses with perisarc, which remained alive for days, were formed; in three of

these experiments several of the spheroidal masses sent out coenosarcal outgrowths. In two experiments a considerable number of coenosarcal outgrowths were obtained from nodules of tissue which had remained alive in comparatively large flattened plasmodial masses, and in one experiment these outgrowths gave rise to hydranths, four in number.

The first experiment, July 9, was much the most successful. In those that followed a common error undoubtedly lay in endeavoring to handle too much tissue. But over and above that the water grew warmer with the advancing season and the Eudendrium colonies perhaps became more abundantly infested with protozoan ectoparasites.

The technique in general of these experiments is especially faulty in that, (1) it allows parts which must die, tentacle and gonophore fragments, to get into the cultures; and, (2) it subjects masses of tissue that evidently need the best environment to the harmful influences of quiet water in a laboratory dish. Small gauze floats kept at the top of a running aquarium were used, but with no success. Very probably if the cultures were placed outside in the harbor water, they would do better. As to methods, the following may be added to what has already been said:

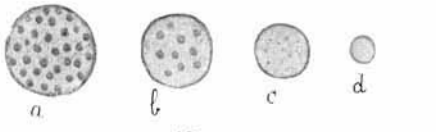
Only clean colonies or parts of colonies should be used. If the whole hydroid is used I believe that colonies without gonophores are the best, and those with female gonophores the worst. Stem tissue would perhaps be better than that of the whole colony. Care should be taken to allow the cells and small lumps to cohere, and not to break up the cohering tissue at first more than is necessary. It is well to get the tissue in comparatively small pieces a few hours after preparation, and in fresh dishes away from the original surface of attachment. If the masses of tissue a few hours after preparation appear soft and pasty, they will probably not live. They should show a good color, absence of the characteristic color indicating presence of dirt or infesting animals. The gauze (silk bolting cloth), usually used runs 50 meshes to 25 mm. A cloth running about 75 meshes to 25 mm. was also used. The sea water was well aërated and filtered. An effort was made to pick out from the cultures all coarser particles, such as pieces of

hydranths and gonophores, that could be seen with the eye. The tissue was usually pressed out and kept in solid watch glasses with a cavity 50 mm. in diameter and 10 mm. deep. These were immersed in crystallization dishes 200 to 250 mm. in diameter or in finger bowls of about 120 mm. diameter. Dishes, instruments, and gauze were thoroughly cleaned, but were not sterilized. Possibly sterilization in hot water would be advantageous. I lay emphasis on the technique of the experiments in the hope that it may be improved by others. With a more certain technique this method of growing hydroids ought to lend itself to the production of hydrids, as I have suggested ('07b, '11b) for sponges.

Histological study of the restitution masses of Eudendrium

Observation record, July 27. At 4:30 p.m. a drop of the Eudendrium tissue was squeezed directly from the gauze sac on to a slide. A supported cover was put on and slide examined at once. The fluid contained quantities of separate cells. In addition there were present a few small masses each consisting of several cells. All the cells were about spheroidal, but they varied a great deal in size. Some contained abundant pigment granules, others a few, and others were quite transparent. Four types could be distinguished, all of which were abundantly represented, but plenty of transitional cells connecting these types were also present. The types are shown in fig. 10, *a*, *b*, *c*, *d*. Cell *a* is well filled with pigment granules which appear brownish red by transmitted light. Cell *b* contains similar granules, but they are few in number. Cells or particles *c* and *d* are transparent and either without pigment or show only a faint granule or two. The slide was kept in a moist chamber and examined at intervals for four hours. The formation of numerous minute masses each consisting of a few cells was observed. These grew large through fusion with one another from hour to hour. At 8 p.m. a few plasmodial masses of considerable size were present, the largest of which measured about $300\mu \times 50\mu$. This was a thin flattened, sheet-like mass having an irregular outline. Its general body was opaque, but the peripheral part was thinner and here it could be seen that all of the four types of cells entered into the composition of the

mass. At this time, 8 p.m., numerous small plasmodial masses grading down to aggregates of a few cells were also present. In many of these too the four types of cells could be distinguished. Finally in the preparation at this time there still remained quantities of free cells. In this preparation there were no bits of tentacles, gonophores, or foreign particles.



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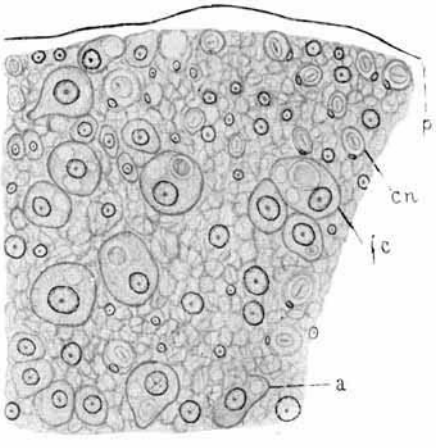

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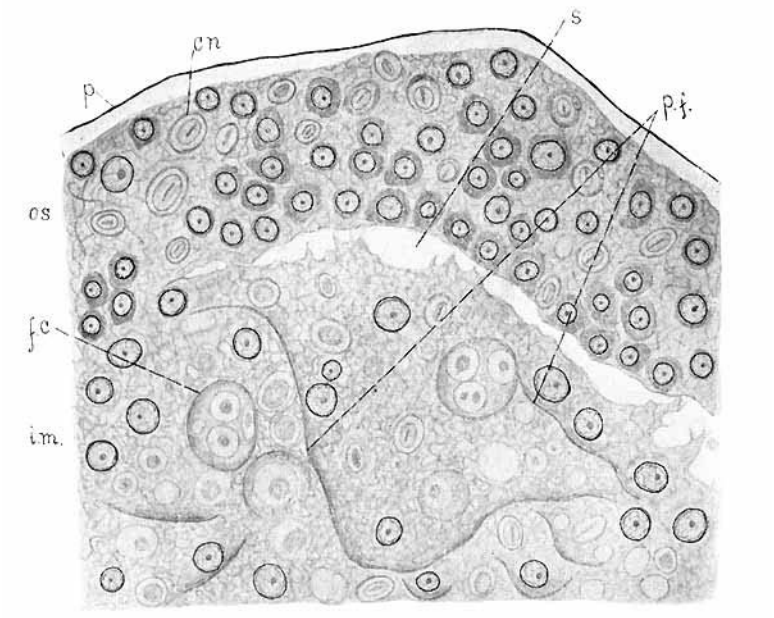
Fig. 10 Eudendrium. Elements of the pressed out tissue. × 700.

Fig. 11 Eudendrium. From a section through a lobe of the mass shown in fig. 7. *cn*, cnidoblast; *f. c.*, free cell; *p*, perisarc. × 1200.

Results from study of sections. The plasmodial mass shown in fig. 7 was about twenty-four hours old when preserved. A part of it had already died, but there were three large lobes of living tissue surrounded by an obvious perisarc. These lobes were found to agree in structure. Part of a section through one of them is shown in fig. 11. In the interior of the lobe there are numerous cells which seem to be free, that is the body is well defined all round. These cells vary in size; the nuclei are relatively large with

abundant nucleoplasm and usually with a conspicuous nucleolus; the cytoplasm as a rule shows vacuoles and solid inclusions in vacuolar spaces. Similar cells are met with which are not sharply delimited all round, but only on one side (cell *a* in fig. 11); on the other side, the cell shading off into the syncytial reticulum. In such a case, I take it, we have a mass which has broken away from the general syncytium on one side, the protoplasm on this side condensing to form a film of exoplasm. Cnidoblast cells, *c n.*, with included nematocysts are also common in the interior of the lobe. Between the cells or groups of cells the protoplasm exists as a vague reticulum, the vacuolar spaces in which are of all sizes. Scattered in the reticulum are nuclei. The external stratum of the lobe in some places is not markedly different from the interior. In other places it shows smaller cells and more nematocysts than the interior. No doubt some of the nematocysts have been carried over from the parent hydroid; possibly others are new formations. The external structure of the lobe is in most places continuous with the interior, but here and there it is separated as in the next mass to be described. The dead part of the mass (fig. 7) consists of a loose granular stuff including some nuclei and nematocysts. The live and dead tissue are not sharply separated, but grade into each other.

Two other plasmodial masses about twenty-four hours old were sectioned. These masses were irregular bodies of the same general character as the one shown in fig. 7. They were however entirely alive, and surrounded everywhere by a distinct perisarc. They proved to be essentially alike in internal structure. Part of a section through one is shown in fig. 12. The body is solid and an outer stratum is almost everywhere separated from an inner mass by a vaguely delimited cleft-like space, *s*. The outer stratum is chiefly composed of comparatively smooth cells, forming four or five layers, and of cnidoblasts. The cells have large nuclei and are in general well defined. There are places however where one can only find nuclei lying in a vaguely reticular protoplasmic matrix. The inner mass is a complex syncytium containing abundant large nuclei and vacuoles with inclusions. Numerous cnidoblasts are scattered through it, and well defined ordinary



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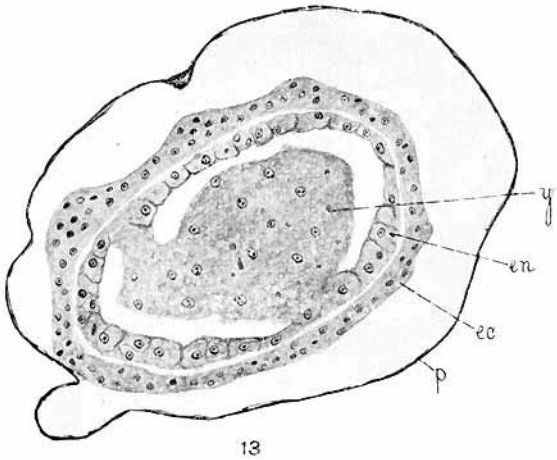

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Fig. 12 Eudendrium. From a section through a restitution mass about twenty-four hours old. *cn*, cnidoblast; *f. c.*, free cell; *p*, perisarc; *p. f.*, protoplasmic film; *s*, space separating an outer stratum, *o. s.*, from the inner mass, *i. m.* × 1200.

Fig. 13 Eudendrium. Section of the restitution mass, with one coenosarcal outgrowth, shown in fig. 8. Section strikes the original mass and does not include the outgrowth. *y*, yolk mass. Other lettering as before. × 350.

cells are found in it here and there. Protoplasmic films, *p f*, are common which mark off cells or areas on one side while on the other side the protoplasmic area has no distinct boundary.

The masses shown in fig. 8, two spheroidal and two with outgrowths, were sectioned. These bodies when preserved were three days old. In fig. 13 is shown a section through the dilated body of one of the masses which had a coenosarcal outgrowth. All the bodies proved to be in essentially the same condition as far as the differentiation of layers is concerned. In them all an ectoderm and entoderm are distinctly differentiated. The two layers are separated by a cleft-like space, there being no distinct supporting lamella. In the small spheroidal masses and in the dilated portions (representing the original shape) of those with outgrowths, there is a central yolk that is still continuous with the entoderm in spots. The yolk mass does not extend into the outgrowths. The perisarc is laminated and in the sections almost everywhere widely separated from the ectoderm. The ectoderm is composed of small cells, probably all interconnected, forming in places one layer, in other places two or three layers. Small nematocysts and stages in development of these are common, and other inclusions also are present in the ectoderm. The entoderm consists of a single layer of large closely packed cells varying from a more or less cubical to a somewhat flattened shape. These cells are uninucleate, the cytoplasm more or less vacuolated and sometimes containing small (developing) nematocysts and other inclusions. The central yolk, *y*, is a granular mass in places composed of small spheres of varying size. In it small spheroidal vesicles containing one or two deeply staining granules are common, doubtless representing swollen and degenerating nuclei. A few small nematocysts are also found in the yolk. The yolk mass although in general separate from the entoderm is perfectly continuous with this layer in spots.

While my observations on the histological structure of the restitution masses, both in Eudendrium and Pennaria, are fragmentary, they are nevertheless definite. From them it would seem that in Eudendrium the solid aggregate formed by the fusion of the isolated cells passes into the condition of a syncytium which

includes partially or perhaps completely free cells (fig. 11). An outer stratum in which cell bodies are well differentiated, and which is several layers deep, now becomes marked off from an inner mass (fig. 12). The outer layer probably represents the ectoderm, while the inner mass represents a yolk-entoderm, which subsequently splits into the definitive entoderm and the yolk. Finally ectoderm, entoderm and a central yolk mass appear (fig. 13), as in the development of a coelenterate planula, Manicina for instance (Wilson, '88), or Eudendrium itself (Hargitt, '04).

PENNARIA. RESTITUTION FROM DISSOCIATED CELLS

Species used. The species used was Pennaria tiarella McCrady (Proc. Elliott Soc., vol. 1, no. 1, p. 153; Nutting, '01, p. 337). Pale specimens with light colored ova and deeply colored specimens with orange ova were abundant together on the floats round the laboratory wharf at Beaufort during August. Both pale and colored forms liberated medusae at dusk, about 7 p.m. The forms appear to represent merely the extremes in a range of color variation (for the varieties at Woods Hole *vide* Hargitt, '00).

Experiment July 26. A vigorous and clean colony was pressed out in the usual way at 4:25 p.m. Quantities of cells of various kinds, especially spheroidal granular cells with more or less pigment, came through; also small cell groups, and bits of tentacles. Fusion of the cells and cell aggregates begins at once and proceeds rapidly. In ten minutes time a mass $500\mu \times 100\mu$ has been formed, in a watch glass kept under the microscope, practically from isolated cells. Such masses change their shape slowly and fuse with one another. The tissue which was pressed out in a watch glass was shaken to the center at 4:35 p.m. At 4:53 it has formed a thin coherent cake. This is now sucked up in places with the pipette, and so broken into pieces about 5 mm. in diameter which are transferred to a bowl of water.

At 6 p.m. the tissue lies on the bottom of the bowl in the shape of thin, somewhat reticular sheets. These are freed from the bottom (they had already begun to curl up round the edge) with small pipette and are transferred with a large pipette to a

fresh bowl. In so doing the sheets are of course broken whereupon the peripheral parts turn white and disintegrate quickly. But the body of the piece remains alive, keeps its color (a reddish tinge), contracts and soon has a comparatively smooth surface once more. Fragments accidentally broken from the sheets are

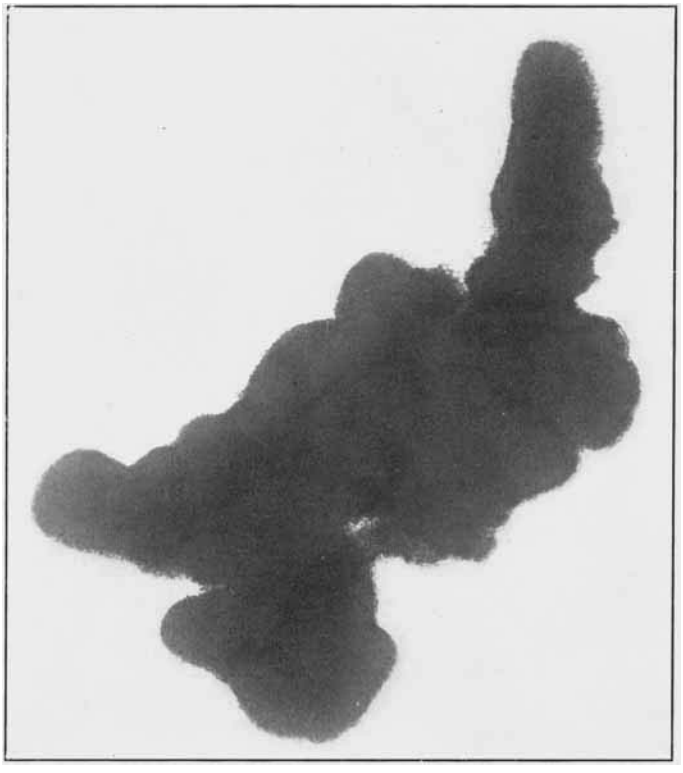
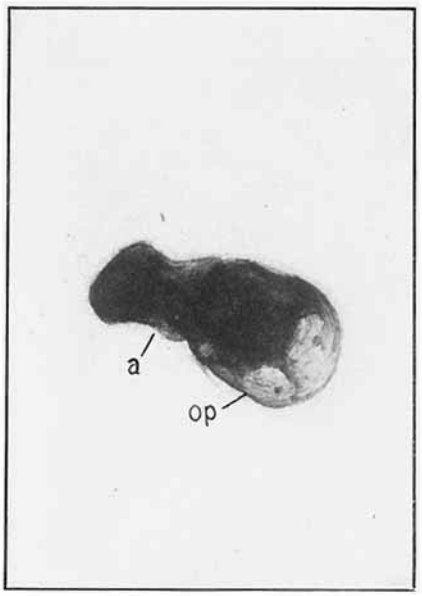


Fig. 14 Pennaria. Restitution mass, four days old. Photograph. × 50.

also transferred, and these also quickly 'heal.' All the masses continue to contract, and by 8 p.m. many of them have a massive shape, although some at this time are still sheet-like. They all have a smooth surface, and the majority of them are in the neighborhood of 1 mm. in diameter. One of the largest is shown in fig. 14.

On July 27 at 9 a.m. the masses are surrounded by a distinct perisarc and with some exceptions are still alive or include considerable live tissue. The color of the live tissue is pink. The smaller masses are of compact shape, spheroidal or ovoidal, and are alive throughout. The larger masses are of a somewhat lobular shape, and while the projecting lobules are alive, a considerable part of the body of the mass is dead or dying. Several are now preserved. Sections confirmed what I have just said as to the distribution of the live tissue. In fig. 20 a section through one of the smaller masses is figured, and it may be seen that the whole mass was alive, and while in general still solid had begun to differentiate the ectoderm and entoderm layers. The masses at this stage are soft and burst easily on rough handling. The water was henceforth renewed with a siphon.

On July 29 two of the masses have developed outgrowths. A photograph of one of them is shown in fig. 15, and the other is represented by a photograph (fig. 16) and a camera sketch (fig. 21), the latter made from the living object. The original mass in both cases was spheroidal, and the thick perisarc, *o. p.*, which surrounded it, still persists. The mass, as sections of similar bodies show, has differentiated into ectoderm and entoderm layers which surround a central cavity containing the remains of yolk material. The body shown in fig. 21 has developed one long outgrowth *c* and two short ones, *a* and *b*, just protruding at the opposite end from the original perisarc. In the long outgrowth the ectoderm and entoderm are thicker than elsewhere. The perisarc over this outgrowth is noticeably thinner than that over the original mass, and the ectoderm in a part of the outgrowth has contracted away from the perisarc, remaining connected with it by strands, *ect. s.* after the fashion characteristic of the adult hydroid. The original mass, too, it may be seen has contracted away from the perisarc and has materially changed its once globular shape. The other mass, fig. 15, which has developed only a single outgrowth, represents a slightly earlier stage than figs. 16 and 21. In it the original mass has contracted away from the perisarc, *o. p.*, but remains connected with it by strands of ectoderm. In the outgrowth however the ectoderm has not yet



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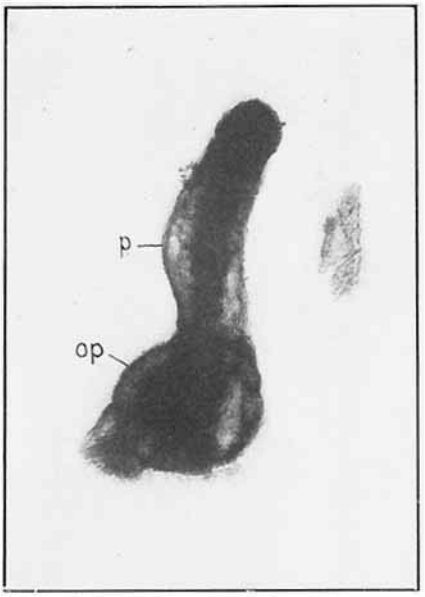

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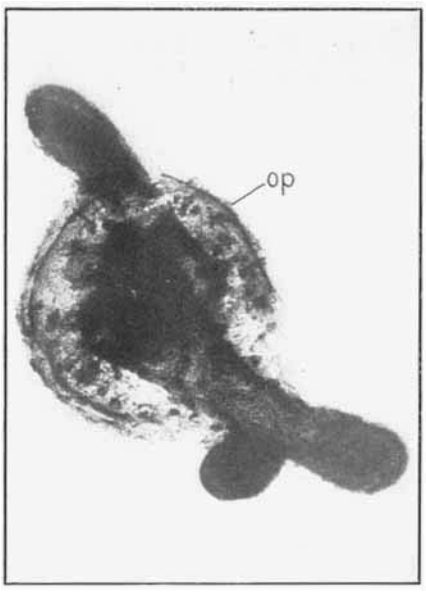
Fig. 15 Pennaria. Restitution mass three days old. *a*, coenosarcal outgrowth; *op*, perisarc of original mass. Photograph. × 50.

Fig. 16 Pennaria. Restitution mass three days old. *op*, perisarc of original mass; *p*, perisarc of long coenosarcal outgrowth. Photograph. × 50.

begun to separate from the perisarcal covering. Both these masses including the outgrowths are firmly adherent to the bottom of the vessel.

On July 29 a large number of the masses are dead. Only the smaller ones together with two or three of the larger survive, and the latter have been injured and evidently are in bad condition. Injury often comes in changing the water, the siphon setting up a current which strains the bodies of the larger masses especially since these are attached to the bottom only throughout a part of their extent. The small masses are more perfectly attached to the glass. It is clear that if one wishes to grow hydroids in this way it is better to produce comparatively small masses instead of large ones such as that shown in fig. 14.



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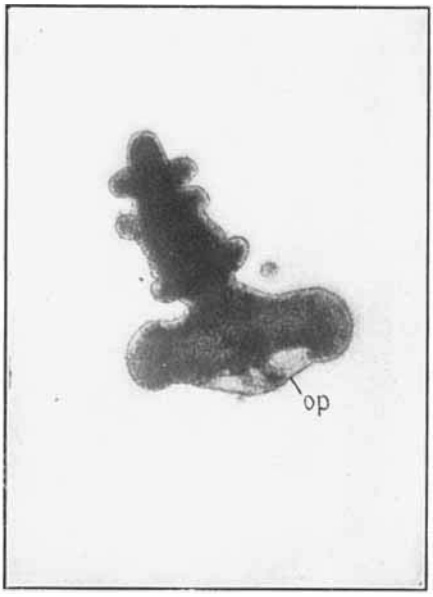

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Fig. 17 Pennaria. Restitution mass, five days old, with two outgrowths, one branched. *op*, perisarc of original mass. Photograph. × 50.

Fig. 18 Pennaria. Restitution mass, two days old, metamorphosed, with hydranth. *op*, perisarc of original mass. Photograph. × 50.

On July 30 one of the masses has developed three outgrowths, each about like the long outgrowth in fig. 16. The mass itself and two of the outgrowths adhere to the bottom, while the third rises obliquely in the water. On July 31 only one of these outgrowths remains adherent to the bottom; the other two rise obliquely in the water. The extremities of the latter have now the character of knobs, reddish brown in color and resting upon lighter colored stalks. This mass continues to develop and on August 1 has reached the condition shown in fig. 19. The ascending outgrowths now bear hydranths, each with the lower filamentous tentacles and the upper short capitate tentacles characteristic of this hydroid. The thick perisarc, *o. p.*, marks out the size of the original mass from which the outgrowths sprouted. The outgrowth x is the one that remained adherent to the glass. In the

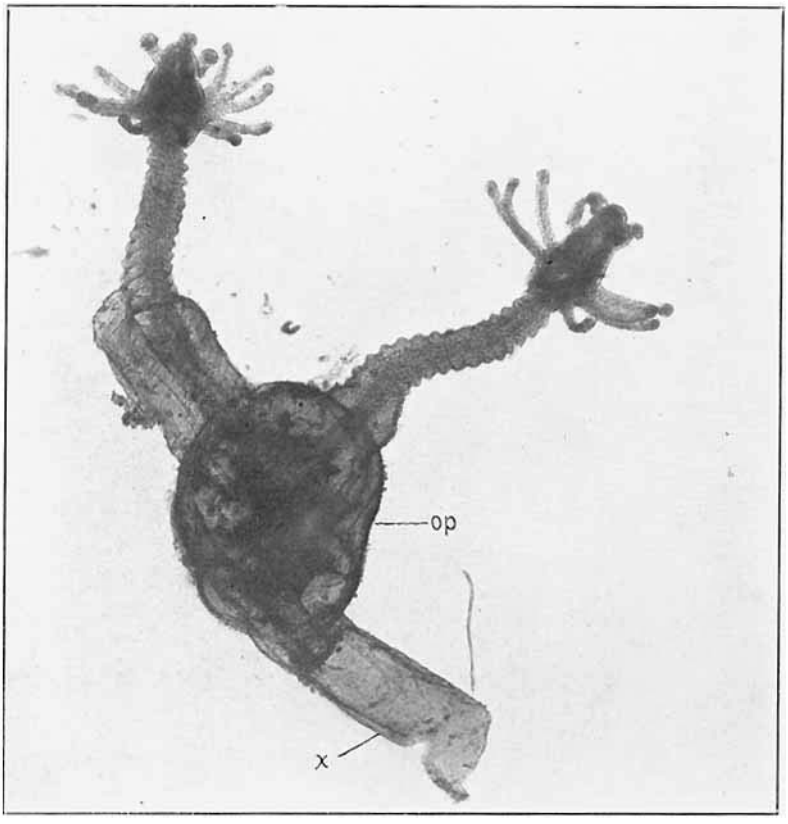

Fig. 19 Pennaria. Restitution mass six days old, completely metamorphosed, with developed hydranths. *op*, perisarc of original mass; *x*, perisarc of outgrowth adherent to glass. Photograph. × 50.

living body the perisarc ended in a closed rounded extremity and contained a coenosarcal prolongation extending throughout its length. When the body was pried from the glass, the coenosarcal prolongation retracted and in the photograph it appears very short.

Experiment August 3. A clean vigorous colony about 5 inches high is selected and only stem material (coenosarc) is used. All lateral branches are cut off, also the base and tip of the main stems. The latter are then cut into pieces about 3 mm. long and

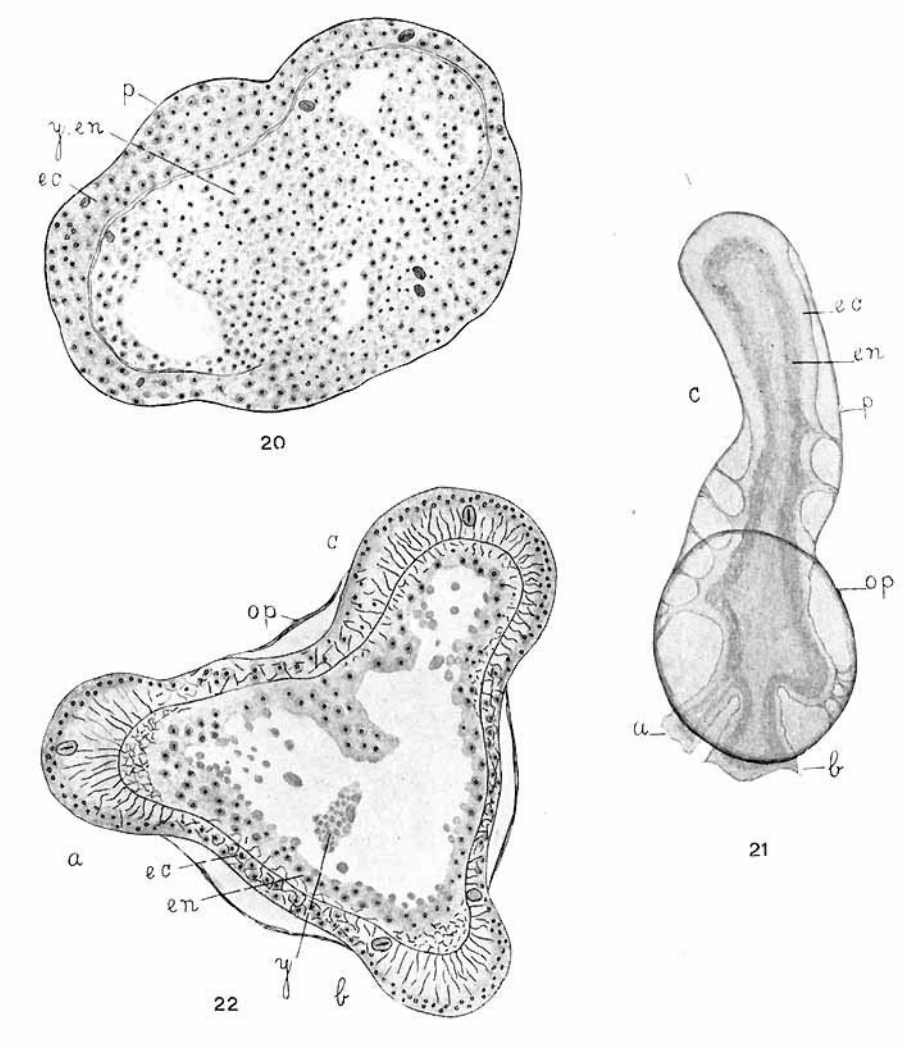

Fig. 20 Pennaria. Section of restitution mass, seventeen hours old. *ec* ectoderm; *p*, perisarc; *y. en.*, yolk entoderm. × 150.

Fig. 21 Pennaria. Same mass as in fig. 16. *a*, *b*, *c*, coenosarcal outgrowths; *ec*, ectoderm; *en*, entoderm; *op*, perisarc of original mass; *p*, perisarc of long coenosarcal outgrowth. × 90.

Fig. 22 Pennaria. Median section of restitution mass two days old. *a*, *b*, *c*, short coenosarcal outgrowths; *ec*, ectoderm; *en*, entoderm; *op*, perisarc of original mass; *y*, yolk material. × 150.

these are pressed through gauze in the usual way. The tissue thus obtained is pure coenosarcal tissue broken up into separate cells and minute aggregations of cells. The tissue is pressed out at 3:10 p.m. Fusion goes on rapidly, and in twenty minutes round the edge of the collection of tissue small bars and plates, 1 to 3 mm. long, have been formed. At 4:50 p.m. the material exists as a thin cake about $\frac{1}{2}$ mm. thick, of considerable coherency, yet of loose reticular texture. This lies on the bottom scarcely adherent to the glass. It is cut with scalpel and needle into pieces about 4 mm. in diameter. Nine such pieces are prepared and together with some much smaller fragments are transferred with a pipette to a fresh bowl of water.

At 7 p.m. all the pieces have contracted considerably. The plate-like pieces have begun to curl up at the edge and the smallest fragments have already assumed a compact, massive shape. The color is a light pink. On the next morning (August 4) all the masses are still alive and have secreted a distinct perisarc. The smaller are spheroidal or ovoidal in shape, the larger of an irregularly massive shape, measuring up to a length of 3 mm. They are all adherent to the glass. The water is now changed by siphoning, and some of the larger masses rupture. They rupture very easily and it is clear they are too large to thrive. After rupturing, a mass quickly acquires once more a smooth surface within the perisarc.

On August 5, two of the masses have partially transformed. One is shown in section in fig. 22. This mass was originally spheroidal and in the living state, including the perisarc, measured about $\frac{1}{2}$ mm. in diameter. It was firmly attached to the glass, and at 9 a.m. August 5 was still spheroidal. At 2 p.m. it was observed to be triangular. The triangular character became more marked during the afternoon, and it was plain that the mass was sending out three outgrowths, *a*, *b*, *c*. The body was preserved at 7 p.m. Sections showed that the originally solid plasmodial body had differentiated the ectodermal and entodermal layers.

The other mass on August 5 had developed farther. A photograph of this mass is shown in fig. 18, and a camera sketch made

from the living object in fig. 23. The original mass was spheroidal; its outline is indicated by the thick perisarc, *o. p.* Two short outgrowths, *a* and *b*, had developed, and these together with the original mass adhered to the glass. A third outgrowth *c* ascended in the water and had transformed into a hydranth bearing whorls of short stubby tentacles. The ectoderm and entoderm had developed throughout the mass, and the ectoderm of the tentacles and of the short outgrowths included abundant nettle cells. The hydranth while under the miscroscope, in a watch glass, was frequently active, bending from side to side. The size of the gastric cavity varied with the contraction state but during most of the time was large. Shortly after transferring the mass from the breeding dish to the watch glass and just after fig. 23 was made a quantity of granular material was twice ejected from the mouth, after which the polyp contracted considerably. The water was then changed, and the polyp returned to about the condition shown in the figure. It was then preserved (4 p.m.). The tentacles of the hydranth arranged in three whorls, all look alike, and are of a more or less globular shape. The upper whorls doubtless represent the capitate tentacles of the adult. Possibly the tentacles of the lower whorl elongate and develop into the filamentous tentacles. In the development of the egg polyp, Hargitt ('00, p. 400) finds that the filamentous tentacles appear first, the capitate somewhat later. In the restitution polyp shown in fig. 23 there may have been a slight difference in the time of appearance of the several whorls.

On August 6 another small mass has developed an outgrowth and resembles fig. 15. On the next day it is dead. By this time, August 7, many of the masses including all the larger ones are dead, but some survive and of these three have developed each an outgrowth. One of them is shown in fig. 24, the others are substantially like it. In all three the coelenterate layers have re-appeared; the original mass has contracted away from the perisarc with which it remains connected by ectodermic strands. While the mass shown in fig. 24 was under the miscroscope, these strands retracted into the body. The three partially transformed masses are now preserved.

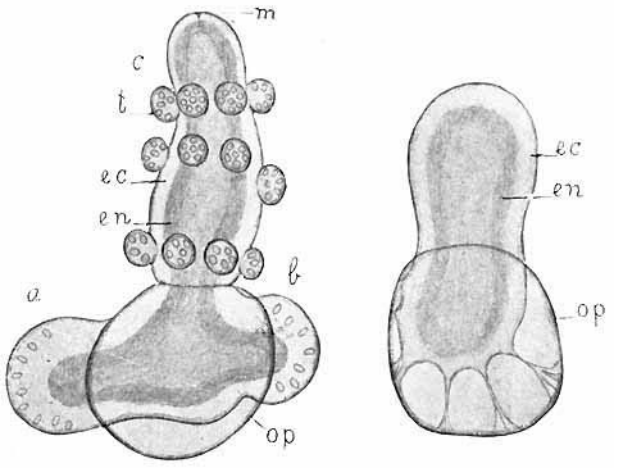

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Fig. 23 Pennaria. Same mass as in fig. 18. *a, b,* short coenosarcal outgrowths; *c,* outgrowth that has become a hydranth; *ec,* ectoderm; *en,* entoderm; *m,* mouth; *op,* perisarc of original mass; *t,* tentacle. × 90.

Fig. 24 Pennaria. Restitution mass three days old, with outgrowth. Lettering as before. × 90.

On August 8 only four of the masses of this experiment are alive. Two are still spheroidal. One has developed an outgrowth and is substantially like fig. 24. The other is shown in fig. 17; it has given rise to two outgrowths at opposite poles, one of which has developed a lateral branch. The original mass has contracted away from the perisarc, remaining connected with it by ectodermic strands in the usual way. On the perisarc algae have settled and these appear in the photograph as rounded spots. In all of these bodies, even in the spheroidal masses, sections showed that the ectoderm and entoderm had developed, and a gastral cavity containing the remnants of a yolk mass was present. The bodies were preserved August 8.

Summary. Two experiments were made. In the first both stems and hydranths were used. In the second only stem tissue was used. A large number of solid plasmodial masses were obtained, and these within a day uniformly secreted a distinct perisarc. In the first experiment all the larger masses gradually died. Three masses $\frac{1}{2}$ to $\frac{2}{3}$ mm. in diameter, differentiated the

coelenterate layers and developed coenosarcal outgrowths. One of these masses went further and developed perfect hydranths. In the second experiment also the larger masses died. Ten smaller masses for the most part about $\frac{1}{2}$ mm. in diameter, the largest reaching a diameter of $\frac{7}{10}$ mm., differentiated the ectoderm and entoderm layers. Of these, eight developed coenosarcal outgrowths, and of the eight one mass produced an actively motile hydranth with whorls of tentacles.

Comparison with egg development. For the purpose of comparison with the restitution hydroids my assistant, Mr. O. W. Hyman, reared Pennaria from the egg. As Hargitt ('00) states, the eggs vary considerably in size, the planulas in size and shape both. Several planulas were measured and it was found that they ranged in length from about $\frac{4}{10}$ mm. to 1 mm., while the cross diameter was about $\frac{2}{10}$ mm. It will be seen that these planulas were not so far removed in bulk from the restitution masses that transformed, although neither in the case of the planulas nor in that of the restitution masses was there any uniformity of size. On the other hand the hydranths produced by metamorphosing planulas are fairly constant in size, and they agree in this respect with those produced by the restitution mass shown in fig. 19.

Histological study of the restitution masses of Pennaria

Sections of the Pennaria stem show that the entoderm is made up of a single layer of large columnar cells tapering towards the base and measuring about 30μ by 15μ. The cells contain very many large spheroidal granules that stain pale blue with haematoxylin. The ectoderm contains an abundance of nettle cells, large and small. In each layer the elements are freely interconnected to such a degree that in many regions at least the structure is that of a reticular syncytium.

In order to study the composition of the pressed out tissue I squeezed pieces of stem in a watch glass of water so that the squeezed out tissue fell on an immersed cover glass. After five minutes strong formalin was added. Much of the tissue thus fixed adheres to the cover, and this when mounted on a slide in water gives clear pictures. In such a preparation, fig. 25, there

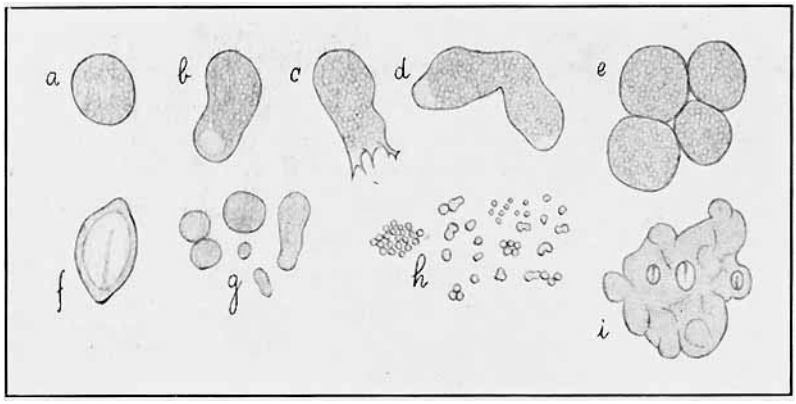


Fig. 25 Pennaria. Elements of coenosarcal tissue. From a preparation fixed five minutes after tissue was pressed out. × 600.

are quantities of large granular cells, many of which are spheroidal as *a*, other with pseudopodia as *c*, some with a larger vacuole as *b*. The granules are generally scattered all through the cell, but as in *d* there may be some clear protoplasm. These cells exist separately but also in small aggregations as *d* and *e*. The cells and contained granules are of about the same size as those seen in sections of the stem entoderm and the cells evidently represent the entodermic elements. In the preparation again are numbers of cnidoblasts of various sizes with included nettle cells, *f*. Other elements, doubtless also ectodermic, are finely granular pale cells varying a good deal in size, *g*, together with small groups of such cells. When the stem piece is pressed, numbers of cells must be ruptured, and the preparation contains quantities of free granules, *b*, doubtless derived in large part from the entoderm cells. These are transparent, and for the most part spheroidal, although often irregular in shape. They seem to stick together and even to fuse. Droplets of translucent substance smaller than the entodermic granules and varying in size down to the vanishing point are common. It seems probable that some of this material spoken of as droplets and granules represents minute fragments of protoplasm that have rounded off. And the question is worth formulating, although I can not answer it: are such minute granular or drop-like bodies, representing por-

tions of broken down cells, incorporated in the restitution mass as it grows? Again in such a preparation one finds some masses, *i*, composed of finely granular material, with cell boundaries here and there vaguely showing, and sometimes with included nettle cells. These are probably lumps of ectoderm.

If such a preparation be made and examined alive in sea water, the same elements are observed. Formalin removes the color from the granules in the entoderm cells, but in the living preparation it may be seen that they have in general an orange tint, ranging from yellow to reddish, although some are colorless. The entoderm cells execute slow amoeboid changes of shape.

When the stem pieces are pressed through gauze and the tissue is at once examined alive in a drop of sea water, it is found to consist of the same elements described above. With this treatment more entoderm cells seem to be ruptured and there are fewer groups of cells. The coenosarc is almost entirely broken up into the elements *a*, *f*, *g*, *h*, of fig. 25. When the entire hydroid is cut up and pressed through gauze, again the same elements are found if the tissue is examined at once, although possibly more aggregates of cells occur than when stem tissue alone is used.

If the drop of live tissue pressed out through gauze, or squeezed out without using gauze, be kept under observation, it may be seen that small masses are soon formed which include entoderm cells, cnidoblasts, and the pale cells that probably are of ectodermic origin. As these masses grow in size it becomes impossible, owing to their opacity, to study their composition while alive.

As stated already, fusion between the cells of the pressed out stem tissue goes on so rapidly that in twenty minutes times small bars and plates, 1 to 3 mm. long, can be drawn off with a pipette. Some of these were preserved and sectioned and it could be seen that such masses were solid bodies of fairly uniform structure showing no stratification into incipient layers. The superficial part does not differ from the interior. The structure throughout is that of a cellular syncytium, that is in certain regions no cell boundaries can be seen, the protoplasm here appearing as a syncytial mass containing scattered nuclei, while in other places cell boundaries are visible. Even where cells are marked

out it is probable that they are interconnected with one another and the rest of the mass by protoplasmic strands. The cells that are marked out vary in size and shape. Here and there cells, usually in groups, may be recognized by the contained granules as the original entoderm cells. Only a small fraction of the mass however is now made up of such cells, and yet the entodermic elements composed a very large part of the tissue when the fusion masses began to form. It is plain then that the entoderm cells after fusion to form, or rather help form, the plasmodial masses, undergo a transformation which effectually precludes us from recognizing them later. The large cnidoblasts formed a conspicuous set of elements in the tissue when fusion began, and these are to be seen in very considerable numbers scattered throughout the plasmodial mass at the stage under examination (twenty minutes old). A comparison between the sections of this and later stages indicates that the bulk of the nematocysts carried over from the parent gradually disappear during the development of the plasmodial mass. If this is so, it is a question of some interest what becomes of the cnidoblast cell itself? Does it share in the formation of the regenerative tissue? The point is worthy of special study, including as it does the idea of the de-specialization of a highly differentiated element.

The protoplasm of the cell and syncytial areas in this stage is for the most part finely vacuolated so as to present a reticular appearance to a high power. The nuclei are in general large and contain abundant nucleoplasm. The mass at this time seems to have no surface film apart and distinct from the superficial syncytial and cell areas. Finally in connection with this stage it may be said that owing to the transformation which the entoderm cells undergo after fusion, it does not seem hopeful to attack from purely histological evidence the question as to whether ectodermic and entodermic elements become segregated, the ones on the outside, the others in the interior of the mass. There is of course always a possibility that this occurs, but it seems remote.

Somewhat older plasmodial masses formed by the fusion of stem tissue pressed through gauze were studied. These were preserved 1 hour after fusion had begun, and were considerably

larger than the mass just described. Sections show however that they have essentially the same structure. Cells or protoplasmic areas with the entoderm granules are still recognizable here and there. Perhaps most of such cells lie in the interior but some are found at the surface. This is not a point of importance for the question as to the possible segregation of ectodermic and entodermic elements, since as I have already explained a very large part of the entodermic material can no longer be recognized as such. It is clear that many of the areas or elements of the plasmodial mass now without granules, and with a vacuolated protoplasm, must have been formed from entoderm cells.

The solid plasmodial mass does not long remain unstratified. As already said the masses uniformly secrete a perisarc within about one day after fusion begins, and this in itself is probably evidence that the superficial layer has assumed something of an epithelial character. Masses preserved July 27, about seventeen hours old, were sectioned. Some of them were healthy and alive all through, and fig. 20 represents a section through such an one. In this body an outer layer, *ec.*, the ectoderm, has separated from an inner mass, *y. en.*, the yolk entoderm. The latter as later stages show gives rise to the definitive layer of entoderm and a central yolk mass, as in the planula development of Pennaria (Hargitt, '00, '04). Both ectoderm and yolk entoderm are cellular syncytia in which free elements or apparently free elements are included. In both the ectoderm and yolk entoderm, some of the large nematocysts carried over from the parent, are present. On one side of the body it will be seen the differentiation into layers has not yet been carried out, the ectodermal region here shading off into the inner mass. The isolated and irregular cavities in the yolk entoderm doubtless represent the beginnings of the gastric cavity. In other masses of the same lot preserved at the same time the shape was lobular, and only the projecting lobules were alive, while the more central part of the body was dead or dying. Sections showed that in the lobules the layers were present, in about the same stage of differentiation as in fig. 20.

Restitution masses from Experiment August 3 that were preserved nineteen hours after fusion began were sectioned. These proved to be in about the condition shown in fig. 20.

A mass two days old, from Experiment August 3, was sectioned, and a median section is represented in fig. 22. The mass was originally spheroidal, but gave rise to three short outgrowths *a*, *b*, *c*. In this body the ectoderm and entoderm are well differentiated. The yolk entoderm of the earlier stage has obviously given rise to the entoderm, *en.*, and to more centrally located yolk material, *y*, and the latter has been nearly absorbed. In places the entoderm is still continuous with yolk elements. The best sections show that the entoderm is still a reticular syncytium, but the cell bodies are distinctly outlined in places. In the most distinct regions they have a columnar shape as in the adult. The entoderm is now well stored with the spheroidal granules found in the adult cells. The ectoderm also appears to be in reality a reticular syncytium, but cell bodies are clearly differentiated and regularly arranged in the regions of the outgrowths. They have here an elongated, columnar shape. Some large nematocysts, apparently such as were carried over from the parent, are present in the ectoderm. A very few such nematocysts are found in the entoderm, and these seem to be in a phase of dissolution.

Other masses from the same experiment (August 3) that were four and five days old were sectioned, and the results may be briefly given. While the mass is still spheroidal and before it has developed outgrowths, it may differentiate an ectoderm, entoderm and central yolk. By the time a well defined layer of entoderm is present, the yolk mass is small in amount and consists of scattered spheres or small groups of spheres. In such spheroidal masses the ectoderm and entoderm have the character of reticular syncytia. As outgrowths develop, both ectoderm and entoderm assume the character of columnar epithelia, especially in the outgrowths themselves.

LEPTOGORGIA. FUSION OF DISSOCIATED CELLS

The species used was Leptogorgia virgulata, the 'sea feather' that is common, especially under piers, in Beaufort harbor. Some introductory experiments were made under my direction by my assistant, Mr. O. W. Hyman. He established the fact that when Leptogorgia is cut into small pieces, and these pressed in gauze sacs

in the usual way, the tissue is broken up into small masses and separate cells. By the subsequent union of such masses and cells, smooth balls up to and over 1 mm. in diameter are formed. These remain alive for days in laboratory dishes, but do not transform.

A record of two subsequent experiments is here given.

Experiment August 9. Pieces 4 to 5 mm. long are cut from the upper end of a yellow colony. The horny axis occupies about one-fourth the total diameter. These pieces are simply squeezed with forceps in a watch glass of water. The tissue exudes freely from the cut ends. Much of it is stringy. With pipette it is dispersed and so broken up. It is then shaken to the center of the watch glass and the glass transferred to a bowl of water, 11:15 a.m.

Some of the tissue is now examined under a supported cover. It is made up as follows: (1) Ciliated cords and masses, varying in size from large to minute are abundantly present. These are doubtless pieces of mesenterial filaments with mesenterial tissue. (2) Motionless masses of loosely packed cells, also varying considerably in size are abundant. (3) Isolated cells and groups of a few cells are abundant (fig. 26). In fig. 26, *a* represents a characteristic small cell group made up of a few spheroidal cells with sharp outlines, some full of highly refractive granules, some merely containing a good many such. Similar granules form a compact mass at one end of the cell group, but this mass lacks a bounding pellicle. Separate spheroidal granular cells, *b*, resembling the constituents of *a* are common, and they may have pseudopodia. There is an abundance of small spheroidal masses of glassy protoplasm, *c*, the larger with one or two granules. Finally there are plenty of isolated granules, *d*, such as are found in the granular cells. In the category *c* the smallest elements must surely be fragments of cells or bits of intercellular connectives that have rounded off.

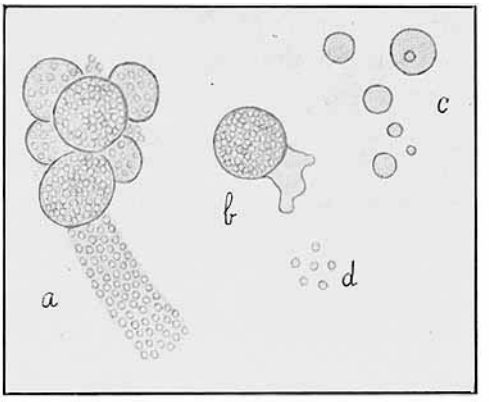
The preparation under the microscope was watched for about an hour, and it was observed that fusion took place involving all the classes of constituents above enumerated. At 12 m. several of the larger ciliated masses were motionless, the surface bearing instead of cilia numerous small pseudopodia and transitional stages from cilium to pseudopod.

The tissue left in the bowl was examined at intervals. The masses grew evidently through fusion with one another and through incorporation of the granular cells and other elements. A typical mass at 2 p.m. is shown in fig. 27. The mass is full of granules like those of fig. 26, and in it the outlines of some spheroidal cells, like fig. 26 *b*, are distinguishable. Round it are similar granular cells and very many small masses of glassy protoplasm some with a few granules, some without any, ranging in size from mere points up nearly to the diameter of the granular cells.

By 9 p.m. the process of fusion had gone so far that spheroidal masses with smooth surface, from about ½ mm. in diameter downwards, were present. A number of these were now picked out and transferred to fresh sea water. In the formation of these masses about ten hours transpired. It is evident that during this time some regressive differentiation of the fusing lumps of tissue to a simpler condition took place. What the character of these changes were and how uniform they were, it would be interesting to know.

The further history of the masses is briefly as follows. On August 10 they were alive. At this time they are perfectly opaque, and the smooth surface shows abundant fine flagellum-like pseudopods. Many of them are surrounded by a deposit of whitish material. This on examination proves to be made up of spheroidal cells of various sizes and degrees of granulation, which evidently have been slowly given off from the mass. This giving off of cells continues during the next day. It is probably evidence of a bad condition of the mass, and it is noteworthy that some of the masses do not exhibit it.

All of these bodies were preserved on August 11, and several were later sectioned. While still alive something could be learned of their histological structure by gently crushing them under a cover glass. On doing so some of the contents streams out in the shape of small spheroidal masses. The larger of these are like the granular cells (*b*) of fig. 26. From such they range down to minute particles of glass-like protoplasm just large enough to be seen at a magnification of 600. Of the intermediate sizes some are full of granules, others contain one or a few, while still others are



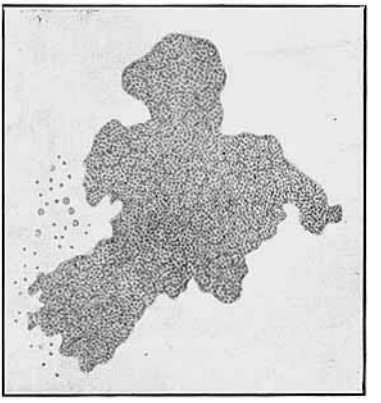


Fig. 26 Leptogorgia. Cell aggregate and free elements of pressed out tissue. × 1200.

Fig. 27 Leptogorgia. Fusion mass two hours fifteen minutes old. × 150.

without granules and glass-like. The granules in all these bodies are mostly of one size and yellowish. Such an examination by no means necessarily implies that in the natural condition the mass is composed of spheroidal cells. Rather, I assume that, on crushing, the cell bodies are separated and, where such exist, the intercellular strands are broken. A quick contraction would then make all the protoplasmic masses spheroidal. A body that has been slightly crushed under a cover glass in this way may heal. One such was kept for two hours in a moist chamber, and at the end of this time the body healed perfectly and was once more surrounded, as it originally was, by a smooth surface pellicle.

Sections showed that these bodies did not have a uniform composition. In one (ball 1) the structure was as follows: There is a surface film but sections give nothing definite as to its composition. The interior is solid and shows no stratification into layers (ectoderm and entoderm). In many regions one finds protoplasm studded with nuclei but no cell boundaries can be made out. In other places there are small cells, rounded or angular that are very closely packed. In still other places while the tissue is compact the cells are slightly separated by unstained substance, probably fluid. In such places the cell bodies are distinctly outlined and intercellular strands of protoplasm are freely present.

In a few small areas there is a scanty accumulation of mesogloeal jelly imbedded in which are some strands and small masses of nucleated protoplasm which are freely interconnected. The jelly stains blue with haemalum (or haematoxylin) and with a 2 mm. objective appears homogeneous. These several observations show that the mass is a syncytium in different parts of which cell bodies are differentiated in various degrees.

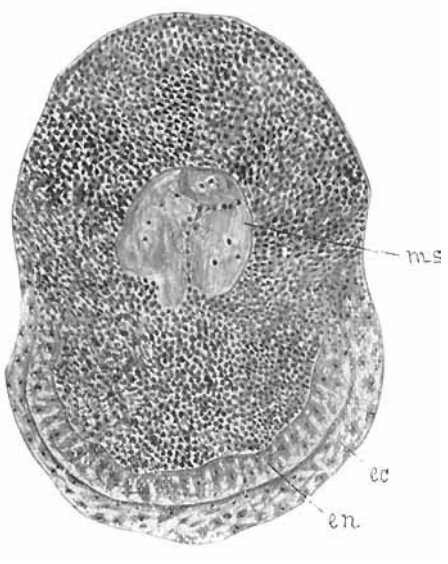


Fig. 28 Leptogorgia. Median section of a fusion mass two days old. Layers interpreted as ectoderm, *ec*, and entoderm, *en*, have developed. *ms*, mesogloeal jelly. × 350.

Another mass (ball 2) has the structure shown in fig. 28. There is a surface film which appears as a mere line. The general mass consists of the syncytial cellular tissue described for ball 1, but round half of the body two layers, apparently the ectoderm and entoderm, are differentiated. These layers are distinctly differentiated, although there is no mesogloeal jelly between them. At about the middle of the body they fade away into the general mass. The entoderm consists of more or less columnar elements,

the ectoderm of irregular cell bodies separated by a good deal of fluid and freely interconnected. Near the center of this ball is a considerable collection of mesogloeal jelly, of the same character as that described for ball 1.

In still another case (ball 3) while a part of the body resembles ball 1, at one end of the body the structure is that shown in fig. 29. We find here an accumulation of finely granular, lightly stained material, *cg*, which is quite different from mesogloeal jelly and is probably a fluid that has coagulated in the fixation process.

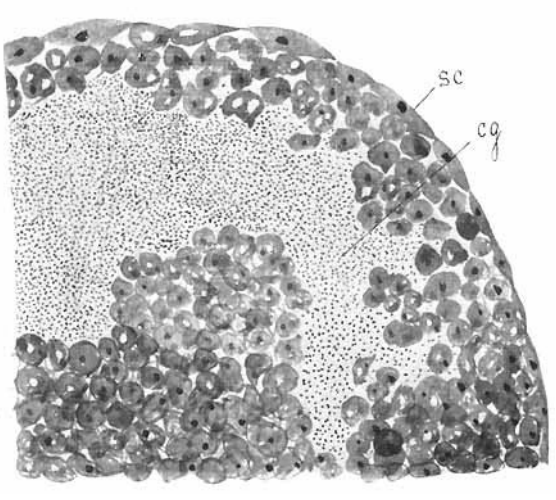


Fig. 29 Leptogorgia. Part of section through a fusion mass two days old. *cg*, coagulum; *s.c.*, superficial cells. × 1200.

The same ball contains some mesogloeal jelly near the center. In the region round the coagulated fluid the cells are loosely packed. They are more or less rounded and intercellular connections are practically absent. Vacuoles are common in these cells and a conspicuous nucleolus is frequently to be seen. In this region the surface layer is formed of flattened cells, *s. c.* In this ball there is no differentiation of ectoderm and entoderm layers.

Interpreting the results of this study of sections, it seems probable that ball 1 represents an earlier stage, and ball 2 a later one in which the coelenterate layers have begun to differentiate.

In ball 3 the condition shown in fig. 29 is perhaps to be correlated with the gradual extrusion of rounded granular cells which goes on in the case of some of the masses during life, as already recorded. Such a condition is one, perhaps, into which the dense syncytial cellular structure passes when the struggle for life is going against the body. It may of course only be a mortuary change, viz., a step in a process of gradual dying.

The results of this experiment, while very inconclusive, suggest that the bodies formed by the fusion of cell masses and cells would regenerate into new individuals if placed under good conditions, possibly hung out in gauze bags in a part of the harbor where the current is good.

Experiment August 10. Small pieces of a Leptogorgia colony were pressed out through gauze at 4.30 p.m. The tissue that streams through the gauze is finer than that obtained in the experiment just recorded. It is made up of the elements shown in fig. 26 and of small opaque lumps of tissue, mostly spheroidal and many of them ciliated, which are commonly three or four times the diameter of one of the granular cells (fig. 26 *b*). The living tissue and the spicules are separated as far as possible.

Fusion goes on and by 7 p.m. masses of irregular shape are present. These are transferred to fresh sea water. The next morning a number of smooth balls have been formed, some of which have incorporated spicules. These are kept for a couple of days during which they show no external signs of differentiation. Sections showed that these balls had essentially the same structure as those of the preceding experiment.

Experiment to test the regenerative powers of a fusion mass when inserted in the body of the parent species. August 10. Six of the Leptogorgia fusion masses produced in the experiment of August 9 were inserted in the parent species in the following way. A piece of an orange colored Leptogorgia, about five inches long, was slit lengthwise down to the horny axis. The slit so made was pushed open and the fusion masses dropped in with a pipette in a row. Ties were then made round the piece of Leptogorgia closing up the covering layer of polyps over or partly over the fusion masses.

On August 11, 10 a.m., the ties are removed. The slit has not healed but the edges have curled in. The whitish fusion masses

may be seen in the slit. They have fused with one another in some degree, the number of masses now being four. The piece of Leptogorgia looks healthy; the polyps are well expanded. At 7 p.m. the whole preparation is preserved. The questions are: Have the fusion masses undergone any histological differentiation? Have they established union with the Leptogorgia?

Sections through one of the masses showed that the body had grown deeper into the slit and had established connection with the Leptogorgia on one side of the slit. This connection included perfect continuity with the entodermic lining of a coelenteric cavity which had been laid open, and also with the entoderm of several small coenenchymal canals in the neighborhood. The whole fusion mass is solid and somewhat club-shaped at its outer end where it shows a stratification into an outer stratum and an inner core. The thickness of the outer stratum is considerable including several layers of cells. The other masses did not penetrate so deep into the slit. They were found to be in continuity with the superficial layer of the Leptogorgia, but had not established connection with the interior of the latter.

The indication from this experiment, which was merely meant as a tentative one, is that the fusion masses if allowed to grow would have become part of the Leptogorgia colony.

ASTERIAS. FUSION OF THE DISSOCIATED CELLS OF THE IMMATURE GONAD

Experiment August 5. Gonads 25 mm. long of the common starfish, Asterias arenicola, were cut into pieces about 5 mm. long and these were pressed through gauze at 12 m. Abundant separate cells and small cell aggregates stream through the gauze together with some larger pieces of gonad. The latter are picked out, and the remaining material is shaken to the center of watch glass. A drop of the material is now examined under the microscope. Many of the cells whether free or combined in small aggregates are coarsely granular. Both cells and aggregates show fine pseudopodia. The cells and the aggregates quickly combine and in a few minutes the field of the microscope presents the appearance shown in fig. 30. There are numerous small masses, such as *a*, which have been formed by the fusion of cell aggregates

and separate cells; and there is an abundance of free elements. Among the latter coarsely granular cells like *b*, with and without pseudopodia, are conspicuous. There are also many clear glass-like cells (*c*) ranging down to bits just visible. Free granules resembling those of the granular cells are abundant. The masses (*a*) make the impression of being aggregations of the granular cells (*b*), but doubtless other elements enter into their composition. Abundant fine pseudopodia, occasionally branched, cover the surface of such masses.

By 1 p.m. the tissue in the watch glass has combined to form a thin and extensive reticular plate, produced by the gradual

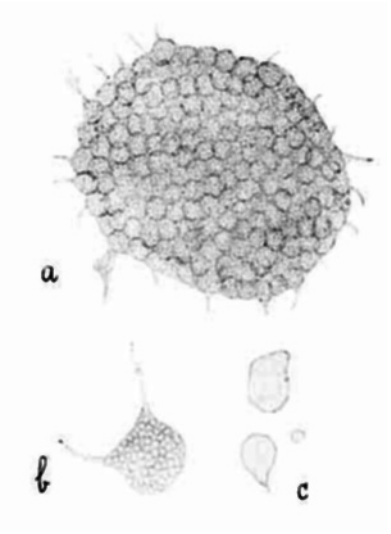


Fig. 30 Asterias. Small fusion mass and free elements of pressed out gonad tissue. *a* × 600. *b* and *c* × 1200.

fusion of masses of many shapes. The reticulum in general is attached, though feebly, to the glass, but pieces 1 to 2 mm. wide have been broken off and are free. At 7 p.m. the whole reticulum is broken up into pieces of about this size, and all transferred to fresh sea water. On the following day a number of such pieces had contracted into smooth, massive bodies. But all pieces died in a day or two.

Experiment August 16. Gonads 20 mm. long were used. The gonads were cut into pieces and these simply teased up with needles in a watch glass of sea water. The pieces are thus broken into small masses, cells, and fragments, essentially like those shown in fig. 30. Fusion commences at once as in the former experiment, the isolated cells and the masses both throwing out pseudopodia. The granular cells in particular are observed to become interconnected by delicate and complex pseudopodial networks. The formation of pseudopodia by the granular cells may go on to such an extent that the granular substance of the cell body almost disappears in the network of pseudopodial strands. This e periment was not carried farther.

Sections showed that the gonads used in these experiments were in the indifferent stage. The germinal epithelium lining the follicle is more than one layer deep, and many of the nuclei are large and rich in chromatin. The epithelium has proliferated to such an extent that the lumen is nearly filled with cells. In sections these are compressed, with rather vaguely granular cytoplasm and nuclei which are smaller than in the lining cells. When the living gonad is slightly pressed, these cells exude and appear as the spheroidal granular elements described above.

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