DEVELOPMENT OF SPONGES FROM DISSOCIATED TISSUE CELLS

By H. V. Wilson

Professor of Zoology, University of North Carolina

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This investigation was carried on at the Beaufort Laboratory of the Bureau of Fisheries during the summers of 1907 and 1908. An outline without illustrations of the results has been published in the Journal of Experimental Zoology (On Some Phenomena of Coalescence and Regeneration in Sponges, vol. v, no. 2). In papers read before the Fourth International Fisheries Congress (Washington, September, 1908) and the American Society of Zoologists (Baltimore, December, 1908), I made brief mention of the results and in connection therewith exhibited specimens and photographs. It now seems desirable to publish the facts, with illustrations, in sufficient detail for the account to be useful as a guide in future investigations.

MICROCIONA PROLIFERA.

This species, known as the red oyster sponge, is common in Beaufort Harbor and is the form I have chiefly used in my experiments.

DESCRIPTION OF SPECIES.

Diagnosis.—Incrusting at first, but later forming lobes, and eventually becoming a complex branched body. Color, red. Skeleton in incrusting type a basal horny plate with short upright plumose columns. Skeleton of branched sponge a reticulum of spiculo-fiber. Characteristic megascleres are: (1) Smooth style, 400–160μ long, 8–16μ thick; (2) small spinose style, 80μ by 6μ. Microscleres are isochelæ, 12–16μ long, and toxas 16–40μ long, both, but especially the latter, scantily present.

Verrill and Smith have pointed out that the habitus varies greatly, and have indicated the chief types. The sponge may form thin incrustations, especially on oyster shells and on wharf piles. Such incrustations may be entirely without lobes, or may bear a few projecting lobes as is the case with the specimens shown in figure 2, plate 1. Older specimens are not infrequently found in which the formation of the lobes has gone on with accompanying branching and anastomosis, such growth eventually producing an intricately branched sponge body (fig. 1, pl. 1). Specimens of this type may reach a height of 150 mm.

Structure of incrusting type.—In the incrusting specimens the skeleton consists of a horny basal plate bearing closely set vertical horny columns from which the larger spicules (megascleres) project. A section through such a sponge is shown in figure 5, plate 1. From near the apex of each horny column

a few large, smooth, and slightly curved styles project, forming a well-marked tuft. These styles measure 400–160μ long, 8–10μ wide. The longest styles lie nearest the apex of the column and some of them project beyond the surface of the sponge. Mingled with the mature styles are younger spicules of the same type, but slenderer and shorter. Projecting from the sides of some of the larger horny columns are a few small styles, 80 by 5–6μ, some of them distinctly spinose, others with few and feeble spinulations.

The origin of the plumose columns may be studied in sections like figure 5 and may be here briefly sketched. A single long smooth style is formed with its rounded end buried in the basal horny plate, the spicule projecting vertically upward. Spongin accumulates round the base of such a spicule, forming a small mound. The spicule elongates and is, moreover, carried outwards by the elongation of the spongin mound at its base. While this is going on the other spicules of the column develop around and beneath the first-formed one. Small spinose styles are found here and there projecting, independently, upwards from the basal horny plate of the sponge. These are doubtless incorporated in some neighboring horny column that starts a vigorous growth, coming to lie on the side of such a column.

In the incrusting sponges there are only a few microscleres, scattered through the trabeculæ of the interior and in the dermal membrane. They are small isochelæ, about 12–14μ long, and toxas 16–24μ long. The pores are scattered irregularly over the dermal membrane. They open into large spaces (subdermal chambers) lying beneath the membrane. The oscula are small apertures, often 1–2 mm. in diameter, found here and there over the surface. They lead into canals which extend in a horizontal direction, branching as they go, directly beneath the dermal membrane. Thus the large cavities found beneath the dermal membrane (fig. 5) are of two kinds, some belonging to the afferent and some to the efferent system. The sponge tissue of the interior or parenchyma is reduced to a set of anastomosing trabeculæ lying between the two canal systems. In the trabeculæ are situated the small spheroidal flagellated chambers. The trabeculæ contain numerous granular amœboid cells (amœbocytes), but these are especially abundant in the layer of parenchyma which lies directly upon the basal horny plate. This basal layer of parenchyma (fig. 5) lacks flagellated chambers. Imbedded in the trabeculæ or basal parenchyma are abundant sperm masses and some small ova. Young sponges of this incrusting type are frequently found to contain numerous larvæ in various stages of development.

Structure of a sponge with lobes.—The incrusting sponge as it grows older throws out lobular outgrowths that are more or less cylindrical. The sponges shown in figure 2 exhibit several such lobes. Lobes of this sort have an extensive skeleton which consists of a reticulum of horny spiculo-fiber breaking up near the dermal surface into independent terminal branches. The latter are arranged more or less vertically to the surface and support the dermal membrane. Their structure is essentially like that of the horny columns of the young sponge. It is obvious that such a skeleton arises through the continued growth and anastomosis of the vertical horny columns of the young sponge. With the elongation of the columns to form fibers, many styles come to be entirely included in the horny substance.

The spiculo-fibers in the interior of the lobes consist of abundant spongin together with included and projecting styles. The included styles are chiefly of the smooth type, but the small spinose styles are also found occasionally included. Typical included styles measure 280μ by 10μ, 260μ by 10μ, 160μ by 8μ. The head is sometimes slightly enlarged, the spicule becoming a subtylostyle. The projecting (echinating) styles are few and scattered, spinose or smooth, the two types intergrading. The spinose type has numerous distinct though small spinulations on the shaft, and a minutely tuberculate, slightly enlarged, head. Spicules with only a few scattered spines occur, and finally quite smooth spicules with head end simply rounded and not enlarged.

The terminal branches of the skeletal framework also possess included styles. Such branches break up each into a spreading tuft of long styles. Smaller lateral styles, projecting obliquely, some spinose, some smooth, are also present.

Quantities of young megascleres (very slender) are found throughout the sponge. The microscleres are scantily present. They include isochelæ 12–14μ long, and toxas 30–40μ long. The pores, oscula, canals, and trabeculæ of sponge parenchyma in such a lobe have essentially the same character as in the incrusting type. Amœbocytes are abundantly present throughout the lobe.

Structure of large branched specimen.—Comparison makes it obvious that large branched specimens, like that shown in figure 1, arise through continued growth and anastomosis of lobular outgrowths of younger specimens. Any part of such a sponge therefore repeats the structure of one of these outgrowths, although there are details of structure in which the older sponges differ from the young. Thus the spiculo-fibers in the former are much thicker than in the latter. The megascleres, too, are thicker, and the small echinating styles are abundant. The larger megascleres may be 12–16μ thick and the head end minutely spinulate. The echinating styles are chiefly spinose and about 80μ long, but smooth ones sometimes larger are also present. Microscleres, which are only scantily present, include isochelæ 12–16μ long, and toxas 16–40μ long.

Microciona prolifera Verrill and Smith, Report on the invertebrate animals of Vineyard Sound, Report U. S. Fish Commission 1871–72, p. 447, 1874. H. V. Wilson, Sponges collected in Porto Rico in 1899, Bulletin U. S. Fish Commission, vol. xx, 1900, pt. 1, p. 396, 1902.

METHOD OF OBTAINING DISSOCIATED CELLS, FUSION OF CELLS, AND FORMATION OF PLASMODIA.

A branched specimen of *Microciona* in good condition is cut with scissors into pieces about one-fourth inch in diameter. The pieces are then strained through fine bolting cloth, such as is used for tow nets. A square piece of cloth is folded like a bag around the bits of sponge and is immersed in a saucer of filtered sea water. While the bag is kept closed with the fingers of one hand it is repeatedly squeezed between the arms of a small pair of forceps. The pressure and the elastic recoil of the skeleton break up the living tissue of the sponge into its constituent cells, and these pass out through the pores of the cloth into the surrounding water. The cells streaming out through the cloth present the appearance of red clouds. They quickly settle down on the bottom of the dish like a fine sediment. By using the branched specimens of *Microciona* large quantities of this "sediment" may be had. The lobes of incrusting specimens or even the sheet-like body of such specimens may be cut up and used, but naturally the dissociated cells are obtained in comparatively small quantity.

If a drop of the "sediment" so obtained be examined at once on a slide with a high power the preparation is seen to consist of myriads of separate cells together with a few spicules. There is a certain resemblance to a blood preparation, which at once suggests itself, sea water occupying the place of the plasma. The cells (fig. 21, pl. IV) fall into several classes. The most conspicuous and abundant are spheroidal, densely granular, reddish bodies about 8μ in diameter. These cells are obviously the unspecialized amœboid cells of the sponge parenchyma (amœbocytes). They put out hyaline pseudopodia that are sometimes elongated, more often rounded and blunt. There is also a great abundance of partially transformed collar cells, each consisting of an elongated body with slender flagellum. The cell body is about 8μ long, hyaline, and without a collar, the latter doubtless having been retracted. The flagellar end is thick and rounded, and contains the nucleus, the body tapering away to a point at the opposite end. The flagella are long and hyaline, and at first are vibratile, the cells moving about. Soon however the flagella cease to vibrate. The third class of cells is not homogeneous. In it I include more or less spheroidal cells ranging from the size of the granular cells down to much smaller ones. Many of these are completely hyaline, while others consist of hyaline protoplasm containing one or a few granules.

Fusion of the granular cells begins immediately and in a few minutes' time most of them have united to form small conglomerate masses which at the surface display both blunt and elongated pseudopodia (fig. 22, pl. IV). These masses soon begin to incorporate the neighboring collar and hyaline cells. One sees collar cells sticking fast by the end of the long flagellum to the conglomerate mass (fig. 22). Other collar cells are attached to the mass by short flagella. Still again only the body of the collar cell projects from the mass while there is no sign of the flagellum (fig. 24). Similarly spheroidal hyaline cells of many sizes fuse with the granular conglomerates.

The small conglomerate masses first formed early begin to fuse with one another, while they still continue to incorporate outlying free cells. The space under the cover glass thus soon becomes occupied with numerous small balls or masses (fig. 24, pl. IV), which are of a syncytial nature. As the sequel shows, these masses continue to unite and eventually restore or regenerate the sponge. They may be spoken of therefore as masses of regenerative tissue, and the observations already described make it plain that they are composed chiefly of the spheroidal granular cells or amœbocytes, but that other cells, collar cells in particular, enter into their composition.[a]

The small syncytial masses of regenerative tissue produced in the way described attach with some firmness to the substratum. In order to watch their further history they must be kept healthy, and with this point in view it is advisable to proceed in the following fashion from the beginning. After the cells squeezed out from the sponge have settled over the bottom of the dish, the water is poured off and fresh sea water added. This should be done shortly, 10 to 15 minutes, after the cells have been squeezed out. By this time the fusion of cells has progressed so far that the tissue exists in the shape of innumerable small conglomerate masses with free cells between. The tissue is easily handled. It may be sucked up with a pipette and then strewn over cover glasses, slides, cloth, watch glasses, shells, etc.

For the purposes of observation it is best to strew the tissue sparsely over slides and covers. But if one wishes really to breed sponges, it is better to strew the tissue more thickly over slides or clean oyster shells. The slides, covers, whatever is to be used, are placed in a large dish filled to about the depth of 2 inches with clean sea water. The tissue is dropped from the pipette. It sinks down through the water on to the slides, to which it at once begins to attach. Attachment is at first easily broken and for about half an hour all disturbance of the water must be avoided. At the expiration of that time the slides should be gently removed from the water and held for a moment in such a position that they drain. The draining off of the water causes the tissue to sink closer to the substratum, to which it makes a firmer attachment. The object with its coating of sponge tissue is now gently replaced in a dish of fresh sea water, where it should lie for about 24 hours. During this period the water should be changed several times, or the object may be kept in a running aquarium, in which it should be protected from any considerable agitation of the water. After a day the attachment of the

[a] For a brief discussion of the question as to the fundamental nature of this regenerative tissue see my paper: On some phenomena of coalescence and regeneration in sponges, Journal of Experimental Zoology, vol. V, 1907, no. 2, p. 250–252.

tissue to the substratum is so firm that the object (slide or shell) may be removed to an out-of-door live box.

The form of live box I have used has a wooden frame 3 feet by 2 feet by 18 inches. The sides, top, and bottom are all made of coarse galvanized-wire netting. There is a door of some size in the top. Round the edge of the box there is a wide strip of wood which projects like a shelf and serves to keep the box floating. After a trial of several places I have found that the best situation in which to keep such boxes is under a wharf where the sponges are somewhat protected from the sun, and where the current is fairly strong and the water therefore clean. In the live box are some crossbars of wood. To these are attached the small galvanized-wire boxes in which are put the objects coated with the sponge tissue. The latter boxes afford an additional protection to the growing sponges. They are especially useful for slides. Shells may have a hole bored through them with a drill and be suspended directly by wires from the crossbars. If the small box is to be made for slides, it will be found convenient to proceed as follows:

Take a rectangular piece of galvanized-wire netting and fold the edges up, thus making a long, shallow box wide enough for an ordinary slide. Prepare a piece to serve as the top. Immerse the box and tie the slides to the bottom. The slides should be exposed to the air as little as possible. After the top has been tied on, the boxes, each with a number of slides, are suspended from the crossbars in the live box. The slides may be removed, if it is desired, from day to day and examined under the microscope in a glass dish of water. Thus the gradual transformation of the coating of sponge tissue into a functional sponge may be followed.

In the course of a week it will be found that the slide is covered with a thin incrusting sponge provided with pores, oscula, canals, and flagellated chambers. If slides or shells on which sponges have been started in this way are kept suspended in the live box for one to two months, they grow thicker and develop the characteristic species skeleton. Sponges were grown very successfully in this way during the past summer by my assistant, Mr. R. R. Bridgers. Among the hundred or so sponges which survived accidents during two months many had at the end of that time developed reproductive bodies (egg or asexual embryos?) and several had developed lobular outgrowths like those of the specimen shown in figure 2.

As already stated, for the purposes of observation it is best to scatter the tissue sparsely over covers or slides. And these may be kept in laboratory dishes or aquaria. Differentiation goes on at a decidedly slower rate than in preparations placed in the live box.

Some stages in the later history of the conglomerate masses first formed (such as that shown in fig. 24) are shown in figures 3, 4, and 6 of plate 1. The conglomerate masses exhibit amœboid changes of shape and throw out pseudopodia all over the surface. Many of the pseudopodia are fine, filose processes, others bleb-like, while others are processes of some size, covered themselves with delicate small pseudopodia. Neighboring masses fuse together. The resultant masses may be rounded or irregular or have the character of networks. Figure 3 is a photograph of a cover-glass preparation. The

sponge cells were strewn over the cover, and the preparation preserved 40 minutes later. Independent syncytial masses, some rounded, some irregular, are present. The formation of networks has begun. Between the masses, easily seen at this magnification (×12), are abundant very minute masses and free cells. In figure 6 is shown, more highly magnified, one of the small syncytial masses of a preparation like figure 3. Other smaller syncytial masses appear in the neighborhood. Figure 4 is a photograph of a cover-glass preparation preserved 10 minutes after the sponge cells were strewn over the cover. The culture drop that was sown on the cover was very thick, and in details this preparation differs from the more common forms. The sponge cells have combined in part to form individual masses but these have very early begun to unite with one another to form extensive reticula. Free cells and minute masses are very abundantly scattered between the masses that are large enough to be distinct at the magnification used.

As regards the further history of the collections of syncytial masses, such as are shown in figure 3, the details of behavior vary, being largely dependent on the amount of tissue which is deposited in a spot and on the strength of attachment between the mass of tissue and the substratum. Very commonly fusion of the masses, large and small, goes on until coarse reticula are produced. Figure 8 gives a good idea of such a reticulum. The figure is a photograph slightly larger than natural size of a typical slide preparation. The small syncytial masses gradually continued to fuse with one another until a reticulum was formed composed of cords for the most part 1–2 mm. thick. The sponge tissue was strewn over the whole slide except at the ends, and practically all of the tissue was absorbed into the reticulum. The cords are compact and, except where they adhere to the substratum, rounded. Their structure is that of a dense syncytium, the outermost layer of which forms a smooth limiting membrane. After the formation of such a reticulum the peripheral cords begin to flatten out, spreading over the slide as thin incrustations which completely fuse with one another. This is the condition of the preparation shown in figure 8. The flattening out of the rounded compact cords continues, gradually involving the more centrally located parts of the reticulum. Eventually the whole reticulum is transformed into a thin, even incrustation which completely covers the slide. A slide preparation in this condition is shown in figure 10, the incrustation interrupted along the lines where the ties were made around the slide.

The sponge tissue strewn over the slide (or substratum in general) does not always form a reticulum of cords such as that just described. It often aggregates around separate centers, forming distinct masses which may be rounded or irregular in shape. Such masses are dense with smooth surface and in structure are quite like the cords. It often happens that on the same slide part of the tissue combines to form a reticulum and part to form discrete masses. This was the case with the preparation shown in figure 7. The separate masses flatten and transform into incrustations as do the cords, and the incrustations as they spread commonly unite as described above.

A third variation may here be mentioned. The small dense syncytial masses of sponge tissue, instead of combining to form an open reticulum, may unite so as to give rise to an expansion interrupted with minute rounded apertures. Such an expansion will be referred to as a perforated plate. This condition is illustrated by parts of the slide

preparations shown in figure 8, plate II, and figure 17, plate III, and by the cover-glass preparation, figure 9, plate II. The tissue in this shape flattens and spreads quite as it does in the other types.

These three types, reticula, discrete massive aggregations, and perforated plates, may all be found on the same slide or shell. Moreover, formations that are transitional between the three types are common. The differences are differences of detail. The important fact is that the sponge cells quickly unite to form small, dense, syncytial masses, and that fusion between these goes on until collections of large size (fig. 7, 8, 9, 17) are produced. The larger collections, like the smaller, have the structure of dense syncytia, but unlike the smaller (compare fig. 3 and 6) have a smooth limiting membrane. The larger collections, like the smaller, exhibit amœboid changes of shape, although these are perhaps slower than in the small masses.

The collection of dense syncytial tissue, whatever its shape or size, bears some striking points of resemblance to such an organism as a myxomycete, and such collections may conveniently be called plasmodia. The essential features of the plasmodial state are its simple dense syncytial structure and its slow amœboid power to change shape and position. A plasmodium has only a temporary and chance individuality. It may fuse with others or be subdivided. It is merely a lump or collection of syncytial regenerative tissue.

METAMORPHOSIS OF PLASMODIA.

The flattening of the plasmodial masses, reticula, or perforated plates, and their transformation into thin incrustations constitute a part of what may be called the metamorphosis of the plasmodium. The histological details of the metamorphosis may be reserved for a later study. Only the conspicuous and easily observed steps in the process will be here enumerated.

The first obvious step in the metamorphosis is the appearance of collenchyma (simple connective tissue consisting of branched interconnecting cells) at the periphery of the mass. The collenchyma begins to appear just before or coincidently with the flattening out of the plasmodium. It may be observed in the living mass. With the formation of the collenchyma, a distinct thin epidermal membrane becomes lifted up from the deeper parts of the plasmodium (fig. 31, pl. V). By the time the plasmodium has been transformed into an incrustation (fig. 10, pl. II), the peripheral collenchyma with the overlying epidermal membrane exists everywhere.

Somewhat later flagellated chambers begin to appear in great abundance, and canals develop as isolated spaces which come to connect with one another. A stage in the development of the canals is shown in figure 11, which represents a part of a typical slide preparation kept two days in the live box. The opaque regions indicate where the dense plasmodial tissue lingers more or less unaltered. The canals extend horizontally through the incrustation, and are so arranged as to form radial systems. Each system is composed of a few, usually three or four, main canals. At the center where the main canals meet, an osculum is later formed. Such systems are then efferent systems. The finer

branches of the canals are at this stage imperfectly developed, and the flagellated chambers have scarcely begun to differentiate. Examination shows that the radial systems interconnect with one another.

The distribution of the radial efferent systems is well shown in figure 12, plate II, and figure 13, plate III, both of them photographs of entire slide preparations, the former taken with transmitted, the latter with reflected light. The preparation shown in figure 12 was kept six days in the live box. The flagellated chambers and canal systems are well developed. The movements of the flagella belonging to the collar cells and the currents passing out of the oscula were directly observed with the microscope.

A slightly later stage in the development of the canals is shown in figure 14, representing part of a slide preparation that had been kept eight days in the live box. Three of the efferent radial systems appear. A higher magnification would reveal an osculum at the center of each system. The oscula are sometimes mere apertures in the dermal membrane, but they may also terminate short tubes (oscular tubes) which ascend vertically from the incrustation. The terminal ramifications of the efferent canals are well differentiated in this preparation. Pores are scattered over the dermal membrane. The afferent canals are not conspicuous. Between the efferent canals lie immense numbers of flagellated chambers. How abundant the flagellated chambers are in these young sponges may be inferred from figure 19, plate III, which represents a small part of a cover-glass preparation in about the stage of figures 12 and 14. The chambers are thickly crowded between the efferent canals.

When the plasmodia have metamorphosed and the canals and chambers have developed, the skeleton makes its appearance. In sponges that have been kept a few days in the live box one observes spicules (styles) some of which are strewn horizontally through the body, others of which ascend more or less vertically, projecting from the surface. The latter are arranged both singly and in small tufts. The skeleton in this condition is shown in figure 15, plate III, which represents part of a slide preparation kept eight days in the live box. At this stage the horny columns are exceedingly small, consisting of minute aggregations of spongin round the bases of the ascending spicules. All the spicules characteristic of the species are present. As to the size of the spicules, the chief point of difference from the adult condition lies in the slenderness of the smooth styles. Actual measurements made at this stage of growth are as follows: Larger smooth styles, 200–250μ by 5μ; spinose styles, 72μ by 5μ; isochelæ, 14μ long; toxas, 40μ long. The incrustation at this time is very thin, about $\frac{1}{8}$ mm. thick.

If the preparations are kept in the live box they gradually thicken, and the skeleton continues to develop. Figure 16, plate III, represents a vertical section of a preparation that was kept six weeks in the live box. In removing the incrustation from the glass plate on which it had grown, it was somewhat torn. The incrustation is about $\frac{1}{3}$ mm. thick. There is a distinct basal horny plate. The vertical horny columns are conspicuous. The spicules characteristic of the species are all present, and the smooth styles are as large as those found in many normal incrusting specimens. The smooth styles actually measured in this preparation 250–340μ by 8–10μ. On comparing figure

16 with figure 5, which represents a vertical section of a normal sponge, it will be seen that the regenerated and normal specimens are essentially alike.

In regenerated sponges that are kept one or two months in the live box reproductive bodies make their appearance. In some cases they are found strewn through parts of the incrustation in great numbers, precisely as in the normal sponge. One of these bodies is represented in figure 16. I have not worked out the origin of the reproductive bodies, and so am unable to state whether they arise from eggs or masses of cells.

Some of the *Microciona* slide preparations that were kept one to two months in the live box grew to be 1 mm. thick in regions, and developed lobular outgrowths such as those on the sponges shown in figure 2, plate 1. In a preparation before me such lobular outgrowths vary in height from 1 mm. to 10 mm. and in thickness from 1 mm. to 4 mm.

It is clear from the preceding account that *Microciona* can be perfectly regenerated by this method of growth from dissociated cells.

The question may arise how large or how small a mass of the plasmodial syncytial tissue will transform into a sponge. The question would seem to be a purely physiological one, for the idea of morphological individuality is not applicable to the plasmodial tissue nor indeed even to the sponge itself. Fusion or subdivision may constantly occur both among the plasmodia and in the case of the perfected incrusting sponge, whether large or of microscopic size.

The upper limit to the size of incrustations formed by the fusion of plasmodia is obviously not determined by intrinsic laws of differentiation, but by the success or absence of success with which the different regions of each incrustation meet in the struggle for food and against enemies.

The lower limit can not be stated. Small plasmodia, instead of fusing, may flatten and metamorphose into tiny sponges only a fraction of a millimeter wide. The two cover-glass preparations represented in figures 18 and 20 show numerous such small plasmodia. Experience in rearing sponges grown in this way shows that the very little ones are at a disadvantage. They frequently die and disappear when larger incrustations under the same conditions continue to live and grow. There must of course be a lower limit to the size of the tissue mass which can directly (without further growth) transform into a sponge having osculum, canals, flagellated chambers, etc. Doubtless a mass of tissue below a certain minimum and outside the body of the parent could only become part of a perfect sponge by fusing with some other mass. Inside the body of the parent such a mass would have the ordinary opportunity of growth that falls to the lot of metazoan cells, and conceivably might increase of itself to the size of an asexual reproductive mass (gemmule).

LISSODENDORYX CAROLINENSIS, New Species.

DESCRIPTION OF SPECIES.

This sponge is common in Beaufort Harbor under the wharves. Habitus changes with age. Sponge exists first as a thin incrustation on shells, piles, etc. With continued growth it throws up ascending lobes 10–20 mm. high, which frequently overlap in an intricate way. Eventually a large, amorphous mass may be produced, incrusting at its base but the body of which has been formed by the

continued fusion of overlapping lobes. The free surfaces of such masses bear projecting lobes like those of the younger stages, and doubtless the mass continues to increase in size by the growth and fusion of these lobes.

Color, white, frequently with a green or blue cast. Sponge is firm and brittle and generally dirty. It is much infested with worm tubes and overgrown with hydroids and polyzoa.

The whole surface is abundantly covered with tubular translucent papillæ perforated with numerous pores. Papillæ may be simple or slightly branched, often bifurcating. They are contractile and may almost entirely disappear. When dilated they are about 3–5 mm. long and 1 mm. wide. Oscula 1–2 mm. in diameter are scattered over the surface of the incrustation and often develop at or near the ends of lobes. They are sometimes mere apertures in the dermal membrane, but more often are raised up on short collenchymatous tubes. The surface in all stages of growth exhibits numerous ramifying and anastomosing canals which extend just below the dermal membrane. Pores are abundantly scattered over the dermal membrane and, as above stated, over the tubular papillæ.

Spicules: (1) Style, smooth and slightly curved, 160–180μ by 5–7μ; (2) tylote, smooth, 160–180μ by 5μ; (3) sigmas, 20–36μ long; (4) isochelæ, 12–24μ long. Internal skeletal framework a loose irregular reticulum of styles, commonly polyspicular, which may in places develop into spiculo-fibers. Spongin seems to be absent. In wall of larger canals tylotes are found. The peripheral or ectosomal skeleton includes (1) tylotes in radiating loose bands which support the dermal membrane, (2) tylotes which project radially from the dermal membrane singly or in tufts of a few, (3) tylotes strewn horizontally in the dermal membrane.

The microscleres are sigmas and isochelæ. The latter are isochelæ arcuatæ (Levisen, 1893), viz, have at each end a median tooth with two lateral alæ, and the axis is strongly curved. In the interior especially sigmas are found, although round the larger canals there are some isochelæ. In the dermal membrane both isochelæ and sigmas are abundant.

Wall of the pore papillæ contains abundant tylotes strewn horizontally, and a few isochelæ.

FORMATION OF PLASMODIA.

The following experiments show that the dissociated *Lissodendoryx* cells can carry on the process of fusion with the consequent formation of plasmodial masses of considerable size. In one experiment the masses began to die early. In another experiment they gave no signs of dying but remained inactive and did not metamorphose. It is more difficult to get this species-tissue free from dirt than that of *Microciona*. Again the absence of a horny skeletal framework (which by its elastic recoil would tend to scatter the sponge cells) may make it more difficult to dissociate the cells in a healthy condition. Or the failure of the plasmodial masses to go on and metamorphose may be ascribed to a less hardy nature of this species-tissue.

Experiment record, August 11, 1907.—Specimen from under laboratory pier was cut up into pieces, and the pieces strained through bolting cloth into Minot watch glasses. The sponge tissue comes out in clouds made up of cells and minute groups of cells. Practically no skeleton is intermixed. As the tissue settles to the bottom, it is shaken into center of watch glass, and is then strewn with pipette over cover glasses in saucers.

The tissue behaves quite as in the case of *Microciona*. The cells and small cell groups display the same amœboid phenomena, and attach to the glass. They fuse and in the course of a day give rise to plasmodial masses, some rounded, some irregular, others in the shape of networks, essentially as in *Microciona*. The plasmodial masses

were kept in the laboratory one day longer. They remained unchanged and were then discarded.

Experiment record, August 22, 1907.—Specimen from Gallant's Point wharf was cut up and pieces strained as above. The tissue was treated in the same way. The cells and cell masses carried out the preliminary steps in the fusion process, but the tissue soon began to die.

STYLOTELLA HELIOPHILA, New Species.

DESCRIPTION OF SPECIES.

This *Stylotella* is the most abundant sponge in Beaufort Harbor. Common on the bottom in shallow water attached to shells, also under wharves attached to piles, stones, etc. Habitus varies. Sponge incrusts the shell or other substratum and grows up in shape of lobes. These may be quite independent of one another. More commonly the ascending lobes fuse where they touch, and thus a more compact mass is produced reaching but rarely exceeding 100 mm. in diameter, in which the original lobes remain conspicuous. The oscula are for the most part at the ends of the lobes or at the ends of tapering more or less conical outgrowths from the lobes. Pores scattered over dermal membrane. Surface is diversified in appearance, owing to the canals which course in the ectosome, and is very generally roughened with minute conulose elevations ½ to 1 mm. high. Color, orange, sometimes with a greenish cast.

The only spicule in the sponge is a smooth style 120–350μ by 4–8μ. Spicules of interior are scattered irregularly. The arrangement may in places approach the condition of a reticulum, or the spicules may combine to form vague spiculo-fibers or tracts. Spongin seems to be entirely absent.

At the surface are abundant more or less radially arranged styles, some of them slightly projecting, in places combined to form vague tufts. In some regions the ectosomal styles are about horizontal, often forming loose tracts which fray out in a brush-like fashion at the end.

FORMATION OF PLASMODIA.

The following experiments show that the dissociated cells of *Stylotella* will unite to form plasmodial masses. The behavior of the tissue is slow and feeble as compared with *Microciona*. In the actual experiments the plasmodial masses did not transform. The tissue is certainly not hardy and dies easily. Possibly it needs the better aeration of the outside water. The syncytial masses produced during the gradual degeneration of this species in aquaria[a] have never transformed for me in laboratory aquaria, but have transformed into functional sponges when removed to the harbor.

Experiment record, August 9, 1907.—Specimen of *Stylotella* kept one day in aquarium was cut into pieces, and the pieces strained in the usual way into large watch glasses. The dissociated cells settle on the bottom and are strewn with pipette over cover glasses. The tissue behaves in essentially the same way as the *Microciona* and *Lissodendoryx* tissue. Small masses are quickly formed, and these establish connection with one another, thus producing fine plasmodial networks. Part of a cover-glass preparation showing such a network is represented in figure 33. The cords of the network have a dense syncytial structure and are $\frac{1}{6}$ to $\frac{1}{3}$ mm. wide.

A number of such cover-glass preparations were made and kept in laboratory dishes. On some covers the plasmodial networks remained unchanged and after a day or two

[a] Wilson, H. V.: A new method by which sponges may be artificially reared, Science, n. s., vol. xxv, no. 649, 1907.

died. On other covers the networks gradually contracted so as to produce thicker sheets of tissue. These in part were continuous and in part perforated with gaps which represented the spaces of the earlier reticulum. The preparations died in this condition. On still another set of covers the plasmodial networks continued to contract, and in the course of a couple of days had so contracted as to be in the shape of numerous distinct, spheroidal masses, many of which were in the neighborhood of 1 mm. in diameter. These, too, died after some days without further change.

Experiment record, August 23, 1907.—Stylotellas were cut up and strained in the usual way. Only the basal denser parts of the sponge were used. The tissue was spread over the bottom of saucers (50 mm. diameter), and these were soon transferred to large crystallization dishes of sea water. On the following morning the tissue covered the bottom of the saucers partly in the shape of reticula, partly in the shape of continuous incrustations having a ridged and exceedingly irregular surface, and partly as small isolated masses of spheroidal or irregular shape. These various kinds of plasmodia developed no further, but gradually died.

RESULT OF INTERMINGLING DISSOCIATED CELLS OF MICROCIONA AND LISSODENDORYX.

As the following experiments show, when the dissociated cells of these two species are intermingled, they do not fuse with one another, but fusion goes on between the cells and cell masses of one and the same species. Perhaps if the mixture were made under conditions such as those which make cross fertilizations possible that normally will not occur, better success might be had. As I have said elsewhere,[a] the more promising task is to find allied forms, the tissues of which will fuse under natural conditions.

Experiment record, August 9, 1907.—Dissociated cells of *Microciona prolifera* and *Lissodendoryx carolinensis* were prepared in the usual way in separate watch glasses. In each case the cells and small cell masses began to fuse quickly. The bulk of the tissue, including all the coarser masses, was then removed with a pipette from each watch glass. There were thus left only the very smallest masses and separate cells strewn over the bottom. These were dislodged with pipette and collected in center of watch glass. The two collections of tissue, the one of *Microciona*, the other of *Lissodendoryx*, were then brought together in the same watch glass, and were thoroughly intermixed by use of the pipette.

The *Microciona* tissue is bright red, the *Lissodendoryx* tissue greenish. The contrast of color is very marked between masses of any size. Between cells or very minute cell masses the difference in color is of course much less conspicuous. The mixture of tissues in the watch glass was kept under constant observation, but the behavior of individual cells and of the most minute cell masses was disregarded. The mixture of tissues was spread evenly over the bottom of the watch glass, and looked like a fine sediment. Fusion began, and the bottom was soon covered, no longer with a continuous "sediment" but with discrete small masses, some red, some green. Pseudopodial activity was observed at the periphery of both kinds.

[a] Wilson, H. V.: On some phenomena of coalescence and regeneration in sponges, Journal of Experimental Zoology, vol. v, 1907, no. 2, p. 253.

Fusion of the small masses continued. In general red mass fused with red mass, and green mass with green mass. Nevertheless fusion was also observed in some instances between red and green masses, the two putting out pseudopodia on the confronting surfaces, and the masses later coalescing bodily. Such fusions, as the further history of the watch glass showed, must have been temporary or the combined masses soon died. For as fusion progressed and the masses increased in size, the distinction between red and green tissue became more evident. In the course of one to two days the red tissue went through the preliminary step of metamorphosis, flattening out in shape of small thin plasmodia, which established connection with one another. The green (*Lissodendoryx*) tissue remained in compact masses. In figure 32 the two kinds of masses are shown at this stage of development. The *Lissodendoryx* tissue is stippled, the *Microciona* is unstippled. It will be seen that, while the *Microciona* and *Lissodendoryx* masses are sometimes closely applied, they are distinct bodies.

The *Microciona* masses increased in size, and on August 16 had completed the metamorphosis, viz, had flagellated chambers and some canals. The *Lissodendoryx* masses gradually diminished in number, doubtless dying. Those that survived until August 16 were of about sizes shown in figure 32, but had flattened out somewhat.

In this experiment fusion took place between the cells and cell masses of the same species. Whatever fusion there was between the two kinds of tissue was insignificant in amount.

Experiment record.—In preparing for the last experiment, as stated above, the coarser masses were removed from the watch glasses containing respectively the two kinds of tissue. A quantity of these coarser masses of *Microciona* tissue was now (Aug. 9, 1907) thoroughly mixed with a like quantity of similar *Lissodendoryx* tissue in a watch glass. Fusion went on, and, as before, between masses of the same species. The red (*Microciona*) masses increased greatly in size, and on August 12 had the shape of irregular plasmodia, which were flattened and thoroughly adherent to the glass, the different plasmodia more or less interconnected. In figure 29 some of the interconnected plasmodia are represented (unstippled) at this stage. The green (*Lissodendoryx*) masses resulting from continued fusion did not become so large. Many of them disappeared (died or failed to attach well and were washed off?) during August 9 to 12. Those that remained on August 12 were compact and not flattened. In this condition they appear (stippled masses) in figure 29. It will be seen that in some cases they lie in close contact with the *Microciona* plasmodia, and may even be surrounded by the latter, but no real union between the two kinds of bodies exists. By August 16 the *Microciona* plasmodia had metamorphosed completely, viz, were thin incrustations with flagellated chambers and canals. A good many of the *Lissodendoryx* masses were still left on this date, some in shape of fairly thick compact masses, others flattened out and thin. None had metamorphosed. Possibly if the mixture were made in dishes at once exposed to the water of the harbor, better results might be had.

Experiment record, August 9, 1907.—Dissociated cells of *Microciona* and *Lissodendoryx* were prepared in the usual way and the two tissues thoroughly mixed in equal

quantities in a dish of sea water, as soon as possible after the extrusion of the cells, and while only separate cells and fine cell masses existed.

The mixture was then quickly strewn with pipette over eight cover glasses immersed in sea water. The formation of small compact masses, some red, some greenish, in about equal number, ensued. These grew by fusion with their own kind. After a couple of days the *Microciona* plasmodia were thriving, but the *Lissodendoryx* masses had decreased in quantity.

By August 12 the condition of one of the covers was as follows: The *Microciona* masses were thin and incrusting and had begun to metamorphose, viz, had flagellated chambers. The *Lissodendoryx* tissue was in the shape of compact masses, many now beginning to disintegrate, but others spheroidal, smooth, and healthy looking. In places small spheroidal masses of *Lissodendoryx* tissue remain embedded in the metamorphosed *Microciona*. The total amount of *Lissodendoryx* tissue is very small as compared with that of *Microciona*.

The second cover-glass preparation was on August 12 for the most part like the one just described. But in exceptional places the condition was that shown in figure 28, where the *Lissodendoryx* tissue is again represented by stippled and the *Microciona* tissue by unstippled areas. The *Lissodendoryx* tissue forms a somewhat large, flattened, but not very thin mass, which is evidently still in the original dense syncytial condition. On it the *Microciona* tissue has settled in the shape of spheroidal masses, also in original dense syncytial state. Near by a partially metamorphosed *Microciona* plasmodium is shown. Here there has evidently been a relatively long-continued opportunity for fusion between the dense syncytial masses of the two species, but no fusion has occurred. By August 16 the *Microciona* masses have flattened out over the underlying and still unchanged *Lissodendoryx*, and have in part fused with one another.

The remaining cover-glass preparations of this set on August 12 offered nothing different from conditions found on the two covers just described. On August 16 they were all about alike, the *Microciona* plasmodia metamorphosed, the *Lissodendoryx* masses still unchanged. Small compact masses of the *Lissodendoryx* tissue are found here and there in the metamorphosed *Microciona*. They probably die and disintegrate.

RESULT OF INTERMINGLING DISSOCIATED CELLS OF MICROCIONA AND STYLOTELLA.

In endeavoring to bring about fusion between these two kinds of tissue, the same methods were followed and essentially the same results were obtained as for *Microciona* and *Lissodendoryx*. The cells and cell masses of each species tissue fused with one another, and there was an absence of fusion between the tissues of the two species. The *Stylotella* tissue is brown and easily distinguishable from *Microciona* tissue.

The following experiment on plasmodial masses of some size is recorded as perhaps of value for guidance in future work:

Experiment record, August 26, 1907.—Plasmodial masses of *Microciona* and *Stylotella* were placed in contact about 9 p. m., to test whether they would fuse. The *Microciona* plasmodium was of reticular character and had begun to curl up round the

edge. Three small irregular flattened *Stylotella* masses were selected, and were placed upon the *Microciona*.

The condition of these plasmodia on the next day at 3 p. m. is shown in figure 30. The *Microciona* reticulum has contracted into a compact ovoidal body. The *Stylotella* masses have fused with one another and form the upper irregular mass lying upon the *Microciona*. The two tissue masses are tightly adherent, but there is no fusion. Other similar attempts to bring about fusion between plasmodial masses of the two species were made with the same negative result.

EARLIER EXPERIMENTS ON MICROCIONA CHRONOLOGICALLY ARRANGED.

For the use of those who may carry on investigations such as are reported in this paper I here append some of the earlier experiments leading up to the method finally practiced. The general account given for *Microciona* is based on a large number of experiments made in the latter part of the summer of 1907 and during the summer of 1908. About 200 specimens of *Microciona* were grown by this method during the two summers. The work of 1908 was under the direct charge of my assistant, Mr. R. R. Bridgers.

Experiment record 1, August 2, 1906.—Question involved: If regenerating tissue that is formed in a degenerating sponge is forcibly freed from the sponge and broken up, will the elements recombine outside the sponge body? They do.

A branched specimen of *Microciona* that had been kept in an aquarium long enough for degeneration to have begun was used. In this state the sponge tissue had died in or retreated from the superficial parts of the lobes, which however contained a core of bright red and dense live tissue. The same tissue forms here and there irregular masses on the surface. I have shown experimentally that in *Stylotella* masses of similar tissue have the power of developing into perfect sponges. The tissue therefore is regenerative tissue.

Lobes of the sponge were teased in a watch glass of filtered sea water with needles in such a way as to liberate and break up the regenerative tissue into cells and small cell agglomerates. Many of the cells are more or less spheroidal and contain granules and spheroidal inclusions of varying size. Many inclusions are reddish and the cell may in consequence appear of an opaque red color. Such cells while under observation throw out hyaline colorless pseudopodia, some rounded, some fine and elongated. An infinite number of smaller cells, some with granular or spheroidal inclusions, some nearly or quite hyaline, are also to be seen.

The cell agglomerates are opaque. They are probably made up of both spheroidal and smaller cells. They certainly include numbers of the spheroidal type. Round the periphery pseudopodial activity was watched. The pseudopodia were for the most part rounded, but some were elongated and pointed. Whatever locomotory motion the mass makes is slow and feeble.

By gently shaking the watch glass the cell agglomerates were brought together, and repeated instances of fusion between the masses were observed.

Experiment record 2, August 3, 1906.—Question involved: Can masses of regenerative tissue such as were produced in experiment 1, be made to unite and to form outside the sponge body smooth gemmule-like masses such as are produced in sponges allowed to degenerate slowly in aquaria? Yes.

A branched *Microciona* in which degeneration had begun was selected. The regenerative tissue forms a core in the lobes and discrete masses here and there. Pieces of the sponge were teased in sea water and the regenerative tissue broken up as before. The cells and cell agglomerates were gently forced with pipette to center of watch glass. Fusion of cells and masses, with amœboid phenomena, began at once, and in half an hour quite large irregular masses existed. In the course of a few hours the masses grew enormously through continued fusion. From this time on they adhered firmly to the glass, retaining irregular plasmodium-like shapes, and the growth was inconspicuous. To bring them together once more and induce further fusion they were on the following day forcibly freed with pipette and needle, and to clean them of cellular débris and bacteria were transferred to a tumbler (covered with bolting cloth) in which they were kept actively moving under a fine glass faucet for about 30 minutes. In the course of this violent agitation a good many masses were lost. Those remaining in the tumbler became in the next few hours noticeably rounder and smoother at the surface. From this experiment 18 more or less spheroidal masses were obtained, some of which measured ½ mm. indiameter. They were similar to the small plasmodial masses produced in this species when the sponges are allowed to degenerate slowly in aquaria.

Experiment record 3, July 17, 1907.—Question involved: When regenerative tissue is removed from a degenerating sponge and induced to form masses of some size, will these masses transform into perfect sponges? Result was negative.

A branched *Microciona* that had been kept in aquarium some days was used. Degeneration had set in and regenerative tissue formed as above. Lobes were teased in watch glasses of sea water. The cells and minute cell masses settle down on the bottom like a fine sediment, resembling in appearance small invertebrate eggs. Some fusion quickly takes place. The material is then brought together in the center of the watch glass, where it forms a loose aggregation about 10 mm. in diameter and 1 mm. or less thick. This is left for half an hour for further fusion to take place and is then immersed in a crystallization dish of sea water. The mass of sponge tissue adheres to the bottom of the watch glass. Two such watch glasses (Minot glass) were prepared. About an hour later, to induce further fusion and concentration, the tissue was freed from the bottom of the glass, and the various masses brought together in as dense a heap as possible.

About two hours later the condition of the aggregate was as follows. The appearance is essentially like that of the rough excrescences of regenerative tissue which occur on the surface of *Microciona* when the latter degenerates in aquaria. Much of it is simply an amorphous mass of dense, syncytial, sponge tissue. But this tissue has a tendency to round off into compact smooth nodules or lobes or free rounded masses.

Many of the latter, often ranging from 400μ down, cohere and make up loose masses of any shape which may be several millimeters in diameter.

Fragments of the entire aggregation about 1 mm. in diameter were now hung in a small bolting-cloth bag which was suspended in a live box floating in the harbor. The bag used was rectangular and flat, 60 mm. by 20 mm. on the side and about 2 mm. deep. The two sides of the bag were held apart by wooden strips, and the bag was divided into two compartments. In each compartment several fragments were placed. The bag was opened July 23, and it was found that the masses had not grown or attached. Some had died. The rest were spheroidal and embedded in a mass of débris.

The result of the experiment does not really indicate that the tissue masses were nonregenerative. A later experiment shows that similar masses obtained from a fresh sponge can actually regenerate. Possibly the masses of regenerative tissue obtained from the degenerated sponge require more careful handling.

Experiment record 4, July 19, 1907.—Results: (1) When the tissue is teased out of a fresh sponge in which no degeneration-regeneration phenomena have occurred, the cells and cell masses combine; (2) the compact masses of tissue so obtained transform into sponges when removed to the harbor.

A branched specimen of *Microciona* kept only one day in aquarium, and as yet showing no signs of degeneration, was used. Sponge was cut in pieces and pieces teased with needles in watch glass of filtered sea water. Cells and minute cell masses were liberated in great quantity. These began to fuse, displaying amœboid activities. About one hour later the fusion is aided by gently forcing the tissue to center of watch glass with pipette. The bulk of the tissue is thus gathered at the center, where it forms a loose heap about 7 mm. in diameter. One hour later the general aggregation in the center consists of rounded and irregular masses and lobes. The watch glass is now immersed in crystallization dish of sea water. Three such watch glasses were prepared. In one a good many small skeletal fragments of the teased sponge were left in the aggregation of tissue. In the other glasses an effort was made to remove all skeletal fragments.

The aggregated heaps of sponge tissue, each consisting of many loosely adhering rounded or irregular masses of compact tissue, were divided up some hours later into seven lots. Each lot was put into a compartment of a bolting cloth bag, and the bags suspended in live box. On July 23 the bags were opened and the sponge tissue in each compartment was found to consist of distinct and separate masses, many of which gave signs of development. Some of these masses were now kept in crystallization dishes of sea water containing *Ulva*, others were returned to the bags. On July 29 a final examination showed that many of the masses in the bags had completely metamorphosed, viz, had oscula, flagellated chambers, and canals. Other masses had no conspicuous oscula or canals, but had partially metamorphosed. None of the masses kept in laboratory dishes had completely metamorphosed.

In metamorphosing some of the masses had flattened out greatly, spreading as incrustations over the bolting cloth, the meshes in which were thus completely covered over. Others had remained as compact masses. One such is shown in figure 26. In

this sponge we find a conspicuous system of subdermal cavities, an oscular tube (near one end), and radially projecting spicules. Embedded in the sponge is a considerable fragment of the skeleton of the parent (near pointed end).

Skeletal fragments derived from the parent were present in several but not in all of the masses of tissue which metamorphosed completely. The presence of such a fragment is then not a necessary condition to complete metamorphosis. Nevertheless, the impression from numerous experiments is made on my mind that where the mass of tissue is of some size and does not flatten out into an incrustation the chance of metamorphosis is increased if there is present a piece of the original skeletal framework. This may possibly act as a mechanical support.

Experiment record 5, July 19, 1907.—Result: Tissue teased out of fresh normal sponge quickly attaches to substratum and spreads out as thin sheet. Will such sheets metamorphose, without passing through condition of compact gemmule-like mass? Indications are that they will.

In the preceding experiment when the bulk of the tissue was gathered into center of watch glass, a large number of small masses remained adherent to the bottom. These soon flattened out into thin, irregular plasmodia which were watched for an hour, and were observed to change shape and establish connection with one another. One such plasmodium is shown in figure 25.

These plasmodia were kept under observation and it was found that they did not contract into compact masses but spread as thin sheets over the bottom of the glass. In this condition they were removed to the live box in the hope that they would metamorphose. The plasmodia on July 21 exhibited a slight but significant change. They were no longer homogeneous in appearance, for in many places a distinct surface membrane existed which was separated from the opaque general mass by a space filled with branched cells and colorless transparent matrix. In other words collenchyma had made its appearance. In a day or two some of the plasmodia had what appeared to be flagellated chambers and the beginnings of canals. The conditions in the live box were not good. Sediment was deposited in the watch glasses and the plasmodia did not develop further, eventually dying.

Before disappearing they diminished in size, and became once more homogeneous in appearance (a regressive series of changes). Some of them sent up solid massive processes into the water, as if growing away from the sediment. Others contracted again into minute compact rounded masses. Some of these lose their compact character and break up into separate cells, for the most part spheroidal, that are loosely held together.

Experiment record 6, July 20, 1907.—Question involved: Will smooth rounded gemmulelike masses formed by teased-out sponge tissue transform in laboratory aquaria? They did not.

In the preceding two experiments the contrast in behavior between tissue masses which while small quickly made firm attachment to the glass, and such as did not attach to the glass or were prevented from doing so, was marked. The former spread over

glass as thin incrustations. The latter contracted and fused with one another, forming massive bodies and loose aggregations of such bodies. Massive collections of tissue of this sort, as has been recorded, metamorphosed in the live box.

The effort was made to see if such massive collections of sponge tissue would not metamorphose in laboratory dishes. Small massive bodies were selected which for one reason or another had remained quite free, viz, unattached to substratum. These were 500–700 μ in diameter. They had a dense syncytial structure, were homogeneous in appearance, and had a quite smooth surface—in short, were very gemmulelike. They were kept in laboratory dishes with *Ulva*, but would not transform, behaving then in like manner to the masses of regenerative tissue that form in a degenerating sponge or are produced outside the sponge body.

Several similar masses were put on July 1 in *Ulva* dishes. Some made a slight step toward metamorphosis, in that they flattened out at points of the periphery, here spreading for a short distance over the substratum. Regressive changes then occurred, and on July 27 the bodies were again spheroidal and smooth.

The conclusion seems to be that when the sponge cells have once united into a compact mass of any size, this mass is slow and as it were reluctant to transform. Particularly is this so if the mass of tissue has been free long enough to acquire a smooth surface. It has by this time apparently passed into a quiescent physiological state. For such a mass to set up differentiation, the stimuli coming from the open water (excellent aeration and movement of water probably) are necessary.

Experiment record 7, July 21, 1907.—Question involved: Will compact masses formed by the continued union of tissue teased out of the sponge metamorphose in live box? The masses began but did not complete the metamorphosis. Essentially same experiment as 4.

Fresh *Microciona* tissue was teased up and the teased-out tissue allowed to fuse. The bulk of the tissue was collected in center of dish, where numerous compact masses commonly 0.5 to 1 mm. in diameter were formed. Most of these were more or less united to form larger aggregates.

Two of the compact masses were hung in bolting-cloth bags in the live box on July 22. One was smooth, spheroidal, 800μ in diameter. The other was a composite mass of same character as that shown in figure 27, about 3 mm. long, 2 mm. wide, and 0.5 mm. thick. It included a small fragment or two of the old horny skeletal framework. On July 29 the smaller mass had split into two, each of which was a flattened incrustation firmly attached to the bolting cloth. The two incrustations were opposite, and it was evident that the original mass had attached to both surfaces of the bag. The larger mass had likewise split into two, both of which had flattened out and attached to the cloth. These masses went no further in metamorphosis, but eventually died.

Experiment record 8, July 30, 1907.—Result: Teased-out tissue quickly combines to form small masses. These fuse if brought in contact. If not too large they then flatten in peripheral region which spreads over substratum. On same day tissue pressed out through bolting cloth was found to behave in same way.

Fresh *Microciona* was teased up. The teased-out tissue was brought together so that many small spheroidal masses were formed free of all fragments of the old skeletal framework. About a dozen such masses were then brought together with needle and pipette. They fused, giving rise to the lobed mass shown in figure 27. The width of the whole mass is slightly less than 1 mm., the thickness about 0.5 mm. It contains no skeletal fragments, although close at hand lies a bit of the old skeletal framework. The outlines of the lobes gradually disappeared and on the same day the mass had assumed a simple rounded, subspheroidal shape. It incorporated the outlying piece of skeleton and made attachment at points of the periphery to the substratum. Before the end of the day the peripheral part of the body was extended out over the glass in the shape of a thin sheet, showing pseudopodial activities at its edge, where the incorporation of outlying cells and small masses went on. Doubtless this preparation would have completed the metamorphosis had it been kept.

Experiment record 9, July 30, 1907.—Result: Teased-out tissue strewn over cover glasses formed plasmodia which metamorphosed completely. Pressed-out tissue behaved in similar way.

Fresh *Microciona* tissue was teased out and centripetalized in watch glass, and then strewn over cover glasses. Small masses were formed which flattened and fused and soon formed a continuous thin plasmodial sheet. The covers were kept in laboratory dishes of filtered sea water, and the water was changed several times a day. On August 4 flagellated chambers were distinct and the flickering movement of the flagella could plainly be seen with a Zeiss 2 mm. objective. By August 5 well-developed canals were present, and oscula on short upwardly projecting tubes. The discharge of the current from the oscula was watched. On August 1 pressed-out tissue obtained by straining through bolting cloth was prepared and treated in same way with same result.

Experiment record 10, August 1, 1907.—Result: Pressed-out tissue, when it is strewn thickly enough to form plates, etc., 0.5 to 1 mm. thick, does not transform in laboratory aquaria, but the tissue tends to separate from the substratum and contract into massive shapes. Such collections of tissue will transform in the open water. The firmer the attachment to the substratum, the greater is the chance which the collection of tissue has of metamorphosing.

Pieces of *Microciona* were strained through bolting cloth. The tissue thus pressed out was strewn thickly over fine bolting cloth fastened to coarse galvanized wire netting and immersed in dishes of sea water. Irregular plasmodia formed which combined for the most part into fine networks, such as that shown in figure 23, *a*. Isolated masses, rounded or irregular, such as *b* in figure 23, were also formed. By the next morning the plasmodia had changed their character. Concentration of the tissue toward separate centers had occurred, and thus the fine networks had broken up into coarser networks, perforated plates, and more or less compact masses. Various such collections of tissue are represented in figure 23, *c–j*. They are all in the neighborhood of 0.5 to 1 mm. thick and adherent to the cloth. The tissue has considerable rigidity, although without skeleton. Thus it may project up in shape of vertical lobes 1 mm. high, or as vertical

ridges or walls 1 to 2 mm. high, or arches may be formed which rest upon the substratum only at the ends.

By the next day concentration, viz, the aggregation of tissue toward certain nodules or bars and the transformation of coarse reticula into compact masses, had gone farther. It is evident that the masses of tissue were too thick to flatten and spread, and thus the opposite tendency, a tendency to separate from the substratum and contract into massive shape, came into activity. In such concentration the edge of a plate or reticular expansion often curls up, as in *g*, figure 23.

On August 3 the plasmodial tissue was still in the shape of networks, plates, and masses attached to the cloth. The cloth, which was fastened to wire netting, was now hung out in the live box, to give the plasmodial tissue a chance to metamorphose. Two such pieces of cloth, each about 4 inches in diameter, were hung out. There was probably some unintentional difference in the handling, for on one piece all the tissue died, while on the other much of it had by August 10 metamorphosed completely into incrusting sponges with oscula, canals, etc.

On August 3 eight compact small masses, some about 1 mm. in diameter, others 2 to 4 mm. long and about 1 mm. thick, were hung out in bolting-cloth bags. These did not do well. The bags silted up. The sponge masses flattened and spread to some degree over the cloth, but did not metamorphose.

Experiment record 11, August 24, 1907.—Result: In this experiment the attachment of the tissue to the substratum was forcibly interfered with. But it sometimes happens that when no such interference has been made the tissue nevertheless contracts into massive aggregates. It may be said in general that in the history of the early formed plasmodial masses a critical moment arrives when the masses will either flatten out and begin metamorphosis or go on contracting and uniting to form massive aggregates. Such massive aggregates will not transform in confinement. The formation of massive aggregates is furthered by strewing the tissue thickly and by breaking the early attachment to the substratum. The attachment to the substratum is strengthened, I believe, by the use of flat surfaces, such as slides. When the slide or similar body is drained the tissue sinks closer to it and is mechanically somewhat flattened. This aids attachment.

Microciona tissue was pressed out through bolting cloth into a saucer. Bottom of the saucer (50 mm. in diameter) was covered with the tissue. Saucer was left to stand 30 minutes, by which time the tissue had attached in some degree. Water was now poured off and fresh sea water added. The tissue was then dislodged with pipette from the bottom and concentrated toward the center. Saucer now immersed in a large dish of water. Fusion quickly takes place and bottom becomes covered with a fine reticulum and small masses.

Local contraction goes on and in some hours the tissue appears in the shape of coarse reticula, perforated membranes, or isolated compact masses (about as in fig. 23). To hasten or insure the formation of small compact masses it is only necessary to cut off a small part of a coarse reticulum or plate. When so freed from the substratum, the

tendency to contract becomes active and the irregular little sheet gradually draws and rolls up to form a ball.

The tissue in general (reticula and sheets) was on August 27 broken up with pipette into portions from a few to 20 mm. wide and 0.5 to 1 mm. thick. All these continue to contract and curl up. On August 30 these masses were more compact and still quite free from the substratum. Although kept for several days they remained unchanged and did not attach.

Experiment record 12, August 28, 1907.—Question involved: Does a mixture of very fine pressed-out tissue and fairly coarse tissue offer any advantage, for the growth of sponges, over coarse tissue alone? In general it does. The fine particles as they metamorphose tend to fasten down the larger masses.

Pressed out *Microciona* tissue was prepared in abundance. In the course of 30 minutes it was freed with pipette from bottom of dish and collected in center. It was then strewn over slides. Fusion had gone on rapidly and the bulk of the tissue was already in the shape of rather coarse lumps. On some slides only this coarse tissue was strewn. On a second set of slides, after the coarse tissue had been strewn, a quantity of very fine particles was deposited on and between the coarser masses. On all slides the tissue during the next two to three hours attached and underwent the preliminary steps toward plasmodium formation. During the next few hours there was a marked difference between the two sets of slides. Where coarse tissue alone had been strewn local contraction brought into existence masses (spheroidal, irregular, reticulated, etc.) of considerable size and thickness and without much interconnection. Where coarse and fine tissue had been strewn there was formed a continuous network of small, thin, flat plasmodia exhibiting local enlargements and thickenings which represented the coarse masses produced by the early fusion of the tissue. The indication was that the second set of slides would metamorphose first. Nevertheless both sets went ahead in the metamorphosis at about the same rate.

In this instance the coarser and comparatively massive collections of tissue continued to retain their attachment to the substratum. This is not always the case. In handling large numbers of such preparations during the following summer my assistant, Mr. Bridgers, found that the practice of strewing very fine particles of tissue over the preparation that had just been made was often useful. It sometimes happens that the reticula or perforated plates formed by the tissue that has been strewn over the slide or shell begins to separate from the substratum, curling up at the edges. If very fine tissue has been strewn over the slide, it forms small, flat, and thin plasmodia, which fasten down the larger ones. As already said, if one wishes to get sponges, it is important that the plasmodial masses make firm attachment to the substratum before the preparation is put in the live box.

Experiment record 13, July 2, 1908.—Question involved: What difference in behavior is there between tissue pressed out of a fresh sponge and tissue pressed from a sponge kept several days in the aquarium? Tissue obtained from the sponge kept in aquarium is slow to metamorphose, but can do so in the open water.

A large branched *Microciona* was selected. It was divided into a few parts. One of these was chopped up and strained. The extruded tissue was sown on slides. The preparations developed quickly and were put in live box July 3. On July 4 canals had developed in them.

Two days later the other pieces of the *Microciona* were chopped and strained and the tissue sown on slides. The tissue quickly collected in shape of rounded and irregular masses. These do not combine with one another to any extent and do not flatten out. The tissue remains in this condition for a couple of days in the laboratory. Some of the preparations were then hung in the live box. Much of the tissue died, but a considerable number of the masses flattened and metamorphosed. Other preparations were kept in laboratory dishes for a few days longer. They underwent no visible change.

ADDENDUM.

April 17, 1911.

I am fortunately able to take note of the progress that has been made in this field of inquiry while the foregoing paper was in progress of publication.

Müller, working on the Spongillidae,[a] confirms my account of the behavior of dissociated cells in sponges. The phenomena are essentially the same in these sponges as in the marine forms I have studied, and Müller has been able to rear perfectly formed Spongillas in this way. He has kept some of his Spongillas alive in confinement as long as seven weeks. It is to be hoped that he will find the time to carry on a detailed histological study of the cellular changes involved in this method of regeneration, a side of the subject on which my own observations are very fragmentary.

Müller has also been able, again working on the Spongillidæ,[b] to confirm the essential points in my investigation (intimately linked with the present and leading up to it) on the formation of masses of regenerative tissue in sponges that are kept in confinement.[c] Müller finds as I did that in sponges kept for a considerable time in confinement a slow process of regressive differentiation takes place, resulting finally in the production of masses of a simplified or "embryonic" tissue. Such regressive differentiation would fall under the currently employed rubrics "involution" (Barfurth) and "reduction" (Driesch and Eugen Schultz).

The early steps in the process (contraction of body, gradual suppression of canals, dissolution of flagellated chambers into their constituent cells which become despecialized, division of the body in this simplified state) all seem to be identical in the Spongillidæ and in *Stylotella*, the marine form which I especially studied. The differences concern the later stages and consist (1) in the absence of any extensive death of the sponge body in the Spongillidæ and (2) in certain interesting histological features of

[a] Müller, Karl: Versuche über die Regenerationsfähigkeit der Süsswasserschwämme. Zoologischer Anzeiger, bd. XXXVII, nr. 3–4, 1911.

[b] Müller, Karl: Beobachtungen über Reduktionsvorgänge bei Spongilliden, nebst Bemerkungen zu deren äusseren Morphologie und Biologie. Zoologischer Anzeiger, bd. XXXVII, nr. 5, 1911.

[c] Wilson, H. V.: A new method by which sponges may be artificially reared. Science, n. s., vol. XXV, June 7, 1907.

the small simple masses finally produced in the two forms. In *Stylotella* these bodies are aggregations of syncytial protoplasm quite without cell boundaries, and studded with nuclei that are optically all alike. In the Spongillidæ discrete cells can be distinguished in them, apparently of two kinds. Müller finds that the reduced choanocytes are engulfed and digested by some of these cells, the granular elements. As to this question, concerning the persistence or absorption of the choanocytes, I was not able to reach a definite conclusion.

It is important that Müller was able to get one of his reduction masses to transform into a sponge, and so really to prove that the tissue composing such masses is regenertive tissue and that the masses are therefore not stages in a series of purely mortuary changes, the bizarre character of which, in the case of slowly dying protoplasm, must be familiar to many. Possibly the method I employed in handling the *Stylotella* masses, and which permitted them to transform, whereas in laboratory aquaria they uniformly refused to do so, might prove applicable to the Spongillidæ.

As Korschelt and Heider remark in the latest installment of their textbook[a] (p. 486), it is probable that such bodies occur widely in the sponges. The peculiar capsules formed on the surface of *Spongelia* kept in aquaria and described as early as 1886 by Thomson[b] are in all likelihood bodies of this kind. Thomson recognized them as such, and speaks of them "as a histological modification in response to a change in the environment," and again "it seems possible that they may thus secure the persistence of the organism in unfavorable environment." Maas (*vide infra*) has found them in calcareous sponges. Lendenfeld mentions[c] that he has observed similar formations in *Reniera* and *Sycon*. Urban[d] has recently studied their origin in the Calcarea (Clathrinidæ). Müller raises the question whether it is proper to designate these bodies as "artificial gemmules." I agree with him in finding the terminology unsatisfactory. It draws attention away from the fact that what is formed is a *tissue*, a simplified, regenerative tissue. This may take the shape of small spheroidal masses scattered through the interior of the old sponge, in which case the resemblance to the gemmules of the Spongillidæ, or better, to such simpler ones as are formed in the Chalinidæ, is marked. But identically the same tissue may collect in masses scattered over the general surface of the sponge. And here, while some of them may be spheroidal and small, usually they are flattened and of an irregular shape with lobes, suggesting a lobose rhizopod or myxomycete plasmodium.[e] There are no facts which indicate that such masses regularly subdivide into small spheroidal bodies. Thus in the one case the regenerative tissue collects to form masses, the size and shape of which vary greatly, probably being determined by local conditions, while in the other case, in the Spongillidæ, a reproductive

[a] Korschelt und Heider: Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Thiere. Allgemeiner Theil, 4te. lief., 2te. hfte., 1910.

[b] Thomson, J. Arthur: On the structure of Suberites domuncula, Olivi (O. S.), together with a note on peculiar capsules found on the surface of Spongelia. Transactions Royal Society of Edinburgh, vol. XXXIII, pt. i.

[c] Lendenfeld, R. von: Zoologisches Centralblatt., bd. 14, 1907, p. 631.

[d] Urban, F.: Zur Keuntnis der Biologie uud Cytologie der Kalkschwämme (fam. Clathrinidæ Minch.). Internationale Revue der gesamten Hydrobiologie und Hydrogaphie, bd. 3, 1910.

[e] Wilson, H. V.: A new method by which sponges may be artificially reared. Science, n. s., vol. XXV, June 7, 1907.

body of very definite character is produced, the shape, size, and covering layer of which are all fixed as species characteristics. It seems permissible to regard the first case as the habit, still probably universal among sponges, out of which in certain groups a definite gemmule-forming habit sprang phylogenetically.

Various important and stimulating observations on certain steps in the process of regressive differentiation that takes place in sponges when they are kept in confinement or have been subjected to overfeeding, to the cold of winter, or to foul water, have been recorded by Metschnikoff,[a] who cites also from his predecessors Carter and Hæckel, and others, especially Lieberkühn,[b] Masterman,[c] Bidder,[d] and Weltner.[e] A detailed study of the cellular changes that take place in this process has recently been made by Maas.[f] Maas some years ago announced[g] that when calcareous sponges are exposed to sea water deprived of its calcium, the living tissue breaks up into cords and rounded masses. Whether such masses were able to transform into sponges he was not able to say, although he suspected that such was the case. At the same time (December, 1906), at the New York meeting of the American Society of Zoologists I described the phenomena as they occur in *Stylotella* and exhibited the degeneration-regeneration masses, some of them completely transformed into sponges. And in the Proceedings (Science, May 17, 1907) I published a note to the effect that such masses can be produced and that they will transform into perfect sponges. Later in the year Maas published a communication[h] touching upon this subject in which he announced that the rounded masses of cells produced in the degenerating *Sycon* are able to transform into functional sponges. Apparently the calcium-free water leaves the sponge protoplasm in a state that makes further development difficult, for it is clear from Maas's recent paper[f] that the *Sycon* masses are very slow to transform. Maas's statement with regard to the transformation, moreover, leaves it uncertain as to whether this process is completed or not. The masses in question after some weeks increased in size, developed a gastral cavity, and differentiated new spicules (op. cit., p. 100).

Maas in his recent investigation finds, as I described in 1907, that as the reduction progresses a stage is reached in which the sponge flesh consists of trabeculæ made up of several kinds of cells all interconnected to form a syncytium. Maas goes on and traces the history of the several kinds of cells and finds that a process of phagocytosis occurs. Certain granular amœbocytes incorporate and digest the choanocytes and other cells, a mass of these constituting the last stage in the process, the nodule of

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[a] Metschnikoff, E.: Spongiologische Studien. Zeitschrift für wissenschaftliche Zoologie, bd. XXXII, 1879.

[b] Lieberkühn, N.: Beiträge zur Entwickelungsgeschichte der Spongillen. Archiv für Anatomie und Physiologie, J. Müller, 1856.

[c] Masterman, A. J.: On the nutritive and excretory processes in Porifera. Annals and Magazine of Natural History (6), vol. 13, 1894.

[d] Bidder, G. P.: The collar cells of Heterocoela. Quarterly Journal of Microscopical Science (2), vol. 38, 1895.

[e] Weltner, W.: Spongillidenstudien II. Archiv für Naturgeschichte, jahrg. 1893, bd. I. Spongillidenstudien V, ibid, jahrg. 1907, bd. I.

[f] Maas, Otto: Ueber Involutionserscheinungen bei Schwämmen und ihre Bedeutung für die Auffassung des Spongienkörpers. Festschrift zum sechzigsten Geburtstage Richard Hertwigs, bd. III, 1910.

[g] Maas, Otto: Ueber die Einwirkung karbonatfr. Salzlösungen auf erwachsene Kalkschwämme und auf Entwicklungsstadien derselben. Archiv für Entwickelungsmechanik der Organismen, bd. XXII, hft. 4, December, 1906.

[h] Maas, Otto: Ueber die Wirkung des Hungers und Kalkentziehung bei Kalkschwämmen und anderen kalkausscheidenden Organismen. Sitzungsberichte der Gesellschaft für Morphologie und Physiologie in München, 1907.

regenerative tissue. The amœbocytes have a heterogeneous origin, some representing the wandering cells of the normal sponge, many more representing transformed (reduced) pore cells. It may be remarked that our knowledge concerning the very existence of specialized pore cells is exceedingly inadequate except in the case of the ascons. I have, for instance, been utterly unable to find them in monaxonids such as *Stylotella* and *Reniera*.[a] It is evident then that the process of regressive differentiation can not pursue quite the same path in *Stylotella* that Maas maps out for the Calcarea. The large question involved is of course: Do the several kinds of cells, preserving their nature, struggle with one another for the mastery, certain kinds or one kind absorbing and digesting others, and so growing and forming the regenerative mass? In support of this idea it is to be noted that Maas and Müller agree in finding that the choanocytes are absorbed and digested by amœbocytes. Or when the flagellated chambers, the canal epithelium, the epidermis perhaps, all break up into cells which wander away from one another and help to form the mesenchyme-like syncytial tissue of the reduced trabecula, does the protoplasm of all these cells undergo a reconstruction, a sort of rejuvenescence, whereby they all pass into the condition of the unspecialized, generalized plasm of that species, the masses of this plasm fusing intimately to form the regenerative tissue? This is the interpretation of the facts which I have favored in my paper on the subject.[b]

Turning now to the phenomena that follow upon the sudden violent isolation of sponge cells, their rapid fusion to form masses physiologically similar to those produced in the slow process of regressive differentiation that goes on in confinement, the same question meets us. Amœbocytes, hyaline elements, and choanocytes all combine to form the plasmodial masses.[c] Do the amœbocytes absorb and digest the other elements? Or do all the cells as a result of the shock pass into the generalized protoplasmic state and persist as parts of the regenerative mass? A careful histological study might enable one to answer this question. Meantime it seems to me that the latter hypothesis receives support from my recent observations on the fusion of isolated cells in hydroids.[d] In hydroids the body is made up of two specialized layers and there are comparatively few cells present which correspond in this matter of regenerative ability to the amœbocytes of sponges. I have found that a *Eudendrium* colony may be cut into pieces and pressed out after the fashion described in this paper, and so broken up into cells, minute cell masses, and possibly cell fragments. Fusion between these elements goes on and plasmodial masses are formed which secrete a perisarc. Such masses throw out hydrorhizal outgrowths which in successful cases develop perfect hydranths. The same phenomena were observed in *Pennaria* when only the stem was cut up, the regenerative mass being thus exclusively derived from the cœnosarc. In these cases it might, to be

[a] Wilson, H. V.: A study of some epithelioid membranes in monaxid sponges. Journal of Experimental Zoology, vol. IX 1910.

[b] Wilson, H. V.: A new method by which sponges may be artificially reared. Science, n. s., vol. XXV, June 7, 1907.

[c] Wilson, H. V.: On some phenomena of coalescence and regeneration in sponges. Journal of Experimental Zoology, vol. V, 1907.

[d] Wilson, H. V.: On the regenerative power of the dissociated cells in hydroids. Proceedings of the American Society of Zoologists, Science, n. s., vol. XXXIII, Mar. 10, 1911.

sure, be contended that ectoderm cells eventually recombined to form ectoderm, and entoderm cells to form entoderm. The obivous facts are that the cells all combine to form a solid aggregate in which ectoderm, entoderm, and a central yolk mass later differentiate after the general fashion of cœlenterate planulas. The probable interpretation of these facts seems to be that the cœnosarcal cells when thus violently treated pass into an indifferent, generalized state. In this state they recombine to form a mass of undifferentiated tissue comparable to a heap of blastomeras, in which differentiation and growth later occur.

DESCRIPTION OF PLATES.

PLATE I.

Microciona prolifera.

FIG. 1. Branched specimen. ×⅔.

FIG. 2. Two specimens incrusting on shells. Lobular outgrowths have developed. ×⅔.

FIG. 3. Cover-glass preparation photographed in alcohol by transmitted light. Sponge cells were strewn over cover and preparation preserved 40 minutes later. The cells have combined to form small masses, and many of the latter have united to form networks. ×8.

FIG. 4. Cover-glass preparation photographed in alcohol by transmitted light. Sponge cells were strewn over cover and preparation preserved 10 minutes later. Sponge cells have combined in part to form individual masses, in part continuous reticula. ×8.

FIG. 5. Vertical section of normal sponge, incrusting type. Photograph from stained balsam mount. ×30.

FIG. 6. One of the small syncytial masses of a preparation like figure 3, stained with hæmatoxylin. Balsam mount. Photographed by transmitted light. ×160.

PLATE II.

Microciona prolifera.

FIG. 7. Slide preparation photographed in alcohol by reflected light. Plasmodial masses have partially transformed into thin incrustation. ×½.

FIG. 8. Preparation similar to figure 7, but plasmodium had more the character of a reticulum ×⅔.

FIG. 9. Cover-glass preparation photographed in alcohol by transmitted light. Plasmodium partially transformed into incrustation. ×1⅔.

FIG. 10. Slide preparation photographed in alcohol by reflected light. Slide covered with continuous thin incrustation developed from plasmodia. No canals or flagellated chambers as yet. ×1½.

FIG. 11. Slide preparation kept two days in live box, photographed in alcohol by transmitted light. Canals have appeared, but the system is not complete, especially as regards the terminal ramifications. ×3½.

FIG. 12. Slide preparation kept six days in live box, photographed in alcohol by transmitted light. Canals well developed. Dark spots are barnacles. ×1½.

PLATE III.

Microciona prolifera.

FIG. 13. Slide preparation photographed in alcohol by reflected light. Canals have appeared ×2.

FIG. 14. Slide preparation kept eight days in live box, photographed in alcohol by transmitted light. Canal system well developed. Dark spots are barnacles. ×4.

FIG. 15. Slide preparation kept eight days in live box. Photograph made by transmitted light from balsam mount stained lightly in hæmalum. Canal system well developed. Characteristic spicules have appeared. ×20.

FIG. 16. Vertical section of slide preparation kept six weeks in live box. Photograph from stained balsam mount. Characteristic skeleton has developed. Reproductive bodies present. ×73⅓.

FIG. 17. Slide preparation photographed in alcohol by reflected light. Plasmodial masses have partially transformed into thin incrustation. ×⅓.

FIG. 18. Cover-glass preparation photographed by transmitted light. Plasmodia formed by continued union of sponge cells have transformed into incrustations. ×1⅔.

FIG. 19. Small part of cover-glass preparation of metamorphosed plasmodium, showing canals with very abundant flagellated chambers and scattered spicules. ×100.

FIG. 20. Cover-glass preparation photographed by transmitted light in alcohol. Most of the plasmodial masses have metamorphosed into incrustations. A few, two especially, persist as thick rounded bodies which appear as very dark areas in the photograph. ×1⅔.

PLATE IV.

Microciona prolifera.

FIG. 21. Freshly dissociated cells (pressed out through bolting cloth). From a living preparation. Camera, Zeiss 2 mm. Comp. Oc. 6. ×666⅔.

FIG. 22. From preparation shown in figure 21, but 10 minutes later. Many cells have combined to form masses. Camera, Zeiss 2 mm. Comp. Oc. 6. ×666⅔.

FIG. 23. Plates, reticula, and compact masses of the pressed-out tissue. ×1⅓.

FIG. 24. From the preparation shown in figures 21 and 22, about one hour after cells were pressed out of sponge. Mass of regenerative tissue formed by fusion of smaller masses. Camera, Zeiss 2 mm. Comp. Oc. 6. ×666⅔.

FIG. 25. Plasmodium in shape of perforated plate formed by pressed-out tissue. ×13⅓.

PLATE V.

FIG. 26. *Microciona prolifera.* Sponge with oscular tube, subdermal cavities, etc., developed from mass formed by gradual fusion of teased-out tissue. ×13⅓.

FIG. 27. *Microciona prolifera.* Lobed mass formed by continued fusion of teased-out tissue. ×60.

FIG. 28. Plasmodia of *Microciona* and *Lissodendoryx*. *Lissodendoryx* tissue stippled. *Microciona* tissue unstippled. ×16⅔.

FIG. 29. Plasmodia of *Microciona* and *Lissodendoryx*. *Lissodendoryx* tissue stippled. *Microciona* tissue unstippled. ×16⅔.

FIG. 30. Plasmodia of *Microciona* and *Stylotella*. The upper irregular mass is the *Stylotella* tissue, which rests upon the ovoidal *Microciona* mass. ×16⅔.

FIG. 31. *Microciona prolifera.* Small plasmodial mass in early stage of metamorphosis. Collenchyma has been differentiated in several places at the periphery. ×80.

FIG. 32. Plasmodia of *Microciona* and *Lissodendoryx*. *Lissodendoryx* plasmodia are stippled. *Microciona* plasmodia in unstippled water color. ×30.

FIG. 33. *Stylotella.* From a cover-glass preparation, showing plasmodial masses combined to form a reticulum. ×16⅔.

Fig. 1.

Fig. 2.

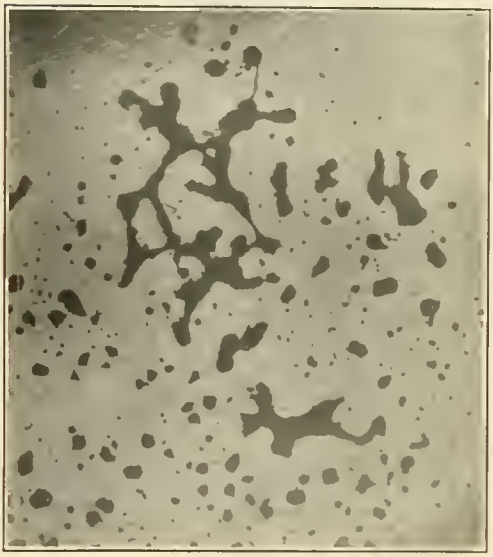


Fig. 3.

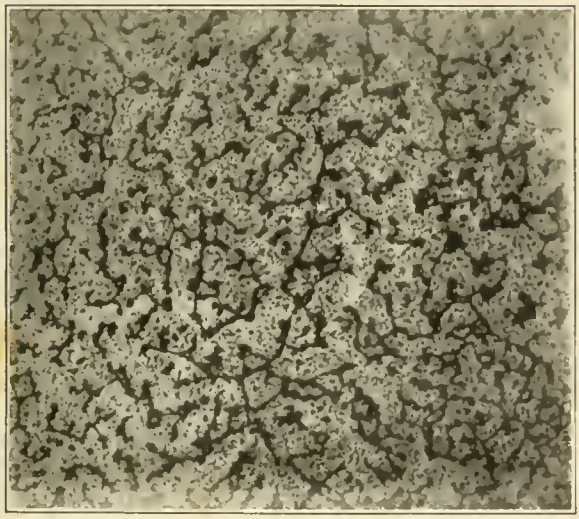


Fig. 4.

Fig. 5.

Fig. 6.

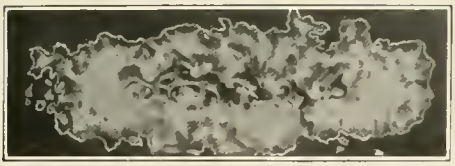


Fig. 7.

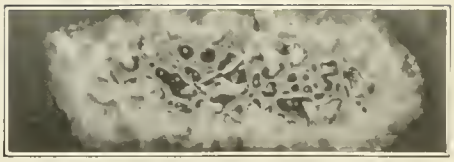


Fig. 8.

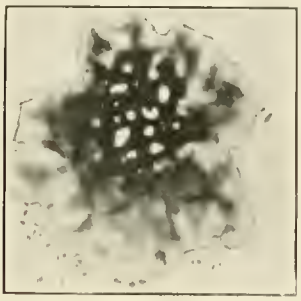


Fig. 9.

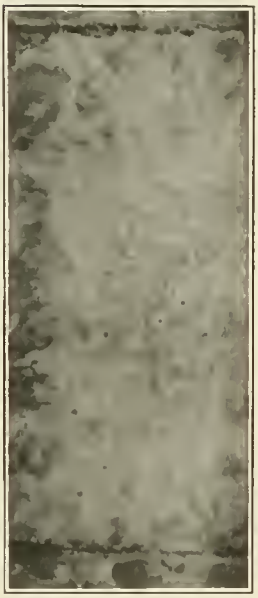


Fig. 10.

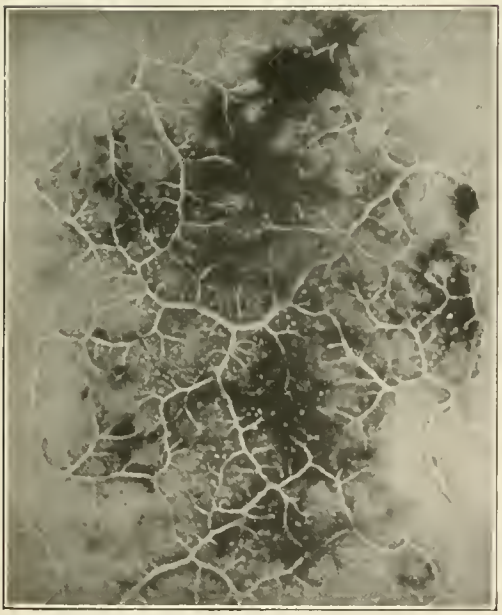
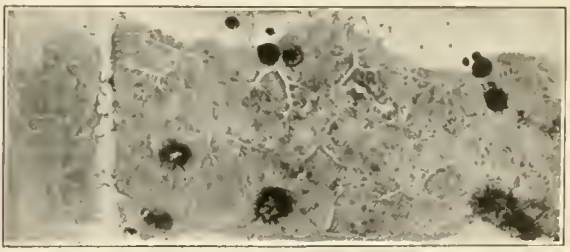


Fig. 11.



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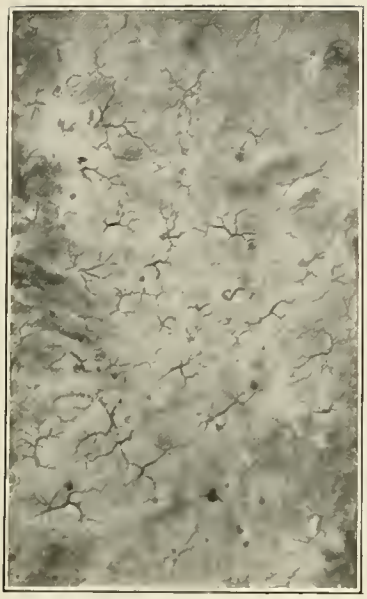


Fig. 13.

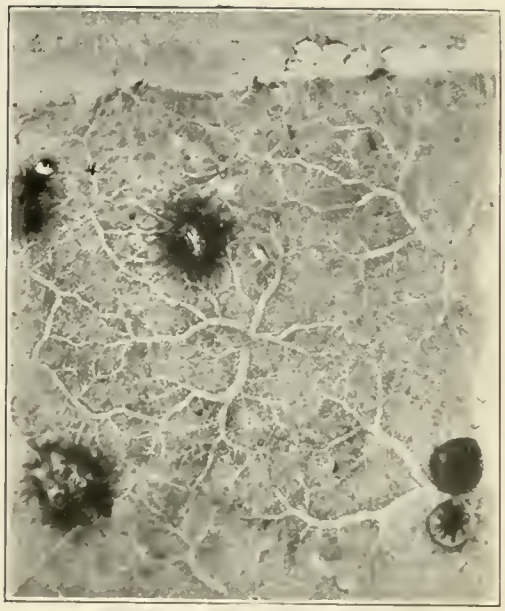


Fig. 14.

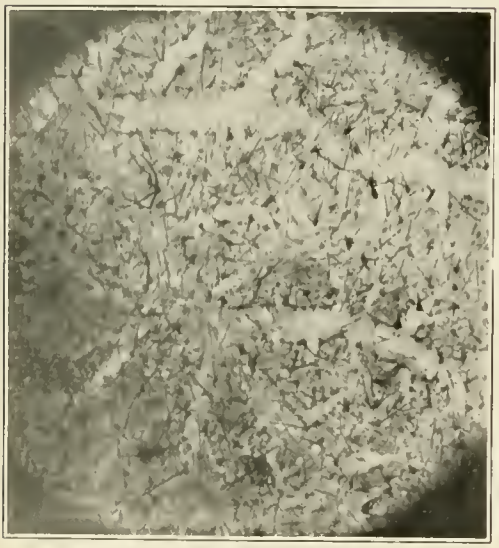


Fig. 15.

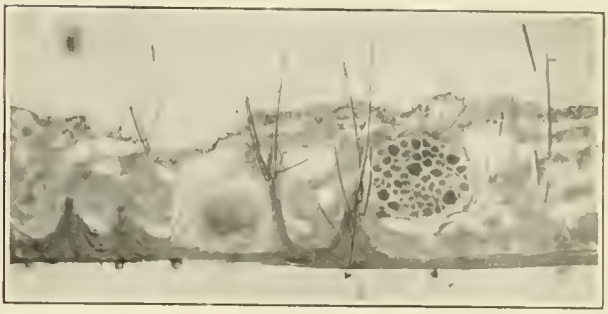


Fig. 16.

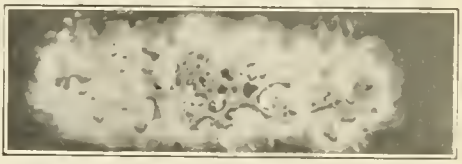


Fig. 17.

Fig. 18.

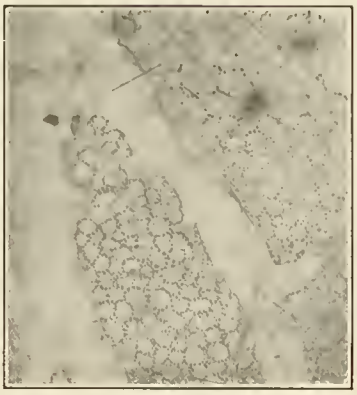


Fig. 19.

Fig. 20.

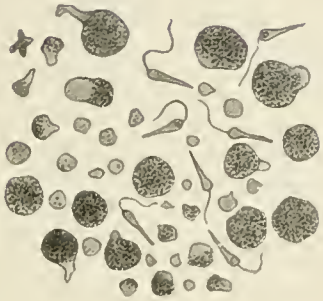


Fig. 21.

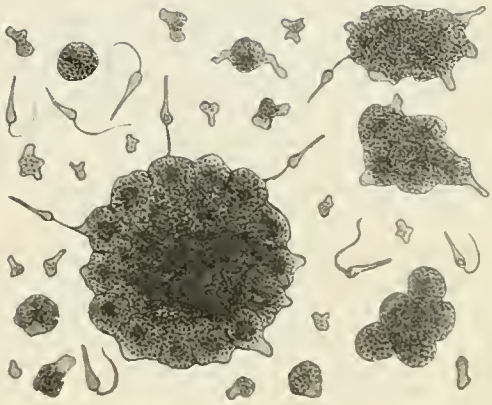


Fig. 22.

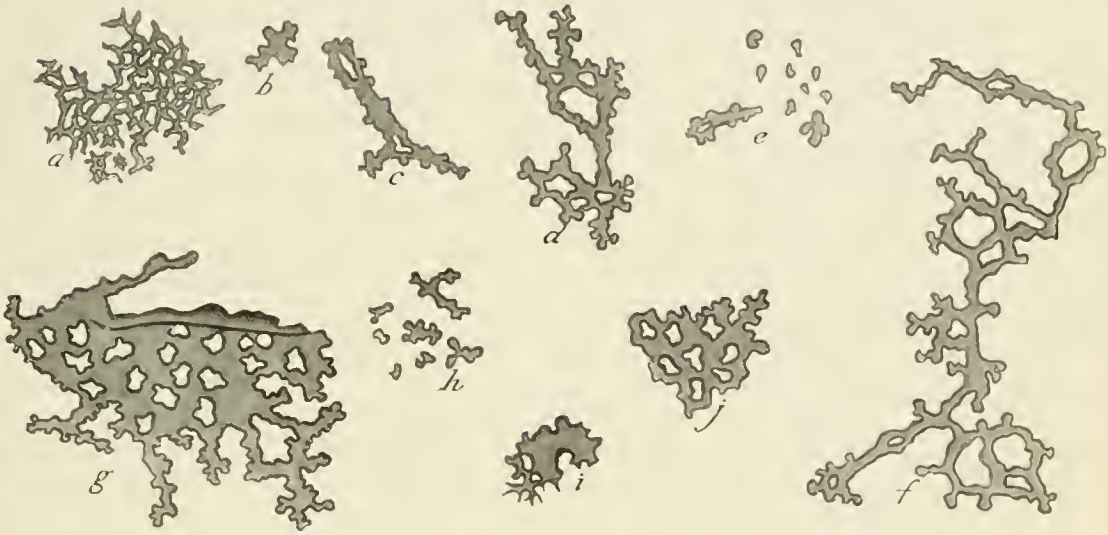


Fig. 23.

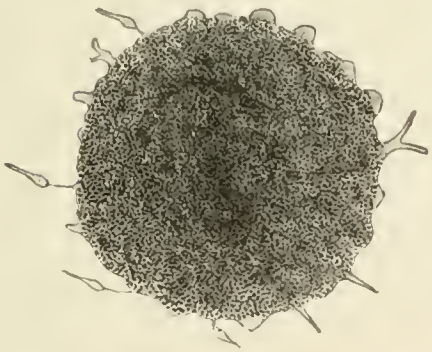


Fig. 24.

Fig. 25.

Fig. 26.

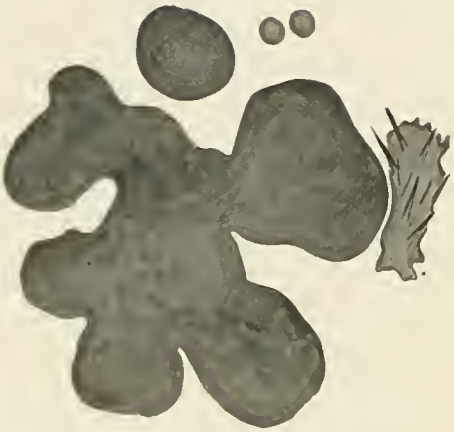


Fig. 27.

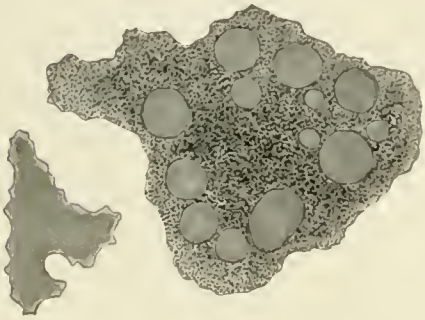
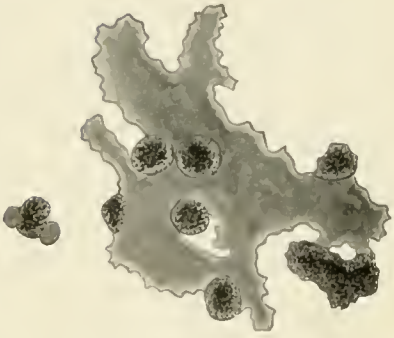


Fig. 28



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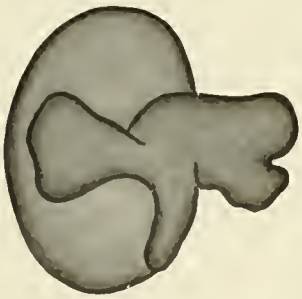

Fig. 30.

Fig. 31.

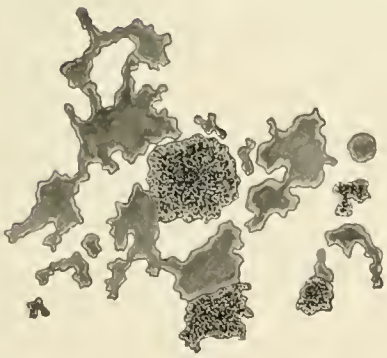

Fig. 32.

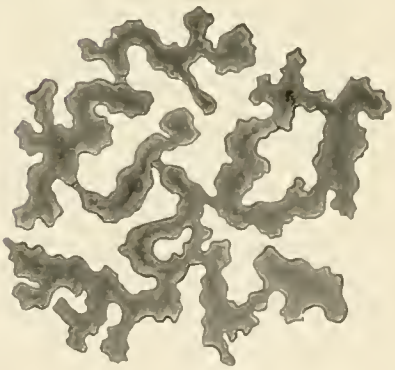

Fig. 33