A NEW METHOD BY WHICH SPONGES MAY BE ARTIFICIALLY REARED.¹

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I have found in the course of an investigation carried on for the Bureau of Fisheries that silicious sponges when kept in confinement under proper conditions degenerate, giving rise to small masses of undifferentiated tissue which in their turn are able to grow and differentiate into perfect sponges. The investigation has been prosecuted during the past three summers at the Beaufort Laboratory. While the degeneration with the formation of the indifferent masses has been observed in several species, it is only in one species, a *Stylotella*, that the process as a whole has been worked out.

This sponge, which is exceedingly abundant in Beaufort Harbor, is a fleshy monactinellid commonly reaching a thickness and height of 10-12 cm. Conical processes with terminal oscula project upwards from the lower body. With this species, which is a light-loving form, I have obtained the best results when outside aquaria, either concrete aquaria or tubs, were used. The method of treatment is briefly this: Into a tub about 60 cm. by 30 cm. and covered with glass, a half dozen sponges, freed as far as possible from live oysters and crabs, are put. They are raised from the bottom on bricks. The tub is emptied, filled and flushed for some minutes three times in every twenty-four hours. Direct rays of the

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sun should be avoided. Tubs answer as well as concrete aquaria, and have the advantage of being movable.

In a day or two the oscula of the sponge disappear, and the surface begins to acquire a peculiar smooth, dense and uniform appearance. Microscopic study reveals the fact that not only the oscula, but the pores also, for the most part close, and the canal system becomes interrupted and in some degree suppressed. The mesenchyme is more uniform, and is denser than in the normal sponge, owing in part at least to the disappearance of the extensive collenchymatous (very watery mesenchyme) tracts of the latter.

The whole sponge may pass into this state and remain without great change for weeks. During this period it shrinks greatly in size, in a given case to one quarter the original bulk. The arrangement of the skeletal spicules becomes much simplified. With the shrinkage in size the sponge becomes more solid, *i. e.*, more of the canal space is suppressed. Some flagellated chambers persist and there are a few small scattered apertures on the surface. The bulk of the chambers disappear as such, the collar-cells transforming into simple polyhedral masses which become scattered singly or in groups in the general mesenchyme. The mesenchyme is a syncytium composed of well-marked cells that are freely interconnected. The sponge in this condition closely resembles *Spongilla* in its winter phase, as described by Weltner.² Presumably water continues to circulate through the body, but the current must be an exceedingly feeble and irregular one.

As a sponge in this condition continues to shrink, it may subdivide and thus a large sponge may eventually be represented by numerous masses, in a given case about 1 cm. in diameter. Now if the sponge in this condition or if one of the masses into which it has split up, be attached to wire gauze and suspended in a live box floating at the surface of the open water of the harbor, the sponge or piece will in a few days grow and redevelop the pores and oscula, flagellated chambers, tissue differentiation, and skeletal arrangement of

² 'Spongillidenstudien, II. Archiv für Naturgeschichte,' 1893.

the normal sponge. Whether in this regeneration the transformed and separated collar cells again unite to form the flagellated chambers, I can not say. I think it very doubtful.

In the two classes of cases just described the sponge as a whole degenerates and slowly shrinks. Cellular death takes place so gradually that at no time is there any obvious corpse tissue or skeletal debris. Much more common and of far greater interest are the following cases. In these a large part of the sponge body dies in the course of two or three weeks, leaving the skeletal network still in place and bearing the brown decaying remnants of the flesh, which, as maceration continues, are washed away. In places, however, the sponge body does not die. Here masses of living tissue are left, conspicuous amidst the dead remains by their bright color and smooth clean surface. These living fragments may be classified into three groups. First, the upper end of an ascending lobe or a considerable part of the body of the lobe may be left alive in its entirety, thus forming a more or less cylindrical mass up to 5 mm. diameter, with a length sometimes two or three times the thickness. The histological condition of these masses is not very different from that of the sponges already described. Such a mass may be said to consist of anastomosing trabeculæ, separated by the remains of the canal system. The mesenchyme composing the trabeculæ consists of discrete cells interconnected by processes to form a syncytium. The flagellated chambers as such have nearly disappeared, although remnants may still be recognized. In them the collar cells have transformed into simple polyhedral bodies that are widely separated. The bulk of the chambers have broken up into their constituent cells, and these are now scattered as elementary parts of the general mesenchyme. When such masses are attached to wire gauze and hung in a floating live-box they transform into perfect sponges.

A second class of surviving remnants includes masses scattered over the general surface of the sponge. These may be spheroidal and small, less than one millimeter in diameter. Usually they are flattened and of an irregular shape with

lobes, suggesting a lobose rhizopod or myxomycete plasmodium. Such masses which may be connected by slender strands are commonly from two to five millimeters in the longest direction. The third class of remnants are found scattered through the body of the dead and macerated sponge, in which they sometimes occupy positions that are obviously favorable for respiration. These bodies are more or less spheroidal and small, their diameter varying commonly from one half to one and a half millimeters. In the most successful cases of treatment, the small masses, internal and superficial, are exceedingly abundant, and the dead and macerated sponge body with its contained nodules of conspicuous living tissue strongly suggests a *Spongilla* full of gemmules.

These living remnants of the sponge (bodies of the second and third classes) execute slow amoeboid changes of shape and position, behaving thus like plasmodia, and they may be designated as plasmodial masses. Microscopic examination shows them to be of an exceedingly simple character, without canal spaces or flagellated chambers. The mass does not consist of discrete cells, but is an aggregation of syncytial protoplasm studded with nuclei. The protoplasm is stored with minute inclusions and is reticulate in arrangement. The nuclei are practically all alike, and there are no signs of persisting collar-cells. Such a mass represents a portion of the original sponge in which the degenerative changes have progressed farther than in the larger remnants. In the latter we find a syncytium made up of discrete cells among which some persisting collar-cells are distinguishable. But in the plasmodial mass the cells have united so intimately that cell outlines have been wiped out, and recognizable collar-cells (or their nuclei) have disappeared. The optical evidence points to the conclusion that the latter help to form the general syncytium, undergoing regressive changes in their differentiation which result in their becoming indifferent parts of this unspecialized tissue.

The plasmodial masses remain alive in the laboratory indefinitely, but do not transform. They attach to the bottom

of the vessel, but so feebly as to be easily shaken loose. In order to see if they would transform when returned to natural conditions, I devised the simple plan of enclosing them in fine bolting-cloth bags which were hung in a live-box floating in the harbor. The bags, rectangular, were divided into compartments about an inch square with the two flat sides nearly touching. In each space an isolated plasmodial mass was inserted, and the bag sewed up. It was found that in such bags the masses were held in place long enough for them firmly to attach to the bolting cloth. Once attached to the cloth they grow, sometimes quite through the wall of the bag to the outer water, and transform into perfect sponges with osculum, canals, pores and flagellated chambers in such abundance as to be crowded.

This ability to undergo—when the environment is unfavorable but not excessively so, regressive changes of differentiation resulting in the production of a simpler, more uniform tissue, is something that is plainly useful, *i. e.*, adaptive. In the simplified state the sponge protoplasm withstands conditions fatal to such parts of the body as do not succeed in passing into this state, and on the return of normal conditions again develops the characteristic structure and habits of the species. That this power is exercised in nature there can scarcely be a doubt, since the conditions that are present in an aquarium must now and then occur in tidepools.

It is probable that the power thus to degenerate with the production of masses of regenerative tissue is general among sponges. I first discovered the phenomenon in *Microciona*, a very different form from *Stylotella* and one in which the skeleton includes much horny matter. And in two other Beaufort species I have succeeded in producing the plasmodial masses. There is every reason for believing that the commercial sponge shares in this ability. If this is so, we have here a means of propagation which with a further development of methods may at some time become economically practicable. In any case it is now possible to study the differentiation of a quite unspecialized tissue, one that is physi-

ologically embryonic, into a perfect sponge at any time of the year irrespective of the breeding season. We may even exercise some direct control over the size of the plasmodial masses, as the following experiment shows.

Microciona was kept in aquaria until the degenerative process had begun. Pieces were then teased with needles in a watch glass of sea water in such a way as to liberate quantities of cells and small irregular cell-agglomerates. These were gently forced with pipette to the center of the watch glass. Fusion of cells and masses, with amoeboid 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 (covering with bolting cloth) in which they were kept actively moving under a fine glass faucet for about thirty 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 eighteen more or less spheroidal masses were obtained, some of which measured one half millimeter in diameter. They were similar to the small plasmodial masses produced in this species (and in *Stolotella*) when the sponges are allowed to remain quietly in aquaria. As already stated, it is only in *Stylotella* that I have directly proved the regenerative power of these masses.

Maas has just announced³ that calcareous sponges (*Sycons*) when exposed to sea water deprived of its calcium undergo

3 'Ueber die Einwirkung karbonatfreier und kalkfreier Salzlösungen auf erwachsene Kalkschwämme und auf Entwicklungsstadien derselben. Archiv für Entwicklungsmechanik der Organismen,' Bd. XXII., Heft 4, December, 1906.]

marked degenerative changes, which may be of such a character that the living tissue quite separates from the skeleton and breaks up into compact cords of cells showing active amoeboid phenomena. The cords further constrict into rounded masses the likeness of which to gemmules is pointed out. Maas states that he is not yet in a position to say whether these masses have the power to transform into sponges, but adds that some of his observations induce him to believe that this is possible.

It is evident that Maas, working on very different forms, has independently met with the same degenerative-regenerative phenomena as are described in this communication, the essential facts of which were presented (together with an exhibit of gemmule-like degeneration masses and young sponges into which such masses had transformed) at the recent December meeting of the American Society of Zoologists. I may add that more than two years ago at the end of the summer of 1904, in my official report (unpublished since the research was still in progress) to the Bureau of Fisheries on the investigation under my charge, I described the degenerative phenomena in *Microciona* and *Stylotella*, i. e., the formation under certain conditions of confinement of minute masses presenting a likeness to gemules, and emphasized the probability that these masses were able to regenerate the sponge. It was not, however, until the summer of 1906 that I was able to demonstrate the truth of this view.

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February 16, 1907.