DEVELOPMENT OF SPONGES FROM TISSUE CELLS OUTSIDE THE BODY OF THE PARENT

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About five years ago I suggested to the Bureau of Fisheries that an investigation to cover the various ways in which sponges reproduce might yield some results of value for scientific sponge culture. I had in mind the high degree of reproductive (technically, regenerative) power possessed by at least certain body cells, as distinguished from germ cells, in sponges.

This great regenerative power of somatic cells in sponges is displayed, as has long been known, in the formation of asexual masses which under proper conditions develop into new sponges. The regenerative masses of this kind that are best known are the gemmules of fresh-water sponges, but similar gemmules have been discovered by Topsent and others in marine sponges. Observations of my own, dating as far back as 1889,[a] indicated that in some marine sponges such asexual masses not only possess the power to transform into sponges, but in so doing pass through a swimming stage not distinguishable from the ciliated larva which typically develops from an egg. In a case of this kind, as I have pointed out (op. cit., 1891), the nature of the body cell as measured by its potentialities is fundamentally like that of a germ cell—it has full regenerative power, including the ability to recapitulate in some measure the ancestral history of the protoplasm. Considerations of this kind led me to doubt whether in all metazoa the protoplasm really did divide sharply into somatic and germinal cells. Rather was the idea encouraged that in the lower metazoa, such as sponges, the cellular elements all retained just so much of the nature of the germ cell (just so much of the specific idioplasm, one might say) as would enable them, under the influence of an appropriate stimulus, to develop either into ova or sperms, or into asexual reproductive masses. Assuming that sponge protoplasm had this eminently plastic character, I conceived that one might discover ways in which to call into unusual activity the reproductive or regenerative power, and so, as it were, to invent new methods of growing sponges.

[a] Wilson, H. V.: Notes on the development of some sponges, Journal of Morphology, 1891; Observations on the gemmule and egg development of marine sponges, ibid., 1894.

The results, at this time, of the investigation that I have been conducting for the Bureau during the past five summers at the Beaufort laboratory justify, it seems to me, the point of view above outlined. Two new methods by which sponges may be grown have been discovered, and both of these methods attest the remarkable regenerative power of the body cells of sponges. That both methods are applicable to the commercial sponge there can hardly be a doubt. Whether at the present time any economic advantage would accrue from the practice of either is perhaps doubtful, in view of the fact that sponges may so successfully be grown from cuttings—a method first practiced by Oscar Schmidt, and further developed in this country by Richard Rathbun, while in recent years H. F. Moore has brought it through a long series of admirable experiments to a high degree of efficiency.

But while the methods which I shall presently describe may not now be of practical utility, they add something to our knowledge of the underlying scientific principles of sponge culture. And it is a truism that such principles are the funds, so to speak, on which the practice of succeeding generations draws in the conduct of economic enterprises. I am convinced that our knowledge of these scientific principles of sponge culture may be vastly increased. Future researches will surely clear up, among other points, the relation between the formation of sexual products (ova and sperms), of asexual masses which transform directly without passing through the swimming stage (ciliated larva), and of asexual masses which imitate the egg development in passing through the stage of the ciliated larva. I may add that such a relation is "cleared up" to the eye of science (in contradistinction to metaphysics) only after the discovery of the actual treatment to which, when the sponge tissue is subjected, it responds by the development of this or that reproductive body. In this instance, as in many such biological problems, the most intimate knowledge of the structure and movements of the cells concerned in the production of each kind of body is necessary. But such knowledge of itself falls short, and remains unsatisfactory until it leads up through experiment to an actual control of the phenomena—to the power which can at will compel the sponge to produce the one or the other kind of reproductive body.

The first of the two new methods to which I have alluded has been described in Science.[a] It is briefly as follows:

If sponges are kept under appropriate conditions in aquaria, the body dies in some regions, but in localities the cells remain alive and congregate to form masses. In the production of such masses the component cells lose their individuality, fusing with one another to form a continuous mass of protoplasm studded with nuclei (a syncytium). Such masses of syncytial protoplasm are

[a] Wilson, H. V.: A new method by which sponges may be artificially reared. Science, June 7, 1907.

easily seen with the unaided eye scattered through the interior or over the surface of the remains of the original sponge. They are frequently spheroidal, but often of an irregular shape, and have the power of slow amœboid movement. In successful cases of treatment these masses, varying from a fraction of a millimeter to a few millimeters in diameter, are exceedingly abundant. The smaller ones of more regular shape at once call to mind the gemmules that are normally formed in such sponges as *Spongilla*. Experiment shows them to be physiologically like such gemmules in that they have the power to transform into perfect sponges. To bring about this transformation it was only necessary to remove the regenerative masses to the open water of the harbor at Beaufort, where they were kept in small bolting cloth bags suspended in a floating live box. The sponge especially worked on was a silicious form, a species of *Stylotella*.[a]

The second of the two methods, a description of which may be found in the Journal of Experimental Zoology,[b] is the more interesting and important. It should be said that the method succeeds best with sponges in which there is a considerable development of horny skeletal fiber. The form especially used in my work has been *Microciona prolifera* Verrill, and it has proved practically necessary to use always the large bushy specimens. The procedure is as follows:

Cut the sponge into small pieces and put them on a square of bolting cloth. Gather the cloth round the sponge fragments in the shape of a bag. Holding the upper end closed with the fingers, compress the bag repeatedly with small dissecting forceps. The bag meantime remains immersed in a little dish of sea water. The sponge cells are squeezed free of the skeleton and are strained through the pores of the bolting cloth. They fall like a fine sediment on the bottom of the dish. Collect the sediment with a small pipette and strew it over glass plates or shells immersed in sea water. The originally separate cells quickly combine with one another, exhibiting amœboid phenomena. The masses so formed go on fusing with one another through the formation of peripheral pseudopodia, and thus the whole surface of the glass slide (or other object used) may become covered with a network of plasmodial masses and cords, which adhere to it with some firmness. After perhaps half an hour the plate should be lifted from the water and cautiously drained. The sponge plasmodia are thus flattened out somewhat and their attachment to the plate is strengthened. Return the plate at once to a dish of fresh sea water, where it should be left with two or three changes of water for a day. By this time the network of plas-

[a] Otto Maas has independently discovered that the cells of calcareous sponges under the influence of reagents exhibit a behavior essentially like that above described. See my account in Science, loc. cit.

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

modia has probably transformed itself in whole or in great part into a thin uniform incrustation. It is best now to transfer the plate to the open water. My practice has been to tie such plates to the inside of galvanized-wire boxes, and to hang the boxes in a large live-box.

In the course of a week it will be found that the incrustation has transformed itself into a functional sponge with pores, oscula, well-developed canal system, and flagellated chambers. The steps in this gradual differentiation may be followed by examining the sponge at intervals under the microscope. The differentiation goes on, but at a slower rate, in preparations kept continuously in laboratory dishes or aquaria. While the sponge incrustation is quite thin, the currents of water and vibrations of the flagella in the flagellated chambers may be observed with a high power. For this purpose small incrustations grown on cover glasses are the best.

Until the past summer it was a question whether sponges produced in this way would continue to grow and would develop the skeleton characteristic of the species. If they would not, it was clear that the method had no value for economic sponge culture. And so, early in July, I again visited the Beaufort laboratory and with the help of my assistant, Mr. R. R. Bridgers, started some *Microciona* plasmodia on glass slides and oyster shells. It was possible for me to remain at the laboratory only two weeks, but Mr. Bridgers took charge of the sponges and continued to start other plasmodia at intervals during July and August, conducting his experiments with great care and skill.

Mishap of course overtook some of the cultures; but scores of them grew perceptibly during the summer and by the first of September a large number had developed the skeleton of the adult with the characteristic spicules and the horny columns projecting up from the basal skeletal plate. What was equally gratifying was that the sponge in many cases had not only spread and thickened and developed the species-skeleton, but had also developed quantities of reproductive bodies. These lay scattered in the deeper part of the incrustation, plainly visible to the eye. I have not yet made a sufficiently precise histological examination of these bodies to determine whether they are egg larvæ or asexual masses. The whole appearance of the sponges grown in this way, some six weeks old, is quite like that of normal *Microciona* of incrusting habit.

Looking from the utilitarian standpoint at this latter method of growing sponges, it is not at all inconceivable that it may at some time be of direct economic value. The ease with which quantities of sponge cells may be had and the opportunity afforded of attaching them to any desired object are considerations which encourage such an idea. Going farther afield from present-day practice and looking to the future, the method suggests itself as one of the possible means of altering the specific characteristics of sponges and improving races. In

a paper presented to the National Fishery Congress of 1898[a] I briefly discussed the possibility of improving sponge races, suggesting as means thereto the breeding of sponges from the egg with accompanying selection, and also the practice of grafting. Now if the cells of two closely allied races were mixed together it is on the whole probable that a composite plasmodium would result which would develop the characteristics of both races. Such a form would be something comparable to a hybrid. I have in fact carried on experiments of this character.[b] The results of my experiments were negative—the cells of each species coalesced, but there was no permanent union between the cells or cell masses of the different species. It should be said, however, that the species used were so unlike that there was at the outset but little chance of coalescence. In a more favorable locality, where a great variety of horny sponges exist, such experiments hold forth some promise.

NOTE.—In connection with the foregoing paper there was an exhibit of microphotographs illustrating some of the more important stages in the development of sponges from cells forcibly removed from the parent body.

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[a] Wilson, H. V.: On the feasibility of raising sponges from the egg. Proceedings of the National Fishery Congress, 1898, in U. S. Fish Commission Bulletin, vol. XVII, 1897.

[b] Journal of Experimental Zoology, loc. cit.