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Some Phenomena of Regeneration in Sycon; with a Note on the Structure of Its Collar-Cells

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V. *Some Phenomena of Regeneration in Sycon; with a Note on the Structure of its Collar-cells.*

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[Plate 8.]

Contents.

Summary.

1. These experiments were conducted on *Sycon raphanus* at Naples, in order primarily to repeat H. V. Wilson's work on coalescence and regeneration in monaxonid sponges, with a less specialised calcareous form. His method of straining the chopped-up sponge through fine gauze was used, and by this means nothing came through except cells, singly or in twos and threes. These united together, mainly by means of actively amœboid cells, into small lumps of irregular shape, in which the various kinds of cells were confusedly mixed. This ended the process of reunion—one that does not occur normally in Nature. Next came that of reorganisation—similar in its main features to what occurs during reversal of the layers in the parenchymula larvæ of Calcarea; the dermal cells migrated to the surface to form a flat epithelium round a solid central mass of quiescent collar-cells. The two layers of the sponge-body are now present in their definitive positions, and the subsequent period may be called

one of redevelopment. The regenerate in this stage is very like a normal Sycon directly after metamorphosis, so that this redevelopment is practically the same as the normal post-larval development of the species. Spicules are formed, first monaxons, then triradiates; a gastral cavity and finally an osculum appears. The only differences from normal development are that here spicules arise later (in the larva, be it noted, spicule-formation is certainly precocious), and that the regenerates, though making an attempt to fix, do not succeed in doing so permanently. None of the regenerates reached a heterocœle condition, but one lived and grew as a functioning sponge for several weeks.

2. By other methods, collar-cells were obtained nearly or quite pure. If large bits of unbroken gastral epithelium were taken, they bent back, and eventually rounded up to form perfect hollow spheres, with collars directed outwards. Similar spheres were also formed, but in a different way, when numerous small groups or single cells were taken. These united into solid lumps, which later swelled up to form spheres. Though some lived over a month, yet no other form of tissue was ever regenerated by them.

These spheres can have nothing to do with phylogenetic questions. On the one hand, their form and appearance does *not* prove the existence of a Volvox-like Choanoflagellate ancestor of sponges, being probably due to the oxygen requirements of the cells and to surface tension. But, on the other hand, neither does their failure to regenerate other kinds of cells prove anything *against* a choanoflagellate ancestry; the more ancestral cells may simply have given up their regenerative power to others more suited to the work, as we know has happened in other cases; for instance, the Ascidians, where mesoderm (mesenchyme) has taken over nearly all the regenerative power from the more ancestral ectoderm and endoderm.

3. Examination of the collars has shown that, in *S. raphanus*, at least, the longitudinal supporting rods described by Bidder exist during life as well as in preparations.

1. *Introduction.*

While occupying the Oxford Table at the Naples Station last year, I was looking about for a suitable subject to work at, when Prof. Paul Mayer suggested to me that there were many points of interest in sponges; amongst other things he brought to my notice H. V. Wilson's extremely interesting paper on reunion and subsequent regeneration of isolated cells ('07_2). Fired by this, I decided to try some similar experiments on a calcareous sponge; if possible, one of a more simple and primitive nature than the monaxonid forms with which he had worked.

An admirable material lay ready to hand, in the shape of *Sycon raphanus*, H.,*

* The late Dr. Lo Bianco informed me that other workers have identified this species as *Grantia capillosa*, but as I have always found that the ends of the radial tubes project freely, with no trace of an external membrane covering them, it cannot be a *Grantia*, and agrees very well in all respects with the descriptions of *S. raphanus*.

the only calcareous sponge which can hold its own in the Naples Aquarium when adult, or establish itself there from larvæ. This in itself was one recommendation. Another was its simple structure, and the comparatively large size of its cells. With this I was able to repeat WILSON'S experiments, and succeeded in discovering other points of interest, as I hope the following pages will show.

Before proceeding, however, I must express my best thanks to all the staff of the Naples Zoological Station, who were uniformly kind and helpful, but particularly to Prof. MAYER, without whose valuable advice on matters of technique I should scarcely have been able to accomplish anything at all.

The work was completed at Oxford in the laboratory of Prof. BOURNE.

2. *Union of Isolated Cells, with Subsequent Regeneration of Functional Individuals.*

(a) *Methods.*—To obtain numbers of the cells singly, or only in small groups, and nearly free from skeletal structures and dirt, I used H. V. WILSON'S method of cutting the sponge up small, enclosing the bits in a piece of the finest silk gauze tow-netting obtainable, and squeezing them once or twice from top to bottom with a pair of forceps. The whole process was done under water in a small dish. By this means I got a dense cloud of cells and cell-fragments with only a slight admixture of non-living matter. The cells gradually settled down on to the bottom of the dish as a thick sediment, portions of which I then pipetted off into small dishes with clean water. This water was frequently changed, usually every two days, though I found that four days in the same water did not seem to hurt the regenerating masses.

The fixing fluid used was almost entirely 2 per cent. osmic acid diluted with sea-water to 1 per cent. This preserved the collars and flagella beautifully. Of stains I used picro-carmine and magnesia-picro-carmine, the latter always when it was wished to preserve spicules, and on sections iron hæmatoxylin as well. Corrosive sublimate dissolved in alcohol was occasionally used, followed by borax-carmine.

(b) *Description of the Results.*—The cells which come through the gauze are often completely isolated, though many are still attached to each other in little groups containing up to 15 or 20 cells. The gastral cells in this state usually still possess a flagellum, lashing with all its accustomed vigour, but the collar is nearly always gone, and the body has become spheroidal. Other of the cells are markedly amœboid. These crawl about on the bottom and adhere to any other cells with which they come into contact, whether of their own or a different sort. It is chiefly by their means, I think, that out of the thin layer of cells little lumps and balls are formed, which go on increasing in size up to 24 or 30 hours from the beginning of the experiment. From each of these lumps a functional little sponge may later be formed.

These balls are thus composed of the various kinds of cells mixed up pell-mell; their outline is rough and irregular, though approximately circular. With this the first period, that of simple Reunion of isolated parts, is over, and the second, that of Regeneration (or, better, Reorganisation), now begins. The cells first sort themselves out into their respective categories and take up their proper positions. After five days (reckoning always from the beginning of the experiment) the cells which will form the dermal layer have migrated to the exterior, and united to form a continuous epithelium. This is in most places very thin, without inclusions in its protoplasm, but at intervals there rise from it little humps containing the nuclei, together with some black granules (Plate 8, fig. 1). In the interior is what I shall simply call the inner mass—a number of polygonally compressed cells with many inclusions. Their protoplasm is of a light greenish brown, as opposed to that of the dermal cells, which is colourless and rather dense. These inner cells, as will be proved by their subsequent development, are nothing more than collar cells which have withdrawn both collar and flagellum, passing thus into a quiescent state. Unfortunately the material I preserved of these stages has proved to be neither sufficient nor very good. So I fear that several interesting questions—such as whether the dermal layer of the regenerate is formed from the dermal cells of the "parent," or from its porocytes or amœbocytes, and then how the rearrangement is effected—must be left for the present unanswered.

Reorganisation is now complete, and the subsequent processes, being much like what occurs normally after metamorphosis, may be called Redevelopment.

In what I regard as the next stage (though the different appearance might possibly be due merely to the presence of a larger number of dermal cells in proportion to the whole surface), a space, filled with a perfectly colourless and transparent fluid, arises between dermal layer and inner mass (fig. 2). Freed from the pressure of the dermal layer, the outermost gastral cells can now protrude as rounded lumps. On focussing up, the polygonal outlines of the dermal cells can be seen, each containing a clear refractive body, presumably the nucleus (fig. 3, *a*). In optical section (fig. 3, *b*), there are often sharp angularities in the interior contour of the dermal layer, especially at what I take to be the junctions between the cells (*x*, fig. 3, *b*). At the time I did not look closely for any connections between outer layer and inner mass, and saw none, but it has since occurred to me that some of these angularities may have been the tapering bases of very fine, almost invisible threads of protoplasm (*cf.* G. F. Andrews, "Spinning Activities of Protoplasm," 'J. Morph.,' vol. 12, '97).

In the next stage, at all events, connections are very much in evidence (fig. 4). The space between the two layers has increased in width, and is traversed by numerous protoplasmic sheets and threads of diverse shapes and thicknesses. Most go straight or diagonally across from dermal to gastral cells, but some pass from one dermal cell to another, and some anastomose irregularly. These strands are

sometimes quite thick, and faintly tinged with greenish-brown; they then contain a good many granules, and seem quite fixed and still. Others, the thinner ones, look colourless, contain very few granules, and are continually changing in thickness, shape, and position. On focussing up (fig. 4, *b*), the cell outlines seem to be no longer clearly defined. The nuclei are obvious; round them are a number of smallish granules, and these granular areas are connected by anastomosing strands in which the granules are smaller and fewer. The intermediate protoplasm is quite hyaline.

The next step is the production of spicules. Of these, the monaxons were formed first, just as in the larva. Fig. 5 shows the first spicule I saw. Though it was already quite large there were no others in the regenerate. One end was pointed, the other clubbed and enclosed in a granular spiculoblast. After this, fresh monaxons formed in quick succession, and grew rapidly in size. Fig. 6 shows a 13-day individual with at least 12 spicules, most of which have grown so large as to have pierced the dermal layer with one or both ends, which thus project free into the water.

Not until this stage did the production of triradiate systems begin. These had their rays curved with the curvature of the body, though later one or two rays might pierce the dermal layer and project freely (a rather abnormal condition with triradiates). After this, formation of all the sorts of spicules went on continuously, as in the adult.

After a supporting skeleton had been thus prepared, the regenerating animal was ready to form its gastral cavity. To this end the whole organism gradually enlarged, and a space arose in the centre of the inner mass, which up till now had remained solid and inert. This space increased in size, the cells of the inner mass arranged themselves in a single epithelial layer round it, and eventually proved their true nature by putting forth flagellum and collar, and beginning their normal activities once more. At this stage (fig. 7), although the collar-cells were thus functional, there were no pores, and the osculum was not open, though its position might be indicated (as in the larva) by the absence of collar-cells over a circular region.

Whether the beating of the flagella has any share in bursting it open or not, the osculum is subsequently opened. Meanwhile, the body elongates somewhat, and pores originate over its surface, and we have what is, in essentials, an Olynthus. I say in essentials, for in detail there are many differences between this and the normal Olynthus (post-larval or Ascon stage) of Sycon. In the first place, the normal Olynthus is fixed by one end, and secondly, it is of beautiful regularity, both in its general form and in the arrangement of its spicules (Maas, '00). My sponges, on the other hand, were in this stage never fixed. They had, however, made a previous attempt at fixation, which I may perhaps describe here. Just after the beginning of spicule formation the dermal layer on the lower side began to stick

to the glass, extending over it in thin, irregularly-pointed sheets and sharp tongues, just as is usually seen at the fixation of sponge larvæ. By this means they held pretty firm, though they could be easily loosened by a gentle stream from a pipette, the adherent tongues curling up, contracting, and rounding off when freed from the bottom. They remained fixed only three or four days, however; sooner or later the dermal layer drew in its processes and became as smooth as before, and the young sponges lay free on their sides at the bottom of the dish (*cf.* p. 171, for similar phenomena in Reniera, and p. 175 for a discussion). Neither were my Olynthi remarkable for symmetry of shape or skeleton. They were merely bags of roughly cylindrical form, with a circular osculum taking in one end. The arrangement of the spicules conformed in a general way to the normal, but here and there they projected singly, or in great bunches, in peculiar and quite asymmetrical fashion. The normal young Sycon has a beautiful double oscular crown of long monaxons, one row projecting forward as a palisade, the other radially as a chevaux-de-frise. The crowns of my sponges were sadly lacking in order and beauty, though a distinct attempt was made to carry out this double-rowed arrangement. All these deficiencies, however, do not make this stage any less in essentials an Olynthus—that is, a functional sponge consisting of a simple two-layered sac with pores, osculum, and skeleton.

Unfortunately, most of my sponges died just before this stage. Of those that reached it, some were preserved, some soon died, and only one (that shown in fig. 8) succeeded in surviving for any length of time. It grew considerably in length and slightly in breadth, but was then attacked by bacterial parasites. These it got rid of, as far as I could make out, by throwing off the anterior third of its body, which had been especially infested. The posterior part lived happily for about a fortnight more, but was then again attacked by the same parasite, and this time succumbed.

It had lived since the beginning of the experiment for 10 weeks and 2 days, and for over 5 weeks had been a functional sponge. It had been the whole of this time in a small dish, so that, under more favourable conditions of aëration or food supply, it is at least probable that such a sponge would develop further, become heterocœlous, and finally take on the characters of an adult Sycon. The only difficulty I can see lies in the failure to fix. However, the experiment remains to be tried.

For purposes of reference I append the times observed for the various processes described:—

	Time, in days.	
	Shortest time observed.	Longest time observed.
Solid balls of cells	$\frac{3}{4}$	1
Two layers, with space between	4	5
Two layers, the space traversed by strands	7	10
First monaxon spicules	10	15
First triradiate spicules	14	25
Formation of gastral cavity	17	30
Death	30	72

(c.) *Similar Experiments on* Reniera rosea.—By way of control and comparison, I made similar experiments on *Reniera rosea*, a monaxonid sponge belonging to the same order as that used by WILSON. It takes its

name from its fine dark rose-red colour, lodged in spherules in the amœboid and flagellate cells. The adult sponge is a colony with little individuality of its members; and, doubtless correlated with this, the regenerating masses varied in size to a degree not seen in Sycon, where they were fairly uniform. Here they ranged from a few cells up to sausage-shaped masses 4 or 5 mm. in length.

The first differentiation took place as in Sycon—a single layer of clear dermal cells forming round an opaque red central mass. The chief subsequent difference was in the mode of fixation. As in Sycon, the dermal cells crept over the bottom in tongue-like processes, but this creeping went on so as to produce quite a large border, many cells wide. This border was colourless, and consisted of a double flap of dermal epithelium, between whose two layers there were various other cells. Here, as well as in the inner mass, numerous spicules were soon secreted. They were at first single and isolated, but after a time became cemented together into a scaffolding in the way characteristic for the species.

No central cavity ever arose in the inner mass, but numerous flagellated chambers were soon found internally: the strange thing, however, was that I never saw any trace of the chimney-like oscular tubes possessed by the normal adult, though several of my regenerates lived over 50, and one or two over 80 days.

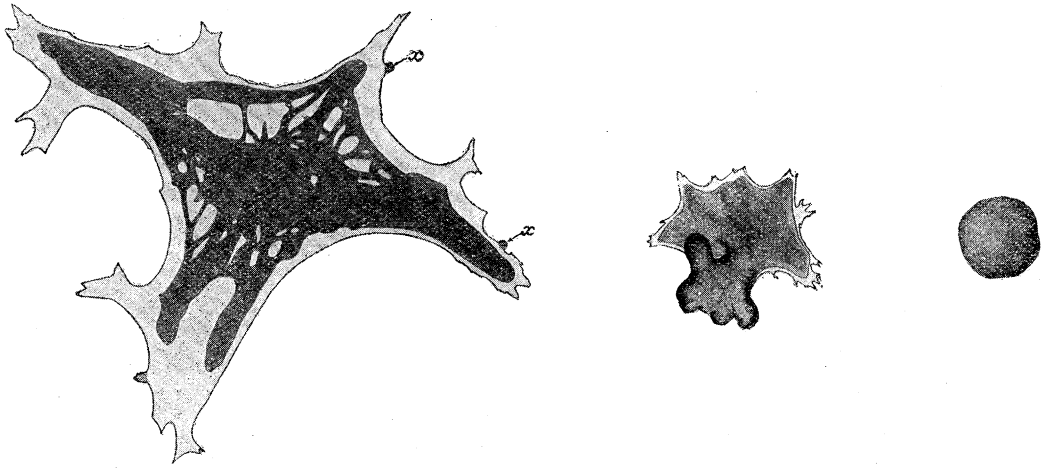
The gradual manner of their death was quite interesting: The parts in contact with the water simply rotted away bit by bit, leaving their skeleton free to view. This bare scaffolding, however, was directly continuous with the skeleton of the living central part, which appeared perfectly healthy, except that it continued thus slowly disappearing till nothing was left but a framework of silica and spongin.

The most curious thing about the regenerates, however, was the way in which they were continually changing their shape. When they are fixed and spread out on the bottom of a dish (text-fig. 1) there are two forces at work that can affect their shape. The first is surface tension. We must suppose that at first this was acting alone, so producing the spheroidal form of the young regenerates. The second is the force exerted by the dermal cells, which apparently tend to crawl on and on as far as they can: the effect of this is to spread the organism out over the substratum.

In one large mass I followed the course of events from the time of fixing. At first, it seems, the tendency of the dermal cells to crawl onwards is very strong, for the clear outer area grew broader and broader, extending in several directions at once, and thus flattening the whole sphere down to a mere crust. Things at last went so far that the red inner mass was torn through in several places, leaving clear gaps where there were only dermal cells (text-fig. 1).

This state was reached by the regenerates in from two to three weeks, and maintained (with considerable alterations of outline) for a few days to a fortnight. Eventually, however, as in Sycon, the sponge began to unfix itself, becoming usually quite free at the last; even if, as sometimes happened, it remained fixed along the lower surface, the broad clear layer with its sharp tongues was drawn in to form a close-fitting sheath, and the whole animal took on a more or less cylindrical shape with rounded contours. The regenerate I have been describing, for instance, had next day become roughly Y-shaped, the left-hand arm of text-fig. 1 having elongated considerably at the expense of the other two. On the next day again, it was nearly a straight line; one of the three arms had disappeared. The force exerted by the dermal cells, too, was decreasing, for the inner mass had no gaps left in it, and was getting thicker. On the following day a total change had come over it (text-fig. 2). It was only attached by one quite small area, and even here the clear border was very small. The rest of it was free from the bottom, and stuck diagonally upwards as a thick cylinder, towards the top increasing in size and giving off short fat finger-shaped processes. Over this part the dermal layer was evenly contoured, and fitted so close as scarcely to be distinguished with a $\frac{1}{3}$-inch lens. Next morning, still less of the clear border was in evidence, and the processes of the erect part were shorter and fatter. By the next day, the dermal cells had ceased to exert any effort, and the mass was completely free, an irregular ovoid, the dermal layer fitting close all over it. Finally, on the day after that, the continuous action of its surface-tension had reduced it to an almost

perfect sphere (text-fig. 3). A comparison of this with text-fig. 1, drawn to the same scale, will give an idea of the creeping and adhering powers of the dermal layer.



TEXT-FIGS. 1, 2, AND 3.—Three Drawings of a Regenerate of *Reniera rosea*, all of the same magnification (× 17), at intervals of 3 days, to show its changes of shape.

TEXT-FIG. 1.—After 17 days. Condition of fullest extension. The dermal border is very broad and is produced into sharp processes. Gaps are to be seen in the inner mass. At *x x* are little thickened lumps of darker colour, representing contracted portions of the dermal layer.

TEXT-FIG. 2.—After 20 days. Much contraction has taken place. Where still attached to the bottom, the dermal border is quite thin; and no gaps are left in the inner mass. The bulk of the contents are no longer adherent at all, but project freely upwards as a lobulated mass, over which the dermal layer fits tight.

TEXT-FIG. 3.—After 23 days. Now no longer adherent at all. The action of surface-tension has resulted in the formation of a nearly perfect sphere, the dermal layer fitting close all over it.

Curiously enough, after 18 days as a sphere, this same sponge began once more to attach itself; it never got far, however, but rounded off again, and shortly died.

To test the effect of the surface-tension, I chose another mass which was well spread out, with broad dermal layer edged by numerous sharp tongues of protoplasm, and forcibly detached it by squirting with a pipette. Immediately on getting loose, the dermal border decreased considerably in breadth, and the sharp processes were all drawn in, leaving no longer pointed promontories and deep bays, but a contour only slightly more irregular than that of the inner mass. This appearance had scarcely changed after an hour, but by next day the dermal layer was everywhere close-fitting, and the whole had become less flattened, now consisting of a perfectly smooth-contoured ovoid with two rounded humps at one end. After two days, however, it had fixed again, and remained so for over a month, though changing its outline and its degree of extension all the time.

(d) *Discussion.*—The developing embryo of any animal reaches at one time or another a stage in which the general plan of the adult is laid down, subsequent changes consisting almost entirely in the amplification and full expression of this plan, or of processes which occur regularly in the adult. The exact moment when this condition is reached may sometimes be difficult to determine where the whole development takes place within the mother's body, but, in animals with a free-swimming larva, this stage is usually the post-larval, or, more accurately, that which

follows immediately on the metamorphosis. In regeneration, and often in budding, the animal seeks to reach this stage by the shortest way it can find. Since it is not starting from an ancestral state (as it is in the unicellular ovum), it is not necessary, or even convenient, for it to pass through ancestral stages. Hydroids, Polyzoa, and Ascidians (notably Clavellina) will furnish us with examples.

The regenerating Sycons here are no exceptions. There is no suggestion of any larval stage—the jumble of cells rearranges itself as soon as possible with the dermal cells outside the gastral, then resembling in its main features the normal pupa-stage immediately after invagination. Its subsequent development is like that of the pupa in almost every way. Indeed, the state of the balls during rearrangement is not at all unlike that of a young sponge actually during metamorphosis, not, however, of Sycon itself, in which no reversal of the layers takes place, but of one with a parenchymula larva, such as *Clathrina blanca*. Here, to quote Minchin ('00, p. 69): "The reversal . . . . is effected partly by dehiscence, . . . partly by diapedesis, the individual amœboid cells struggling through the ciliated layer to the exterior." In the regenerates there can, of course, only be diapedesis of single cells. The details of the rearrangement I have not been able to follow. In one point at least, however, it differs from the similar process at the metamorphosis of Clathrina—in the time required. In Clathrina it is less than 24 hours—and this is an unusually long time for reversal. Here, rearrangement takes nearly three days after reunion is completed.

Of the three processes—reunion, reorganisation, and redevelopment—that occur during the establishment of a functional Sycon from isolated cells, the first, as far as we know, does not take place in nature at all, the second does not take place in Sycon, though something very like it occurs in Clathrina (and probably occurred in the ancestors of Sycon), while the third is extremely like what takes place normally in Sycon after the metamorphosis.

It should be noted that, at the close of the period of reorganisation, the two layers of the sponge-body, though in their definitive relations, are still sharply separated. This absolute separation of the two layers is very obvious in the young Sycon just after fixation (Maas, '00), and its occurrence at such a stage of development is apparently very widespread among sponges (Maas, '94), if not universal (Minchin, '00, p. 71:—in *Clathrina blanca* the porocytes are said to remain in the interior at metamorphosis, never coming to the surface with the rest of the dermal cells). Besides this, Maas ('01) has made the interesting discovery that it recurs in the development of the buds of Tethya, which appear to arise solely from archæocytes. Such a condition is never found in Tethya or Sycon when adult, and it differs from the contraction stages seen in Clathrina (Minchin, '00, p. 30), as porocytes are not recorded from the centre of the mass of gastral cells. Neither is there on general grounds any reason for regarding such a condition as reminiscent of an ancestral state—much more probably it is simply a stage which it is convenient or needful to

reach as a point of departure for further development, developing and regenerating sponges, like men, rising "on stepping-stones of their dead selves to higher things." The simplest way to get order out of the chaos of the reunited cells is for the various kinds of cells to sort themselves into their respective categories, not to crystallise out at once into the adult organisation. In regeneration, as in normal development, there is true epigenesis, though not complicated as it is there by ancestral reminiscences and larval acquisitions.

The three points in which the process of redevelopment differs from normal post-larval development merit some further discussion. The first is the much longer time taken by the regenerates. They had only produced a gastral cavity in 17–30 days, while the metamorphosed larva is already an Olynthus long before.

This can only be due very slightly, if at all, to the external conditions, for the larvæ will develop well in a very small volume of water (SCHULZE, '78; MAAS, '00). It must rather be put down to the abnormality of their internal state. In normal development, one of the great objects is speedy attainment of a self-supporting condition, and natural selection will have been at work for countless generations, making the sequence of events as rapid as possible. But in the regenerates we are dealing with an artificial situation; and even at the close of reorganisation, the regenerate is not wholly similar to the metamorphosed sponge, for its cells are adult, while those of the normal young Sycon do not assume the adult form for some time (MAAS, '00). Rapidity of development having been acquired in the larva for special reasons, we must not be surprised if it is not found in this "redevelopment" of adult tissue, for the conditions, though in general similar, are by no means identical.

The second point of difference is the relative (as well as absolute) lateness of spicule-production. In the regenerates none were formed till reorganisation was well over, 6 to 10 days after the two-layered state was reached, while in egg-development they begin to appear directly after metamorphosis, or even in the free-swimming larva. Similar arguments apply here: in normal development the sequence of events has not only been accelerated, but their order has been sometimes transposed —certain elements are precociously developed when their early presence makes attainment of the adult self-supporting form more rapid. Among sponges, the spicules are thus precociously developed in many cases, of which Sycon is undoubtedly one. Like rapidity, precocity has been acquired by, and for, the larva only, and it is not to be expected that it should crop up in regenerates composed of reunited adult cells.

A curious point of *resemblance* to the larval processes is seen in the way triradiates appear only after numerous monaxons have been formed. Curious, for the cells here are adult cells, and have been producing both sorts of spicules simultaneously for a long time past. The only explanation which occurs is that the absence or insufficiency of spicules is a "formative stimulus" to the production of monaxons only, and *their* presence in its turn a formative stimulus to the production of

triradiates—which would imply that, in the growing adult, the production of monaxons was always as it were slightly ahead of that of triradiates.

The third point of difference is the absence of permanent fixation in the regenerates. After fixing themselves in much the same way as do the larvæ, they seemed to be actually forced off the bottom by the growing spicules. Why this should happen here and not in the larva is hard to see, unless it be that in the larva, correlated with its existing polarity, there is some controlling factor which prevents the formation of spicules at one pole (the pole of invagination), while in these irregular masses there is at first obviously no polarity, and so perhaps nothing to prevent the spicules from protruding at any point on the surface. A polarity is revealed later in the preparations for the osculum, but would thus not be determined till after spicule formation.

As regards the results of other authors, two papers only—Wilson ('09) and Müller ('11_1)—have appeared on this subject since Wilson's first paper ('07_2). Wilson has extended his experiments by proving that in Microciona these products of coalescence grow up perfectly normal, and may become sexually mature, confirming the established view that the amœboid cells of sponges are potential germ-cells, and further showing that the strange and violent treatment suffered by the cells does not injure them in the performance of any of the functions of life. Müller has repeated the experiments on fresh-water sponges (*Spongilla lacustris* and *Ephydatia Mülleri*) and has obtained results very similar to Wilson's, adding, however, the interesting fact that active division of many cells goes on even during the period of reunion. He believes (though he cannot be quite sure) that the collar-cells take no part in regeneration, while Wilson is not very explicit on the subject, though he finds that they are certainly taken up into the masses during reunion. In Spongilla the reunited masses seemed to be composed almost entirely of large amœbocytes. In the Sycon masses, the collar-cells of the original sponge certainly persist to form the collared epithelium of the regenerate. This is correlated, doubtless, with their much greater relative bulk in Sycon than in the more specialised types with small and scattered flagellated chambers, where an equal current can be produced by fewer cells.

This utilisation of all the old materials is probably more primitive; in Spongilla the decreased importance of the collar-cells, together with the acquisition of the power to gemmulate, has resulted in a regenerative process which is half-way to gemmulation, the amœbocytes taking the largest share, while in Sycon we have, in part at least, an actual rearrangement of old units. This method is also more interesting; for it gives clear proof that the characteristic form of a species can arise as well by rearrangement of dissimilar specialised units as by differentiation of similar unspecialised ones. In the latter case (gemmule development in Spongillidæ, regeneration from pieces of stolon in Clavellina, &c.), Driesch's dictum that the fate of each unit is a function of its position in the whole holds good. Here, almost the

reverse is true: the differences in constitution among the units determine the positions they take up in the whole.

3. *Behaviour of Isolated Cells and Fragments of the Gastral Layer.*

(a) *Methods.*—I had made various attempts to separate the gastral from the dermal cells, but without success, till I hit upon the idea of using coarser gauze for the straining of the sponges. These I teased, but only roughly, beforehand, and the result was that while the free-creeping amœboid cells came through isolated as usual, the epithelial gastral layer passed through for the most part in fairly large pieces—up to 50 or 60 still-united cells at once. These larger masses naturally fell to the bottom quicker than the single cells, and so by pipetting off the lowest layer after a short time, I got fairly pure gastral cells. By repeating the process they could be procured still purer.

By picking out large fragments of gastral layer under the microscope and isolating them, results were obtained similar to those got in the first way, proving that in that differential method gravity had effected a pretty thorough separation of the gastral elements.*

(b) *Description of the Results.*—I will first describe the fate of the large fragments of gastral layer. When first isolated they reversed their curvature, so that the collars projected from a slightly convex surface. This points to the interstitial substance between the collar-cells or the epithelium as a whole being in a state of tension and possessing a certain amount of elasticity. This inference is supported by the observations of many workers on Demospongiæ, where flagellated chambers isolated by teasing are often found with the flagella on the exterior surface —turned completely inside-out.

After 24 hours the fragments had healed their wounds and rounded themselves up to perfect little hollow spheres, composed of a single uninterrupted layer of choanocytes, whose flagella, all actively beating, were to my surprise directed outwardly. Of singular beauty and perfect transparency, they looked at first sight like a choanoflagellate Volvox. The movements of the flagella, though often violent, were, however, not co-ordinated, so that the sphere, instead of swimming actively about like a Volvox colony, was only capable of a slow and aimless rolling, scarcely shifting its position on the bottom of the dish. That slight and continual movements did exist was proved by arranging the spheres overnight round the edge of the

* I have fears that the differential action of gravity in itself (though certainly a true and probably the main cause) does not account for all the separation; I think that in some way most of the amœbocytes must have been prevented from passing through the gauze—whether by exceptionally thick jelly in this batch of sponges or what, I cannot tell. My reason for this belief is that in one dish the bottom layer was not pipetted off for nearly three hours—time and to spare for all cells to have settled—and yet here the phenomena were essentially similar to what I shall have to describe for the rest. I had intended to repeat and vary the experiment, but was unluckily taken ill and could not utilise my last three weeks at Naples.

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water in a watch-glass: in the morning all would be together in the centre, though the slope was far too gentle for them to have rolled down had they lain quiet.

In some of the dishes of gravity-separated material (only among those that had been pipetted off very soon, or had been twice separated) spheres had also formed by the next day—spheres exactly like those I have described, except that some of them had one or two amœboid cells sticking to the epithelium. In no case did I see anything that could be called a pore, either intra- or inter-cellular—the collar-cells formed a smooth unbroken spherical shell. But, though so similar, their origin had not been the same. An hour after straining there had been plenty of flagellar movement in the dishes; the cells were in small masses, which scarcely adhered to the bottom (unlike masses containing many amœboid cells, which are very sticky). The number of cells in these masses was a good deal less than in the spheres of next day, so that they must have united by threes and fours before swelling themselves out to spheres.

Whatever their origin, the fate of the spheres was always the same. After some time they would begin to contract, the wall of the sphere getting thicker and sometimes slightly wrinkled. After this they might pursue one of two courses (resembling each other in that the central cavity was in both quite obliterated): either the surface would remain more or less smooth, and the result be a solid ball, or else little protuberances would appear, the furrows between which gradually grew in, so that the former sphere was converted into an irregular, lobate, solid mass. In either case the flagella of the cells left at the surface still beat continuously, though ever more slowly as time went on. The solid stage once reached, however, there was no long respite. In a few days the flagella ceased beating, the whole surface became uneven, and death ensued.

The formation of normal spicules and the essentially normal end-result in the first experiment described in this paper, as well as the fortnight's healthy existence of the spheres of collar-cells in this, render it to me very probable that the collar-cells of Sycon do not possess the power of regenerating other tissues—if they had, they would have used it here.

Besides perfect spheres, other structures formed in some of the dishes, especially those where the cells were more crowded. Here, after 24 hours, no spheres were seen—only solid cell masses, mostly of considerably larger size than those that had given rise to spheres in one day. In the course of time, however, these masses too underwent a form of what I may call the "blowing-out process." First of all the cells in contact with the water put forth collars and flagella, so that there was now a solid lump of irregular shape and contour, entirely composed of collar-cells, but with the outside layer alone active. The next step was the true "blowing out." At one point usually in small masses, at two, three, or more in others, especially the bigger ones, a cavity would be formed, covered on one side by a single layer of active

choanocytes. This layer formed part of a spherical surface, and was precisely like the perfect spheres already described, except in its incompleteness, its cavity being bounded on one side by the solid remainder of the mass. The single layer was at first often less than a hemisphere, and that of small radius; but as time went on it increased its radius and approximated in shape more and more to a full sphere. Rarely, and only in small lumps, the whole mass of cells would be thus drawn out into a single layer, so that a perfect sphere, just like those previously described, was formed. Usually, however, the furthest stage reached was one like that shown in fig. 9, where, although the single layer has attained to full three-quarters of a sphere, yet the solid part is nearly as large, and, of course, contains many more cells. This solid half exerts a certain influence on the shape of the single layer, which, as it nears the point of junction, departs slightly from the spherical, inasmuch as the radius of its curvature increases.

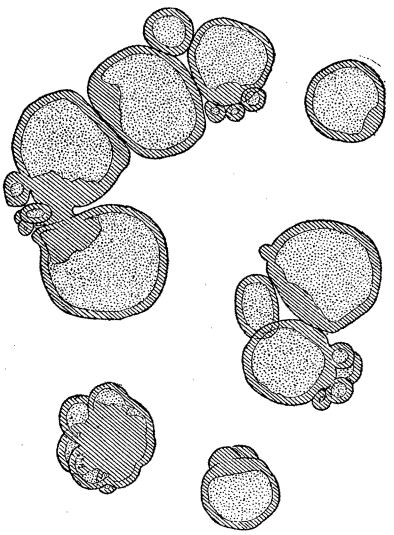
The formation of several foci, so to speak, of blowing out in the same mass led to very various appearances, some of which are given in text-fig. 4. Besides the perfect sphere and the type shown in fig. 9, we have first single spheres with one or two slight thickenings, either external or internal, on their walls; then there are masses more or less solid inside with a number of small hemispherical cavities round their circumference; and, lastly, large spheres with numerous very small spheres being produced on one side. In a big mass, several of these large spheres, each with attendant satellites, would often be formed, and might be clearly marked off from each other, though still actually adherent. In all cases, however, the plan was essentially the same—a solid mass of cells, with collar and flagellum only at the surface, portions of which would gradually swell out into single-layered spheres or parts of spheres, composed of active collar-cells with their flagella directed outwards.

For these, as for the others, shrinkage and death eventually set in, though they remained healthy somewhat longer than the perfect spheres.

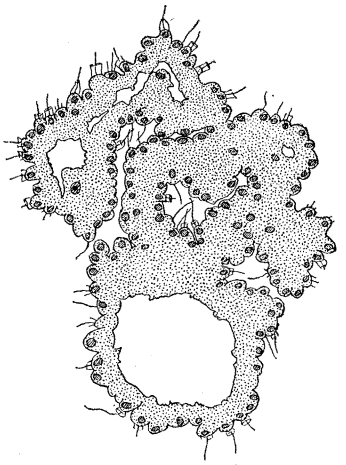
I append the earlier and latest times (in days) observed for the various stages in the different dishes of material:—

	For spheres.		For "blown-out" masses.	
	Earliest.	Latest.	Earliest.	Latest.
All spheres	?	1	—	—
Some blown out	—	—	1	4
Most blown out	—	—	3	6
Some contracting	5	7	5	18
All contracting	7	14	6	24
Some solid	6	18	6	24
All solid	7	21	7	26
All dead	8	24	8	30

(c) *Further Remarks on the Collar-cell Regenerates.*—Sometimes the blowing-out process was not quite what I have described, but there would be a growing-out of irregular finger-shaped lobes, and a growing-in of canals from the outer world, as shown in text-fig. 5. This, like the normal blowing-out, results in an increase of surface, and so enables more collar-cells to resume active life. For be it observed that a choanocyte with active collar and flagellum was never seen except in contact with the external medium. I could never find any putting forth their flagella into a closed cavity like that within a sphere, even where a mass of latent gastral cells bounds a whole side of a large cavity as in fig. 9. Text-fig. 5 shows this clearly: practically all the nuclei, and thus the distal, collared ends of the cells are arranged on the exterior, or round cavities which can be traced to the exterior in this or in other sections, while the completely closed cavities have no nuclei in their walls.



TEXT-FIG. 4.



TEXT-FIG. 5.

TEXT-FIG. 4.—From various Preparations. × 165. Diagrammatic outline Drawings to show some of the Forms assumed by the Regenerating Masses of Gastral Cells.

TEXT-FIG. 5.—Section of a Regenerating Mass of Gastral Cells. × 435. Semi-diagrammatic. To show how nearly all the nuclei have become grouped along surfaces in contact with the external medium. The most central cavity appears closed, but in reality opens to the exterior in another section. The cavities with no nuclei in their walls are completely closed. Most of the cells in contact with the water are active. The large nucleus at 10 o'clock is that of an oöcyte.

These closed cavities are seen in sections to contain a thin network of coagulated fluid, so that there must be organic matter dissolved in their contents. A similar coagulable fluid is recorded in the interior of many sponge larvæ.

At the close of the "shock period" it would seem that the impulse to put forth collar and flagellum again is often felt simultaneously by a number of contiguous cells, for in sections of still entirely solid masses of the second or third day there will often be one quite large patch where all the cells are active and flagellate, while over the rest of the surface such cells are few and far between.

Once the outermost cells had become thus active again, it was very noticeable how they formed a definite epithelial layer, resembling the single layer found in hollow spheres, and totally different from the confused mass of the rounded quiescent cells within (figs. 11, 12). In this layer the cells were attached to each other laterally, the optical section of the plane of attachment being a nearly straight line. There seemed to be no interstitial matrix or secretion. Whereas the cells in a normal gastral layer may be described as columnar, and are all more or less of the same height, in these epithelia they may be low and much laterally produced (fig. 12), or their shape may be very variable (fig. 11).

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The inner mass consisted of "latent cells," rounded or compressed, sometimes densely packed (fig. 13), sometimes quite loosely scattered (fig. 12). Their nuclei, especially in dense-packed masses, were often distinctly smaller than those of the functional outer cells, and a considerable proportion were bean-shaped, as at *x*, fig. 13, whereas those of active cells were always rounded.

The wall of hollow spheres was an epithelium of precisely similar nature to that round solid masses (fig. 10). All of these spheres' stability was given by the adhesion of each cell to its neighbours; there was no supporting membrane nor any interstitial substance visible with the highest powers. There was often a certain amount of cell *débris* sticking to the inner side of the epithelium, and, more rarely, latent choanocytes or sometimes other sorts of cells.

In the solid parts, however, there was often a more considerable admixture of other cell-types. Oöcytes are frequent (*e.g.* the large nucleus on the left, in text-fig. 5, and the big granular cell-body in fig. 13). Amœbocytes, sometimes with outstretched pseudopodia, can also be found; in two or three cases I have seen what appears to be phagocytosis of gastral cells (presumably damaged) and of cell-fragments by amœbocytes. Dermal cells, too, may be present; in one solid mass, where the collar-cells were just becoming active over most of the surface, one part was overspread by an epithelium of obvious dermal cells (fig. 13), exactly as in the normal regenerates (figs. 1, 2). This shows clearly the strong tendency of the dermal cells to migrate to the exterior and unite with each other into an epithelium. On the whole, the presence of other kinds of cells besides choanocytes seemed to retard the blowing-out of the solid masses into spheres.

(d) *Discussion of the Collar-cell Regenerates.*—At first sight it seems very tempting to suppose that these spheres, forming what we may almost call a choanoflagellate Volvox, are reminiscent in some way of an early stage in the sponge phylum. Add reproductive cells, and we should have something very like what Minchin ('00, p. 160) would hold to be the probable intermediate link between Protozoa and Sponges. However, an explanation can equally well be sought in the direct action of the peculiar circumstances in which the cells were placed; and if it can be shown that their peculiarities are in all probability the result of a reaction to external stimuli, then their value as phylogenetic evidence falls nearly or quite to zero. Let us see what the influence of the conditions would probably be.

When isolated, the collar-cells almost invariably lose their collar, but not their flagellum, which is retained for some time, and lashes violently. In addition, though not exactly amœboid, they can alter their shape very considerably. By these means they are brought into contact with other cells, and then tend to adhere (*cf.* in this connection H. V. Wilson ('07$_2$), on the fusion of flagellated sponge larvæ). Thus balls and lumps are formed out of the originally scattered units. The cells in the centre of these masses withdraw their flagella and become "quiescent" just as the collar cells of Clathrina do when the sponge contracts, or as the larval flagellated layer does during the pupal state.

After a time, practically all the cells are thus collected into masses. Now at last they are at peace—what we may call the shock period is over. Those in contact with the water put out collars once more. But what is to happen to the central ones? They are accustomed to be in contact with a liquid: in the normal pupa this can only be accomplished by their arranging themselves round a central space, the water in

which can be kept changed through the pores and osculum, which are formed by and in the dermal layer. They cannot get directly to the exterior because of the dermal layer which surrounds them. Here, however, there is no dermal layer to bar them from the exterior water, or to make any arrangements for keeping fresh any liquid in a central space. So what happens is that the outermost cells put forth their collars, on the outside; the cells immediately below the surface, feeling the water near them, seek to expand into it. This they do presumably by forcing themselves up between the surface cells by active movements. As more and more cells migrated to the surface, a cavity would be produced directly below the outer layer, and would go on increasing in size as described in § 3 (*b*). The spherical shape finally assumed is presumably the direct result of surface tension.

The behaviour of the large sheets of collared epithelium can, I think, be more or less explained, first, by the elasticity of the epithelium as a whole, and secondly, by the strong tendency to cohesion shown by the cells. If we imagine a slightly-curved sheet of strong gutta-percha forced, with reversal of its curvature, into a thimble, it would press outwards against the thimble's walls. The flagellated epithelium of each radial tube of Sycon is, I should say, in somewhat the same state as the gutta-percha in the thimble—exerting an outward pressure on the chamber walls. This would help to keep the chamber stiff and open. The firm outline, without folds or crinkles, and the rather tense look of the widely-open chambers could scarcely be explained unless the lining epithelium were in some such state of tension, with the collared surface compressed, the dermal surface stretched. Then, when a piece of it was freed it would (as actually observed) bend back to a greater or less extent according to the degree of tension it was in. The small spherical chambers of Demospongiæ often turn right inside out. Here in Sycon the curve is but slightly reversed. Even this, however, would bring some cells at the edges into contact. These would stick together, and then, as I conceive it, those at the free margin would not feel normal with one side as well as their base free, and so, altering their shape continually, they would touch and stick to other cells, thus causing the fragment to approximate more and more to a closed sphere. This, of course, would increase the tension if the properties of the fragment remained the same as those of the whole. But it seems more probable that when released from normal conditions, the gastral cells, after the first mechanical springing-back as a whole, would lose their original physical state and group themselves spherically, because then all the forces acting on them would be equally distributed.

Thus these spheres afford no support to the choanoflagellate theory of sponge affinities. Some people, however, might use these experiments to attack that theory, because, they would say, the collar-cells, though living for a sufficient time, cannot, or at least do not, regenerate any other tissues; if sponges are descended from choanoflagellates, the collared cells must historically have given rise to all the other sorts, and should have retained the power. This argument, with

variations, has actually been employed by MAAS ('10, p. 125). As used by him it rests on three main supports:—(1) If you separate the two halves of the Sycon amphiblastula (by means of Ca-free water), the granular half can give rise to a normal Olynthus, while the flagellated half closes up to form a "blastula," which may live for over a week, but regenerates nothing (MAAS, '06. He gives no figures of the "blastulæ," but they must bear a general resemblance to my spheres, only without collars). (2) In bud development (Tethya, MAAS, '01), the collar-cells are the last cells to be produced. Their early appearance in the larva is then precocious, due only to the need for locomotion. (3) In reduction or involution (MAAS, '10, Sycon and other sponges), they are the first cells to degenerate. This is borne out by MÜLLER ('11_2) and WILSON ('07_1), both of whom think it almost certain that no collar-cells survive in extreme reduction; and by THOMPSON ('88), if, as seems probable, the "capsules" he figures are "artificial gemmules," like those produced by WILSON. URBAN ('10) is not so decisive on the question, but WELTNER'S observations ('07) on hibernating Spongilla point the same way.

I venture to say that the argument is not valid. In the first place, bud- or gemmule-development is almost universally considered as showing fewer ancestral features than egg-development. For one thing, it has certainly been acquired later in the history of the race; and secondly, when a detailed comparison is possible between the two, as in Polyzoa and Ascidians, we can be quite sure that the course followed in the buds is the less primitive.

Among Ascidians, indeed, the process of budding is quite different in different species, while the egg-development is very constant throughout. The course of regeneration is usually very similar to that of budding; in Ascidians (Clavellina) and Polyzoa, apparently identical. In other cases, such as the regeneration of the lens of the Urodele eye, the method is obviously not primitive. The processes of reduction, too, do not seem to be ancestral. From work I am now doing it appears that in the reduction of whole individuals of Clavellina, the ectoderm becomes a simple shell, and all the internal organs are reduced to one closed vesicle, somewhat lobed or branched, while the mesenchyme cells remain unaltered. Yet no one is going to say that therefore the mesenchyme of Clavellina is more primitive than its gut—that its endoderm was acquired later than its mesoderm. Returning more particularly to the spheres, we may say (and the argument applies equally well to MAAS' "blastulæ" of larval flagellated cells) that their failure to regenerate proves nothing as to the phylogenetic history of the animals to which their cells belong. There are many groups in which regenerative functions have been delegated to a younger tissue. Clavellina will once again serve as an example. Here the mesodermal blood-cells (mesenchyme) have taken over nearly the entire regenerative power from the phylogenetically older ectoderm and endoderm. There is a further parallelism; for in embryonic development the younger tissue is produced by the older, though in regeneration the older remains sterile, having handed over its

productiveness to the younger. If we take the parenchymula larva of Ascons as primitive, the upholders of a choanoflagellate ancestry would say that the same thing has happened in the phylogeny of the sponges as in the ontogeny of Clavellina.

This is not the place to enter into a discussion of the ancestry of sponges, but I would like to lay stress on the following points; well known though they are, they only become convincing when all are considered together, and it is seen that each supports and fits in with the other.

(1) In sponges, and especially in Calcarea, a series can be traced in which the relative total bulk of the collar-cells and, to a certain extent, their absolute individual size, gets less and less, while they become confined to an ever smaller part of the inner (exhalant) surfaces of the sponge. In Calcarea certainly (and probably in other sponges) the steps in phylogeny must have been very like those in this series, and in the same direction; for the "higher" forms are not merely more complicated, but also more efficient in the arrangement of their collar-cells (Miss Sollas, 'Camb. Nat. Hist.'); and in their development they pass through stages closely resembling the simpler forms (Maas, '00, etc.).

(2) The larvæ in which there is least precocious development belong to some of the simplest forms in this series (an additional reason for supposing that these forms are primitive and not degenerate); and they consist at first wholly of flagellated cells (potential collar-cells) with a small number of archæocytes (potential germ cells).

(3) In the larvæ of the more specialised forms alone do you find archæocytes taking over the production of collar-cells (Spongilla, Evans, '99, etc.), and then only in part.

(4) In gemmules and buds, however, archæocytes usually produce all the collar-cells.

(5) In such forms as Proterospongia (*fide* Savile-Kent, '80) and Volvox, the reproductive individuals lose their characteristic organs before beginning to divide.

In addition, the "somatic cells" of Volvox, according to Klein ('Ber. Nat. Ges. Freiburg,' vol. 5, '90), have not normally got the power of further division once the germ cells are formed, and are predestined to death from this moment. There do not seem to be any experiments on the regenerative powers of Volvox, but even if the somatic cells should prove capable of regenerating the germ cells, they have become at least partially sterile as compared with the more primitive Volvocidæ where each cell normally reproduces the whole colony, and they serve to show that because certain cells are sterile we cannot therefore infer that they are not the most ancestral in form and structure.

With further division of labour and specialisation it is easily conceivable that a condition would be reached like that which exists in sponges (supposing them descended from Choanoflagellates), namely, that the reproductive cells would not even temporarily (as in Volvox and Proterospongia) retain their ancestral form, while

in the cells that still kept the ancestral structure, the original unrestrained fertility would give place to a partial sterility (as has occurred in Volvox), and this to total infertility as in the collar-cells of sponges to-day.

But we are straying from the point, which is that, however curious and beautiful these spheres are in themselves, yet neither their structure nor their fate has any bearing upon the still-vexed question of the ancestry of sponges.

[*May* 10, 1911.—Since the above was written my attention has been drawn to certain figures and descriptions of Savile-Kent's, upon which these observations of mine may at last throw some light.

Savile-Kent, in the 'Manual of the Infusoria' (1880–82), vol. 1, pp. 178–194, and vol. 3, Plate 9, figs. 15–21 and 24–28 (see especially pp. 183–185 and 189, 190), describes certain structures composed entirely of collar-cells, some of which bear the closest possible resemblance to my "pseudo-blastulæ." He, in pursuance of his theory as to the protozoan nature of sponges, interprets them as stages in the development of the "ciliated gemmules" (free-swimming larvæ).

Every collar-cell, he supposes, runs through a definite cycle of development: starting as a simple flagellate cell, it later develops a collar, and, finally, withdrawing both collar and flagellum, becomes amœboid. Following out this idea, he can bring practically any observations on the structure of the larvæ into line with his theory; for instance, in the amphiblastula he simply assumes that one half has advanced further in this cycle than the other. He first describes (his fig. 23) a larva of *Grantia compressa* entirely composed of flagellated cells (a parenchymula, in fact); this he explains as a very early stage. In reality, it presumably belongs to some other species which normally possesses a parenchymula larva. As a later modification of this type he figures a hollow, oval body composed entirely of normal collar-cells (his fig. 25), exactly like one of my spheres, except for being rather ellipsoid in shape. There can be very little doubt that this body was accidentally produced from adult collar-cells. (Its oval outline could be explained in various ways.)

Then he figures, besides normal amphiblastulæ, others in which the anterior half looks perfectly normal, but the posterior half seems to be composed of a hemisphere of adult collar-cells; the outer ends of these cells protrude considerably beyond what would be the normal outline of the non-flagellate half (his fig. 26). Here it is quite possible that a small sheet of collar-cells, detached in teasing up the sponge, had touched a larva, adhered to it, and bent back over it to produce this curious picture.

In both the above cases the cells figured by Kent are obviously adult collar-cells—they have the characteristic body shape and the collar of the right proportionate size. It is for this reason that I think these figures must be regarded as accurate representations of what he saw. This being so, they must have been produced in some such way as my hollow spheres.

Savile-Kent figures other larvæ (his figs. 24 and 28), in which elongated

prismatic cells, of the regular larval type, have their extremities adorned with collars. In addition, these collars are relatively very small; and the general appearance is thus quite unlike that of any described protozoan or metazoan collared cell. These small collars on larval cells have either remained overlooked from that day to this or else are but phantasms of the too eager eye.

Besides this, KENT described in Halichondria, a monaxonid sponge, spherical aggregations of adult collar-cells, these aggregations being solid, not hollow. He figures one of but three cells (his fig. 18), others of nearly a hundred (his fig. 20), and writes that "numbers of them were liberated on cutting sections" of the living sponge. These masses are very like the solid collar-cell masses in my experiments; but it would be strange if, as he implies, such were liberated immediately on the sponge being cut. He also figures other "earlier" stages of the masses—first without flagella or collars, then with flagella only, and says that they often project freely into the canals. However, until some work has been done on the power of aggregation in the collar-cells of Silicea, it would be waste of time to speculate further as to the nature of these problematical bodies.]

4. *Note on the Collar-cells of* S. raphanus.

The existence of longitudinal supports in the collars of Calcarea, described by BIDDER ('95) in *Sycon compressum*, has been denied by all subsequent observers until last year, when URBAN ('10) redescribed them in various sponges.

My own observations (made several months before seeing URBAN'S paper) are very fragmentary; but as they are positive, proving that BIDDER'S rods certainly exist in life in some conditions of the collar, I venture to append them here.

With a $\frac{1}{12}$ inch apochromat and even a No. 4 ocular, it was quite easy to see, in sections cut with a razor from the living sponge, that the collars were not homogeneous but longitudinally striated, just as BIDDER has described them in preserved material. The striations are slightly darker than the rest of the collar, and run at regular intervals straight up from base to summit (figs. 14 and 16). On looking at the collars from above, the milled or beaded edge figured by BIDDER was very clear. Cells from the choanocyte spheres and masses of § 3 (*b*) frequently presented the same appearance; and in the right-hand cell of fig. 15, from a solid choanocyte mass, we see the rods present in a fixed and stained preparation.

The rods, however, are not always visible. I believe, though I will not yet affirm, that they are always present in the extended collar; but during its retraction they certainly seem to disappear. If, as I suppose, the cells in fig. 15 are in various stages of contraction, it seems that the ribbing disappears first from the basal part of the collar, fading finally from the distal part as well, but leaving it with a thicker and more obvious free rim than usual. This thickened border to nearly retracted collars is often seen in life as well. However, more observations are needed on this point.

It only remains to add that in some sections of young *S. raphanus* I observed in

many collar-cells a structure just like that figured by BIDDER ('95, text-fig. *b*, left-hand cell—his so-called "Iris")—a faint-staining ring round the unstained centre of the intra-choanal area. I saw no radial arrangement in it, however. In the same sections I was able, like HAMMER ('09), to assure myself that the flagellum terminated in a definite basal granule lying on the outside of the nuclear membrane.

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Fig. 7.—An 18-day regenerate. Osm. + M.P.C. Decalcified. × 415. Slightly diagrammatised. A gastral cavity has now been formed, lined by a single layer of collar-cells. The nuclei of the dermal cells are larger and stain more deeply than those of the gastral cells. A patch of gastral cells is drawn in surface view, and the outline of their optical section is indicated. The gap in this represents the place at which the osculum will later be formed.

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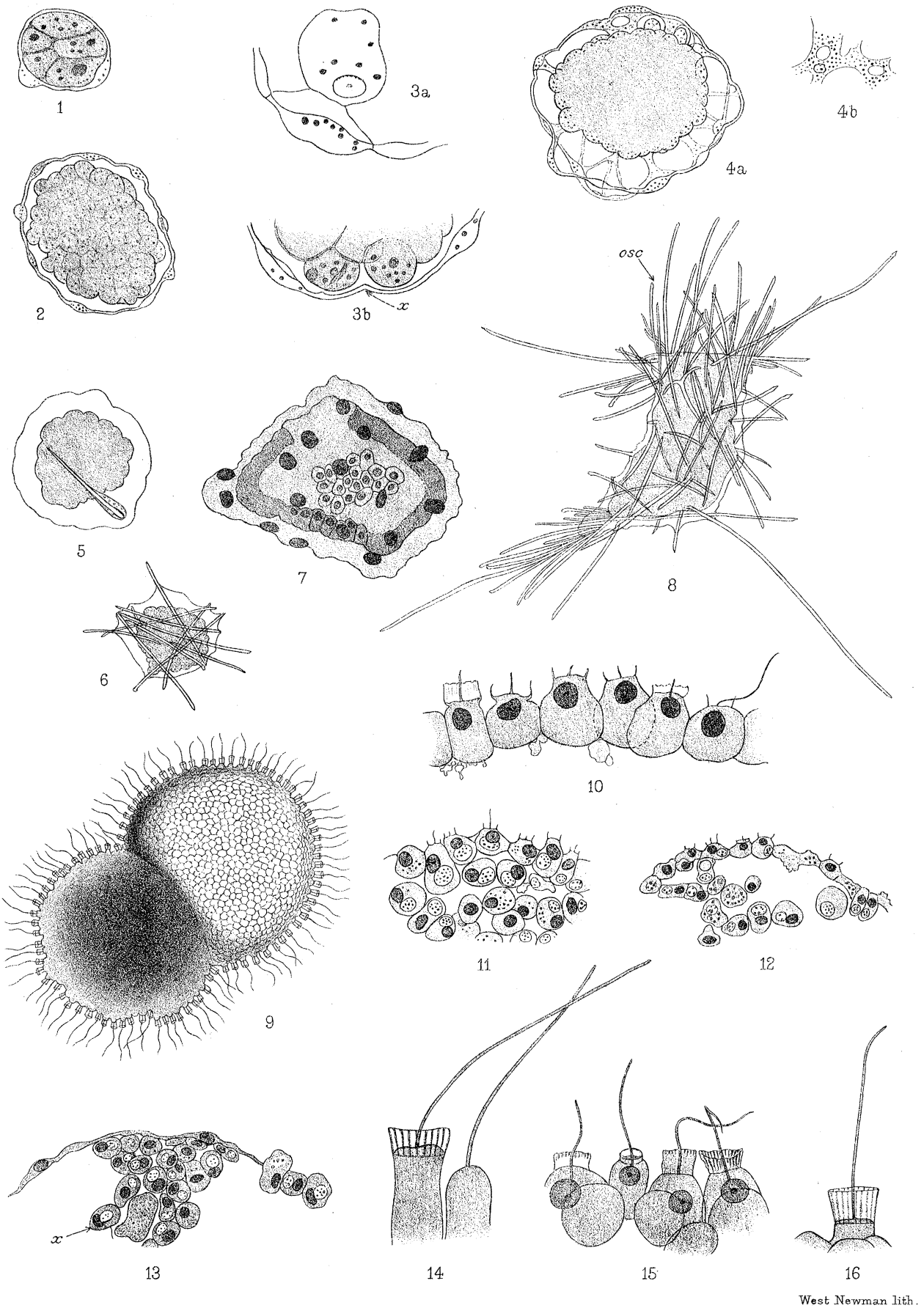

West Newman lith.

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Fig. 10.—Part of a section through a hollow sphere of collar cells. Osm. Iron-Hæm. ×1480. All the cells are active, and are attached laterally to one another to form an epithelium, which is more columnar than the usual outer layer in solid collar-cell masses (see figs. 11 and 12). There is no interstitial substance between the cells. A little *débris* is seen attached to the inner ends of the cells. The details of the cytoplasm are omitted.

Fig. 11.—Part of a section of a 1-day solid collar-cell regenerate. Osm. M.P.C. ×770. The outer cells are united into an epithelium, and are mostly provided with collar and flagellum. They vary considerably in height. The inner mass consists of quiescent cells, which are here fairly, but not very, close packed.

Fig. 12.—Part of a section through an 8-day solid collar-cell regenerate. Osm. M.P.C. ×625. The inner quiescent cells were quite loosely packed. The outer cells were mostly active, and formed a continuous epithelium. They were much extended laterally, their height being usually less than their breadth.

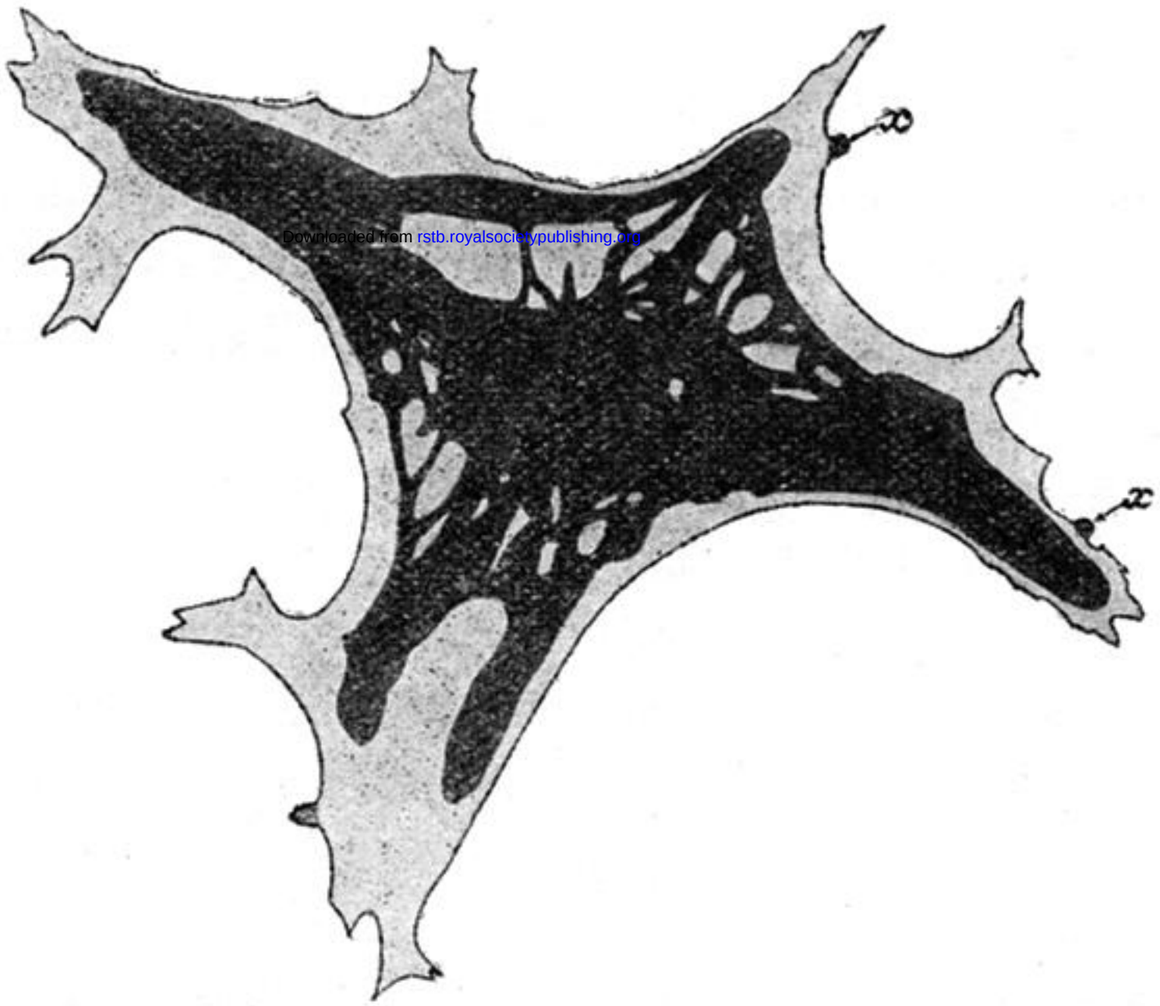
Fig. 13.—Part of a section through a 1-day solid collar-cell regenerate, which also contained a considerable number of other kinds of cells. Osm. M.P.C. ×770. The inner mass of quiescent cells is quite densely packed. Internally the body of an oöcyte is cut; and at *x* one of the collar-cells has a bean-shaped nucleus (only seen in quiescent cells). The outer layer in this region is mostly formed of a flat epithelium of true dermal cells. On the right, however, are three collar-cells, but their nuclei are not yet distal, nor have they put forth collar and flagellum.

FIGS. 14–16.—Collar-cells of *Sycon raphanus*.

Fig. 14.—Two collar-cells of an adult, after two hours under the cover-slip. Alive. ×1900. The one on the right has completely retracted its collar, and its distal end is hemispherical. The collar of the other is present, but considerably retracted. It shows rib-like thickenings.

Fig. 15.—From a solid collar-cell mass. Osm. M.P.C. ×1180. Collars in various states of retraction. In one, ribs are present as in life; in two others, indications of ribbing are seen round the upper edge; and the last is nearly retracted, showing a firm, thick distal edge with no signs of ribbing.

Fig. 16.—From an adult. Alive. ×1380. Collar only slightly retracted: ribs clearly seen.

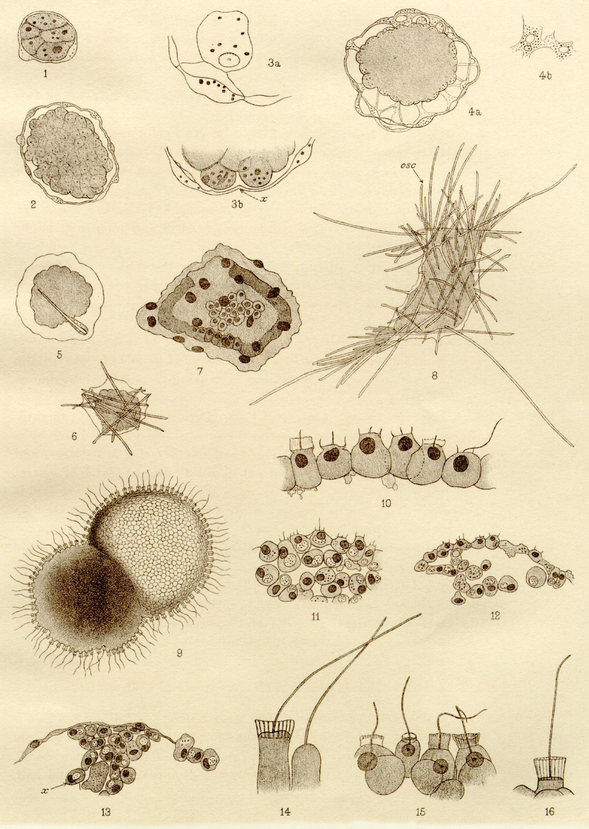


TEXT-FIGS. 1, 2, AND 3.—Three Drawings of a Regenerate of *Reniera rosea*, all of the same magnification (× 17), at intervals of 3 days, to show its changes of shape.

TEXT-FIG. 1.—After 17 days. Condition of fullest extension. The dermal border is very broad and is roduced into sharp processes. Gaps are to be seen in the inner mass. At *x x* are little thickened lumps f darker colour, representing contracted portions of the dermal layer.

TEXT-FIG. 2.—After 20 days. Much contraction has taken place. Where still attached to the bottom, he dermal border is quite thin; and no gaps are left in the inner mass. The bulk of the contents are no onger adherent at all, but project freely upwards as a lobulated mass, over which the dermal layer fits ght.

TEXT-FIG. 3.—After 23 days. Now no longer adherent at all. The action of surface-tension has resulted n the formation of a nearly perfect sphere, the dermal layer fitting close all over it.



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