On the Production *of* Spores *in the* Radiolaria. By Professor L. Cienkowski. (With Plate XVIII).

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Almost all that we know of the developmental history of the Radiolarians renders it extremely probable that the Capsule takes an important part in the reproduction of these organisms. Besides the oft observed multiplication of the Capsule by division, science possesses some other evidence which seems to be of great importance. Joh. Müller[1] saw in the interior of an Acanthometra a swarming of small monad-like vesicles, which moved about for a time, and then changed themselves into Actinophrys-like structures. Since the subsequent history of these monad-like bodies was not made out, the suspicion of the intrusion of some parasitic organism could not in this case be set aside.

Still more in favour of the existence of motile Radiolarian germs are the facts brought forward by Haeckel, in his celebrated work.[2] He saw, firstly, in Sphærozoids, the contents of the Capsules break up into many vesicles. and, secondly, in Sphærozoum, he observed masses of vesicles which exhibited a vibratory movement. What was especially convincing that these structures belonged really to the Radiolaria, was the circumstance that these vesicles contained the same wetstone-like crystalline bodies which are seen abundantly mixed up in the Capsule contents of the compound Radiolaria. Lastly, the groups of amœboid vesicles with movable flagellum-like processes observed by Schneider in Thalassicolla Capsules must be mentioned.

The above observations make it, then, exceedingly probable that the Radiolaria reproduce by motile germs which are developed from the Capsule contents.

To the investigation of these phenomena, as also to the development of the still questionable yellow cells, were my energies chiefly directed, when I had the opportunity, in the past winter (January to the middle of March), in Naples and Messina, of examining living Radiolaria. It soon became apparent that the Acanthometræ and the simple Radiolaria, *e.g.*, the Aulacantha, so common in Naples, are little suited to the investigation. I therefore devoted myself mainly to the colony-building forms. I investigated especially Collosphæra and Collozoum.

The structural relations of the compound Radiolaria are in

[1] 'Abhandlungen der Berlin Akademie,' 1858.

[2] 'Die Radiolarien,' pp. 141, 147, Taf. 33, fig. 9; Taf. 35, figs. 11, 12.

all that is essential nearly exhaustively treated in Müller's papers and Haeckel's classical work, so that I may be very brief about this point. The Sphærozoids and Collosphærids present us with aggregates of Capsules, which are held together by a common mass of Protoplasm.

The Capsules are separated by a certain interval from one another; the Protoplasm binding them together consists of Alveoli (vesicles), of various sizes, between and on to which sarcodic threads and networks are disposed. I always found the capsules supported on the surface of the Alveoli, often lenticular, compressed, and enclosed by a radiating layer of Protoplasm, which also spreads itself over the Alveoli, and passed over continuously into the sarcodic envelope of neighbouring Capsules. Besides those Alveoli which carry capsules there are many smaller, which are free from Capsules. Lastly must be mentioned the Yellow Cells, which are scarcely ever wanting, and are found scattered in various positions, so that the chief soft elements of the compound Radiolaria are characterised by them.

The *Collosphæræ* which I investigated in regard to their developmental history belong to the two species already described—*C. Huxleyi* of Müller, and *C. spinosa* of Haeckel.

As has been long known from Müller's and Haeckel's works, Collosphæra possesses a fenestrated shell, which encloses a Capsule with protoplasmic investment. In the first named species the shell is smooth and furthest removed from the Capsule (figs. 2—4); in *C. spinosa*, it is beset with short spines (figs. 7, 8). The contents of the Capsule is, in both species, homogeneous—here and there of a faint violet colour, and contains a central oil-bubble. The Yellow Cells I have generally found united in a mass within the shell, although some also adhered to the surface of the fenestrated shell outside (figs. 7, 8). The young capsules are naked, embedded, without any shell), in a radiated protoplasmic sheath, not emarginated by any sharply marked envelope (fig. 1). In this stage they often divide themselves by fission into two halves. Not until maturer age does the Capsule obtain a resisting membrane, and become enclosed in a fenestrated shell (fig. 2).

The changes which now further take place in the Capsule consist herein, that their entire content breaks up into a quantity of little spheroids (fig. 6 *c*). I was able to see this complete itself on the stage of the microscope in a single day in the case of *C. Huxleyi*. After a few hours many delicate vesicles appeared in the contents, which later broke up into smaller bodies (figs. 5, 6). Unfortunately it is not possible to carry the "cultivation" in this species further whilst on the object-slide. However, in *C. spinosa,* luckily it was possible to get a step further. In some vigorous specimens which I caught at Naples in February, nearly all the Capsules were filled by an immense number of small spheroids (fig. 7). These Collosphæra colonies were laid in large flat vessels filled with sea-water, and in order to prevent the water becoming bad, bits of ulva and other green Algæ were placed in with them. After one day I found, instead of the common sausage-shaped or spherical colonies which I placed in the vessel, masses of yellow granules which when taken out with a glass tube and examined under the microscope proved to be Capsules of *C. spinosa*. The alveoli to which they had been adherent were entirely gone, only a trace remained here and there of the radial protoplasm, sticking on to the Capsules. The Capsules were thickly squeezed together. At the first glance the specimens seemed in the act of dying, and I was just going to throw them away, when I observed in several Capsules a tremulous movement of the enclosed corpuscles, which in a short time manifested itself in nearly all the Capsules of the mass, and ended with a copious outpouring or "swarming."

I could now quite comfortably observe a part of the material with the higher powers under a covering glass (care being taken to prevent its pressing too heavily) as well as in hanging drops.[1]

In nearly every Capsule monad-like organisms vibrated, the liberated ones swam meanwhile actively in all directions round about. By the side of capsules, whose contents still remained homogeneous, not differentiated, lay those which were full of as yet quiescent, others full of moving, corpuscles. From one capsule I saw the latter issue forth in mass at one point (fig. 8). In some cases I believe I have quite clearly observed how they passed through the fenestræ of the shell.

Let us now pay somewhat closer attention to the little bodies swimming here and there around, which I shall henceforth speak of as zoospores. The Collosphæra zoospores are ·008 mm. long, oval, somewhat obliquely trimmed away at the smaller end, which carries two long cilia (figs. 9, 10). In all the zoospores I found a crystalline little rod, ·004 mm. in length, rounded at either end, or brought to a point, which often projected out somewhat from the body. Add a few oil drops, and you have almost all that one can make out of form-elements in the naked protoplasmic bodies of the zoospores.

[1] Prof. Cienkowski alludes to the use of a chamber such as that described in my paper in this number of the Journal.—E. R. L.

Among the swarms of swimming zoospores lay many motionless ones dispersed. They were round or angular, with drawn-out points; their contents had the same composition as those of the motile zoospores; in addition one or more constrictions could be seen in them. Apparently they were developmental stages of the zoospores, obtained as they were in formation from the contents of the Capsule (figs. 13—15). The same appearances I obtained again in the other specimens of Collosphæra, which I allowed to remain in the vessel for further "cultivation." The movement of the zoospores lasted over twenty-four hours, then they dissolved away, leaving the little rod and the oil bubbles behind. My efforts to cultivate the zoospores in various ways, in order to bring them to a further stage of development, ended always in negative results. In spite of this, and although the further fate of the swarming cells remained undetermined, I believe I may consider them as zoospores. In favour of this view speaks their formation from the Capsule contents, which I, at any rate in the first stages, was able directly to observe on the object-slide in *C. Huxleyi;* further, the bits of protoplasm caught in the act of constriction (ending in fission), which already contained the little rod which one so often finds in great quantity in the undivided Capsule-contents. These facts, as well as the normal appearance of the Capsule-content, make the supposition that we had here to do with parasitic monads, not admissible. When we have once obtained the conviction that swarm cells belong to the developmental cycle of the Radiolaria, some of the earlier statements, especially those of Haeckel as to Sphærozoum, acquire a high significance. The vibrating vesicles with wet-stone shaped bodies, which this naturalist found in *Sphærozoum,* were very probably identical structures with the zoospores of *Collosphæra.*

The second form of the colony-building Radiolaria investigated by me was the common *Collozoum inerme.* The results here obtained agree completely in the chief points with the earlier results obtained by Haeckel. In some points they extend these, and on account of the facility with which one can observe what goes on in the Capsule-contents, they are well fitted to support not unimportantly the view here put forward.

The Capsule is also in this case in the young stage devoid of an envelope, and imbedded in a radiant protoplasmic layer. They multiply by division, taking on the biscuit form or elongating in a worm-like form, and bending and then dividing by several constrictions into separate parts (figs. 20, 23).

Just as the Collosphæra-capsules secrete a hard membrane before the formation of zoosphores, so do also the Collozoa. Their Capsules acquire a sharp contour, and grow considerably larger. Their content contains besides the oil-bubbles not unfrequently a number of small crystalline rods, which are quite like those which we found in the Collosphæra-zoospores. These little rods seem, however, to be of no importance in the further development of the contents, since Capsules which behave themselves similarly not unfrequently occur in the same colonies with or without the little rods. The beginning of the differentiation of the contents is inaugurated by its breaking up into cuneiform areæ arranged radially around the oil-globules (figs. 16, 17). To be sure, this arrangement is by no means without exception, since the divisions of the Capsule-contents as often form irregular or spherical masses. The differentiation now steps further in advance; the large protoplasmic areæ break up into a number of small bodies, which are again able to divide themselves by constriction (fig. 18).

When one squashes a Capsule in this stage, naked content-balls are seen to escape of various sizes, which are already entirely made up of small corpuscles. The indifferent behaviour of the oil-globules in the breaking up of the Capsule-content becomes here apparent. They lie partly enclosed in the balls, partly free between the masses round about. Where only one oil-globule was present, I have always found it outside the aggregate of small spheroids. As in Collosphæra so also here, the complete differentiation of the contents is indicated by the commencing contraction of the Capsule. The colonies obtain in consequence a coarsely punctate aspect, occasioned by the sharp contours of the Capsules and the yellow cells which bedeck them. One after another the Alveoli disappear, and the radiant Protoplasm almost entirely; the Capsules become thereby generally so closely pressed against one another that they appear flattened out like a parenchyma-tissue whose intercellular spaces are filled up by Yellow Cells (fig. 19). In these masses of Capsules, as already the experience of Collosphæra showed us, the differentiation of the Capsule-contents, *par excellence*, takes place, although this may begin even in the normal habitus of the colony.

So far the analogy of what takes place in the Collosphæra is so close that it would be exceedingly strange if the last stage—the out-swarming of the corpuscles formed from the Capsule-contents—were to be wanting here. Unhappily, I was obliged by illness to break off my researches at this point, and so to leave also undecided the question as to how, from the differentiated Capsule-contents, the whole colony takes its rise. Here I will only notice two observations which make the direct development of the Capsule from the radiant Protoplasm very probable. The first fact was found by Stuart.[1] In *Collozoum inerme* Stuart saw that a simple lump of thickened protoplasm became the seat of the development of new individuals. In this case small fat-drops are secreted from the clear protoplasm, which later unite themselves in a central drop; further, there follows a division of the protoplasm into a clearer outer layer and an inner darker, which develops itself into the Capsule. The youngest stages of the latter were recognised as such by the presence of small polyhedral crystals, which are characteristic of the species investigated. I had no opportunity of proving these statements.

The second fact, which appears to show that the capsules develop out of the radiant Protoplasm, I have myself often enough observed in Collozoum inerme. In place of the common layer of protoplasm which surrounds the capsules I often saw many vesicles, thickly pressed together, which possessed all the appearance of young capsules (fig. 24*a*). They were of various forms, often drawn out into sharply pointed processes, contained one or more oil-globules, and were caught in active division (fig. 24, *b*, *c*). Around the entire mass of these little vesicles, bedecking the old Capsule, was spread a thin viscid coat, the remnant of the enclosing protoplasm of the Capsule. After some days I found the little vesicles in question in a cultivated Collozoum, scattered about on the surface of the colony, and rounded off. Further their development did not allow me to follow it.

I conclude this notice with some remarks relating to the Yellow Cells. The writers who have busied themselves with Radiolaria regard the Yellow Cells as integral parts of these organisms, making not the slightest doubt about it. If we ask, however, what ground this conviction rests upon, all we get as an answer is, that the Yellow Cells always are present in most Radiolaria. The fact alone that in a given species the number of these said cells is subject to the greatest variations, and not seldom sinks to a very few, as well as that we possess no knowledge of the way in which they form themselves, is in itself sufficient to raise our suspicions as to whether the yellow cells really belong to the Radiolaria. And if we take into consideration with what a remarkable constancy some parasitic organisms insinuate

[1] 'Göttinger Nachrichten,' 1870, No. 6.

themselves into the developmental cycle of other organisms, it will not, perhaps, appear to be over rash if we raise the question whether, indeed, these Yellow Cells are to be considered at all as integral parts of the Radiolarian body? As a matter of course, developmental studies alone can bring light here.

In the first attempt to follow the origination of the Yellow Cells in the protoplasm, the observation did not appear to be encumbered by any serious difficulty. As is known, the Yellow Cells multiply by division, and one finds them of various sizes. Easy also is it to find in the body of the Radiolarian examined, naked, yellow-coloured, protoplasmic specks, which one would feel inclined to regard as the first steps in the development of a Yellow Cell. It has turned out, however, from more careful observations, that the particular Radiolarian observed had taken in as food yellow Tintinnoids, and that the yellow colour of the protoplasmic specks arose from the undigested food. I failed to discover any single fact which proved the direct origin of the Yellow Cells from the protoplasm of the Radiolaria. In order to get nearer to the question in another way, I availed myself of the interesting fact discovered by Schneider, that the Capsule of Thalassicolla, when extruded from its shell, had the power of building up anew the Radiolarian body. I thought in this way to be able to follow the formation of the Yellow Cell step by step, and the more so since Schneider succeeded in nursing the regenerated Thalassicolla up to the point of development of Yellow Cells. In my researches, which I also carried on with *Thalassicolla nucleata* (the blue coloured variety), the extruded capsules certainly did go so far as to produce new pseudopodia coloured with blue particles, only I had not the luck fully to follow out the process of regeneration. The only new fact which I have found relating to the Yellow Cells is this, that in Collozoum which for some time lay in sea water (over a week), the Yellow Cells proceeded gaily to grow even when the protoplasm and capsules of the whole colony were already completely decomposed. In this condition there appeared around the Yellow Cell a somewhat tough viscid membrane, which completely enclosed it (figs. 25, 26*h*). From this sheath the growing cells escaped very slowly, forming a new envelope which in turn became discarded also. This formation of a sheath occurred in the same cell several times; the escape from the sheath took place so slowly that it was not possible to observe it directly. The liberated cell grew, acquired a ragged outline, and multiplied itself finally by division This property of the Yellow Cells to grow and reproduce themselves after the death of the organism to which they are supposed to belong, and then the remarkable quantity of starch which they produce according to the important discovery of Haeckel, which I can confirm, are phenomena which, though certainly not decisive as the signification of the Yellow Cells yet appear very surprising if belonging to the life-history of the Radiolaria.

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NOTE.—Iodine stains, as Haeckel rightly states ('Jenaische Zeitschrift,' 1870, p. 534; 'Quart. Journ. of Micros. Science,' January, 1871), most of the corpuscles enclosed in the yellow cells, blue. In order to obtain the reaction clearly, I have first extracted the yellow pigment with alcohol and then acted upon the Radiolarian several times with strong iodine tincture. With the chloride-of-zinc-iodine solution the coloration comes out more quickly and intensely.

NOTES *on the* GENUS DOLIOLUM. By EDWARD L. MOSS, M.D., F.R.C.S.I., Assistant-Surgeon R.N.

(*Read before the Dublin Microscopical Club.*)

THERE can be but little doubt that the distribution of ocean surface life is considerably influenced by the great systems of oceanic circulation which recent researches have disclosed. Such circulation probably tends to assimilate the inhabitants of adjacent ocean basins, and helps to explain the remarkable similarity in the faunas of even widely separated seas. But unless surface animals can live with equal facility at considerable depths, a sort of retentive selection will be exerted on those that drift in through narrow straits into seas like the Mediterranean, where the surface waters have an average inward flow, and where the denser outward current is in depths removed from the direct influence of light and air.

Like other Pelagic Tunicates, the genus Doliolum possesses a wide geographical range; but nevertheless, if I can judge by the results of my own "fishing" during voyages over more than a hundred thousand miles of Atlantic and neighbouring seas, it is far from being as widely spread as many of its relatives. Salpa, Appendicularia, and Pyrosoma, are not uncommon in the Mexican Gulf and Caribbean Sea, but Doliolum has never been captured in either. I have met with isolated specimens off Cape Clear, Cape St. Vincent,