The Entry of Zooxanthellæ into the Ovum of Millepora, and some Particulars concerning the Medusæ.

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With Plate 18.

During the present winter Prof. S. J. Hickson kindly gave me an opportunity of examining the material from Jamaica in which he discovered the female Medusæ of Millepora ('99). The results of my inquiry are mainly concerned with the manner in which the symbiotic zooxanthellæ, found to exist in the free ova of that genus, infect the germ cells. I also wish to record the occurrence of free male medusæ amongst the free female medusæ collected in the above locality, and to make some remarks upon the oogenesis.

The Infection of the Ovum by Zooxanthellæ.

In the earliest stages of the female medusa that came under observation the germ cells are arranged around the manubrium of the medusa, as a single layer, in most instances, in dome-shaped fashion. Each cell has well-defined boundaries, and possesses a large nucleus with intensely-staining chromatin nucleolus (fig. 1). The substance of the manubrium is vacuolated and scattered; in it lie numerous zooxanthellæ. At the points where ova arise it would appear as if a gradual dissolution of the cell membrane took place between the cell destined to become the ovum and the cells in contact with it. The nuclei of these sister-cells must undergo dissolution before such a plasmodium is formed, for I have never observed them in the growing ovum, which, moreover, is often bordered by cells in which the nucleus is but faintly discernible (fig. 2). The ovum continues its growth, in this manner at the expense of its sister cells, until at or about the time of liberation of the medusæ the last are incorporated; and I have observed that one or two of their nuclei, at this stage, have in some cases persisted in the cytoplasm of the egg (figs. 3, 8, 9, 10). Finally the ova encroach upon the vacuolated substance of the manubrium until it is reduced to very small proportions (fig. 12), when they are set free.

In the material examined it was clear that the vacuolated substance of the ova in the free medusæ, in all cases, contained numerous zooxanthellæ; but they were equally absent from the ova of the numerous unliberated medusæ that had been inspected. However, after a long search some half a dozen medusæ were found which, I think, afforded conclusive evidence that zooxanthellæ pass in numbers from the manubrium into the ovum, and that this invasion may commence at a period prior to the liberation of the medusæ.

The ovum, until it has attained a considerable size, is sharply divided off from the vacuolated substance of the manubrium. Eventually a period is reached when its compact cytoplasm becomes continuous with the central meshwork (fig. 8). This stage could be commonly observed, and zooxanthellæ be seen round the margin of the egg, but never in its compact substance. Sometimes the egg cytoplasm displayed small incipient vacuoles.

The subsequent period, during which vacuolisation of the cytoplasm of the ovum occurs, and during which the zooxanthellæ are incorporated, could be found only on one piece of Millepora, where half a dozen medusæ had reached this stage (fig. 9). Their ova showed a variable amount of vacuolisation, which, so far as I could make out, is in great part inaugurated at the inner borders of the egg substance; as if in some manner the vacuolated portion of the manubrium with its contained zooxanthellæ were drawn in.

That the medusæ are liberated at this stage can be concluded from their mature appearance within the ampullæ, and from the fact that one free medusa (fig. 10) was met with which exhibited an essentially similar structure.

The other free medusæ examined, possessed ova, more completely vacuolated, and with more numerous zooxanthellæ. The egg-cells showed various phases of an encroachment upon the manubrial substance, which eventually was almost entirely reduced (fig. 12), the ova at the same time becoming rounded off and similar to the extruded ova that were examined.

During all this period the zooxanthellæ exhibit their normal appearance, and it can be observed that they divide fairly frequently within the ovum. These cells have been figured by Moseley ('81) who was able to examine fresh material. He remarks that they closely resembled those of other Hydroids. They contained irregular granules of a bright gamboge-yellow colour, the cell-contents frequently dividing into two, and sometimes, more rarely, into four. In the older portions of the colony the pigment was of dark-brown hue. I show their structure as displayed in stained specimens (fig. 18). The spherical nucleus exhibited a mass of closely-packed chromatin granules. A pyrenoid was always present, the clear space around which, in most cases, gave the reaction for starch. The pigment-bearing granules varied in number and size, did not always stain to the same degree, and in some cases had a little starch associated with them. The cell membrane did not respond to cellulose tests. I observed in a few cases division of a cell into four. Their average diameter was somewhat over 9 μ.

That zooxanthellæ pass into the ovum from the parental tissues appears to be undoubtedly the case from the foregoing evidence; but the part these play in the future economy of the animal remains to be solved. It may be that their enclosure is more accidental than physiologically necessary, for the bulk of the foreign cells in the canals and tissues of the colony may come from the surrounding water at a subsequent period. An apparent increase in the substance of a mature ovum (see below) might be an indication of their activity. At all events, there is suggested, an approach to a more complete symbiotic union than that which exists in Convoluta roscoffensis, for instance, where it has been definitely shown ('07) that infection takes place after the animal is hatched, for there the animal undoubtedly plays, in the long run, the part of a parasite with respect to the alga, as under no known conditions were algæ found to escape alive from the body of that turbellarian.

The Medusæ.

Male medusæ of Millepora have up to the present been found only in material from Torres Straits and from Funafuti ('91), and none of these had been extruded from the colony, though some lay free in the ampullæ. However, along with the liberated female medusæ from Jamaica were two free male medusæ, one of which is figured (fig. 17). The most noteworthy feature of the anatomy at this stage is that the margins of the umbrella are furnished with some batteries of nematocysts. They are the largest of the smaller of the two varieties of stinging cells peculiar to Millepora. The manubrium is practically devoid of zooxanthellæ.

The anatomy of the female medusa has been figured and described by Hickson ('99), and is exhibited to some extent in figs. 8 and 9. In the later stages of the medusæ, within the ampullæ, the substance of the umbrella becomes thinned out centrally into an excessively fine membrane (fig. 9).

The structure of the liberated female medusa was exhibited fairly well in a few cases. A portion of a medusa, shortly after liberation, is shown in transverse section (fig. 10). A stage is also figured in which the eggs are evidently completely developed and ready for liberation (fig. 12), and, at this period, it will be seen that the manubrium has been reduced to very small proportions. An enteric cavity persists, but no mouth. The margins of the umbrella contain four or five batteries of the largest nematocysts of the smaller variety, and also numbers of the smallest forms. The swellings carrying these batteries are the reduced tentacles figured by Duerden ('99) from living specimens in his aquarium.

In the majority of cases the free ova did not differ in their structure from those of the last-mentioned stage (fig. 12), their substance being uniformly vacuolated. However, two specimens were exceptional, showing numerous islands of compact cytoplasm in the alveolar ground-mass. One of these contained what, I think, may be the cleavage-nucleus in process of division (fig. 15). Its islands were free from chromatin. The other had some half a dozen chromatic bodies in as many of the islands (fig. 16), and probably represented a later period in the history of the egg prior to segmentation. As both were of more than average diameter, the presence of numerous areas of compact substance suggested that an accession of material had in some way taken place. Perhaps, as mentioned above, this, if the case, may be brought about by the activity of the included zooxanthellæ.

Concerning Oogenesis.

a. Growth of the oocyte.

The origin of the germ-cells could not be traced out owing to the peculiar fact that the material containing the earlier stages of the female medusæ always exhibited individuals which were at approximately the same period of their development. No doubt the germ-cells, as in the case of the male elements ('91), move into the dactylozooids and gasterozooids, which subsequently undergo modification into medusæ. However, they were always found in the above material, as a single, or in parts double, layer of superficial cells, upon the dome shaped, or at times conical, manubrium of a completely formed medusa.

The cells of this layer vary but little in size, and contain a proportionately large nucleus (fig. 1). The chromatin forms a close reticulum, and is mostly concentrated at the nodes. There is a single deeply staining chromatin-nucleolus.

In the material containing the subsequent stages the ova were practically all approaching the end of their growth period, or in some later phase. The smallest of the two or three exceptional instances (fig. 2) apparently exhibited the oocyte as a plasmodium resulting from fusion of the cytoplasm of several oogonia, the nucleus of the oocyte, however, alone persisting. Some oogonial cells (fig. 2) were in process of fusion with the ovum, and in others the nucleus was becoming indistinct. The germinal vesicle had increased in size, its chromatin now forming open branching strands. The nucleolus was present. In the next smallest (fig. 3) the nucleus was larger, showing to better advantage the branching chromatin strands; the single nucleolus persisted unchanged.

In what I took to be the succeeding stage to the foregoing two cases the nucleolus was never present in the germinal vesicle; which latter body seemed to have reached its limit of expansion. The elongate chromatin strands (fig. 4) were fairly numerous, lying mostly in the peripheral portion of the nucleus, and often exhibiting a ragged outline.

In the greater proportion of growing ova examined the chromatin strands were less conspicuous, fewer in number, and lay in contact with the nuclear membrane, or but a little distance from it (fig. 5). However, they could decidedly be traced into centrally situated, intermingling, achromatic strands, their staining capacity undergoing a gradual diminution towards the interior. In many ova the germinal vesicle was at first sight homogeneous and devoid of chromatin (fig. 6). Though in some such cases the most careful research revealed no chromatin, yet as a rule minute feebly staining strands could be found projecting here and there from the nuclear membrane. Practically always a more or less distinct achromatic reticulum was to be made out in these clear nuclei.

The next phase exhibited by the nucleus was associated with the termination of growth on the part of the ovum. The chromatin, absent from the last of the preceding stages, can again be discerned, and reappears as minute feebly staining granules, which are often clearly at the nodes of an achromatic reticulum (figs. 8, 9). In ova of free medusæ these granules become deeply stainable (fig. 10).

The oogonia that do not develop into ova are practically all absorbed into the substance of the ovum; before this takes place their nuclei fade away (fig. 2), and lose their identity in a manner which I could not determine. Though in the later stages oogonial cells not in process of fusion with the oocyte may exhibit a homogeneous nucleus (fig. 7), yet the nucleolus in these stains deeply to the last. In three ova, belonging to just liberated medusæ, I have observed the nucleus of one of these cells persisting in the cytoplasm (fig. 10). The nucleolus appeared to be broken down into smaller granules. A few similar nuclei could be observed in the vacuoles of the manubrium (fig. 10).

The early stages in the development of the Cœlenterate egg have formed the subject of a memoir by Trinci ('06), wherein he describes several types, and gives an exhaustive discussion of the work that has been done in this direction up to the present. Prior to the growth of the egg it would seem that many cœlenterates reveal a synapsis stage, that is to say, the reticulate nucleus is converted into a closely coiled spireme, which Maas ('97) found resulted from the union of distinctly double chromosomes in the case of Periphylla and Atolla. The chromatin thread then breaks up, as growth commences, into numerous strands, which may practically disappear, except here and there alongside the nuclear membrane (Phialidium), or which may persist

(Tiarella). In the former instance the chromatin shows an increase towards the approach of maturation. The nucleolus is single in the beginning in all cases, but in one type this body may subsequently fragment and undergo changes in staining properties, losing its affinity for basic dyes. Whether single or multiple the nucleolus vanishes before maturation of the egg. In very many cases chromatic bodies make their appearance in the cytoplasm close to the nuclear membrane, suggesting, particularly when the vesicle becomes achromatic, that chromatin is cast out of the nucleus. Bearing the foregoing facts in mind, we may briefly review the phenomena in Millepora.

A synapsis stage such as figured by Trinci ('06) for Tiarella and Phialidium was not observed; however, the subsequent appearance of branching and solitary chromatin strands in the expanding nucleus, found a close parallel in Millepora.

In Millepora the chromatic strands gradually lose their staining capacity until the nucleus is practically, if indeed not absolutely, achromatic. About the time when the ovum has absorbed all its sister cells, the chromatin reappears as minute, diffuse granules.

The single nucleolus vanishes at an early period in the growth of the oocyte.

There is nothing suggestive of an expulsion of chromatin into the cytoplasm during the growth period, though of course an extrusion of non-chromatic substance could go on undetected. The achromatic phase of the vesicle is only temporary.

B. Subsequent phenomena.

Until the cytoplasm of the ovum is completely vacuolated its nucleus remains situated centrally, exhibiting deeply staining granules, uniformly throughout its substance, at the nodes of a fine achromatic reticulum (fig. 10). A nucleus which had begun to assume an oval shape, and was moving to the surface of the egg, was of a like structure, but some of its chromatin granules were of increased size, and as a whole seemed to have contracted away from the nuclear surface for some little distance (fig. 11). I could distinguish no nuclear membrane in this case.

In ova that were ready to be spawned, or had actually been so, the germinal vesicle, now without any semblance of spherical form, and lacking a definite membrane, though contrasting sharply with the cytoplasm, lay close to the exterior, beneath a slight depression on the surface of the egg. It exhibited some remarkable features. The granules of chromatin were confined to certain areas and in many cases were of large size and few in number (figs. 12, 13). In several instances there were found, scattered about in the nucleus, in half-a-dozen or so groups, evidently without any regular arrangement, deeply-staining fragments which had a tendency to exhibit a form suggestive of minute tetrads (fig. 14). No achromatic structures were revealed, but, as at other times, the necessity for a supply of suitably fixed material, on which to confirm the appearances presented, was felt. In several ova deeply-staining granules, similar to those in the nucleus, were observed here and there in the cytoplasm, suggesting strongly that these latter may be cast out to some extent.

Ova that had been liberated possessed a rather uniformly-vacuolated structure, but in a certain instance compact cytoplasmic areas lay scattered throughout the vacuolated substances. This ovum had not the peripheral nucleus recorded of the previous stage, but beneath a slight depression in the surface of the egg, just where one would expect to find it, there was a patch of the more compact protoplasm containing two groups of numerous chromosomes (fig. 15). No traces of a spindle could be seen between them; the axis of such, if it existed, would be tangential and not radial. Another ovum had a similar general structure, but contained some half-a-dozen chromatic bodies in as many of the islands (fig. 16). Five of these were compact and reticulate in structure, the sixth being formed of a number of open intermingling strands.

The limited number of free ova examined, and the possibility of their having been subjected to abnormal conditions, owing to such causes as the concentration of the water in the aquarium, precludes a rigorous discussion of the foregoing facts. However, it is interesting to note that the changes recorded are not at all remotely paralleled in certain forms such as Distichopora ('94) and Pennaria ('04), where a remarkable behaviour of the nucleus during the maturation period has been recorded. In Millepora we have, as in those cases, a migration of the nucleus to the periphery with a dissolution of the nuclear membrane, and an apparent casting out of chromatin into the cytoplasm until hardly any remains. In those forms the nucleus may lose its identity completely, which I have not observed in the limited specimens at my disposal. I think it possible that the maturation phenomena are in a like manner obscured in Millepora. The stage represented in figure fifteen I take to be the first division of the cleavage-nucleus, while figure sixteen presents a further stage in which several nuclear divisions have taken place in the as yet unsegmented egg.

The above-mentioned anomalous behaviour of cœlenterate ova at maturation is discussed fully by Hickson ('94) and more recently by Hargitt ('04, '06). A paper by Lillie ('06) bearing on differentiation in the egg, normal and artificial, may be found of particular interest in this connection.

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EXPLANATION OF PLATE 18,

Illustrating Mr. Joseph Mangan's paper on "The Entry of Zooxanthellæ into the Ovum of Millepora, and some particulars concerning the Medusæ."

Fig. 1.—Oogonial cell from the medusa of Millepora at a period prior to the formation of ova. × 1000.

Fig. 2.—An early stage in the growth of an ovum. The nuclei of the surrounding oogonial cells undergo dissolution, fusion with the ovum taking place subsequently. × 500.

Fig. 3.—A somewhat larger ovum. The nucleolus still persists; the chromatin reticulum has been resolved into many open and branching strands. × 500.

Fig. 4.—The nucleus of an ovum which had absorbed the majority of its associated oogonial cells. Peripherally are numerous chromatin strands. The nucleolus has disappeared. × 500.

Fig. 5.—The nucleus of an ovum of the same period, with fewer chromatin strands and with central achromatic reticulum. × 500.

Fig. 6.—A similar nucleus from which chromatin is practically absent. × 500.

Fig. 7.—An oogonial cell persisting at this period with homogeneous germinal vesicle and sharply-defined nucleolus. × 500.

Fig. 8.—Transverse section through a female medusa prior to liberation. The boundary of the ovum is ill-defined, its substance free from vacuoles and from zooxanthellæ. Chromatin is present as diffused, faintly staining, granules, which form the nodes of an achromatic network. × 200.

Fig. 9.—Vertical section through an ampulla containing a female medusa. The margins of the umbrella are connected by an excessively fine membrane. The ova are undergoing a process of vacuolisation, and zooxanthellæ are being admitted. The nucleus, shown in the ovum to the left, contains diffused chromatin granules forming the nodes of an achromatic network. × 160.

Fig. 10.—Transverse section through a free female medusa. The ovum is only slightly vacuolated, and contains but few zooxanthellæ; most other ova of free medusæ were much more advanced in these respects. Below the nucleus there is seen in the egg cytoplasm the degenerate nucleus of an oogonial cell, and to the left of the ovum two such bodies are in the substance of the manubrium. The chromatin granules of the ovum nucleus stain quite deeply at this stage. × 200.

Fig. 11.—An ovum nucleus from a completely vacuolated egg. It had lost the spherical contour of the preceding stages, and was situated rather peripherally. The deeply staining chromatin granules form the nodes of an achromatic reticulum, and are absent from a small superficial area of the vesicle. × 500.

Fig. 12.—A transverse section through a liberated female medusa. The three ova are completely vacuolated, contain numerous zooxanthellæ, and have their nuclei situated at the surface. The germinal vesicle has lost its spherical shape; its chromatin granules, some of which are of large size, are collected to a great extent in one portion of

the nucleus. The substance of the manubrium has been for the greater part, encroached upon by the ova. × 120.

Fig. 13.—A nucleus from an ovum of preceding figure. × 500.

Fig. 14.—The nucleus of an extruded ovum, with a portion of the egg cytoplasm showing four zooxanthellæ. In the central portion of the nucleus exists an aggregation of variously shaped chromatic bodies. × 500.

Fig. 15.—Portion of an extruded ovum, showing two chromosome groups in a region of more compact cytoplasm. × 500.

Fig. 16.—An unusually large extruded ovum, with islands of unvacuolated cytoplasm; two of these containing compact chromatic bodies, and a third more open chromatic strands. × 200.

Fig. 17.—A liberated male medusa. The manubrium is surrounded by countless minute spermatids. On the umbrella are slight swellings bearing some large nematocysts. × 120.

Fig. 18.—A zooxanthella, showing nucleus, pyrenoid, granules, and cell membrane. × 2000.