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STUDIES IN AUSTRALIAN ATHECATE HYDROIDS.

No. II. Development of the Gonophores and Formation of the Egg in *Myriothela australis*, Briggs.*

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(Plates xlii-xliv, and Figures 1-4.)

Introduction.

Previous to the publication of Benoit's exhaustive researches into "L'Ovogénèse et Les Premiers Stades du Développement chez La Myriothèle et chez La Tubulaire,"[1] our knowledge of the more salient features in the development of the gonophores of *Myriothela* was based on the works of the earlier investigators, in particular Allman (1875), Korotneff (1880 and 1888), and Hardy (1891). Although Labbé (1899), in his work on *Myriothela* and *Tubularia* studied the oogenesis of these genera in more or less detail, he assumed that the origin of the germ-cells and the development of the gonophores were sufficiently well established to enjoin no further investigation into these problems. Consequently he confined his attention almost exclusively to the mode of formation of the egg, but from his observations was unable to reconcile this with the normal cycle of oogenesis in the Metazoa.

Unfortunately Jäderholm's excellent description of the structure and histology of *M. austro-georgiæ*[2] does not include an account of the development of the gonophores in this diœcious species. He has confined himself solely to figuring the immature male and female gonophores as they appear in longitudinal sections. This is all the more to be regretted in view of the fact that the Australian species of *Myriothela, M. australis* and *M. harrisoni*,[3] are also diœcious, whereas the northern forms, *M. phrygia* and *M. cocksi,* are characterized by their monœcious condition. Benoit has observed and described for the first time the process of fertiliza-

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* For No. I see "Records of the Australian Museum," Vol. xvi, No. 7, 1928, p. 305.

1 Benoit.—Archiv. de Zool. Exp. et Gén., lxiv, 2, 1925.

2 Jäderholm.—Wiss. Ergebn. d. schwedischen Südpolar-expedition, 1901-1903, v, 8, 1905.

3 Briggs.—Rec. Austr. Mus., xvi, 7, 1928.

tion in *M. cocksi.* The ripe spermatozoa escape from the cavity of the male gonophore and passing down the gastric cavity in the peduncle of this gonophore enter the gastric cavity in the peduncle of the female gonophore, and so reach the cavity of the female gonophore containing the mature ovum which, at this stage, has not yet been expelled.

Since the male and female gonophores of *M. australis* and *M. harrisoni* are always carried on separate individuals, the process of fertilization in these species appears to demand a different method to the one described by Benoit for *M. cocksi.* The presence of an apical aperture in both the male and female gonophores of *M. australis* and *M. harrisoni* is, I think, to be correlated with the method of fertilization, and I venture to suggest that in these Australian species the ripe spermatozoa are able to make their escape through the opening at the distal pole of the gonophore and so reach the surrounding water. These sperms carried by the currents to the female gonophores enter through the apical aperture and effect the fertilization of the mature ovum. Should this view of the method of fertilization in the Australian representatives of the genus *Myriothela* prove to be the correct one, then a similar condition of the distal pole would be expected to exist in the male and female gonophores of *M. austro-georgiæ.* Jäderholm's figures, however, depict the gonophores of his Antarctic species as spherical structures without a trace of any such apical opening, although his Figure 1 on Plate iii shows a slight but definite thickening of the ectoderm at the summit of the female gonophore. I regard this gonophore as immature, since the sub-umbrellar cavity appears to be filled with cytoplasmic areas and primary oocytes. In this event it undoubtedly represents a stage before the ectoderm invaginates to form the velar aperture.

A perusal of the early literature dealing with the important question of the development of the gonophores in *Myriothela* immediately reveals a mass of conflicting data as well as a number of contradictory views not only on the origin of the primitive germ-cells but also on the formation of the early developmental stages. The problem has been made all the more difficult because the first investigators failed to recognize that there were two distinct, yet very closely related species, *M. phrygia* and *M. cocksi,* on which to base their observations. This fact may account in some degree for the discrepancies noticed in the following summaries from the writings of Allman, Korotneff, and Hardy. Their conclusions are here set forth at considerable length in order that the stages in the development of the gonophores of *M. australis* may be the more readily understood when compared with those in the northern forms.

I. According to Allman's account,[4] the first appearance of the gonophore in *Myriothela* shows itself as a small evagination from

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[4] Allman.—Phil. Trans., clxv, 1875.

the endoderm of the blastostyle. This grows outwards into the ectoderm, carrying with it the endoderm, but separated from the former by the supporting lamella. The extremity of this endodermal diverticulum soon becomes excavated by a spherical cavity which Allman calls the *gonogenetic cavity*. The evagination increases in size, and continues to press the ectoderm before it. The gonogenetic chamber becomes crescentic in shape, while a minute orifice makes its appearance in the summit of the chamber. The ectoderm, however, remains imperforate.

In the female gonophore the floor of the gonogenetic chamber projects further into its interior in the form of a hollow conical spadix. This chamber now becomes filled with a plasmatic mass consisting of a multitude of very small nuclei, each enclosing a minute nucleolus, and immersed in a minutely granular protoplasm. As development proceeds, each nucleus becomes surrounded by a differentiated mass of protoplasm, and the cavity of the gonophore is filled with bodies which possess all the characteristic features of true ova. These continue to increase in size, pressing against one another and thus coming to acquire a polyhedral form; then they gradually become looser and assume finally an oval shape. The male gonophore resembles the female in all points except in being about half the size of the latter, and Allman "could detect no difference as to the origin between the matter which in one case is to be differentiated into ova, and that which in the other is destined for the formation of spermatozoa."

II. Korotneff's early work on *Myriothela* published at Moscow and printed in the Russian language is not available to me; I am, therefore, indebted to Benoit's comprehensive treatise for the following summary of Korotneff's views on the origin of the primitive germ-cells in *Myriothela*.

Korotneff, before any indication of evagination is apparent, recognized the initial cells concerned in the development of the sexual products as large, granular, endodermal cells, cubic or polygonal in outline, which occupy a position against the supporting lamella. These cells divide, forming a large mass which causes the supporting lamella and ectoderm to bulge outward so as to produce a bud entirely endodermal in origin. As the bud increases in size, the ectoderm covering it becomes very thin and reduced to a single layer of epithelial cells. The peripheral cells of the bud then elongate and form an outer covering which is the *Anlage* of the future envelope of the egg. The central cells, cut off from the adjacent cells by a non-cellular layer, multiply and are concerned entirely with the formation of the egg. The male reproductive cells, like those of the female, are also endodermal in origin. Korotneff, according to Benoit, concludes with the remark: "Nous pouvons dire avec certitude absolue que les products génitaux mâles et femelles se développent aux dépens d'éléments embryonnaires de l'endoderme."

Later, in 1888, Korotneff published a second paper, "Contribution à l'étude des Hydraires,"[5] in which he completely reverses his opinion concerning the endodermal origin of the primitive germ-cells, and derives them from the ectoderm of the blastostyle. From an examination of stages earlier than those he had previously worked with, Korotneff describes the formation of a hollow endodermal evagination which penetrates deeply into the ectoderm. The latter is composed of three layers of cells—(1) epithelial, (2) embryonal, and (3) muscular. The embryonal layer gives origin to the reproductive elements, and consists of a mass of cells whose central one, distinguished by its very large size and conspicuous nucleus, represents the primordial germ-cell. This divides to form the germ-cells and is completely surrounded by the vitelline cells.

III. Hardy has described a process of budding in *Myriothela*.[6] The buds arise at the junction of the stolon and body where they may be observed in various stages of development. The first stage is a modification of the character of the ectoderm cells which lose their defined character, proliferate, and a bulging mass of amorphous tissue results. At the same time the thick supporting lamella becomes absorbed, and the endoderm cells likewise proliferate and take on an amorphous character. There is thus formed a kind of blastema in which the limits of ectoderm and endoderm are indistinguishable. The blastema increases in size pushing the perisarc before it, and ultimately forms a rounded egg-like mass attached to the parent-body by a short thick peduncle. The bud soon loses its connection with the body of the parent, but remains attached to the perisarc by a sucker-like arrangement at the aboral pole until it is fully formed.

According to Hardy the formation of the gonophore is, in its earliest stages, essentially similar to this method of budding. Thus the gonophore is a true bud derived from a blastema formed by the fusion of the ectodermal and endodermal elements. The ectoderm of the gonophore-bearing region becomes thickened owing to the accumulation of the primitive germ-cells. Then the basement membrane is absorbed or ruptured, and the endoderm pushes its way into the ectoderm so that the group of primitive germ-cells comes to lie on one side of the evagination. In the next stage the germ-cells become cut off from the maternal tissue by the formation of a supporting lamella. Very soon the male gonophores become distinguished by the rapid proliferation of their generative elements.

IV. Benoit, working on *Myriothela cocksi* (Vigurs) from Roscoff and L'île Ti-sao-son, concludes that the first appearance of the gonophore *always* begins as an evagination from the endoderm of the blastostyle, and penetrates deeply into the ectoderm which, however, remains absolutely inactive and takes no part in the forma-

[5] Korotneff.—Archiv. de Zool., Exp. et Gên., vi, 2, 1888.
[6] Hardy.—Quart. Journ. Micro. Sci. (n.s.), xxxii, 1891.

tion of the gonophore at this stage. The *Glockenkern* (nodule médusaire of Benoit) arises from an interstitial cell whose position has already been definitely established in the axis of the evagination. This cell occupies the apex of the endodermal evagination, and divides to form a rounded mass of cells which become cut off from the rest of the endoderm by the formation of a thin non-cellular layer. The *Glockenkern* increases in size by the absorption of nutritive material from the gastric endoderm, and its cells force their way still deeper into the ectoderm. At this stage a split occurs in the cells of the *Glockenkern* so that a small central cavity is formed which later becomes the sub-umbrellar cavity of the gonophore. At first spherical, this cavity becomes flattened and finally crescentic in shape due to pressure exerted by the underlying endoderm which now gives rise to the spadix. By the proliferation of the endoderm-lamella there are formed the rudiments of the circular canal and the radial canals. At this stage the differentiation of the gonophores into male or female is established. The male germ-cells arise from the epithelial layer covering the spadix. The nuclei of these cells divide directly to form the spermatogonia. Then the germinal mass increases in size, while the epithelium in the upper part of the cavity comes into close contact with the endoderm-lamella to form the epithelium of the sub-umbrellar cavity. Owing to the very small size of the germ-cells Benoit experienced considerable difficulty in following through the stages of spermatogenesis, but he was able to recognize primary and secondary spermatocytes, spermatids, and spermatozoa provided with very long tails. Spermatogenesis begins in the periphery of the germinal mass and proceeds progressively towards the inner part in the neighbourhood of the spadix.

The female gonophore is readily distinguishable from the male gonophore by reason of the much smaller number of sexual products. The female germ-cells give rise by mitosis to the oogonia which multiply and reach a considerable size forming the primary oocytes. The manubrium appears very much later in the female gonophore than it does in the case of the male. Moreover, the female gonophore is less highly specialized since it develops neither circular nor radial canals. The gastric endoderm of the spadix in the female gonophore is always well developed, presenting numerous villi which almost completely fill the gastric cavity of the gonophore.

Development of the Gonophores in Myriothela australis.

The fully-developed blastostyle has a narrow base of attachment to the proximal end of the hydranth, and a club-shaped extremity on which is borne a cluster of capitate tentacles. The blastostyle has no mouth, but contains an extensive gastric cavity communicating with the general body-cavity of the hydranth. In the male, the blastostyle bears terminally some six to nine capitate tentacles, while in the female the swollen head is provided with eight to ten tentacles.

In *M. australis* all the gonophores on a blastostyle are of the same sex, and throughout any one individual the sex of the gonophores is uniform. The mature gonophores are spherical in form, supported on narrow cylindrical peduncles which spring without any definite arrangement from the sides of the blastostyles. The immature gonophores are borne on the proximal part of the blastostyle with the mature ones towards the distal extremity. In the female there are usually three or four mature gonophores near the distal end and some six to eight immature ones on the proximal side of these. In the male the gonophores are more numerous though slightly smaller than those in the female, up to fifteen occurring on a single blastostyle.

Development of the Male Gonophores.

Owing to the fairly advanced state of the material at my disposal, I am unable to give an account of the first appearance of the gonophore which, according to Benoit, always begins as an evagination of the endoderm of the blastostyle. This penetrates deeply into the ectoderm, and the interstitial cell at the apex of the evagination divides to form a rounded mass of cells which becomes cut off from the rest of the endoderm by the formation of a thin, non-cellular layer.

This condition in the development of the gonophore is represented by the earliest stage in my material (Pl. xlii, fig. 2). The gonophore appears here as an endodermal evagination consisting of a mass of cells which has penetrated deeply into the ectoderm and become cut off by the formation of a definite, non-cellular layer. The ectoderm surrounding this mass of cells remains stratified and heavily charged with large, oval nematocysts, except over the outer surface of the evagination where the ectoderm is reduced to a single layer of epithelial cells. The pressure exerted by this penetration causes the ectoderm to bulge outwards so that even at this early stage in the development of the gonophore the ectoderm appears slightly raised above the general surface of the blastostyle.

The *Glockenkern,* thus established, increases in size and a split occurs in the cell-mass where a small central cavity is formed. As this enlarges, the cells become arranged in a single layer surrounding a spherical chamber which constitutes the *Anlage* of the subumbrellar cavity (Pl. xlii, fig. 2). This very soon becomes flattened and appears semicircular in section with its floor composed of a layer of cells which quickly become differentiated from those occupying the two lateral wings (Pl. xliii, fig. 1).

At this stage, owing to the rapid growth of the gonophore, the ectoderm is forced outwards and forms a distinct projection on the surface of the blastostyle. The gonophore, thus coming to project completely on the exterior, is covered by ectoderm which is reduced to a single layer of cubical cells over the summit, but which remains as a stratified layer rich in nematocysts around the base (Pl. xliii, fig. 1).

The gastric endoderm now begins to proliferate rapidly, forming numerous villi that project into the gastric cavity and reduce considerably the extent of its lumen. As this cavity continues to enlarge, the endoderm cells become heavily charged with nutritive spheres which form a very important nutritive material for the development of the gonophore. The outgrowth of the gastric endoderm gives rise to the manubrium. This forces back the cells on the floor of the sub-umbrellar cavity, thus reducing the cavity to a narrow cleft of crescentic form with its horns prolonged laterally over the sides of the manubrium.

Owing to the internal pressure produced by the outgrowth of the spadix, the endoderm-lamellæ occupying the roof of the sub-umbrellar cavity commence to separate in the axis of the gonophore. At this point the endoderm-lamellæ eventually become widely separated leaving a distinct gap (Pl. xlii, fig. 1). In *M. cocksi,* Benoit has described a similar separation of the endoderm-lamellæ, but the gap forms a funnel which becomes filled with a non-cellular substance. In *M. australis,* at this stage, the gap remains open while the endoderm cells surrounding it proliferate and arrange themselves in two layers. It is during this period in the development of the gonophore in *M. cocksi* that the cells become excavated to form the circular canal, but in *M. australis* the cells remain solid, forming a compact mass, and the circular canal fails to develop (Pl. xlii, fig. 1).

At the same time the cells occupying the two lateral wings of the sub-umbrellar cavity form an epithelium which becomes closely applied to the endoderm except in the axis of the gonophore. Here the cells of the sub-umbrellar epithelium enter the gap between the endoderm-lamellæ and come into close contact with the ectoderm (Pl. xlii, fig. 1). Throughout the whole of its extent the sub-umbrellar epithelium is separated from the other cell-layers by a very thin layer of supporting lamella.

The outgrowth of the manubrium also affects the cells on the floor of the sub-umbrellar cavity, causing them to form a crescent-shaped mass over the surface of the spadix. From the cells of this layer are derived the male reproductive elements. An examination of the gonophore at this stage discloses that it has assumed a spherical form and developed a narrow cylindrical peduncle by which it retains its connection with the blastostyle (Pl. xlii, fig. 3). At the distal pole of the gonophore the ectoderm has become raised into a small circular patch composed of deep columnar cells lying directly above the gap between the endoderm-lamellæ (Pl. xlii, fig. 1).

The first stage in spermatogenesis begins in the mass of cells covering the spadix and is accompanied by a rapid multiplication of their nuclei. Then the cytoplasm breaks up and comes to surround each nucleus, forming the spermatogonia in the central part of the mass (Pl. xliii, fig. 1). These cells are quite unlike those of the gastric endoderm, thus offering a marked contrast to the condition that exists in *M. cocksi.* In this species Benoit found that the central part of the germinal mass presented an appearance very like that of the cells in the gastric endoderm due to the presence of large quantities of nutritive material.

The layer of spermatogonia increases in extent, and by rapid division the spermatogonia give rise to the primary spermatocytes (Plate xlii, fig. 3). From these are derived the secondary spermatocytes which almost completely fill the sub-umbrellar cavity (Pl. xliii, fig. 2). They are very small and measure only 3 μ in width. The gonophore has now acquired its definitive dimensions and has a diameter of 700 μ. At the distal pole, the raised patch of ectoderm lying directly above the gap between the endoderm-lamellæ becomes invaginated at the centre to form a small pit-like depression which breaks through into the sub-umbrellar cavity. The velar aperture thus established is lined by a deep columnar epithelium whose cells are derived from the ectoderm (Pl. xliii, fig. 2).

Owing to the absence of a more advanced stage amongst my material, I am unable to describe the formation of the spermatids and the condition of the ripe gonophore. In the case of *M. cocksi,* Benoit remarks that "La *spermiogénèse* est très difficile à observer; on voit seulement dans un gonophore mâle mûr une infinité de *spermatozoides* formés d'une tête très chromatique et piriforme de 1 μ de longueur à l'arrière de laquelle se trouve un centrosome colorable, d'où part un *flagelle* très long formant l'axe de la queue du spermatozoide qui puet atteindre 20 μ. Ces spermatozoides sont groupés en faisceaux à queues parallèles et dans les différentes directions."

I have no observations on the mode of escape of the sperms from the gonophore, but I venture to suggest that the ripe spermatozoa are discharged through the velar aperture, and not through the gastric cavity in the peduncle as observed by Benoit in his specimens of *M. cocksi.*

Development of the Female Gonophores.

The material at my disposal for the study of the female gonophores, while not so complete as for the males, is sufficient to enable me to give an account of the main features of their development. Several blastostyles bearing female gonophores in their middle and late stages of development have been studied entire and in serial sections cut in two directions, transversely and vertically, with a thickness of 6 μ.

My first stage is already well advanced and appears as a rounded outgrowth from the wall of the blastostyle. The ectoderm of the gonophore is reduced to a single layer of cells over the distal pole, but elsewhere remains stratified and is richly provided with large, oval nematocysts. The cells of the germinal mass are arranged in several layers; those in the outer layer have very small nuclei and form the external epithelium of the future spadix, while the others in contact with the very thin underlying supporting lamella are distinguished by their much larger nuclei. They are the mother-cells of the future reproductive elements and represent the oogonia. As these oogonia increase in number, the cells of the gastric endoderm rapidly divide and form numerous villi which become heavily charged with nutritive spheres.

In the following stage, an evagination of the endoderm cells at the distal end of the gastric cavity forms the manubrium. This outgrowth carries the cells of the germinal mass before it, and the sub-umbrellar cavity consequently undergoes a considerable reduction in size. At the same time the germinal mass is driven forward at its central part so that in vertical section it appears as a crescentic patch of cells covering over the outer surface of the manubrium. The gonophore is now represented by a fully formed spherical body, 250 μ in diameter, united with the blastostyle by a short, stout peduncle. Over the distal pole extends a single layer of ectoderm composed of cubical cells, but the rest of the external wall still remains stratified and contains numerous nematocysts.

As the gonophore continues to increase in size, the oogonia multiply *pari passu* until they come finally to fill the entire space between the sub-umbrellar epithelium and the manubrium. When viewed in vertical section, the oogonia are seen to be arranged in a crescent-shaped mass with the horns prolonged for a considerable distance over the lateral regions of the manubrium. The gastric endoderm presents a very characteristic appearance due to the development of the villi which completely fill the gastric cavity (Pl. xliv, figs. 1 and 4). The cells are crowded with nutritive spheres, probably of a lipoid nature, forming a very important nutritive substance for the development of the gonophore.

The oogonia multiply and increase in size to give rise to the primary oocytes which press closely against one another and assume a polygonal form. Each primary oocyte (Pl. xliv, fig. 3) consists of a large cell, 24 μ in diameter, with a large, eccentrically-placed nucleus of 12 μ diameter. The nucleolus is 4 to 5 μ in diameter, and is formed of dense chromatin. Around the nucleolus is a clear zone beyond which is a fine network of threads carrying numerous chromatin granules close against the nuclear membrane. Some of these granules appear to have passed out from the interior of the nucleus, since they occur in considerable numbers either attached to the outer surface of the nuclear membrane or at some distance from it in the cytoplasm.

Although the female gonophore has not yet acquired its definitive dimensions, it will be convenient at this stage to discuss the formation of the egg which now involves the fusion of the primary oocytes in the sub-umbrellar cavity. In *Myriothela*

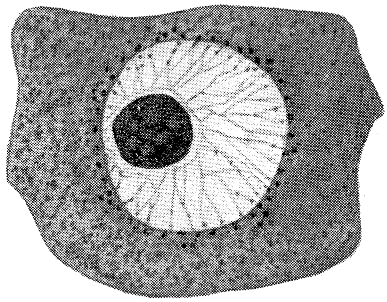

Figure 1.
Myriothela australis, Briggs. Primary oocyte.

australis, egg-formation begins at a stage when the major portion of the gonophore is occupied by the gastric endoderm whose numerous villi completely fill the gastric cavity. The primary oocytes are similar in size and structure, forming a mass of cells which is bounded above and at the sides by a very thin layer of sub-umbrellar epithelium, and below by the supporting lamella and the endoderm of the spadix (Pl. xliv, fig. 4).

Formation of the Egg.

I.—*General.*

The study of egg-formation in the members of the genus *Myriothela* raises several important questions, including the history of the cytoplasmic inclusions of the egg during oogenesis. The problem, which has been made the subject of extensive researches by Allman, Korotneff, Labbé and Benoit, presents a number of difficulties since the formation of the definitive egg in *Myriothela* may be brought about in three distinct and apparently unrelated ways. Firstly, the formation of the egg from a plasmodium has been observed by Korotneff (1880), Labbé, and Benoit. Secondly, the formation of the egg from plasmodial areas has been described by Allman, Korotneff (1888), Labbé, and Benoit. Finally, the formation of the egg by plasmolysis has alone been noted by Labbé.

The first account of the process of egg-formation as it occurs in the case of *M. cocksi* (Vigurs) appeared as early as 1875 in Allman's monograph "On the Structure and Development of *Myriothela*." When the primary oocytes have acquired their full size they begin to undergo a fusion and may be seen united to one another by irregular pseudopodia-like extensions. By the shortening and thickening of these processes the masses are drawn together and become fused into a common protoplasmic mass with numerous nuclei scattered through its substance. The fusion commences among the primary oocytes in the immediate vicinity of the spadix, and continues until eight or more oval masses are formed in the sub-umbrellar cavity. These separate protoplasmic masses increase in size, then coalesce with one another, and finally form a single large plasmodium which entirely fills the cavity of the gonophore.

According to Korotneff's earlier work, a cell situated at the summit of the spadix acquires a more granular cytoplasm which differentiates it from the other cells. This cell increases greatly in size then fuses with the neighbouring cells, the peripheral ones fusing last of all to form the definitive egg.

Later, Korotneff (1888) recognized two kinds of cells in the gonophore: the germinal cells and the vitelline cells. Of these germinal cells, one forms the large *mother-cell* distinguished by its extremely large nucleus, while the others increase in size but their nuclei tend to degenerate and disappear. The nuclei of the vitelline cells become transformed into fat or yolk globules. The definitive egg is formed by the fusion of all these various elements.

Hardy's brief reference to egg-formation throws no fresh light on the problem. He is content to state merely his observations as follows: "In the female gonophore at some period, often relatively late, two or three of the generative cells become larger and more prominent than the others. The period at which this happens does not appear to be fixed, and whatever factor it may be, whether something inherent or accidental, that determines which of these struggling cells shall obtain the mastery and eat up its fellows, it sometimes does not come into play until the gonophore has become a well-formed structure. But it is quite late in the history of the gonophore, when the structure is large and already swollen with yolk, before these two, three or four cells, which, so to speak, have succeeded in attaining to the final heat, decide who is the winner."

Labbé[7] considers that the formation of the egg may be brought about in three ways—(*a*) from a plasmodium, (*b*) from plasmodial areas and (*c*) by plasmolysis.

The formation of the egg from a plasmodium occurs at a stage when the gonophore is filled with free oocytes. These have an active amœboid movement and their pseudopodia fuse in such a manner that vacuoles are left between them. Some of the nuclei now increase in size while the others degenerate. Finally, only a single nucleus remains and this forms the germinal vesicle of the egg. The other nuclei degenerate and produce the "Pseudozellen."

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[7] Labbé.—Archiv. de Zool., Exp. et Gén., vii, 3, 1899.

The most frequently observed method of egg-formation occurs from the fusion of several plasmodial areas. These increase enormously in size by the formation of vacuoles. All the nuclei, with the exception of one or two which become very large, give rise to the "Pseudozellen." The nucleus which persists becomes the germinal vesicle. The complete fusion of the plasmodial areas produces the mature ovum.

Labbé has also observed a third method of egg-formation by plasmolysis. In this case the oocytes which surround the spadix and those which occur on the periphery of the gonophore are not involved in the plasmolysis, and by their fusion give rise to the formative plasma of the egg. In these oocytes the nuclei degenerate with the exception of a single one which becomes the germinal vesicle. The other oocytes undergo plasmolysis and form the nutritive material for the egg.

In *M. cocksi* (Vigurs), Benoit describes egg-formation as taking place by two totally distinct methods:

I. The egg arises from a primary oocyte situated in the axis of the gonophore close to the spadix. The cell increases in size, then fuses progressively with the neighbouring cells to the right and left until there remains only an outer layer of oocytes which are finally incorporated to form the definitive egg.

II. The egg arises from several primary oocytes situated at the distal end of the spadix. These cells increase in size and form several distinct cytoplasmic zones. Each of these becomes a plasmodial area by its fusion with the neighbouring oocytes. The plasmodial areas, to the number of five or six, are at first completely separated by thin, non-cellular partitions extending from the spadix to the epithelium of the sub-umbrellar cavity. As these partitions recede towards the periphery, the plasmodial areas fuse into the large definitive egg which is surrounded by a thin envelope of mesoglœa.

Benoit concludes that the primary oocytes which fill the sub-umbrellar cavity of the female gonophore have been formed *in situ* and are not amœboid cells. The "pseudocellules" are derived from the nuclei of the primary oocytes that fuse to form the definitive egg. In the early stages of egg-formation, the degeneration of the nuclei is rapid and complete, producing spherules or very small granules. In the later stages of the formation of the egg, the nuclei undergo an incomplete degeneration and are always recognizable, forming later the vitelline material of the mature ovum.

The formation of the egg in *Myriothela* reproduces the normal cycle of oogenesis of the Metazoa. In the young gonophore, the floor of the *Glockenkern* is formed from the primitive germ cells which by multiplication give rise to the oogonia. These always divide by mitosis and the last generation forms the primary oocytes which fill the cavity of the adult female gonophore. All the primary oocytes finally degenerate with the exception of a single one which forms the definitive egg.

II.—*Egg-Formation in Myriothela australis.*

We have left the development of the female gonophore in *M. australis* at a stage when the major portion of the gonophore is occupied by the gastric endoderm whose villi completely fill the gastric cavity. The primary oocytes are similar in size and structure, forming a mass of cells which is bounded above and at the sides by a very thin layer of sub-umbrellar epithelium, and below by the supporting lamella and the endoderm of the spadix (Pl. xliv, fig. 4).

The first appearance of egg-formation occurs among the primary oocytes situated in the lower layers of the cell-mass which occupies the region directly above the distal extremity of the spadix. Here two primary oocytes come into close contact and their cytoplasm fuses to form a cytoplasmic mass in which the two nuclei, after undergoing a slight degeneration, persist for a time. This fusion between the cytoplasm of two primary oocytes, initiated at the apex of the manubrium, soon extends to the oocytes surrounding the lateral regions of the spadix, and the sub-umbrellar cavity gradually fills with a number of small cytoplasmic areas (Pl. xliv, fig. 1). These in turn increase in size, not by the formation of vacuoles, but by accretion either of new primary oocytes, or of the previously-formed, small cytoplasmic areas.

The larger areas thus produced have well defined outlines, and in each the eccentric nucleus is discernible as an oval body enclosed by a slightly wrinkled, deeply-staining, nuclear membrane (Pl. xliv, fig. 5). Surrounding the nucleus is a very fine layer of cytoplasm containing scattered basophilic granules. At this stage the gonophore is represented by a spherical body, 600 μ in diameter, completely surrounded by a single layer of cubical ectoderm cells, and united with the blastostyle by a long, narrow, cylindrical peduncle.

As the primary oocytes continue to fuse with the cytoplasmic areas, their nuclei undergo an immediate and complete degeneration, and soon these areas have absorbed all the oocytes with the exception of those in the outer part where they remain for a short time as a kind of covering layer. When viewed in vertical section, the areas are seen to be arranged in some eleven to twelve distinct masses which cover the apex of the manubrium and extend for a considerable distance over its lateral regions. When the last of the primary oocytes fuse with the cytoplasmic areas, their nuclei remain recognizable for a time then undergo an incomplete degeneration, and represent the "Pseudozellen" of the mature ovum.

The gonophore has now acquired its definitive dimensions and has a diameter of 800 μ. At the distal pole, the cubical cells of the outer wall have deepened and formed a raised circular patch of ectoderm which now becomes invaginated at the centre to form a small pit-like depression. This breaks through into the sub-

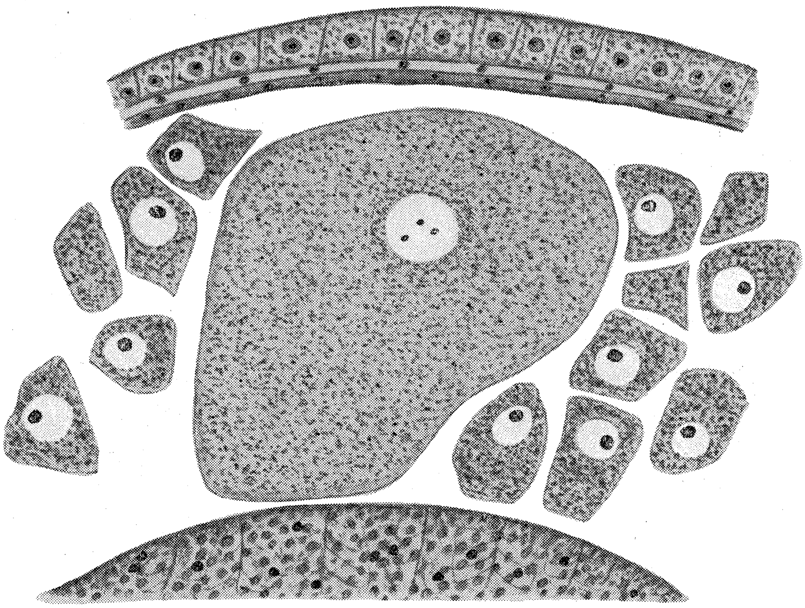

Figure 2.
Myriothela australis, Briggs. Cytoplasmic area and primary oocytes in the sub-umbrellar cavity.

umbrellar cavity, and the velar aperture thus established is lined by a deep columnar epithelium whose cells are derived from the ectoderm.

The final absorption of the primary oocytes into the cytoplasmic areas is very shortly followed by the fusion of the areas themselves, so that there are present in the gonophore some five or six large plasmodial areas completely separated from one another by thin, non-cellular partitions which extend from the spadix to the epithelium of the sub-umbrellar cavity. By means of these partitions, the sub-umbrellar cavity is divided into a number of chambers each enclosing a plasmodial mass which has the appearance of a complete egg with its nucleus and nucleolus.

The last phase of egg-formation involves the withdrawal of these partitions and the fusion of the plasmodial areas. This begins at the surface of the spadix, and as the partitions recede towards the periphery of the gonophore, the plasmodial areas come into close contact with one another and immediately fuse together (Pl. xliv, fig. 2). At the same time the egg increases in volume and exerts a strong pressure on the spadix which is finally driven back com-

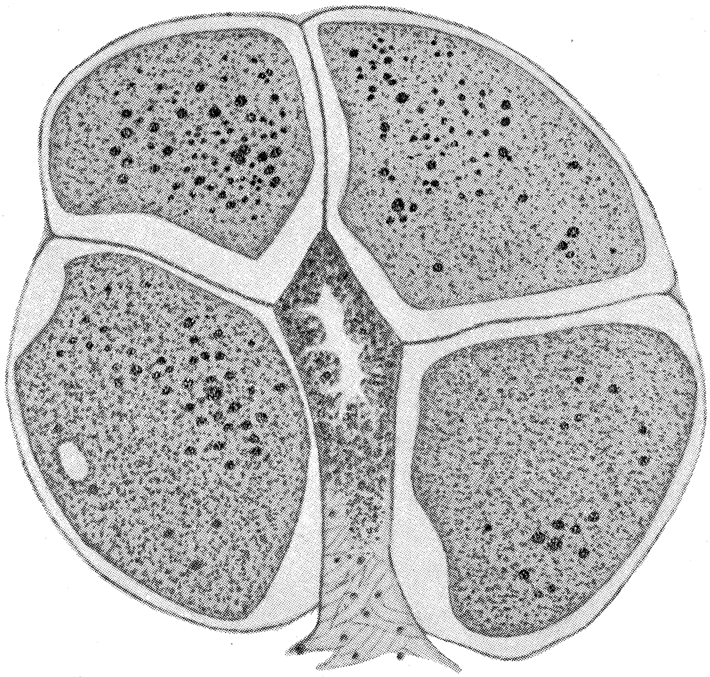

Figure 3.
Myriothela australis, Briggs. Four plasmodial masses completely separated by thin, non-cellular partitions which extend from the spadix to the epithelium of the sub-umbrellar cavity. The outer wall of the gonophore has been omitted.

pletely into the gastric cavity of the blastostyle. At that moment the non-cellular partitions have entirely disappeared, the plasmodial areas have fused into a single, compact mass, and the large definitive egg has completed its growth and occupies the whole of the interior of the gonophore (Plate xliv, fig. 2).

The cytoplasm of the mature ovum contains numerous basophilic granules derived from the complete degeneration of the nuclei of the fused primary oocytes. The "Pseudozellen" ("Pseudocellules" of Benoit) arrange themselves close to the periphery of the cell where they have arisen from the slightly degenerate nuclei of the primary oocytes which were the last to fuse with the cytoplasmic areas. The cytoplasm becomes charged with yolk. This constitutes the deutoplasm of the egg, although this term is equally applicable to other secondary products of the protoplasm.

III.—*The Pseudozellen (Pseudocellules).*

The cytoplasmic inclusions of the egg comprise the yolk bodies and the Pseudozellen (Pseudocellules). Labbé and Benoit have recognized the true nature of the Pseudozellen in *Myriothela,* since both these writers describe them as the slightly degenerate nuclei of the primary oocytes which were the last to fuse with the plasmodial areas. This interpretation has also been put forward by Doflein (1896),[8] Gröneberg (1897),[9] and Pérez (1913),[10] whose works on the athecate genus *Tubularia* clearly support this view.

With regard to the formation of the yolk, Labbé holds that the Pseudozellen undergo degeneration accompanied by a process which he terms karyolysis, and so give rise to the nutritive material of the egg in the form of yolk globules or yolk bodies. In a summary of his views regarding the fate of the Pseudozellen, Labbé states: "Si nous résumons cette question des Pseudozellen, nous voyons que ce sont des éléments morts: oocytes entiers en plasmolyse, ou noyaux d'oocytes dégénérés; et que, très vraisemblablement, ces Pseudozellen de l'œuf des Hydraires jouent, comme l'ont pensé la plupart des auteurs, le rôle de globules vitellins ou de balles vitellines, constituant par conséquent les éléments de réserve de l'œuf mûr." From Labbé's account it would appear that the Pseudozellen degenerate and form the deutoplasm of the mature ovum.

Benoit contends that the nuclei of the primary oocytes are transformed into a refringent mass of chromatin which divides into a number of small spheres each surrounded by a clear zone. By the rupture of the membrane of the Pseudozelle, these spheres are set free in the centre of the egg where later they increase in size and multiply by division to form the vitelline material of the mature ovum. Benoit, however, does not accept Labbé's conclusion that the nutritive material is composed of "éléments morts," but holds that it is "un matérial *bien vivant,* que nous verrons se multiplier par accroissement, suivi de division."

The formation of the yolk, according to Benoit's point of view, involves the breaking up of the Pseudozellen into spherules of varying sizes. The large spherules then subdivide into a number of small spherules which erupt into the cytoplasm of the egg where they form a reserve supply of vitelline material. In his chapter on the fertilization of the egg of *Myriothela,* Benoit returns to the question of yolk formation and again stresses the presence of these spherules which constitute the vitelline material, formed by the division of the Pseudozellen.

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[8] Doflein.—Zeit. f. Wiss. Zool., xlii, 1896.
[9] Gröneberg.—Zool. Jahrb., Abth. Anat. xi, 1897.
[10] Pérez.—Bull. Sci. France et Belgique, xlvi, 1913.

In the unfertilized eggs of *M. australis*, the Pseudozellen lie in the outer part of the cytoplasm where they undergo no further change and remain easily recognizable as distinct nuclei, each with a clearly-defined nucleolus. A similar condition of the Pseudozellen has been described in the unfertilized eggs of some species of *Tubularia*. Owing to the absence of more advanced stages I am unable to follow the subsequent history of the Pseudozellen, but Pérez's description of their fate in *Tubularia* shows that their ultimate transformation only really begins in advanced embryos after the endoderm has become established. As a result of segmentation, the majority of the Pseudozellen are relegated to the endoderm where they are finally digested. In the ectoderm, where they are fewer in number, the Pseudozellen remain almost unaltered until driven out to the vicinity of the free surface. Even in the endoderm there are some Pseudozellen which persist in an almost unaltered condition, but these are finally forced into the gastric cavity and then to the exterior when the actinula is set free. In conclusion Pérez states: "Il semble donc que les pseudocelles n'apportent à l'œuf qu'un supplément assez médiocre de matériaux nutritifs, et qu'elles ne doivent pas être, sans restriction, comparées aux réserves vitellines ordinaires."

IV.—*History of the Yolk.*

The question of yolk formation is of paramount importance in the study of oogenesis. The material at my disposal, however, does not permit of a thorough examination of the cytoplasmic inclusions, especially the mitochondria, of the egg owing to the use of unsuitable fixatives such as 70% alcohol and sublimate-acetic-alcohol.

From the statements of Labbé and Benoit quoted in the previous section it will be seen that both these authors derive the yolk from the division of the Pseudozellen. Benoit, in fact, has given a most circumstantial account of his observations on the subdivision of the Pseudozellen into spherules which erupt into the cytoplasm and form a reserve supply of vitelline material.

No signs of any changes in the Pseudozellen such as those observed by Labbé and Benoit were found in the eggs of *M. australis*, and as far as I am able to determine there is never any observable connection between the Pseudozellen and the yolk bodies.

Evidence on yolk formation in other Hydroid Zoophytes has been derived from the study usually of a single form, and the results at the present time are very contradictory. Hargitt (1913)[11] in his studies on the "Germ Cells of Cœlenterates" has paid particular attention to the formation of yolk in the eggs of *Campanularia flexuosa*. When first observed, the yolk bodies are in

[11] Hargitt.—Journ. Morphol., xxiv, 1913.

greatest number near the periphery of the egg, but as these bodies become more abundant they are present near the nucleus as well. Hargitt argues that the yolk is built up in the cytoplasm out of material which has come from the nucleus, or from the material in the cytoplasm through the aid of material which has come from the nucleus. His explanation is that perhaps an enzyme from the nucleus passes into the cytoplasm and there elaborates and synthesizes the food brought into the egg. Hargitt's observations on *C. flexuosa* show that the nucleolus fragments and entirely disappears. This great activity of the nucleolus coincides with the appearance of the deutoplasmic bodies. From this he concludes that the nucleolus is a dynamic centre concerned primarily with the nutritive activities of the egg-cell. Hargitt, however, does not assume that all the material which forms yolk bodies comes from the nucleus; on the contrary, he believes that a great amount of it comes into the cytoplasm from the food stream in the gastric cavity of the gonophore and never enters the nucleus.

Recent work on yolk formation in other groups of the Metazoa, particularly the Arthropoda and Mollusca, indicates the existence of two main types of yolk—true non-fatty yolk, and fatty yolk. At the present time the evidence as to the origin of the true yolk is not conclusive but points to the formation of this type either from the mitochondria or from the nucleolar extrusions. The fatty yolk apparently arises directly from the Golgi elements. In his account of the cytoplasmic inclusions of the egg of *Ciona intestinalis* Harvey (1927)[12] has attempted to show an intimate relation between the yolk of *Ciona* and the Golgi apparatus.

In the absence of specially fixed material of *Myriothela australis,* I am unable to offer any observations on the mode of formation of the yolk, and in the following notes merely record changes in the latter during and after its formation. The yolk first appears in the periphery of the cytoplasmic areas before the final absorption of all the primary oocytes has been completed. As the cytoplasmic areas begin to fuse into the formation of the plasmodial areas, the yolk rapidly increases in quantity and presents the most outstanding feature in these areas owing to its intensive staining reaction compared with that of the surrounding cytoplasm.

There are two main types of yolk in the egg of *M. australis*: (*a*) small simple yolk spheres which vary greatly in size and lie close to the periphery of the egg, and (*b*) compound yolk spheres which form the largest elements in the egg. These occur as a layer around the egg, a little below the surface, and are found to some extent throughout the centre of the egg.

The two kinds of yolk, simple and compound, show their distinctive characters when stained with Ehrlich's hæmatoxylin

[12] Harvey.—Proc. Roy. Soc., B, Vol. 101, 1927.

followed by eosin. These stains reveal, in certain of the spheres, drops or globules which stain more intensely than the ground-substance of the sphere. After the use of Ehrlich's stain the mature simple yolk is uniformly light red in colour while the compound spheres show very dark red globules in a dark red ground-substance.

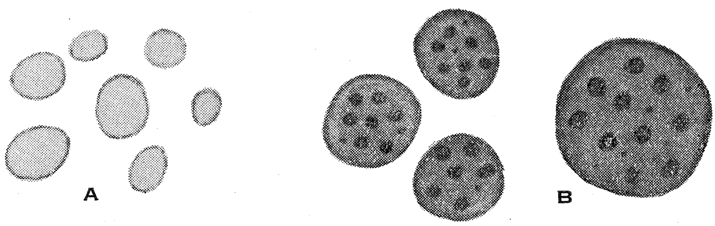


Figure 4.
Myriothela australis, Briggs. A. Simple yolk spheres. B. Compound yolk spheres.

The first elements to appear in the cytoplasm are small yolk spheres, about 3 μ in diameter, which develop into the simple yolk. When the cytoplasmic areas in the sub-umbrellar cavity of the gonophore begin to fuse together, some of the spheres grow more rapidly than the others, so that the uniformity in size is lost. A few now increase in size very rapidly, giving rise to yolk spheres of very unequal sizes. These measure from 6 μ to 9 μ in diameter.

The compound yolk spheres of the mature egg are probably derived from small yolk spheres similar, at first in appearance, to those which give rise to the simple yolk. As the compound spheres continue to grow, small internal globules appear which increase in size until they merge and practically fill the sphere. The size of these spheres varies considerably, some of them measuring 15 μ in diameter and forming the largest elements in the egg.

Although I am unable to determine the precise method of yolk formation, it seems evident that the two kinds of yolk, simple and compound, develop from the cytoplasm or its inclusions, and not from the Pseudozellen as stated by Labbé and Benoit. Whether the yolk spheres develop from pseudochromatin-granules, or from mitochondria, or directly from the cytoplasm itself I am, of course, unable to say, but the presence of simple yolk spheres in the gastric endoderm of the gonophore also suggests a probable source of nutritive material for the growing egg.

Summary.

1. The development of the male and female gonophores in *Myriothela australis,* Briggs is described and figured.

2. All the gonophores on a blastostyle are of the same sex, and throughout any one individual the sex of the gonophores is uniform.

The mature gonophores are spherical in form, supported on narrow cylindrical peduncles which spring without any definite arrangement from the sides of the blastostyles. The immature gonophores are borne on the proximal part of the blastostyle with the mature ones towards the distal extremity.

3. In the female there are usually three or four mature gonophores near the distal end and some six to eight immature ones on the proximal side of these. In the male the gonophores are more numerous though slightly smaller than those in the female, up to fifteen occurring on a single blastostyle.

4. The male gonophore appears as an endodermal evagination consisting of a mass of cells which penetrates deeply into the ectoderm. The *Glockenkern* increases in size and a split occurs in the cell-mass where a small cavity is formed. This enlarges into a spherical chamber which constitutes the *Anlage* of the sub-umbrellar cavity.

5. The outgrowth of the gastric endoderm gives rise to the manubrium. This forces back the cells on the floor of the sub-umbrellar cavity and the endoderm-lamellæ commence to separate in the axis of the gonophore. From the cells on the floor of the sub-umbrellar cavity are derived the male reproductive elements.

6. The first stage in spermatogenesis begins in the mass of cells covering the spadix and is accompanied by a rapid multiplication of the nuclei. Then the cytoplasm breaks up and comes to surround each nucleus, forming the spermatogonia in the central part of the mass. By division, the spermatogonia give rise to the primary spermatocytes. From these are derived the secondary spermatocytes which almost completely fill the sub-umbrellar cavity.

7. The definitive male gonophore has a diameter of 700 μ. At its distal pole the ectoderm becomes invaginated to form the velar aperture which breaks through into the sub-umbrellar cavity.

8. In the female gonophore the cells of the germinal mass are arranged in several layers; those in the outer layer form the external epithelium of the future spadix, while the others represent the mother-cells of the future reproductive elements and form the oogonia.

9. An evagination of the endoderm cells at the distal end of the gastric cavity forms the manubrium. The oogonia multiply and finally fill the entire space between the manubrium and the sub-umbrellar epithelium.

10. The oogonia multiply and increase in size to give rise to the primary oocytes which press closely against one another and assume a polygonal form.

11. The first appearance of egg-formation occurs among the primary oocytes situated in the lower layers of the cell-mass. Here two primary oocytes come into close contact and their cytoplasm fuses to form a small cytoplasmic area. The sub-umbrellar cavity gradually fills with a number of these cytoplasmic areas. These increase in size by accretion of either new primary oocytes, or of previously-formed cytoplasmic areas.

12. The final absorption of all the primary oocytes into the cytoplasmic areas is followed by the fusion of the areas themselves, forming some five or six large plasmodial areas completely separated by non-cellular partitions.

13. The definitive egg is produced by the withdrawal of the partitions and the subsequent fusion of the plasmodial areas. Its cytoplasm contains numerous basophilic granules. The Pseudozellen lie close to the periphery of the mature ovum where they have arisen from the slightly degenerate nuclei of the primary oocytes which were the last to fuse with the cytoplasmic areas. The cytoplasm becomes charged with yolk, which constitutes the deutoplasm of the egg.

14. The definitive female gonophore has a diameter of 800 μ. The whole of its interior is occupied by the large definitive egg which has driven the spadix completely back into the gastric cavity of the blastostyle. The distal pole of the gonophore is occupied by the velar aperture.

15. The presence of an apical aperture in both the male and female gonophores suggests that the ripe spermatozoa escape through the opening at the distal pole of the male gonophore and so reach the surrounding water. These sperms carried by the currents to the female gonophores are able to enter through the apical aperture and effect the fertilization of the mature ovum.

16. The history of the yolk is discussed and the suggestion made that the yolk is formed in *Myriothela australis* directly from the cytoplasm or its inclusions, and not from the Pseudozellen as stated by Labbé and Benoit.

17. There are two main types of yolk in the egg: (*a*) small simple yolk spheres which vary greatly in size, and (*b*) compound yolk spheres which form the largest elements in the egg.

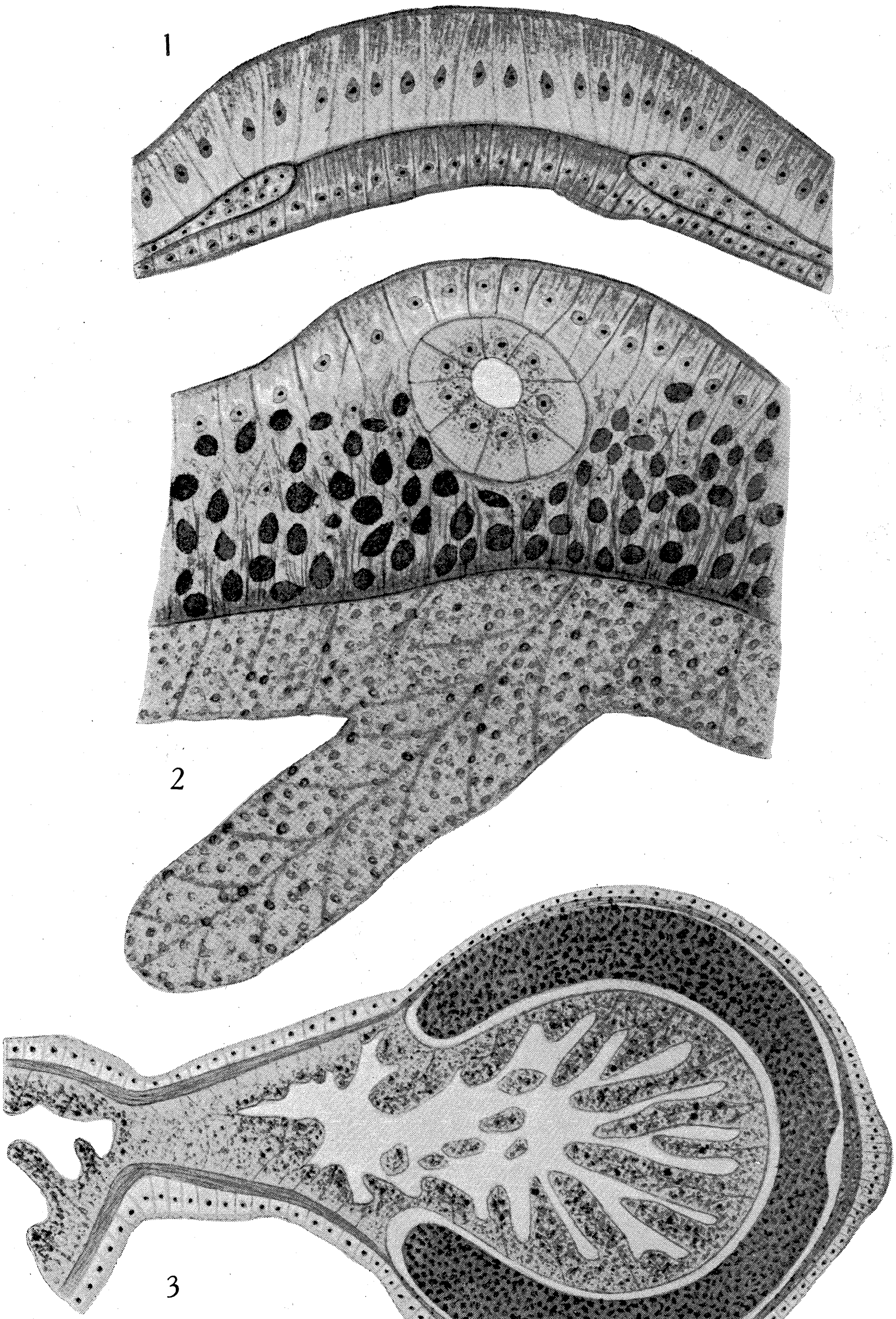
EXPLANATION OF PLATE XLII.

MYRIOTHELA AUSTRALIS, *Briggs.*

Fig. 1. Vertical section through the distal pole of a male gonophore. The ectoderm is composed of deep columnar cells lying directly above the gap between the endoderm-lamellæ. The cells of the sub-umbrellar epithelium have entered the gap between the endoderm-lamellæ and come into close contact with the ectoderm.

Fig. 2. An early stage in the development of the male gonophore, showing a mass of endoderm cells deeply imbedded in the ectoderm and cut off by the formation of a non-cellular layer. These endoderm cells are arranged in a single layer surrounding a spherical chamber which constitutes the *Anlage* of the sub-umbrellar cavity.

Fig. 3. Vertical section through a male gonophore showing its spherical form and narrow cylindrical peduncle by which it retains its connection with the blastostyle. The sub-umbrellar cavity is filled with a mass of primary spermatocytes.



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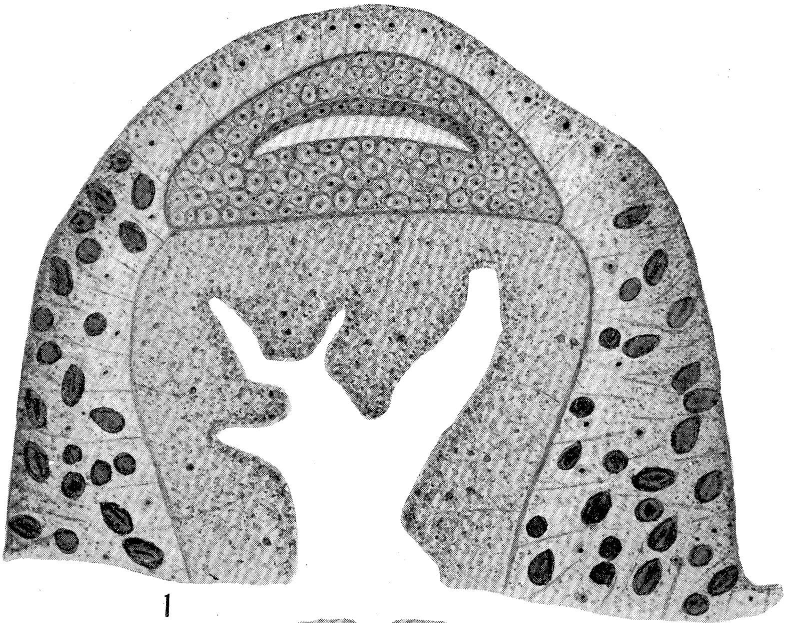
EXPLANATION OF PLATE XLIII.

MYRIOTHELA AUSTRALIS, *Briggs*.

Fig. 1. Vertical section through a young male gonophore in which the sub-umbrellar cavity has become flattened due to the outgrowth of the gastric endoderm to form the manubrium.

Fig. 2. Vertical section through an advanced stage of a male gonophore. At the distal pole, the raised patch of ectoderm cells has invaginated at the centre to form a small pit-like depression which eventually breaks through into the sub-umbrellar cavity. The velar aperture thus established is lined by a deep columnar epithelium. The sub-umbrellar cavity is filled with a large mass of secondary spermatocytes.



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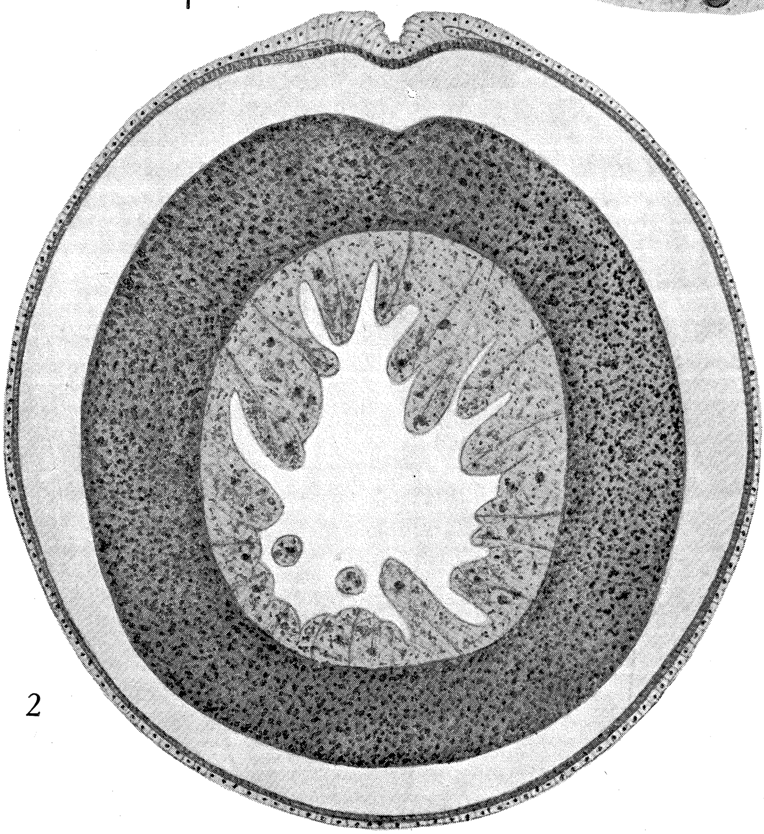

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EXPLANATION OF PLATE XLIV.

MYRIOTHELA AUSTRALIS, *Briggs*.

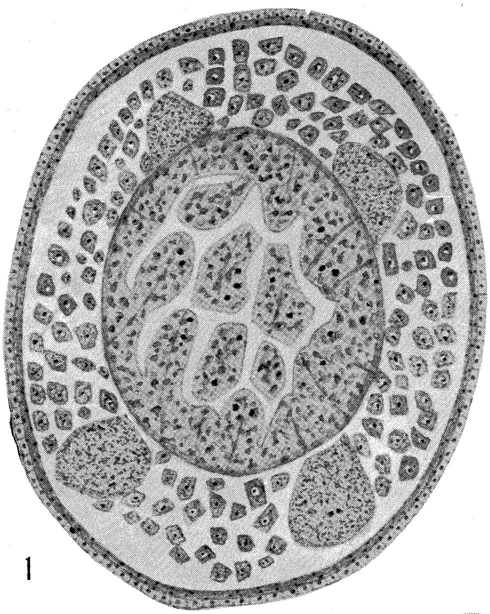
Fig. 1. Transverse section through a female gonophore showing the sub-umbrellar cavity filled with primary oocytes and four cytoplasmic areas.

Fig. 2. Tangential section through a definitive ovum. The withdrawal of the thin, non-cellular partitions is not yet complete; this is indicated by the indentations on the surface of the egg. The cytoplasm is heavily charged with yolk spheres. The outer wall of the gonophore has been omitted from the drawing.

Fig. 3. Primary oocyte from the sub-umbrellar cavity of a female gonophore.

Fig. 4. Transverse section through a female gonophore showing the sub-umbrellar cavity filled with primary oocytes.

Fig. 5. Section through the periphery of a cytoplasmic area. The nucleus is discernible as an oval body enclosed by a slightly wrinkled, deeply-staining, nuclear membrane. Two compound yolk spheres occur in the cytoplasm close to the nucleus.



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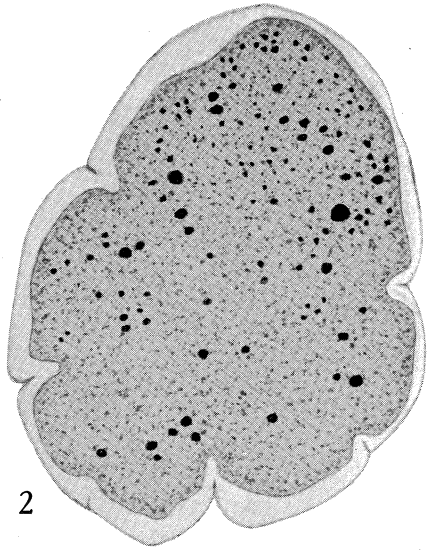

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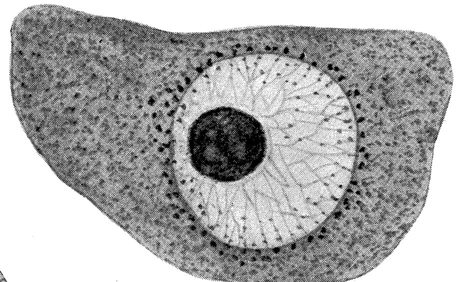

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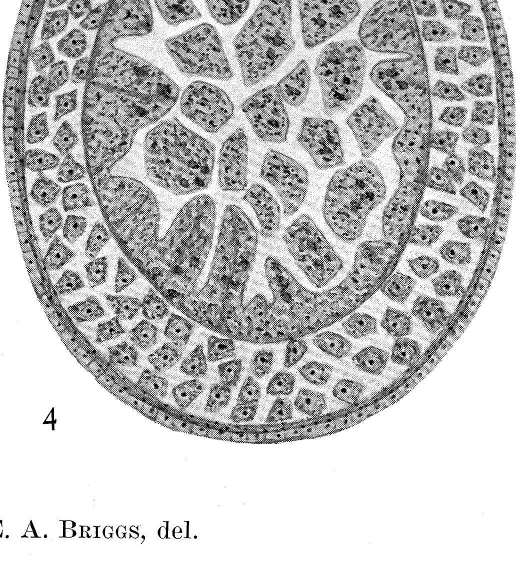

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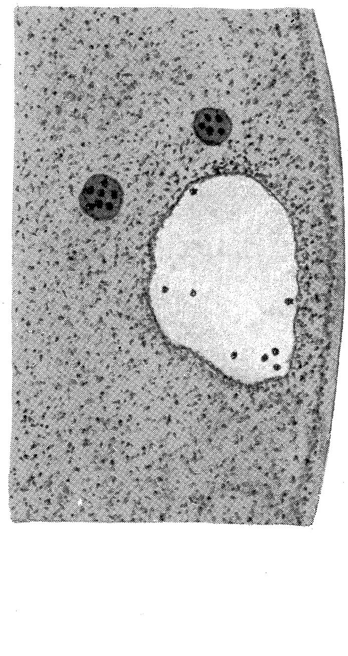

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