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The oogensis of hydra. 1931.

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THE OÖGENESIS OF HYDRA

by

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Submitted in candidacy for the degree

of

Master of Science

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HISTORICAL SURVEY

The oogenesis of hydra has been a subject of biological investigation for two hundred years but even today there are few points on which there is any unanimity of opinion.

Trembley (1744) was the first to describe the egg of hydra. He was succeeded a decade later by Roesel von Rosenhoff and in 1766 by Pallas. The work of these men consisted mainly of superficial observations on the egg, its development into the embryo and the emergence of the young animal.

Ehrenberg ('36) described both the sperm and the egg of hydra.

Kleinenberg ('72) was the first to give a clear account of the interstitial cells, their derivatives and the part they play in the development of the gonads.

Brauer ('91) Describes fully maturation, fertilization and the development of the egg.

Downing ('08), Tannreuther ('08 & '09) and Wager ('09) discover much new and interesting information on oogenesis.

Kepner and Looper ('26) give a concise account of the nutrition of the ovum.

Constant reference will be made to the more important of these authors and their conclusions compared with the results of the present investigation in the various sections of the paper.

A host of authors have contributed to the subject but are of minor interest from our point of view. Those that have been consulted are listed in the bibliography.

THE OOGENESIS OF HYDRA

MATERIAL

A. Sources of Material.

The material was collected from Beaverhill Lake and Hastings Lake, fifty and thirty five miles respectively south-east from Edmonton Alberta. The hydras occur most abundantly on Potamogeton beds in water ranging from two to four feet in depth; some are found both in deeper and in shallower water. Their numbers vary widely from year to year for reasons to be given later. In the fall of 1930 several attempts were made to collect hydras but the results were highly discouraging. As a consequence the work had to be confined to material collected and preserved by Dr. Rowan in former years. Although the material was perfectly preserved and offered the advantages of a variety of fixations, living specimens would have been of great value in settling certain debatable points.

B. Methods of Killing, Fixing and Staining.

The material was killed and fixed in a number of different ways; most of it in Osmic Merkel, Formalin 5%, or Bouin's fluid. Some of it was narcotized with Cocaine and fixed in Formaline 5%. The most satisfactory results were obtained from specimens killed and fixed in Osmic Merkel.

Of the various stains used the following proved most suitable: Heidenhain's Iron Haematoxylin; H. de Winniwarter's Safranin-Gentian Violet and Orange Triple Stain; Heidenhain's Iron Haematoxylin and Safranin; Safranin; and Delafield's Haematoxylin.

Osmic Merkel fixation with Heidenhain's Iron Haematoxylin

was used most. Double staining with Heidenhain's Haematoxylin and Orange G. produces good results for nuclear structures.

C. Species -- general description.

The species of hydra forming the subject for the present investigation is Hydra canadensis, Rowan '30. For the sake of completeness a review of its outstanding characteristics, taken largely from the original paper, follows.

Hydra canadensis is the giant of the hydras, reaching a length of four inches in the fully extended condition: the tentacles, which are constantly six in number, forming five sixths of this length. The development of the gonads commences in early October; sexual reproduction ceases three weeks later. The animal is dioecious. The males mature about ten days before the females. The time of commencement of sexual reproduction, Rowan claims, is the same from year to year regardless of temperature conditions. As the adults die in fall and only the embryos survive the winter, this causes serious fluctuations in their numbers. That is, in years with early, heavy fall frosts, few embryos are produced because most of the adults die off before they have attained sexual maturity.

The gonads are borne on the upper two thirds of the animal's body, the upper margin of the area falling a little short of the bases of the tentacles. Although, superficially, the sexes appear very similar, a number of characteristic features occur by means of which they may be distinguished with a fair degree of accuracy. There is a distinct difference in the distribution of the gonads of the male from that of the female. The eggs and the embryos are scattered over the gonad-bearing region with no

visible regularity, while the testes evince a strong tendency to arise in four, evenly spaced, longitudinal rows. This regularity of distribution, however, is interrupted just at the upper and at the lower margin of the area. The testes appear to be synchronous in their maturation, whereas one female may bear developing eggs and well advanced embryos at the same time. The testes never show the amoeboid or branched shape seen in the early egg, they are more compact. Again, the mature testes have a wider base of attachment than the mature eggs and each testis then bears at its outer or distal pole a small, nipple-like protrusion. With these characters in mind the sexes can usually be distinguished with ease.

The general shape and structure of hydra are well known. It is a diploblastic animal. Its body wall is made up of two main cellular layers enclosing a central digestive cavity, the coelenteron. The layer lining this cavity is the endoderm, composed of large cells, elongate in a direction at right angles to the long axis of the animal. This inner body layer, the endoderm, is separated from the outer body layer, the ectoderm, by a thin, gelatinous, non-cellular layer, the mesogloea. The ectoderm forms the "skin" or covering of the animal and is composed of musculo-epithelial cells smaller than the endoderm cells but elongate in the same direction. The inner ends or bases of these cells rest on the mesogloea. Between the bases lie the small, rounded, interstitial cells which are differentiated in the embryo at some time during the formation of the ectoderm.

Budding is the common method of reproduction in spring

and during the summer. Sexual reproduction is only known in fall.

THE ORIGIN OF THE OVARY

A. Review of the Literature.

Leydig ('54) (as cited by Downing '08) was the first to notice the interstitial cells in the ectoderm of hydra but he failed to recognize them as distinct from the more prominent ectoderm cells. It was Kleinenberg ('72) (according to Downing '08), later, who first discovered their importance and described their origin and fate. He states they form a tissue distinct from the neuro-muscular tissue (ectoderm) and give rise to the nematocysts and reproductive organs. In his paper he gives a clear account of the formation of the ovary from the interstitial cells which has been confirmed by later investigators.

B. *Hydra canadensis*.

The interstitial cells in the non-reproducing hydra lie singly or in small groups among the ectodermal cells. They are fairly evenly distributed over the trunk of the animal. The first indications of the development of an ovary are detected by the rapid division and contemporaneous growth of the interstitial cells. Tannreuther ('08) working on H. sp.? (Brauer)= H. dioecia (Downing), states that the formation of the ovary is first recognized by the rapid growth of the interstitial cells in some region and not by their rapid division. This is not in agreement with my observations on H. canadensis. Here an increase in the numbers of the interstitial cells is observed as soon as any growth can be detected; that is the two processes go on simultaneously. Because the cells stain

differently as soon as growth sets in, this part of the process appears emphasized in stained sections and is therefore probably misleading. In healthy, actively breeding individuals these cells develop over large areas, sometimes forming an almost continuous layer of enlarged cells (ovarian cells) throughout the general gonad-bearing region, (Figs. 1 and 2, Plate 1). In other specimens they may begin as a small patch of cells, probably confined to one side of the animal only, but spreading rapidly. The rate of development naturally depends largely on the physiological condition of the animal concerned. At all events, the territory taken in by the ovary continues to increase and the cells multiply and grow until the organ shows as a low mound with a wide base on the outside of the animal. In this manner usually a number of ovaries develop simultaneously and their boundaries are seldom clearly defined. The development of the ovaries is not synchronous like that of the testes. The same parent may bear at one time eggs or even embryos and show not far from them a newly developing ovary.

The changes the interstitial cells pass through in their transformation into ovarian cells are quite conspicuous. The cells increase in size, the nuclei enlarge and the nucleoli become more prominent. The cells now stain more deeply and the nuclear membrane becomes more distinct. As Downing ('08) points out, the cells closer to the mesogloea grow more rapidly than those farther removed from it.

The ovary has now arrived at a stage where it is ready to give origin to the egg. Tannreuther ('08), working on H. sp.? = H. dioecia (Downing), describes the ovary of this stage as

divided into two distinct regions, namely:" a more central region, which gives rise to the ovum or ova, and a peripheral region which may be considered the temporary ovary, whose cells cease to enlarge and later contribute directly to the formation of the yolk"....."The cells of the central region vary in number and their contents contribute directly to the formation of the ovum or ova. Not merely one but all of these cells continue to enlarge. This increase in size affects the nuclei as well as the cell bodies. The cell walls break down and the cytoplasm which now becomes a common multinucleate mass without any definite outline comes to lie between the enlarged cells of the peripheral region of the ovary and the mesogloea. The egg at this stage of development as stated above is multinucleate."

In H. canadensis such a condition has not been observed. There is no evidence of any such clear cut division of the ovary into regions although the cells closer to the mesogloea are larger than those closer to the ectoderm. But there is here a gradual transition from the one to the other and no sharp line of demarcation exists between the two.

My observations confirm the views of Downing ('08) that the interstitial cells do not migrate toward the region of the ovary during its development, from parts that lie farther removed from it, as was stated by Kleinenberg ('72). Apparently the increase in numbers of the developing interstitial cells which form the ovary is due entirely to an acceleration in the rate of division of the cells that already lie within the future confines of that organ. A migration of the interstitial cells is not involved, for they appear no more abundant just outside

the margin of the ovary than they do in parts farther removed.

An interesting feature repeatedly observed wherever ovarian cells are being rapidly proliferated to form an ovary, is the production of groups of nematocysts. This was also noticed by Wager ('09). They appear to be subject to spells of rapid multiplication at certain centres just like the interstitial cells. Many of these nematocysts appear at the surface of the ectoderm in the normal manner but the majority lie near the mesogloea where they cannot possibly be of any use to the animal for protective purposes. Wager claims they are being "absorbed or digested". Thallwitz ('85) records a strong development of nematocysts in the endoderm covering the gonads of Clava and claims they serve as a protection for these organs against a protozoan parasite. No such reason has been found for their strong development in the case of hydra. Their proliferation may be due merely to the increased rate of division of the interstitial cells.

THE ORIGIN OF THE EGG

A. Review of the Literature.

Authorities, apparently, are agreed as regards the origin of the ovary but a number of different theories are held about the beginning of the egg within the ovary.

Kleinenberg, ('72) (citing from Downing '08) working on H. viridis, holds that after the ovary has reached a certain size, one of its cells, which has so far been identical with the rest of the ovarian cells, begins to grow rapidly and elongates. Its margin becomes marked with short, pointed processes. As these processes grow larger the cell assumes what Kleinenberg describes as the butterfly shape. That is, two winglike masses appear on opposite sides of the main central body giving the cell roughly the appearance of a butterfly. At the same time both the nucleus and the nucleolus increase markedly in size. The processes of the margin of the cell enlarge, and the egg increases still more in size, broadening out, and the winglike lobes with their irregular margins become even more conspicuous. The processes at this stage make up the bulk of the cell, which is now really the egg, and push out into the surrounding tissue. This is the initial amoeboid stage of the egg which proceeds to ingest the ovarian cells about it.

The observations of Tannreuther ('03) have been partly quoted. As we have seen, the central cells of the ovary in his case fuse, forming a multinucleate mass. "All of the nuclei enlarge somewhat, the chromatin assumes the spireme condition and the nucleoli are very prominent. One of these nuclei,

seldom more than one, continues to enlarge and becomes the egg nucleus. The remaining nuclei gradually break down and disappear within the cytoplasm The egg does not begin as a single cell, but as a multinucleate mass which results from the fusion of several cells after the breaking down of their walls. Thus, the cytoplasm which originates from several cells becomes the cytoplasm of the egg."

"The pseudopodia do not grow out between the cells of the ovary, but rather between the ovary as a whole and the mesogloea."

Downing ('08), working chiefly on H. dioecia = H. sp.? (Brauer), observes in addition to the ovarian cells, a new type which he calls "egg cells". His description of these cells follows below. They are supposed to be present in the animal at all times and to form a distinct germinal tissue. Moreover, they are differentiated from the interstitial cells in the embryo and are stamped with unmistakable characters of sex cells. A quotation from his paper follows: "Kleinenberg, working largely on H. viridis, and other investigators, working on some other species, state that the egg appears only after the ovary has achieved considerable size. My studies force the conclusion, however, that the egg cell or cells are always present, even before the proliferation of the interstitial cells begins the formation of the ovary, and not only present but rapidly growing."

"The egg cell which has lain in the midst of the cells of the ovary becomes conspicuous because of its rapidly increasing size, its elongating form and increasingly irregular contour."

"So far as I can find, all investigators, beginning with Kleinenberg, have maintained that the egg is merely an interstitial cell which, after the ovary has begun to grow, increases in size more rapidly than its fellows and assumes new characters. From such unanimity of opinion I hesitate to dissent but in my sections through hundreds of ovaries the egg cells are always distinct, even in early stages, and are derived, in adult life, only from previously existing, similar cells. I submit the opinion, therefore, that in the adult hydra the oogonia (and spermatogonia) are distinctly differentiated a selfpropagating tissue. The early egg cells are slightly larger than the inactive interstitials and have a larger nucleus in proportion to the cell body. The cell outline is spherical whereas the interstitials are polygonal in outline. Adjacent to the nucleus is a small dark ovoid body which stains deeply with gentian violet. There always appears later and frequently at this early stage, a vacuole near the nucleus. The evidence on which I base my conclusion is: 1. That cells with these characteristics are frequently found in mitosis at the point where an ovary is forming. 2. That all gradations from the large undoubted egg to this cell are readily found; but intermediate stages between it and the interstitials are not found."

"Successive stages of the egg development are marked by characteristic shapes. At first it is spherical or nearly so; this spherical phase is much more prolonged in H. dioecia, than it is in H. viridis; shortly after the egg becomes conspicuous in the latter species, before it begins to ingest any quantity

of the surrounding nutritive material it is irregular and amoeboid, but in H. dioecia the spherical shape is long retained and it is only toward the close of its growth that it becomes actively amoeboid. The butterfly shape so long characteristic to the egg of H. viridis is absent in H. dioecia."

Tannreuther ('09) appears to have changed his opinion with regard to the origin of the egg and agrees with Downing in the presence of egg cells in the ectoderm of hydra which give rise to and form the nucleus of the egg. But he maintains that these cells are to be found only during a period of sexual reproduction and do not form a distinct and permanent tissue of the animal. He also claims that no cells can be found in the male that will correspond to the egg cells in the female. The sperms can be traced back definitely to the ordinary interstitial cells.

B. *Hydra canadensis*.

The ovarian cells derive their food from the only source of nourishment, the endoderm. Bearing this fact in mind, it is not surprising to find that the cells closer to the endoderm have proceeded farther in their transformation than those more removed from the endoderm. As a result we find that the inception of the egg always takes place near the endoderm, between the outer ovarian cells and the mesogloea, where the ovarian cells are most mature, (Figs. 3 and 4, Plate 1). Obviously this is also an advantageous position for the developing egg as it lies here closer to the source of food common to both it and the ovarian cells.

Near the centre of the ovary and quite close to the mesogloea may be observed several points at which the ovarian cells begin to fuse. These points of fusion are grouped close together and the cells going into the fusion are, as a rule, slightly elongate, faintly staining and each of them has a large nucleus with a prominent nucleolus. The cytoplasm appears granular. About a dozen or more of these cells come to lie close together at one point and their walls disappear; not simultaneously, for the fusion of the cells is often more complete along one side of the group than along the other. As the cells fuse and their cytoplasm becomes confluent, the nuclei enlarge. The chromatin appears to be in the spireme condition and the large nucleoli are often vacuolate. The chromatin then breaks up and the nuclei degenerate, but at varying rates. In this way a group of independent masses of fused cells form in one ovary, each of them arising as if independent of the others. The central masses of the group grow more rapidly, by continued fusion with additional ovarian cells, than do the more peripheral ones. By this continued absorption of ovarian cells the masses reach a size at which their adjacent walls touch and they now fuse with one another just as each ovarian cell fuses with the mass that it happens to touch. The fusion of these early masses causes this early egg to assume a more connected appearance and it gradually takes on the shape of the early amoeboid egg, before the pseudopodia have developed to any great extent. The amoeboid character, however, is confined to the shape of the egg only, for, although fusion stages between the ovarian cells and the egg are abundant

everywhere, not in a single instance has a stage been found that might be interpreted as a case of amoeboid ingestion. By amoeboid ingestion I understand ingestion as it is effected by an amoeba feeding on a solid particle of food. The process is well known: The cytoplasm of the animal flows round the particle so as to encircle it completely and close around it. This method of ingestion of ovarian cells by the egg of hydra appears to be totally wanting in the present species. For this reason it is probably incorrect to speak of the processes of the egg as "pseudopodia". The term "pseudo-pseudopodia" might be more appropriate. Likewise the term "amoeboid egg" is in this paper used in a restricted sense; that is, it applies only to the shape of the egg and does not imply any active mobility on the part of that organ.

Downing ('08) agrees with Nussbaum in his description of amoeboid ingestion and gives a free translation to describe the process in H. dioecia: "The yolk-filled protoplasmic region of the egg forces its processes into the spaces among the remaining ovarian cells in order to reach them more easily; so the incorporation of the ovarian cells into the egg is probably accomplished by the mobility of the protoplasm. The egg nourishes itself just as a protozoan which with its amoeboid protoplasm surrounds nutrition and takes it into itself." As this process has also been observed and is stressed by other investigators, one can only assume that this must be so in other species of hydra. This interpretation cannot possibly be applied to H. canadensis. If this mode of ingestion takes place in

H. dioecia through "the mobility of the protoplasm" as Downing thinks, it appears that too much individuality is ascribed to the egg of this stage in saying "the egg forces its processes into the spaces among the remaining ovarian cells" to reach them more easily. In H. canadensis the formation of the pseudopodia seems to be more of a passive process. The ovarian cells fuse most actively with the egg at the tips of the pseudopodia (Fig. 4, Plate 1). Each cell added at the tip increases the length of the pseudopodium, bringing it nearer to the cells following it and in this way producing the rapid progress of these organs to the more distant parts of the ovary, so that frequently they completely encircle the trunk of the parent animal at an early period. As the egg invariably lies between the mesogloea and the ovarian cells (Fig. 3, Plate 1), the latter are confined almost wholly to the outer surface of the egg and the sides of its pseudopodia. The great advantage of such a position for the egg will be made clear in the discussion of the nutrition of the egg.

In his description of the early egg and the ovary, Downing states: "The interstitial cells come to be arranged in rows converging toward the centre where the egg lies. The radiate appearance of the ovary is often very marked; in the living hydra it is emphasized by the lines of droplets or granules that are moving toward this central spot. The physiological explanation is evident; the cells have lined up to facilitate the transfer of nutritive material to the eggs and

to carry the excretory products away from this centre of metabolism." Such observations, to be made successfully, demand an extremely delicate technique, a description of which would greatly enhance the value of and lend additional credence to the account. As it is, the "evident" physiological explanation remains a debatable point. The radiate appearance of the ovary has not been observed in H. canadensis and probably never occurs in this species.

It will be noticed that no mention has been made of the "egg cells" observed by Downing in H. dioecia. Although special efforts were made to find these cells in H. canadensis, they have been without success. A few cells that might conceivably correspond to these egg cells were located but a series of stages could always be found to trace them on to the ovarian cells. From the egg cells, larger in size than the interstitials, clear, spherical in shape, containing a large nucleus and nucleolus etc., various cells showing shapes slightly less spherical and staining more and more darkly always led on to the typical ovarian cell as it appears before fusion with the egg. My conclusion is that, at least in this species of hydra, egg cells have yet to be demonstrated.

Tannreuther ('09) claims to be able to produce definite proof that the sperms of the testis are derived from the ordinary interstitial cells. This being the case, it is doubtful whether we are justified to assume a different mode of origin for the egg. If such a condition occurs in any animal it is quite unique and altogether exceptional.

To clear up any doubts with regard to the independent masses of fused ovarian cells that persist around the early egg for some time, an accurate model of an egg of this early stage was constructed (Figs. 5 and 6, Plate 2). It represents a very active egg, for it is composed largely of pseudopodia and shows but a small main body. The actual sections of the egg show the pseudopodia with the pseudocells inside. Amongst the pseudopodia can be seen at least seven individual bodies that have no connection with the egg as yet. These illustrate the independent masses of fused ovarian cells mentioned above. They persist as such until, by an increase in size through fusion with surrounding ovarian cells, their walls touch the main part of the egg. They then fuse with and form part of the egg. As an additional precaution several female specimens were taken intact, stained and cleared for examination. The results proved to be merely a repetition of what the model had already shown..Unfortunately the body tissues of the animals did not destain and clear well enough to enable me to take photographs successfully.

A peculiar feature apparently not observed by former authors is the formation of what I shall term "abortive bodies" as opposed to Tannreuther's "abortive ova". Tannreuther ('08) says:"Abortive ova are often found in sexually reproducing hydra. They consist of a small mass of yolk cells surrounded by a thin egg membrane, and are devoid of a nucleus. The ovary begins as in the normal cases, but instead of one of the nuclei persisting in the multinucleate cytoplasmic cell mass, they all

break down, leaving the common mass of cytoplasm without a nucleus. No pseudopodia are formed. Some of the nuclei of the interstitial cells enter the cytoplasm as in the normal egg and form the yolk. The common mass of cytoplasm with its contained yolk now becomes spherical. The abortive ova do not break through the ectoderm, but are gradually absorbed".

In H. canadensis abortive bodies identical with the bodies described above are occasionally found but they do not arise in a distinct ovary. Such a body merely represents one of the independent masses of fused ovarian cells seen in the model, (Figs. 5 and 6, Plate 2) that has failed to fuse with the main part of the egg, probably because it usually lies somewhat toward the periphery of the ovary where it is not easily reached by the pseudopodia of the egg. An abortive body is illustrated (Fig. 9, Plate 3), it and the embryo of which a portion is seen in the same figure were produced by the same ovary. As Tannreuther says, such bodies are probably, later, resorbed by the tissues of the animal.

Unlike the method of formation of the egg in other hydras, where it arises through the ingestion of ovarian cells by the egg cell or cells, (Downing '08), or through the fusion of a certain number of ovarian cells at one point in the ovary, (Tannreuther '08), in this species the egg starts off as a number of masses of fused ovarian cells; that is, the egg is diffuse to begin with but becomes connected and more compact as development proceeds.

All discussion of the origin of the nucleus of the egg has been carefully avoided up to this stage and for a very excellent reason: although numerous stages of the early egg have been examined, not one of them shows a structure that might with certainty be labelled "nucleus". It appears very significant that of all the authors on the oogenesis of hydra that have been consulted, not one features the early egg showing an unquestionable nucleus apart from nuclei of interstitial cells. Downing entirely omits it; The illustrations of Tannreuther and Wager are drawings and do not appear convincing. For instance, Tannreuther ('08) illustrates (Fig. 3, Plate VII) the early egg with three nuclei. If any one of these nuclei, as figured, is carefully compared with his illustration of the nucleus of the transforming interstitial cell, (Fig. 7a, Plate VII), it will be seen at once that, except for size, they are essentially alike. Similar nuclei can be demonstrated in H. canadensis. However, unless a series of developmental stages of the nucleus can be demonstrated, in which each succeeding nucleus resembles more closely the definite nucleus, our evidence must fail to be convincing.

The earliest stage in which an undoubted nucleus was observed was the stage illustrated by the model, (Figs. 5 and 6, Plate 2). Here the nucleus looks quite normal in every respect but has a diameter only about half that of the nucleus of an egg nearing the end of its growth period.

A number of earlier authors, working on other species of hydra, have observed binucleate eggs. It is claimed that in

the case of such an egg, before maturity is reached, a division takes place dividing the egg in two between the two nuclei and in this way producing what appear to be two normal individual eggs. Examples of such eggs are fairly common in H. canadensis. Except for its binucleate character such an egg looks quite healthy in every respect and its development proceeds in the normal manner. My photographs, (Figs. 11, 12, and 13, Plate 4), were taken from three consecutive sections of a serially sectioned hydra. Knowing this we can judge how close they lie to one another. But even here impending division is evident for the cytoplasm of the egg is beginning to constrict and the course of the constriction lies toward a point between the two nuclei. The result of the constriction will be two normal, independent eggs in one ovary.

From the above it will be seen that although, in minor details, my observations on the inception of the egg agree in one way or another with each one of the three theories presented in the review of the literature at the beginning of this division, as a whole my conclusions differ radically from every one of them. Kleinenberg holds that the egg begins as a single cell which ingests its neighbours and the nucleus of which comes to form the nucleus of the egg. According to this view it would be difficult to explain the occurrence of binucleate eggs, for the egg begins as a single cell which transforms all cells subsequently ingested into pseudocells. The butterfly stage of his developing egg has not been observed in this species.

The egg cell theory of Downing can hardly be reconciled with my results. Neither the egg cells nor the radiate appearance of the ovary have been encountered in the work on this species.

My results agree best with the account of Tannreuther ('08). A noticeable difference appears where he figures the beginning of the egg as a single group of cells that fuse, subsequent development being dependent on the active pseudopodia. In the present case a number of groups of ovarian cells fuse and these groups of fused cells then become confluent. Otherwise the processes are identical.

THE NUTRITION OF THE EGG

A. Review of the Literature.

As has been seen, most authors agree in that the early egg absorbs ovarian cells by means of a process of amoeboid ingestion, the cells disintegrating and their material being dispersed within the cytoplasm of the egg. About the processes of nutrition subsequent to this early phase, opinions differ.

Kleinenberg ('72) (cited from Downing '08) merely records the appearance of and describes the round dark bodies within the cytoplasm of the egg which he calls "pseudozellen". They are the yolk bodies, deutoplasts, of the egg. He makes no attempt to account for their origin.

Downing ('08) states: "The pseudozellen originate in two ways: 1. by the confluence of the small yolk granules formed by and within the egg; 2. by the ingestion of entire interstitial cells when the nucleus of the ingested cell may become a pseudocell. At first the egg is nourished exactly as are the adjacent ectoderm and interstitial cells; material elaborated by the endoderm cells is passed to them all. The egg holds this material diffused throughout it, in the beginning, but later it appears, probably somewhat altered, in granules: these coalesce and make the pseudocells. The same material is taken up by the interstitial cells; it is transformed by them and stored in the nucleus. If now this transformation is complete when the cell is ingested by the egg the nucleus becomes at once a pseudocell; if, however the process is incomplete the

egg must disintegrate the nucleus in order to complete the transformation of its substance into yolk."

Tannreuther ('08) says:"When the egg has reached its growth, it is amoeboid in form with the nucleus near the centre. The egg at this stage of development contains no yolk, but when the pseudopodia are completely formed, the nuclei of the interstitial cells forming the ovary are taken up by the amoeboid egg and become changed into the yolk or pseudo-cells of the egg"....."The transformation of these interstitial cells into yolk is shown in Fig. 7, a-e. The chromatin becomes very granular and forms a band around the inner border of the nuclear membrane. The nucleolus becomes imbedded in this band of granular chromatin and the nucleus has the appearance of a hollow sphere with its wall thickened on one side. After the yolk or pseudocells are formed they divide amitotically".

Kepner and Looper ('26) recognize two phases in the nutrition of the egg of hydra. The first phase is confined to the initial growth of the egg until the time when it has established its maximum contact with the mesogloea. During this time many of the ovarian cells have disintegrated at the periphery of the egg and their contents have been resorbed, so that by the time that the egg reaches its greatest "spread", very often most of the ovarian cells have disappeared and a comparatively small number remain. These authors claim that the pseudocells are not formed from the nuclei of the interstitial cells but wholly from the food derived from the endoderm.

Kepner and Looper ('26) state:"This position seems logical

when we bear in mind the fact that, though many interstitial cells have disintegrated (perhaps most of them) and have been resorbed during the egg's growth, yet, up until maximum surface exposure to the endoderm has been made, no yolk formation has resulted ... Our interpretation is further strengthened by the observation that so long as yolk is making its appearance within the primary oocyte a maximum surface relation to the endoderm is maintained; but when the maximum amount of yolk has been formed the egg retreats from the endoderm as Tannreuther ('08) indicates:..... The second phase of the nutrition of the egg of H. viridis, therefore, ends with the retreat of the primary oocyte from the mesogloea after it has become filled with deutoplasm".

B. Hydra canadensis.

As has been seen before, amoeboid ingestion as described by Downing is absent in H. canadensis. The failure of Kepner and Looper to mention its occurrence in H. viridis may be considered as evidence that they did not observe it in that species either.

The second type of ingestion mentioned by Downing is the only type that occurs in the nutrition of the egg of the present species. It is merely a passive fusion of the ovarian cell with the egg, (Fig. 4, Plate 1). The cell touches the egg and the cytoplasm of the two becomes confluent. This automatically brings the nucleus of the ovarian cell to lie within the cytoplasm of the egg.

During this early period of ingestion, while the egg is just assuming the amoeboid shape, the cells that are ingested undergo degeneration inside the egg. All the cellular structures

disintegrate and the substance of the cell is added to the cytoplasm of the egg in which it is diffused until it is indistinguishable. The egg of this stage does not yet show any of the so-called pseudozellen. The cytoplasm appears colorless and quite granular. In some cases, as the cells touch the egg, they get lighter in color (that is, they take the stain less readily) to resemble the shade of the cytoplasm of the egg. The chromatin collects in little clumps resembling chromosomes just before the cell enters the egg. When inside it is dispersed in the cytoplasm and in this way is no longer recognizable. It is at a later stage that the dark, spherical bodies, which Kleinenberg ('72) terms "pseudozellen", appear within the substance of the egg, apparently built up from the cytoplasm. The nucleoli, which are prominent structures in the ovarian cells, often persist longer than the other structures of the cell and Wager ('09) claims they are sometimes transformed into miniature pseudocells. This is a contention that can neither very well be proved nor disproved.

As we have seen in the review of the literature, Downing recognizes two types of ovarian cells in the ovary. His first type is by far the more abundant. This consists of cells that have enlarged enormously. Many of them are ovoid in shape, others polygonal, depending largely on the state of congestion within the ovary. These cells take the stain readily. They have large nuclei with prominent nucleoli which often persist for a considerable period after the ingestion of the cell by the egg. This is the only type of cells present during the

early growth of the egg. Their disintegration and dispersal on ingestion have been discussed above.

Downing derives the second type of ovarian cells from the first, and apparently quite correctly so. Because the ovarian cells get their food from the same source as the egg, it is conceivable that the results of nutrition might in the two cases be similar. They are identical in size with the first type of ovarian cells but changes in their nuclei are noticeable. The nuclei gradually assume the dark color and spherical shape of the pseudocells and Downing claims that on ingestion they persist in the cytoplasm as pseudocells without any apparent further transformation. It is true these nuclei resemble the pseudocells, yet slight differences from the latter may be observed. They look slightly less compact and their general appearance suggests at once that they may have undergone degeneration. Whether they actually continue in the egg as pseudocells is doubtful. They appear near the egg only when ingestion has practically ceased and it is not likely that they are ingested. They may be resorbed like the abortive bodies.

We do not know what induces the formation of yolk (pseudocells) in the egg. Brambell ('25) shows the relation of the Golgi bodies and the Mitochondria to the formation of yolk in the ovum of the fowl. As the egg of hydra is relatively large and produces a considerable quantity of yolk, research along these lines in this animal might prove very profitable. For such work living material is essential.

Kepner and Looper ('26) state emphatically that the pseudocells make their appearance only after the amoeboid egg has established maximum contact with the mesogloea and so with the endoderm. Until this time all cells ingested are broken down and their substance dispersed in the cytoplasm of the egg. With this statement I cannot agree altogether. The model (Figs. 5 and 6, Plate 2) represents a relatively early stage in the development of the egg, yet it contains both pseudocells and disintegrating ovarian cells. As the egg certainly has not yet established maximum contact with the mesogloea, this occurrence cannot possibly be brought in line with the claims of the above authors. Because numerous pseudocells and disintegrating ovarian cells are both present at the same time we can only conclude that the two processes, the ingestion of ovarian cells and the production of pseudocells, are proceeding simultaneously. This, of course, applies only to a short period just before the egg has established its maximum contact with the mesogloea when, as Kepner and Looper correctly state, ingestion ceases. Even on purely theoretical grounds one might logically expect this mode of procedure. Undoubtedly the egg receives nutriment from the endoderm long before it has established its maximum contact with the mesogloea and there is no obvious reason why it should not commence to build up some of this food into pseudocells before such extensive contact is established.

We have now arrived at the stage where ingestion ceases and the egg is about to retract its pseudopodia. A model of an

egg of approximately this stage was made which illustrates well its complexity, (Figs. 14 and 15, Plate 5; Fig. 16, Plate 6). The egg is molded to some extent by the shape of the parent body. The concave surface lies closely applied to the mesogloea and the pseudopodia project radially from the common central mass. In the animal the convex surface is covered by the ectoderm which bulges out in this region to accomodate the egg. The pseudopodia have been slightly retracted in this egg. The nucleus at this time lies near the centre of the egg. It is spherical and shows three or four prominent nucleoli in the centre with a large number of smaller nucleoli scattered around them. Ingestion has ceased and the egg is now deriving all its food from the endoderm. The pseudocells form rapidly and are scattered throughout the cytoplasm of the egg.

Just what it is that causes the pseudopodia to retract is not evident. It is quite probable, however, that the final retraction is a purely mechanical process. Food is continually passed into the egg by the endoderm, possibly by means of a process of osmosis. The "ectoplasm" of the egg is distensible within certain limits and has to accomodate the food as it arrives. Naturally, as congestion increases within, the egg must assume a more and more spherical form, that is, it seeks to provide the maximum amount of space within a minimum surface area which is a sphere. As this shape is approached obviously the surface contact with the mesogloea becomes reduced. The reduction of contact with the mesogloea automatically diminishes the amount of food material passed into the egg from the endoderm and thus arrests all further growth of the egg.

We must at this point note some differences in the relation between the ectodermal cells which immediately overlie the egg, and the mesogloea in the two species H. dioecia and H. canadensis. Downing claims the ectoderm cells in his species retain their connections with the mesogloea in the region of the ovary and the egg. He says in this connection: "The ectoderm cells are greatly altered in the region of the ovary. Their outer ends are transformed into lamellae which are united by their edges to form the covering of the eggs: their bodies form muscular fibres which are crowded to the sides by the egg like the restraining guy-ropes about an inflating balloon". In H. canadensis the connections between the ectoderm and the mesogloea are broken even in the early egg, except where the ectoderm lies over the spaces between the pseudopodia. As a result the ectodermal covering of the eggs in this species is much thicker than in H. dioecia for there is no strain on the ectoderm cells over the young egg. In the case of the mature eggs the ectoderm cells show a lateral strain due to the bulging produced by the spherical egg and not because of any attachment to the mesogloea.

The nucleus, which lies at the centre of the egg until the ingestion of ovarian cells ceases, grows rapidly. As the pseudopodia are retracted and the egg assumes the spherical shape, the nucleus migrates to the distal pole of the egg and takes a position quite close to the periphery, with only a very narrow line of cytoplasm between it and the outer margin of the egg. Here it reaches its maximum size prior to undergoing the maturation divisions.

THE SUBSEQUENT HISTORY OF THE EGG

MATURATION DIVISIONS

In the absence of living animals no extensive observations could be made on the maturation processes of the egg and the following is merely an outline of what appears to be the sequence of events as judged from fixed material.

When the egg assumes the spherical shape the enlarged nucleus takes up a position at the distal pole of the egg, quite close to the periphery. It now begins to contract forcing a considerable quantity of its nucleoplasm, including some chromatin material, out into the cytoplasm. Whether the nuclear membrane is porous and the nucleoplasm is passed out through it or whether a break occurs in the membrane, could not be ascertained. The nucleus contracts until it has only a small fraction of its former volume. The nuclear material ejected lies around the nucleus and stains darkly making it difficult to see the structure of the nucleus.

Shortly after this two polar bodies are given off, the first slightly larger than the second. It was impossible to infer how much time elapses between the formation of the first and the second polar body. They both remain attached to the egg for some time by means of a narrow strand of cytoplasm. Tannreuther ('08) says they remain attached until after the third cleavage. I have observed them still attached to a late blastula stage, (Fig. 21, Plate 7).

THE STALK AND THE SHAPE OF THE EGG

While handling the specimens prior to embedding them for sectioning it was noticed that the eggs and embryos have a very feeble attachment to the parent body. Extreme care has to be exercised not to lose most of the more mature stages. This confirms the observations of Rowan ('30). The reason for this precarious attachment becomes evident when we examine (Fig. 18, Plate 7). The stalk of the egg, so conspicuous in most other species, is almost wholly wanting in the egg of H. canadensis. The area for its attachment is quite extensive but the egg lies only superficially embedded in the ectoderm of the parent with the result that the slightest jar dislodges it.

The perfect egg is shown in (Fig. 18, Plate 7). there is, however, a good deal of variation in shape. The most frequent is that seen in (Fig. 19, Plate 7) which represents a meridional section of the egg. This shape is still discernable in the early cleavages of the egg but apparently disappears in the later blastula stage when the embryo commonly show an ovoid shape.

Whether eggs like the one shown (Fig. 18, Plate 7) produce spherical embryos (Fig. 22, Plate 8) and those resembling (Fig. 19, Plate 7) produce ovoid embryos (Fig. 23, Plate 8) is difficult to say. Both types of eggs and embryos occur in this one species of hydra.

SPLITTING OF THE ECTODERM AND FERTILIZATION OF THE EGG

Before fertilization the ectodermal covering of the egg splits at the distal pole and retreats. It collects in folds at the base of the egg. This is the time when fertilization takes place. Brauer, Tannreuther, Downing and others agree that a shallow fertilization groove forms at the distal pole of the egg, generally just over the nucleus, bringing that structure nearer to the periphery. The sperm then enters the egg at some point in this groove and fertilization is effected.

A peculiar occurrence was noticed in one specimen and is illustrated (Fig. 20, Plate 7). In the space between the base of the egg and the ectoderm which at this stage has split and slipped back, a large number of sperms have collected. Obviously this is due merely to the fact that the cleft here formed a natural trap for the sperms. It is very questionable whether any of the sperms could enter and fertilize the egg from this region.

EARLY CLEAVAGES, THE EGG SHELL AND THE EGG MEMBRANE

The early cleavages of the egg are total and equal in the spherical eggs. In the more irregular eggs the cleavages are harder to follow but they tend to be unequal.

The variation in the shape of the egg and its probable relation to the shape of the young embryos has been discussed above.

The egg shell is extremely delicate and is spiny. It has been adequately described and figured by Rowan ('30).

The egg membrane gains considerable thickness in the older blastulae and appears quite prominent, (Fig. 17, Plate 6).

SUMMARY

1. The ovary in hydra is formed by the rapid division and growth of the interstitial cells.
2. The ovarian cells nearest the mesogloea are larger than those farther away. The egg arises through the fusion of numbers of ovarian cells at a number of points in the ovary, forming a group of independent masses of fused ovarian cells.
3. The masses constituting the group enlarge and fuse to form an egg which is amoeboid in form only and not in function, for although "pseudopodia" are present amoeboid ingestion has not been observed in *Hydra canadensis*.
4. Occasionally one of the masses fails to fuse with the rest, forming an abortive body which is probably resorbed later.
5. The change of the egg from the amoeboid form to the spherical is effected by a mechanical process.
6. The polar bodies remain attached to the egg until a late blastula stage is reached.

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listed above.

PLATE 1.

Figure 1.

Photomicrograph of a cross section of a female hydra showing the beginning of the formation of an ovary, X57.

Figure 2.

Photomicrograph of a cross section of a female hydra showing a more advanced ovary. X57.

Figure 3.

Photomicrograph of a cross section through an egg showing its position between the ovarian cells and the mesogloea. Note the great thickness of the ectoderm. X233.

Figure 4.

Photomicrograph of a section through the pseudopodia of an egg showing the ingestion of ovarian cells at the outer surface of the pseudopodia. X233.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

PLATE 2.

Figures 5 and 6.

Photographs of a model of the early, diffuse egg of hydra. Individual bodies that have not yet fused with the main part of the egg are clearly seen. The figures show the egg enlarged times 114.

Fig. 5.

Fig. 6.

PLATE 3.

Figure 7.

Photomicrograph of a longitudinal section of a female hydra to show numerous eggs developing in one individual at one time. The figure shows parts of four distinct eggs.
X 57.

Figure 8.

Photomicrograph of a section through the amoeboid egg. The section was cut tangentially to the surface of the animal and shows the strong development of the pseudopodia. X57.

Figure 9.

Photomicrograph showing an abortive body. This body and the embryo seen lower in the figure both developed in the same ovary. X57.

Figure 10.

Photomicrograph of a section through the developing egg to show the numerous pseudopodia. X57.

Fig. 7.

Fig. 8.

Fig. 9.

Fig. 10.

PLATE 4.

Figures 11, 12 and 13.

Photomicrographs of sections of a binucleate egg. The three figures represent three consecutive sections of a serially sectioned animal. Both nuclei appear on figure 12. X233.

Fig. 11.

Fig. 12.

Fig. 13.

PLATE 5.

Figures 14 and 15.

Photographs of a model of the egg of hydra. The retraction of the pseudopodia has just begun. In the animal the concave side of the egg is closely applied to the mesogloea, the convex surface is covered by the ectoderm. The figures show the egg enlarged times 114.

Fig. 14.

Fig. 15.

PLATE 6.

Figure 16.

Photograph of the model seen in Figs. 14 and 15 Plate 5. This view shows the inner side of the egg which is closely applied to the mesogloea in the living animal. X114.

Figure 17.

Photomicrograph of a young embryo. The egg membrane shows lightly removed from the margin of the section. X233.

PLATE 6.

Fig. 16.

Fig. 17.

PLATE 7.

Figure 18.

Photomicrograph of a section of the spherical egg of hydra through the point where it is attached to the parent body. No definite stalk is present. X57.

Figure 19.

Photomicrograph of a section through the more typical egg of hydra.

Figure 20.

Photomicrograph of the base of the egg showing an aggregation of sperms at this point. X233.

Figure 21.

Photomicrograph of an embryo which still has the polar bodies attached to it by means of a strand of cytoplasm. X233.

Fig. 18.

Fig. 19.

Fig. 20.

Fig. 21.

PLATE 8.

Figure 22.

Photomicrograph of a cross section of hydra showing five spherical embryos in one section. X35.

Figure 23.

Photomicrograph of a section of an ovoid embryo. X57.

PLATE 8.

Fig. 22.

Fig. 23.

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