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The Primitive Method of
Yolk Formation in
Stenostoma

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THE PRIMITIVE METHOD OF YOLK FORMATION IN STENOSTOMA.

Stenostoma leucops is the name given one of the simplest species of the genus Stenostoma, family Microstomidae, sub-order Rhabdocoela, order Rhabdocoelida, class Turbellaria, of the phylum Platyhelminthes (flat-worms).

The order Rhabdocoelida presents the greatest diversity in the different methods and forms of the development of the reproductive systems in the various sub-orders and families. Stenostoma leucops has a very primitive form of sexual reproduction, about which very little is known; literature on the subject being exceedingly scarce. Where reproduction is discussed only reproduction by budding and fission is usually mentioned.

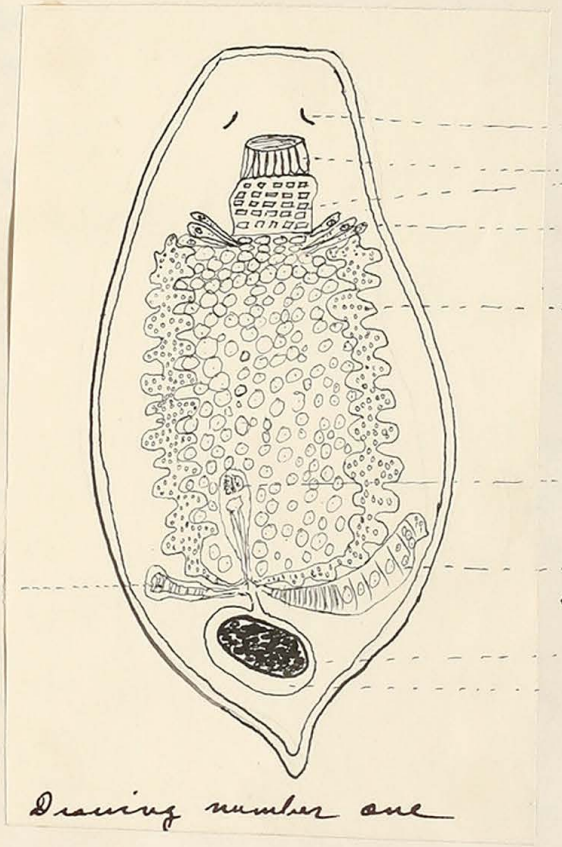
The phylum Platyhelminthes stands next above the Coelenterata in the general succession of animal phyla. These two groups are separated by the great gap that the condition of being diploblastic as over against triploblastic affords. The Coelenterata are, for the most part, sessile animals, having internal cavities which serve chiefly as regions in which food is digested and from which digested food is absorbed. The wall of these animals is made up of only two layers, ectoderm on the exterior and endoderm lining the enteron. Being attached animals they have little need for sense organs, the sense of touch and the smell-taste

senses being the only ones well developed. On the other hand in the flatworms we have a triploblastic condition providing a source of complex body and sense organs. The central nervous system is far above that of the Coelenterata, the musculature much more complex.

The Hydra is a typical example of the Coelenterata and therefore a very simple animal as compared with the flat-worms. The manner in which it reproduces sexually may therefore be considered as primitive. Let us examine the conditions as they exist in Hydra in order to secure a standard of comparison by which we may measure the relative degree of simplicity of some flat-worms as compared with the relative complexity of closely related animals. The first signs of the formation of an ovary in Hydra from the undifferentiated interstitial cells are preceded by a gathering of digested food particles in the region in which the growth is to take place. Then the development of the ovary begins with the enlargement of several neighboring interstitial cells to form the oogonia of the first generation. These cells divide twice, forming the second and third generations of oogonia. One of these third generation oogonia becomes dominant and grows at the expense of its neighbors by ingesting them. The resulting enlarged cell is the primary oocyte. This cell divides giving off the first polar body, and becomes a secondary oocyte. Division again takes place, giving off the second polar body and forming the ootid.

In the meantime the ootid has been forming yolk from the material, in part, supplied by the ingested cells. Another source of yolk has also been provided. The food which has been digested and brought up by the endoderm is also elaborated into yolk by the cytoplasm of the germ cell. The egg is now ready for fertilization by a sperm cell formed in a testis developed in much the same manner as the ovary. Thus in a simple animal we find a likewise simple method of sexual reproduction. Yolk was provided by the action of the germinal cytoplasm on the weaker oogonia and digested food brought in for the purpose.

Turning now to the Flat-worms, let us consider the conditions as found in a typical Rhabdocoele, as Dalyella. The source of the ovary in this animal is the mesoderm. The ovary is a permanent organ persisting after the maturity of the individual, and does not arise and disappear according to whether conditions for reproduction are favorable or unfavorable, as is the case in the Hydra. Developing oocytes of all stages are seen in the ovary. Associated with it is a uterus in which as many as five or six eggs are often seen. There is a vagina through which the fertilized egg passes. Closely associated with these organs there is a chitinous penis and seminal vesicles. Yolk is provided by two glands, which lie on either side of the enteron. Each gland extends along the entire side and is much



Drawing number one

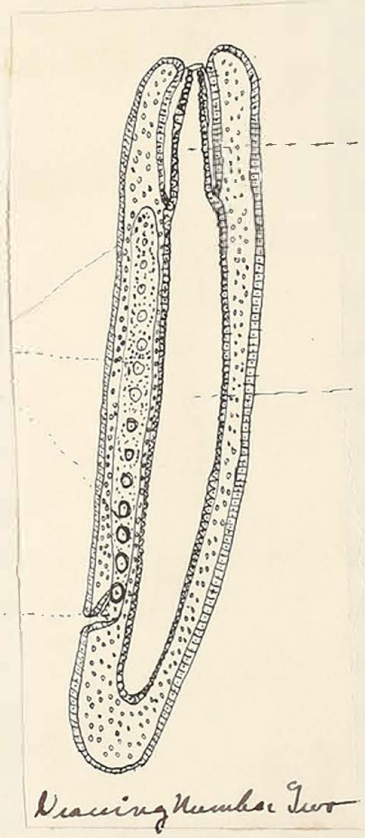
Dalyellia

convoluted. The yolk granules lie within closely packed yolk cells. These yolk cells migrate down to the opening of the gland into the junction between the ovary and the uterus and enter into the formation of the eggs. Each egg is composed of a zygote and many yolk cells which are later consumed as food by the developing embryo.

Dalyella therefore with its complex system of organs presents a highly complex reproductive method as compared with the method in Hydra. It is as far removed from Hydra as the position of the two animals in their different phyla would indicate.

(Drawing number one on page (4) shows diagrammatically the relative position of the reproductive organs of Dalyella to each other and to the general anatomy of the animal).

The Prorynchus, another Rhabdocoele, presents a step down from the complexity found in Dalyella, being not so far removed from the conditions of Hydra. Here we do not find a differentiation into a large number of organs as in Dalyella. There is only one organ which has the double function of supplying both the germinal material and the yolk-cells for the developing egg. This organ is, however, again a permanent one. There is an internal differentiation in function, some of the cells comprising what is known as the "Germarium" and others the "Vitellarium". The former



Drawing Number Two

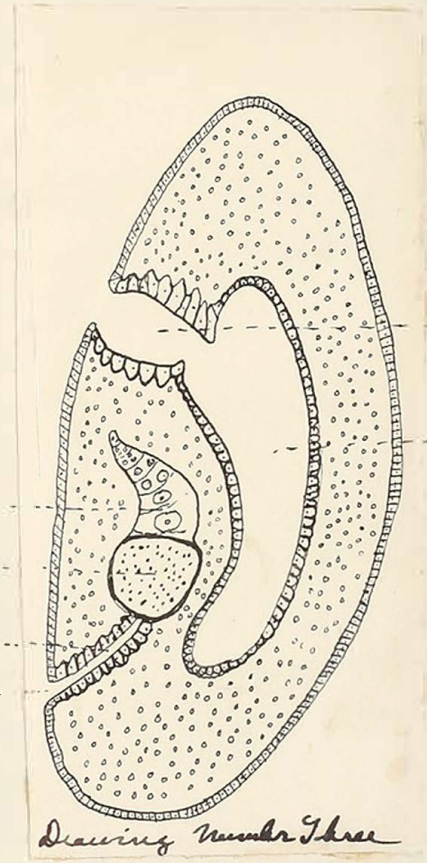
Prorepucbus

term refers to the cells that produce the oocytes and the latter to those that produce the food supply for the embryo. This organ, which is the ovary, lies ventral to the enteron, beginning near the entrance of the pharynx into the enteron and extending not quite as far posterially as the enteron. It terminates ventrally in a vagina. The ovary is filled with all stages of oocytes closely packed with yolk cells.

(Drawing number two shows the general structure of Prorynchus digrammatically).

Still further removed from Dalyella than Prorynchus is the Rhabdocoele, Microstoma, approaching Hydra in simplicity of its reproductive system. Here again we have an ovary associated with a vagina, but we do not find the same conditions of division into "Germarium" and "Vitellarium" as in the case of Prorynchus. The ovary is not a permanent organ, certain mesodermal cells being differentiated to form this organ when the conditions suitable for sexual reproduction are present. The ovary is again elaborated ventral to the enteron and the growth which takes place in the egg causes the enteron to be pushed in and somewhat dorsally displaced. The material for yolk formation is transported to the egg by the mesoderm from the digestive cells of the enteron and elaborated into yolk by the cytoplasm of the oocyte. The ovary is, therefore, invested by the mesoderm.

(See drawing number three, page (8).

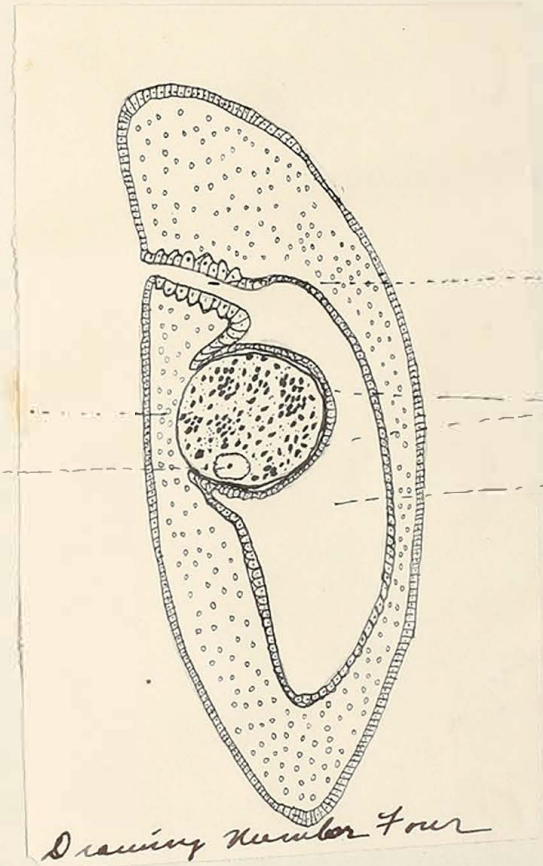


Drawing Number Three

Microstoma

In the preceeding paragraphs we have seen that the Hydra presents a simple form of reproductive system, and that the Rhabdocoeles present a variety of forms ranging form simple to complex. We now come to the discussion of a Rhabdocoele which has a reproductive system as simple as that of the Hydra, and therewith a method of yolk formation more primitive then any heretofore described, so far as I have been able to determine.

The animal to which I have reference is Stenostoma leucops. As in the case of the Hydra the ovary is formed when conditions become favorable for reproduction. As in Microstoma it is formed by a specialization of undifferentiated mesodermal cells. Hydra, it will be remembered, has its oogonia formed from the interstitial cells. So in the case before us we have a departure from the Hydra method, due to the triploblastic condition of Stenostoma as over against the diploblastic condition of Hydra. In the ovaryof Stenostoma there are four oocytes, a number not far from the number of oogonia which start out in Hydra, all but one of which, as we have seen is ingested to form yolk. It is very probable that all four of the oocytes in Stenostoma develop into mature ova. This is a second departure from the Hydra method. We have seen that in Microstoma the ovary is entirely invested in the mesoderm. In Stenostoma, on the other hand, the ovary lies within a pocket of endoderm, being almost completely surrounded

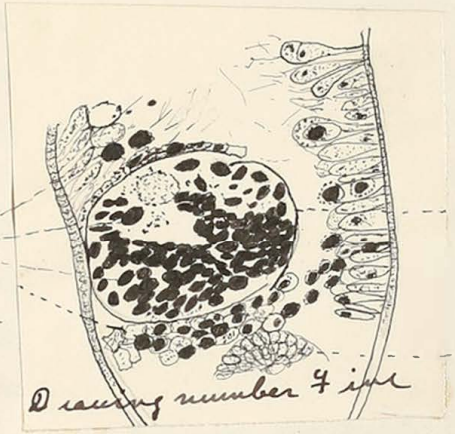


Drawing Number Four

Stenostoma

by it. (See drawing number four, page ten). Von Gaff, probably the worlds authority on Rhabdocoeles says (1913) that the ovary is surrounded by a muscular sheath. I have not been able to confirm this. From the way in which this sheath seems to have cracked in some sections of the slides prepared and studied, it would appear that it is composed of some shell-like or chitinous material, the entire ovary thus entering the formation of an egg. There is apparently no gonoduct, as no trace of one could be found upon careful examination of sections. No opening that could afford a possible exit for the mature egg has been found. This means that we must of necessity infer that death of the parent must preceed freedom of the egg. Von Grafi says (1913), "Die weiblichen gonaden sind als von einer muskulosen Hulle umgehene Follikel ventral vom Darme gefunden worden. Ihre zahlbetregt bis zu 6 und jedes enthalt 3-6 (nach Sekera) Eizellen in verschiedenen Enwirchlungstadien. Die weibliche Geschlechtsoffnung kennt man nicht; die Eier sollen ihre ersten Furchungsstadien im mutterleibe drchmachen und von einer weiblichen Haut oder Schale umgeden, erst durch den Tod des Muttertieres frei werden".

We now come to the unique method of yolk formation in Stenostoma. Careful study of prepared sections of sexually mature animals has revealed the fact that



Stenostoma - section through area of ovary

the oocytes are more or less filled with relatively large straw-colored yolk granules of uniform texture. On all sides of the ovary in the adjacent endoderm, and clearly outside of the ovarian sheath, muscle or shell as the case may be, similar masses of straw-colored material may be seen. What are they? How did they come to be where they are? Their shape, color, and texture clearly mark them as yolk granules. The large number observed and their scattered position in the endoderm all around the ovary, to-gether with the evidence afforded by their constant appearance in all the sections cut through the ovary, or near it, eliminates the possibility that their presence in the endoderm is due to their having been forced out of the ovary through breaks in the sheath. They are not to be found in parts of the endoderm which are not closely associated with the ovary. Further examination of sections has revealed the fact that the surrounding endoderm is in process of disintegration. This state is evidenced by the total lack of nuclear material in some cells, scattered nuclear material in others, and a general breaking down of the cell walls in all stages of transition. Comparitive studies of the relation of the amount of yolk present and the size of the granules shows that the older the egg, the larger and more numerous the yolk granules both inside the sheath and out, and the greater the degree of disintegration;

which indicates that there is a relation between the disappearing endodermal cells and the appearing yolk granules. The enevitable conclusion of such observations is that cells of the endoderm are being transformed into yolk. This strange phenomenon of the destruction of a vital part of the animal to provide a source of material for elaborating yolk is in line with the fact that Stenostoma must totally disintegrate in order to free the mature eggs. Such a source of yolk formation has not been observed elsewhere, in so far as I have been able to find out.

If the source and method of yolk formation just described is correct, how do the yolk granules get into the oocytes? This transfer takes place by the action of the cells. We have an excellent example of such mass transfer in Microstoma. This eats the Hydra and then makes use of the indigestible nematocysts (stinging threads) of the Hydra by transferink them from cell to cell out to the surface of the body and their arranging them at regular intervals over the entire surface of the body, to be used in time of conflict with an enemy. Stenostoma, then, in a similar way transports these yolk granules elaborated from the endoderm bodily into the oocytes.

From the similarity of the straw-colored masses in the eggs and in the endoderm, from the condition of the endodermal cells, and the evidence presented

by Microstoma that such mass transfer can be made, I feel safe in assuming that the endoderm is the source of yolk in Stenostoma.

Such observations as those made above bear out von Graff and those who held Stenostoma a most primitive genus. Gamble (1901) says, "The Rhabdocoela exhibit every stage in the development of a complex reproductive system, from the simple ovaries and testes of a Microstoma or Macrostoma, to the intricate system of ducts and glands of a Macrorhyncus (Proboscidae), in which there is still much to be made out". And here we find an animal of the same sub-order with even a simpler reproductive system than that of the Microstoma! Again he says, "The Rhabdocoelida present the greatest diversity in the development of the reproductive system. The Acoela and the Alloeocoela have the simplest arrangement. Two ovaries are present, and the oviducts, if distinct, are continuations of the ovarian membrane. In most forms a 'bursa seminalis', which receives the spermatozoa of another individual, is appended to the female genital canal. In many of the Alloeocoela, however, a portion of the ovary is sterile, its cells, forming a yolk gland, feed the fertile portion, the whole structure tne being spoken of as a germ-yolk gland". We have seen that this was the case in the Prorynchus, and we have found both Microstoma and Stenostoma of simpler structure. Therefore his

statement should be modified, the Rhabdocoela being in some cases (Microstoma and Stenostoma) more simple than the Acoela and the Alloeocoela.

Our study has brought us to a realization of the wide range of methods by which the same end is attained in a closely related group of animals. If one compare the Rhabdocoeles with any other group of animals, as for instance the Birds, it is astonishing to find that there are so many methods of sexual reproduction in the former closely relayed group, and only one method in the very wide and varied latter group. Such observation indicates that the Rhabdocoele type, once it became established, offered more plastic material for organic evolution, and was accordingly modified in more ways to attain the same end, than was the Avian type when it appeared.

SUMMARY

1. The oocytes of Stenostoma are of mesodermal origin.
2. The ovary of Stenostoma is almost completely invested in apocket of endoderm.
3. The endoderm is the primary source of yolk and is destroyed in order to furnish a food supply for the embryo.
4. Stenostoma's methos of yolk formation is the most primitive found in all Rhabdocoeles, and perhaps even more primitive than that of Hydra, a diploblastic animal.

5. There are four different methods of yolk formation in Rhabdocoeles, namely:

(a). In Dalyella---by permanent glands supplying yolk cells.

(b). In Prorynchus---by a Vitellarium which combines with a Germarium toxform a single organ, the former supplying yolk cells to the oocytes formed from the latter.

(c). In Microstoma---by the cytoplasm of the oocytes from material supplied by the investing mesenchyme.

(d). In Stenostoma---bya process of yolk elaboration involving the destuction of the endoderm.

6. As compared with other groups of animals, as the Birds, Rhabdocoeles offered organic evolution more plastic material wherewith to fashion a variety of means to a single end.