THE

ZOOLOGY OF STORILOCACA APICATA

AND

THE ZOOLOGY OF TURRITOSIS UTRICULA.

BY

SAMUEL RITCHEY HOUSE.

Dissertation

Submitted to the Board of University Studies of the

Johns Hopkins University

in conformity with the Requirements for the degree of

Doctor of Philosophy.

SALT LAKE CITY.

1905
PART I.

THE

EMBRYOLOGY OF STICKLEBAK AFINATA.
THE EMBRYOLOGY OF STOMOTOCA APICATA.

INTRODUCTION.

The material for this research was secured, and the observations on the living forms were made, during the summers of 1903 and 1904 while I was occupying a table at the United States Fisheries Laboratory at Beaufort, North Carolina. Stomotoca is not very abundant in the harbor at Beaufort. I found it there as early as the middle of June. It is most plentiful during July and early in August. A few specimens may also be taken until early in September. The eggs were obtained from medusae captured between July 10 and August 5. The adult animals could not be secured in large numbers; and, owing to the fact that each female lays only a few eggs the material for embryological study was limited. Therefore the greater part of the work the results of which are embodied in this paper was done with living material. All the drawings, with the exception of those of sections were made from camera sketches of the living
forms. Blastulae and planulae ranging in age from five to twenty-seven hours were preserved and sectioned for the study of the various stages in the formation of the endoderm and the other features of development which make their appearance during this period.

I wish to acknowledge my obligations to the Honorable George M. Bowers, Commissioner of Fisheries for the privileges afforded me at the Fisheries Laboratory; and also to thank Dr. Caswell Grave, Director of the Laboratory for help and suggestions. The work was finished in the Biological Laboratory of the Johns Hopkins University. For the interest shown and for kind suggestions offered during my work I am very grateful to Professor W. K. Brooks.

DEHISCENCE.

The eggs are discharged at about five o'clock in the morning. The ectodermal epithelium of the ovaries becomes ruptured, in fact broken down; and by the movements due to the muscular contractions of the manubrium the eggs are set free into the cavity of the sub-umbrella. Then by the rhythmic contractions of the bell they are forced out of the bell cavity into the water outside. While the eggs are being
laid the medusa remains at one spot, unless disturbed, and keeps up a continuous and rhythmic contraction and expansion of the bell and proboscis. Thus as the eggs are liberated, one, two, or three at a time, they are almost immediately passed out with the ejection of the water from the bell cavity. This process of dehiscence lasts for a few minutes during which the medusa remains at the bottom of the aquarium. All the mature eggs are discharged without intermission in the process, unless the medusa is disturbed. In that case it frequently swims to another part of the aquarium and in a short time commences to discharge the eggs again.

The eggs in the ovaries of Stomotoca apicata are usually all deposited at one time. Occasionally a few immature ones are left in the ovaries after the process of dehiscence. Whether these mature and are laid at a later time, or whether they are reabsorbed I am not able to decide.

As stated above, the eggs are laid at about five A. M. On several occasions I observed the process of dehiscence and found that the time was always practically the same. Some medusae were watched all night, July 14. At five o'clock in the morning they began to lay their eggs. They all began
at about the same time and all the eggs were discharged within fifteen or twenty minutes. The time when the medusae are captured and put into an aquarium does not seem to have any influence on the period of dehiscence. I have taken them in the tow at nearly all hours of day and night, and never had them to deposit their eggs except at 5 o'clock in the morning.

THE EGG.

The egg of Stomotoca apicata is spherical and measures 0.14 of a millimeter in diameter. It is devoid of a membrane and the cytoplasm is rather dense and only semi-transparent; however it is not as dense as the egg of Stomotoca rugosa, which is extremely opaque and of a chalky-white color, and also slightly larger. The color of the egg of Stomotoca apicata is a bluish-white.

A point of interest may be mentioned in this connection. On one occasion, having taken a number of Stomotoca in the tow at night, they were picked out and put into a dish of clean sea-water with the intention of allowing them to lay, and using the eggs for study the next morning. It happened that both species of Stomotoca that are found at Beaufort
were represented. There were mature females of both species that deposited their eggs the next morning at the regular period; Stomotoca rugosa has the same time for dehiscence as Stomotoca apicata. Only the eggs of the latter species developed; there being no males of Stomotoca rugosa. The next day when the two species were in the same dish, and both discharged their eggs, only the eggs of Stomotoca rugosa segmented and developed. In this case there were no mature males of Stomotoca apicata. These facts aroused my interest and on several later occasions I placed the two species together with the intention of getting them to interbreed, but did not succeed and therefore I am led to the conclusion that they will not cross even though they are species of the same genus. To my knowledge no other experiments have been made in attempting to cross different species of this group of animals, and I did not have the opportunity to try with any other species than the above named after my attention had been called to the fact that they did not cross when accidentally placed in a dish together.

Polar Bodies.

Soon after the egg is deposited the first polar body
is given off. A few minutes later the second polar body is formed. They remain near the egg for some time; frequently until after the second or third segmentation. The polar bodies are not held by a membrane, as the egg is devoid of such a structure; neither are there any protoplasmic connections visible with a magnification of 212 diameters. Yet for a time they seem to be held near the egg by some means of attraction. The first polar body may segment once or twice. Usually about the time of the second cleavage the polar bodies either disintegrate or pass out into the water and are lost.

FERTILIZATION.

Very little concerning fertilization could be made out on account of the character of the egg. The ova and spermatzoa are discharged into the water and there fertilization takes place. It is impossible to follow the nuclear changes which take place during maturation; or the union of the male and female pronuclei in the living egg because of the density of the cytoplasm, and material could not be secured in sufficient abundance in the various phases for the preservation
of the different stages for sections. There is no visible fertilization-membrane given off after the penetration of the spermatozoa.

CLEAVAGE.

Cleavage is total, equal and nearly regular, especially in the early stages. The divisions occur at short intervals, and the blastomeres soon move away from the center of the egg, thus forming a gradually enlarging segmentation cavity. The cells continue to divide and arrange themselves into a single layer around the blastocoele to form a true blastula. The egg is not divided into an animal and a vegetative pole as the deutochlam and protoplasm are distributed evenly in all parts. But as is customary and for convenience of description I will call the part of the ovum from which the polar bodies are given off the upper pole, and the part of the egg opposite the lower pole.

The first cleavage occurs a short time after the polar bodies are ejected. The plane of division is vertical; the segmentation-furrow begins at the upper pole and gradually deepens until the egg is cut into two equal parts. The egg.
viewed from above, at first shows a nearly circular de-
pression which very soon spreads laterally and begins to
grow down. This first furrow is wide and leaves the blasto-
eres separated some distance from each other as it pro-
gresses downward, as is seen by looking at the egg from
the side (Figs. 4 and 5). This furrow remains open until
the egg is almost separated into two parts: the blastomeres
being connected simply by a narrow protoplasmic film at
the lower pole. Protoplasmic currents can frequently be
seen in this connecting thread. Bunting (’93) describes
and figures in Hydractinia a protoplasmic thread in the two
cell stage in which she also notes protoplasmic movements.
The connecting film in Stomotoca anicata is not as clear
and definite in outline as she shows it in her figure of
Hydractinia. The two cells gradually come in close prox-
imity and in a short time the connection of protoplasm at
the lower pole is broken and the complete two-celled stage
is formed (Fig. 6).
The second plane of division is also meridional and at right angles to the first. This cleavage takes place about fifteen minutes after the first division. These second segmentation furrows start at the centre and move out toward the periphery. During their progress outward there are to be seen globular or oval spaces at their outer extremities. These spaces are large enough to cause openings that extend through the egg as shown in Figure 7. During this cleavage there is a shifting or rotation of the blastomeres from right to left. The second segmentation furrows usually start opposite each other at a point in the centre of the first cleavage furrow, and then are carried apart by the rotation. Or the rotation may have started before the second segmentation began; in that case the second cleavage planes are some distance apart as soon as they make their appearance. Figure 7 shows an egg in the process of division in which rotation has taken place. During the progress of the second segmentation, the egg has fre-
sequently a flattened appearance as seen in the figure just mentioned.

In this stage protoplasmic films or bridges, also, frequently exist for a time after the segmentation is practically complete. They finally are absorbed by the blastomeres which round up forming the completed four-celled stage as shown in Figure 8.

The third cleavage plane is equatorial and divides the egg into eight equal blastomeres; four of which are situated at the upper pole and four at the lower pole of the egg as seen in Figure 9. This is the condition when the condition is regular, and might be described as two four-celled stages of half size superimposed one upon the other, and then the upper set rotated to the left. While the formation of the eight-celled stage was always nearly the same in the eggs that I followed, after the division was completed, the blastomeres did not always retain the same relative positions. Sometimes there occurred a separation of the cells at one side of the equatorial furrow and the blastomeres rolled
apart in such a manner as to form a curved sheet. In others this separation and unrolling of the blastomeres was less definite, and the final arrangement was such as shown in Figure 10.

The irregularity in the relative position of the blastomeres begins with the eight-celled stage and is more or less characteristic of all later stages up to the formation of the blastula. But, while there is diversity of arrangement of the blastomeres, nevertheless I am led to believe that the division of the individual cells is regular and takes just as though the blastomeres always held the same relative position.

The fourth segmentation follows after a short period of time. Figure 11 shows a sixteen-celled stage which is nearly regular, but the cleavage cavity has already been formed within the mass of blastomeres and they are thus pushed away from the centre of the egg. In this stage the cell lineage can still be traced even in the forms that are somewhat irregular. But in subsequent stages the arrangement of
the cells is more irregular and owing to the fragility of the egg it is difficult to follow with accuracy the descent of the cells. Figure 12 shows a later stage in which the arrangement of the cells is more regular than is frequently met with in eggs of the same age.

As stated before, the divisions follow each other at short intervals. Within two hours after the eggs were laid they had undergone the process of maturation and fertilization, and had passed beyond the sixty-four celled stage. The cells continue to divide with the same rapidity, while within them the cleavage cavity is also gradually enlarging. Figure 12 shows a stage in which the cells are more or less definitely placed around the segmentation cavity. The blastoceres finally become very numerous and small, and arrange themselves around the blastocele in a single celled layer forming a true blastula.

BLASTULA.

The blastula is oval in shape, and is but slightly
larger than the unsegmented egg. The average size of several
blastulae that were measured was .18 m* in length and .15
mm. in their largest transverse diameter. The egg before
cleavage measured, as stated before, .14 r. in diameter.
The blastomeres in the blastula stage have become very
numerous and small, and are arranged in a single layer of
epithelial cells. When the larva is about eight or ten
hours old, these peripheral cells develop cilia; probably
each cell has one ciliium. With the development of the cilia
movement commences. At first the motion is slight, but
as the cilia become more numerous, the blastula is enabled
by the ciliary movements to leave the bottom of the aquarium
water which it was heretofore lying and swim about in the
water with a spiral or cork-screw motion which is character-
istic of hydroid blastulae and plerulae. The large end
of the blastula is directed forward and therefore may be
called the anterior end. Whether the anterior part of the
larva corresponds to the upper or lower pole of the egg was
impossible to determine. It is reasonable, however, to infer that there may be no fixed polarity in the larvae of Hydromedusae, for it is well known that normal embryos of small size will develop from fragments of eggs.

FLANULA.

The blastula gradually elongates and becomes narrower forming a larva which is usually about three times as long as broad and known as a planula. From measurements taken of living planulae the average size is about 0.25 mm. in length and 0.07 mm. in the short diameter. These measurements are not constant, the larva becoming somewhat longer at an older age. The anterior end remains slightly larger than the posterior, but the difference is not as great as in the blastula. During the blastula stage the larva stays near the bottom of the dish; when it attains the planula stage it rises and swims about near the surface of the water for a shorter or longer time. This phenomenon occurs about twenty-four hours after the eggs are fertilized.
After several hours the larula gradually settles toward the bottom again and finally the spiral movements cease, due to the loss of the cilia. For a time of varying length after the spiral motion stops the larula glides along or the bottom of the aquarium. About forty-eight hours after the eggs are laid the larva reaches the stage of development in which attachment takes place. In preparation for attachment the larula settles to the bottom, loses its cilia and ceases its movements.

FORMATION OF THE ECTODERM.

The formation of the ectoderm in <i>Stomatococca arctica</i> is simple in comparison with those species in which the segmentation of the egg is unequal, giving rise to macromeres and micromeres; and in which the ectoderm is formed by a rapid increase of the micromeres and overgrowing of the macromeres by the process of erible. In <i>Stomatococca</i> or the other hard the cleavage is equal and at the completion of segmentation the blastomeres have divided into cells of uni-
four size ard are situated in a single epithelial layer around the periphery of the blastula (Figures 16 and 17 show sections of blastulae five and eight and one half hours old respectively). Thus, from their position, all the cells which result from the segmentation of the egg directly may properly be regarded as forming ectoderm; and indeed already at this stage of development be designated as such, were it proper to use the term ectoderm before the appearance of an inner germ layer. The cells of the blastosphere are columnar in shape and at first all are comparatively of the same height; but finally those cells at the posterior end become somewhat taller than the rest. This is the region where the endoderm will be budded off.

**FORMATION OF THE ENDODERM.**

In Stomotoca the formation of the endoderm takes place by unincler ingression, or the "hynetrone" method. The latter term was used by Metschnikoff in contradistinction
multinolar migration. In the multinolar formation of the endoderm he distinguishes four different modes, namely:

1. A primary delamination which takes place by a transverse division of the blastoderm cells, and occurs in the Geryonidae and Eudendrium.

2. A multinolar impression which takes place on all sides (Aeginosis).

3. A secondary delamination which occurs where a larval structure exists, as in Aequala, Phralonema and in most of the hydroid polyps.

4. A mixed delamination in which the endodermal cells originate in part through transverse division or impression; and, also, through subsequent differentiation as a secondary delamination. This last mode of the formation of the endoderm, according to Jatschrioff, occurs in Polyxenia; and is the transitional method between multinolar migration and enibole. In the unincular impression, or "hypotone" process the formation of the endoderm is confined to a comparatively small area at the posterior end of the blastula. This is the method that is followed in the species under
consideration.

About the time the blastula becomes ciliated and begins to swim, usually eight to ten hours after fertilization, the cells at the posterior end of the larva become somewhat taller than those in the other regions; and from these cells relatively few in number, the endoderm arises. The formation of the endoderm in Stomotoca is, in a general way, similar to that described by lettuce off in his "Ehryologische Studien an Medusen" for Clytia flavigula, Clytia viridisans and Ocyronchis Generbauri. The endodermal cells are given off from the lower end of the blastula and are pushed into the blastocelie. At first a single cell may be budded off. Gradually more cells are given off, and those first set free divide; so that by the continuation of this process for an indefinite time, the blastocelie becomes filled solidly from the anterior to the posterior end. Figures 18, 19 and 20 are from sections of blastula in which the formation of the endoderm is in different
stages of progress; and in Figure 21 the endodermal tissue has filled the entire cavity.

According to Metschnikoff, in his description of unipolar ingestion or "hypotrochism," the endodermal tissue arises as a rule by bodily migration of endodermal cells into the blastocoele, and not by a transverse division of the ectodermal cells— the inner parts going to form endoderm and the outer parts remaining as ectodermal cells. In Figure 20, Plate 2 Metschnikoff shows a cell in the process of transverse division; and in Figure 21 of the same Plate the cells are so situated that one can easily infer that they may have arisen by transverse division of a single ectodermal cell. These figures are of Clytia and in his description of the same species he mentions the cell in Figure 20 as the only one that he found in which transverse division occurred. This he seems to regard as an exception, and claims that as a rule the ectodermal cells increase by longitudinal division and migrate into the interior.
Stenoteua was scarce and it is not impossible to have mis-interpreted the phenomena. However, I am inclined to think that the endodermal cells arise by a transverse division of the ectodermal cells, as Jetschkeff shows in the exceptional case of Clytia viridicans. Figure 18 is drawn from the only section I was able to secure from preserved material showing the beginning of the formation of the endoderm, and that was cut slightly oblique, causing some doubt. A section of a little older stage and drawn with higher magnification is shown in Figure 19. Here there are three cells that appear to have just divided by transverse division. Another reason which causes me to think that the endodermal cells arise by transverse division of the original ectoderm cells is the fact that the ectodermal cells in this region are practically as wide as those in other parts of the blastula. This would not be the case if the longitudinal division occurred; for necessarily cell division...
is more prone in the region where the endoderm is given off, and consequently the cells would be narrower. Unfortunately, because of scarcity of material, the exact cellular details of the formation of the endoderm will have to be left for future study.

The migration of the endoderm continues for some hours, and finally the blastocoele becomes solidly filled with this newly developed tissue. At first the cells are crowded together, frequently quite densely, without any definite arrangement except that due to pressure. Then those cells that are situated next to the external al layer change in shape, become columnar and assume the appearance of a more or less distinct layer. Such an arrangement is shown in Figure 22. Later a separation takes place in the centre of the endodermal mass. This is the first beginning of the coelenteric cavity, which gradually increases in size; and finally the endodermal cells become arranged in a single layer around this cavity.
DIFFERENTIATION OF THE ECTODERMAL CELLS.

When the larva is about twenty-four hours old and about the same time that the endodermal tissue begins to arrange itself into the definite inner germ layer, a differentiation commences in the ectodermal tissue. The interstitial cells now make their appearance here and there by crowding in between the bases of the ectodermal cells. These latter cells which heretofore were straight cylindrical structures with their sides parallel to each other, now become more irregular; some assume conical forms, others spindle shapes according to the pressure of the neighboring cells. Also, about this time, or a little later, small oval refractive bodies make their appearance usually in the interstitial cells, occasionally in the ectodermal cells also. These small ovoid structures gradually push their way toward the exterior, and finally come to be situated in or between the ectodermal cells at the surface. They are developed into teratocysts.
When the larva is about forty-eight to fifty hours old it settles to the bottom, loses its cilia, and thus its movements cease. It is now ready to become attached.

The method of attachment in Stenotele differs from that usually described and which is regarded as typical for the hydroid larva; in which case they settle down on the broad anterior end, from which the hydrorhiza are given off, while the opposite end forms the hydranth and develops the mouth and tentacles. The planula of Stenotele instead of settling down on the anterior end, becomes attached by the whole length of the larva. That is, the planula does not become transformed into a hydranth but forms the root; and the first hydranth is given off from the root as a bud. The planula changes its shape about the time it is ready for attachment. The enlarged anterior end is reduced in size and the larva becomes spindle shaped. Then usually about the time the bud which will form the hydranth appears, the
primary root branches, giving off one or two secondary roots; so that when the hydranth is developed it may have two, three or four hydranths, as shown in Figures 27 - 33. The settling down and attachment of the planula of Stomatoc a aricata is very much like that which takes place in Turriforporis nutricula, the development of which will be described in another paper.

Professor Brooks in his work on "The Life-History of Eutira" (1864) has shown that the planulae of Eutira, Turriforporis and Hydactinia form roots and that the hydranths arise as buds from the roots.

DEVELOPMENT OF THE HYDRANTH.

After the larva has become attached it very soon develops a bud, generally at about the centre of the root, which is the beginning of the hydranth. A circle of small projections make their appearance very early around the distal end of the hydranth bud; these are the rudiments of the tentacles and are usually five in number. Occasionally
a hydranth bud is met with which has six tentacular projections and thus gives rise to six primary tentacles. The mouth is now developed, as a slit breaking through the two germ layers, at the apex of the young hydranth in the centre of the whorl of tentacular buds. About a day later more tentacles appear. These secondary tentacles alternate with the primary ones. The secondary tentacular buds do not all appear simultaneously; but are usually added one or two at a time until the second cycle of tentacles is completed and the hydranth has ten tentacles in all. Thus we may have young hydranths with six, seven, eight, nine or ten tentacles according to the stage of development.

Ten seems to be the number of tentacles in the fully developed hydractinid polyp. The oldest polyps that I reared five days old had this number; and Professor Brooks described the hydractinid, which he found on the lower surface of the shell of the living limulus, and which had reduced buds, developed, as having only ten tentacles. The hydranths
that I recall is the laboratory correspond with those found by Professor Broo's and I have no doubt that they are the same species. The primary and secondary tentacles arise from the same level so that they may be said to constitute one chord. The five primary tentacles, however, are longer and project forward; while the secondary ones are shorter and extend backward. The tentacles are well armed with thread cells which are arranged around the tentacles in clusters at short distances from each other, from one end of the tentacle to the other. These groups of thread cells become closer together as the distal end of the tentacle is approached.

A thin delicate perisarc is secreted early in the development of the hydra-th. It adheres closely to the root and stem. It does not extend the entire length of the stem; but stops a little distance below the circle of tentacles. In Figure 31 a polyp is shown in which the ecome sarc has retracted for some distance in one of the hydrotheca and
left the delicate tube of perisarc cavity.

**SUMMARY.**

1. The eggs are laid at a regular time, about five o'clock in the morning. They are set free by the breaking down of the epithelial layer of the ovaries.

2. The egg is spherical and measures .14 mm. in diameter. It is destitute of a membrane when laid, and none is subsequently developed. The cytoplasm is dense and opaque.

3. Fertilization takes place after the eggs are laid; and fertilization takes place very soon. Details of fertilization could not be made out because of opacity of eggs.

4. Cleavage is total, equal and nearly regular, especially in the early stages. Protocellular threads or bridges, connecting the different blastomeres during the early cleavages, are frequently encountered. The segmenting cells arrange themselves around a continually enlarging cleavage cavity.

5. At the completion of the segmentation a true blastula is formed, which develops cilia and sways with a spiral motion. The ovicel blastula elongates and is transformed into blastula.
6. The ectoderm arises directly from the segmentation cells which are arranged in a peripheral layer around the blastocoel.

7. The formation of the endoderm is by unrolled impression. The cells at the posterior end of the blastula bud off the primitive endoderm tissue which migrates into the blastocoel; and later is arranged into the inner germ layer.

8. Larvaceans arise chiefly in the interstitial cells, sometimes in the endoderm, and migrate to the surface.

9. The larva becomes attached by its side and is transformed into the hydrorhiza. The root frequently branches soon after attachment.

10. The hydranth develops from a bud, which is given off from about the center of the hydrorhiza.

11. The tentacles appear early as small projections at the distal part of the hydranth bud.

12. A thin delicate perisarc is secreted around the hydrorhiza and at the end of the tentacles.
PART II.

THE

EMBRYOLOGY OF TURRITOPSIS NUTRICULA.
THE EMBRYOLOGY OF TURRITOPSIS PUTCICULA.

INTRODUCTION.

This work on the embryology of Turritopsis nutricula was begun at the suggestion of Professor Brooks. The material was collected and the observations on the living specimens were made during the summers of 1903 and 1904, while I occupied a table at the United States Fisheries Laboratory at Beaufort, North Carolina. Turritopsis is one of the most common medusae in the harbor during the summer. In the two years that I was there they became abundant in the beginning of July and remained more or less plentiful until I left Beaufort September 13. While the medusae could be collected in fairly large numbers, many of them were immature; they lay only a limited number of eggs. However the material was preserved and sectioned for the study of such facts as could not be made out from the living forms. The work was finished in the Biological Laboratory of the Johns Hopkins
University.

DEVELOPMENT OF THE OVARIAN EGG.

The ova develop in the ectodermal layer of the manubrium. The epithelium becomes very much thickened in four regions; these enlarged areas form the ovaries. The primitive ovarian cells when first differentiated are larger than the ectodermal cells of other parts. Their protoplasm becomes homogeneous and of a finely granular character. The nuclei are less hyaline in appearance; and the nucleolus stains deeply. The primitive ova are first distinguished from the rest of the ovarian cells by the increase in the density of the cytoplasm and the enlarging of the nucleus. The latter becomes very large in proportion to the size of the cell; and acquires a vesicular character. The nucleolus is conspicuous, and a network of chromatin is scattered through the gerinal vesicle.

The primitive ova grow by the absorption of the ovarian cells around them. As growth takes place there is a change
in the character of the cytoplasm. It loses its homogeneous and finely granular nature and develops a supply of deutoplasm in the form of yolk granules. These are large and stain very darkly. They first appear around the germinal vesicle. As they become more numerous by the continual formation of new ones, they are pushed out through the cytoplasm toward the periphery. The formation of the yolk spheres goes on until the ovum is densely crowded with them except for a narrow perinuclear zone, in which the protoplasm retains its homogeneous and finely granular character and forms the ectoplasm of the mature egg. Figures 1 to 6 inclusive show different stages in the development of the ovarian egg and the formation and migration of the yolk granules. Some idea of the extent to which the protoplasm becomes crowded with spheres of deutoplasm can be formed from Figure 6, which is drawn from a nearly mature ovum. In the fully developed egg the layer of ectoplasm is narrower than is represented in this figure.

The yolk granules first appear around the nucleus of the
ovum; and it is not improbable that they are, in part at least, the result of nuclear activity. During the formation of these bodies, the nucleolus shows signs of being in an active condition and may also be connected with their manufacture. In some stages the nucleolus is dense and homogeneous; in others it has one or two clearer globules in its interior. These facts seem to show that it is not in a dormant state; and it is possible that it may be associated in some way with the transformation of the absorbed protoplasm into deutoplasm; at least that the yolk spheres arise directly through the activity of the cytoplasm, independently of any nuclear or nucleolar function, is doubtful. For if this were the case we would expect the yolk bodies to arise in other parts of the ovum than around the germinal vesicle. That this occurs there is no evidence from the study of many eggs. The primitive ovarian cells are all, or nearly all, absorbed and used in the manufacture of the yolk granules by the growing ova, except a layer at the outside which is transformed into the epithelium of the ovary. The cells
of the ovarian wall are small and somewhat flattened. Their nuclei are about the same size as the nuclei of the primitive germ cells, but are less dense. The nucleoli are conspicuous and stain deeply. In general the cells of the epithelium of the ovary are similar, except they are not as much flattened, to the cells in other parts of the ectodermal layer of the subumbrellal. The eggs in the ovary lie next to the mesogloea, that is, there is no ectodermal tissue between them and the supporting layer. The ovarian eggs are irregular in shape due to their being crowded together; but when liberated they become spherical.

DEHISCENCE.

The eggs are imbedded in the ectodermal layer of the manubrium. As the ova grow and increase in size the epithelium of the ovary becomes more and more distended. When they have reached maturity the outer ectodermal tissue of the ovary is under considerable tension. Finally when the time for dehiscence arrives, the outer wall of the ovary is ruptured by the aid of the muscular contractions of the manubrium.
and bell and the eggs escape into the cavity of the umbrella. The process of egg laying is very similar to that described for *Stomotoca*.

The number of eggs deposited by a single female medusa varies considerably. It is usually between twenty and thirty-five. On one occasion an exceptionally large female was taken in the tow; her ovaries were seen to be crowded with eggs. She was put into a separate dish of sea water for the purpose of counting the number of eggs that she would lay. The next morning at the hour the eggs were deposited; and the number was found to be fifty-six, which is unusually large. I made many other counts but this was the only time that the number exceeded fifty. As a rule it is from twenty to thirty-five, only rarely is it as high as fifty. These numbers seem remarkably small when we consider the enormous quantity of eggs that are laid by many of the other animals of the ocean; the number often reaching many millions, as among some of the Echirodermata and Mollusca.

It is a rather curious fact that these animals are
always so very regular in the time for depositing their eggs, which is from five to six A. M. During the two summers that I studied Turritopsis at the sea-shore, great numbers were collected and kept in aquaria. On many occasions I arose early in the morning to observe the act of spawning,—one time they were watched through the entire night,—and always the act of egg laying was seen to commence at about five o'clock or a few minutes after. Very rarely did it take place as late as six o'clock; and on no occasion was the phenomenon observed more than a few minutes before 5 A. M.

This precise periodicity is not confined to Turritopsis, but seems to be quite prevalent among the redusae in general. In Stomotoca aicata, Stomotoca rugosa and a species of Eucythere I find that the eggs are deposited also at a fixed hour, namely, 5 to 5.30 A. M. Professor Brooks found that Lirone and Eutina spawn at about 6 P. M. In Gonionema Perkins found the time to be from 7 to 8 P. M. Bunting found the period of dehiscence for Hydractinia to be about 10 P. M. While Erejtkowsky says that the eggs of
Obelia are laid early in the morning. Metschnikoff also gives the time of spawning of 14 species.

Regular breeding habits have also been found to exist among other marine animals, and may be more general than has been suspected. Wilson in his work on the development of Renilla found that the eggs of that form were always laid at about 6 A. M. In a single case only, he says, the spawning took place as early as 5:30 and it was never observed to occur later than seven o'clock. The pelagic Crustacean, Lucifer, Professor Brooks observed to deposit its eggs at 9 to 10 P. M.

Bunting found that by packing Hydractinia in ice and keeping them at a lower temperature she was able to delay the time of egg laying. On restoring the animals to the normal temperature, the eggs were laid after a short period of time. Perkins found that the periodicity of spawning in Goriorema is definitely affected by changes of light. By placing his Eudusae in a dark place for an hour and then putting them in the daylight apparently normal egg laying again took place.
While I did not try experiments on *Turritopsis* ™ other
with regard to temperature or light, yet the changes of tem-
perature from day to day had no noticeable effect on the time
at which they discharged their eggs, that is, it occurred at
the same hour on warm days and cool days. In like manner
the fact that the aquarium in which the medusae were contain-
ed was kept before a lighted lamp all night had no effect.
on the time of spawning the next morning, which took place
at the fixed period.

THE EGG.

The egg of *Turritopsis* is spherical and is devoid of
a membrane when first laid and none is subsequently formed.
In size it is quite small and can easily be overlooked. If
the water is free from sediment and the dish containing the
eggs is placed upon a piece of black paper the eggs are vis-
able to the naked eye. They measure .116 of a millimeter
in diameter. They are among the smaller of the medusae eggs.
Mitschriikoff gives the measurements of the ova of nineteen
species of medusae; the sizes of which range from .024 \text{m.}
to 1.5 \text{m.} \textit{Cunina proboscidea} having the smallest and
\textit{Polyxenia albescens} the largest egg of the species included
in his list. The egg of \textit{Turritopsis} is just slightly larger
than that of \textit{Rathkea fasciculata} according to the measurement of
\textit{Netschikoff}.

In the substance of the egg two parts are distinguishable; an outer layer of clear \textit{ectoplasm} which consists
of viscid formative yolk composed of \textit{protoplasn} with very
fine granules; and a central mass of \textit{endoplasm} which is dense
and opaque and filled with large, dark granules of nutritive yolk. From the fact that the \textit{endoplasm} is crowded with
these coarse dense granules of nutritive material the egg
is very opaque and the germinal vesicle is not to be seen
from the exterior. Thus the changes which take place during maturation and fertilization, and the nuclear phenomena of segmentation, as well as the formation of the endoderm cannot be followed in the living egg. For this reason
the egg of *Turritopsis* is not as suitable for study during life as those beautifully transparent eggs of *Liriopera* and *Eutirpa* for instance, which allow all the changes that take place within the egg during development to be followed easily.

The specific gravity of the eggs is greater than that of sea-water and consequently they sink to the bottom of the aquarium as soon as they are discharged from the cavity of the umbrella. In opacity the egg of *Turritopsis* is intermediate between the egg of *Stomotoca rugosa*, which is extremely dense and of chalky white color, and the egg of *Stomotoca apicata* which is semi-transparent and appear bluish-white by reflected light. In color the egg of *Turritopsis* is yellowish white.

**Maturati and Fertilizatio**

Because of the opacity of the egg satisfactory observations on the phenomena of maturation and fertilization are impossible during life, except for those changes which
take place on the outside. A few minutes after the egg is laid the first polar body is given off at the upper pole of the egg. The second polar globule follows after a very short interval. These structures are of an ephemeral nature and soon disintegrate or pass out into the water and are lost. Nothing can be made out of their internal structure or of the arrangement of the chromatin with the low magnification which one is obliged to use in the study of the living egg. However I was fortunate enough to get sectors of the early stage of preserved eggs which show the polar bodies in the process of being extruded. The germinal vesicle moves to the periphery of the egg, then a part of its substance is divided off and extruded as the first polar body. In Figure 7, which is a section of an egg that was preserved a few minutes after it had been laid, the second polar body is just being given off. It contains several granules of chromatin scattered through its finely fibrillated substance.
from the egg, but is still held in connection with it by some means of attachment, the chromatin has come together and for a single mass in the centre of the polar globule. The means of attachment of the polar bodies to the surface of the egg is not quite clear, as the egg is destitute of a membrane. It is possible that some of the clear liquid part of the protoplasm may exude from the substance of the egg as the polar bodies are extruded and be the means of holding them to the surface of the egg even during fixation.

As can be seen in the figure, the germinal vesicle during the extrusion of the polar bodies is situated at the very edge of the egg; about half of its bulk extends beyond the general contour of the egg's surface. The yolk granules are crowded around the nucleus with the same density as in other parts of the egg. After the second polar body has been given off, the female pronucleus moves back from the periphery some distance. Here it is met by the sperm nucleus and fusion of the two takes place. Whether there is
any definite spot for the entrance of the spermatozoan or not could not be decided. But I am inclined to think that the male element is capable of penetrating the egg at any part; and that when it has once entered the substance of the egg, the male and female pronuclei are brought together by the attraction existing between the two.

It was impossible to see the discharge of the spermatozoa from the males; neither did I see them enter the eggs. And, as stated before, the eggs are so opaque that the internal phenomena of fertilization could not be followed in the living specimens. There is reason to believe that the sperms are discharged at about the same time that the females lay their eggs. Fertilization takes place in the water immediately following saturation, and segmentation begins in a very short time.

SEGMENTATION.

Segmentation is total and approximately equal. While there is a slight difference in the size of the blastomeres
at times, yet this difference is not constant and they all have the same value in development; that is, they are not divided into macromeres and micromeres. And there is no evidence either from observations of the living eggs, or from the study of sections of preserved material that any of the blastomeres can be localized as forming distinct parts of the future embryo. During the first two or three cleavages the process is usually quite regular, but beyond the eight cell stage the segmentation becomes very irregular and erratic; almost if not fully as remarkable as that described and figured by Haeckel for *Pernarina tiarella* and of which he says: "Between the extremes of the embryonic history from the early cleavage to the formation of the morula are to be found the most erratic and anomalous exhibitions of developmental phenomena which have ever come to my knowledge, if indeed its counterpart has hitherto been known. It is not strange that with the mental pictures of such steady-going exhibitions as are found in the development of annelids, molluscs, etc., one should regard such
...tiosities as are very inadequately represented in the various figures illustrating this paper as abnormal to the degree of being pathologic! And thus it seemed to me when first observed; and as pointed out in the earlier paper, the first batch of eggs were discarded as having 'gone bad.'"

When I first began the study of the development of *Turri-torsis*, the irregularities of segmentation struck me as very peculiar and I was at first inclined to think that they were abnormal. After I allowed the eggs time to progress I discovered that they developed into normal planulae and thus was forced to conclude that this strange and irregular cleavage must after all be normal for the species. On several occasions the attention of a number of other observers who were working in the same marine laboratory was called to this phenomenon, and they also expressed surprise and remarked that they had never seen segmentation presenting such anomalous and irregular features.

Netschnikoff describes and gives a few figures of a very similar condition of segmentation in *Oceania armata*. 
He says: "Wenn bei dem beschriebenen Medusen verschiedenen Abweichungen in der Zustandekommen des vierten Furchungsstadium constatirt werden - ussten, so könnte man doch bei allen eine gewisse Regelmässigkeit auffinden. Ganz abweichend in dieser Beziehung verhält sich Oceania arvata, da bei dieser Meduse die kaum mit einander vereinigter Blastoceren durchaus unregelmässig und ordnunglos nebeneinander liegen. — Das Abweichende in der Embryonalentwicklung der Oceania arvata hört noch nicht so bald auf. Die Furchung setzt sich in unregelmässigster Weise fort und führt zur Bildung unförmlicher Zellenhaufen, in deren Innern eine Furchungshöhle durchsichtig wird. Oft nehmen solche Embryonen eine ganz abenteuerliche Gestalt an, deren Ursache zum Theil darin liegt, dass sie sich durch Theilung vermehren. Diesen Process habe ich an mehreren isolirten Blastula-Stadien beobachtet, so dass ich an dessen Existenz nicht zweifle." In Turritopsis, likewise, the later cleavages take place in most irregular manner and lead to the for-
nation of shapeless and grotesque mass of blastopores in which the cells are frequently held together very loosely. The accompanying drawings unfortunately represent only the most regular forms. This is due in part to the fact that the very irregular forms were at first thought, as stated before, to be abnormal; and partly because it was difficult to make accurate camera sketches of these shapeless masses during life while cleavages were in place rather rapidly.

Whether these embryos multiply by division, as Jetschmikoff stated to be the case with *Oceania armata* and to which he attributed in part the cause of their peculiar shapes, I have no direct evidence; but think that it is very probable that such may be the case. Frequently the blastopores are separated into two distinct masses held together by a small isthmus of cells; even if they do not divide by an internal activity, they rust, occasionally at least, be broken apart by the action of the tides when in the open ocean. Several times the experiment of dividing the egg during the
comparatively early cleavages was tried and the parts were found to continue their development without any hindrance. These experiments will be described more in detail later.

Another point in which the segmenting egg of Turritopsis differs from that of Oceania armata is that it does not form a true cleavage cavity. The blastomeres always form a more or less solid embryo, as shown in the sections of these stages. Occasionally there are small spaces left between the cells; but a true segmentation cavity that later forms a blastocoele is never formed. In this respect also it is similar to the development of Pennaria tiarella as described by Hargitt. As the completion of segmentation approaches, these irregular masses of cells gradually take on a more symmetrical form and finally there is formed an oval embryo composed of a solid mass of cells constituting a morula.

The first cleavage takes place about twenty to thirty minutes after the polar bodies have been given off. It begins at the upper pole of the egg and passed down to the
In Turritopsis the condition is much like that of Rathkea fasciculata, as shown by the last-mentioned observer, in which the connections instead of becoming a very definite bridge remain for a time as a less clearly outlined portion of the ectosarcical material. Proto-lastic
currents may be seen at times in these connecting filaments. Their function does not seem to be clearly known; but it, very probably, is connected with a readjustment of the cytoplasm and the establishment of an equilibrium between the different blastomeres.

Hargitt in his paper on "The Early Development of Lennaria tiarellae" discusses the occurrence of papillae, threads, and bridges; and reviews briefly the observations of a number of other investigators in regard to these phenomena, and the cytoplasmic activities which they have seen to take place in the eggs of a number of animals widely separated morphologically. No definite conclusions are reached as to the functions of these various phenomena, but it is generally thought that they are concerned with fundamental intrinsic changes within the cytoplasm.

These protoplasmic connections are usually composed of the ectosarc only. They are present not only in the two-celled stage, but in several of the following stages as well. As the number of cells increases the connecting fibres be-
come less easily recognized.

The second cleavage occurs about twenty-five or thirty minutes after the first. The plane of division is also meridional and at right angles to the first segmentation. It begins to the centre of the egg next to the furrow of the first cleavage and slowly extends out toward the periphery. When the division the four blastomeres undergo a slight rotation from right to left; and in the centre of the egg between the cells there is, at times, to be seen a small open space or segmentation cavity which may extend through the entire egg as shown in Figure 12.

After a lapse of time equal to that which occurs between the first and second divisions, the third cleavage furrow appears. This plane of division is equatorial and divides the egg into eight blasto-eres. When the segmentation is first completed the two quartets of cells are situated one upon the other and form a more or less spherical whole, as is the usual arrangement in eggs in which segmentation
is equal and regular. This arrangement of the blastomeres, however, is of very short duration, for soon a separation takes place between the cells of the lower quartet and two of them roll away from the plane of separation in one direction; the other two moving out in the opposite direction. In this migration the blastomeres move through an angle of 45 degrees or more, and finally come to lie in such a position as to form a semicircular plate as shown in Figures 13 and 14. The separation and rotation of the cells of one quartet seems to be constant in its occurrence; but the final arrangement of the blastomeres is not always as regular and definite as that shown in the figures. At times they are more loosely and irregularly connected, and may assume relative positions similar to that shown by Metschnikoff for Oceania armata in Figure 34, Plate 1, of his "Embryologische Studien." In the case referred to the blastomeres are so spread out that the individuals, with three exceptions, touch only one of their fellows, thus
resembling a string of beads somewhat coiled.

With this separation and rolling apart, the regularity of arrangement of the cells in the segmenting egg is lost, and the stages from this point on become more and more irregular with each successive division up to the time when the re-adjustment takes place which is the beginning of the formation of the free-swimming embryo.

It is possible to distinguish, during these early cleavage stages, a layer of ectosarc around each individual blastomere. Later as the cells increase in number and become smaller, the ectosarc covering becomes less conspicuous and finally is lost from sight entirely.

After an interval of about one half an hour, the fourth segmentation begins. The divisions of the different cells no longer take place simultaneously; some occur a few minutes before others, but all are completed within a comparatively short time. So far as the cleavage itself is concerned, it is still equal and regular, but the arrangement of the blastomeres is no longer regular or definite. They apparently
follow no law of symmetry, and may come to lie in any position. Figures 15, 16 and 17 show three different forms which the cells of the sixteen cell stage acquire, and various other arrangements of the blastomeres were seen while studying the living eggs which could not be figured for want of space. However the three figures are sufficient to show that the general form of the egg in this stage may be very different. In Figure 15 it is possible to imagine a direct relationship to a preceding form just a little more irregular than is shown in Figure 14. In a form as represented in Figure 16 the descent of the different cells from the individual blastomeres of the eight cell stage is less easily recognized. Figure 17 shows an egg in which all sixteen blastomeres are spread out to form a flat plate one cell thick in the form of a quadrangle. One can easily conceive how this arrangement can have resulted from a regular eight cell stage in which the rotation of the cells of the one quartet was greater than that shown in Figure
13. The flat, spread out position of the cells at once suggests the idea that the egg may have been subjected to pressure. And this might have been the case if the eggs had been studied on a slide under a cover glass; but there is no evidence that pressure was the cause of this plate-like arrangement, for these forms were occasionally found among a variety of other forms while studying the living eggs in a small preparation dish in sea-water with a two-thirds objective. As the eggs present a number of different forms when subjected to the same external conditions, it seems that the cause of these differences must be sought in the nature of the egg itself rather than in any surrounding influences.

The later cleavages follow at intervals of about the same duration as in the preceding stages. The irregularities of arrangement of the blastomeres increase as the cells become more numerous. On account of the smallness of the blastomeres and the extreme opacity of the egg, it becomes impossible to follow the segmentation in detail any further.
Figures 18 - 21 show a few of the later stages of comparatively very regular forms. Figure 20 represents an egg in which the blastomeres are arranged in two main groups held together by a narrow isthmus of only one cell in thickness. Some eggs were separated into three or four thickened clusters that were joined together by small masses of connecting cells. In others there were smaller groups of blastomeres projecting out from the general mass of cells, thus giving the whole somewhat of an ameboid appearance. The term amoeba-like seems to most clearly represent the shape which some of these late segmentation stages assume, for if a simple outline of these remarkable and grotesque forms is drawn it has a general resemblance to an amoeba with thick blunt pseudopods. Whether these irregularities in the shape of the egg during late segmentation, and the tendency of the cells to arrange themselves into more or less distinct lobes is due to an amœboid property of the cytoplasm of the egg, or to a tendency to multiply by division during cleavage, as was suggested by Metschnikoff for Oceania ar-
mata, there is not sufficient evidence to decide. It may be possible that both of these factors act in determining the shape of the segmenting mass of cells. And doubtless the membraneless character of the egg plays a part in these phenomena.

**PLANULA.**

When segmentation is complete a solid embryo is formed which may at first be called a morula. Small spaces occur sometimes between the blastomeres during the different cleavage stages, but they are sooner or later obliterated by the crowding together of the cells. A central cleavage cavity which is later transformed into a blastocoele is not formed; consequently a true blastula does not exist in the development of *Turritopsis*. In this respect it differs very markedly from *Stomotoca* and the majority of hydro-nemusae of which the development has been studied, in which a definite blastocoele is formed that becomes filled
finally with the migrating endoderm cells. When the developing egg is about six to eight hours old, the very irregular shape, which the segmenting mass has assumed, becomes less marked. Gradually the cells become rearranged; the lobes and processes which previously were so conspicuous are now drawn into the main mass of cells, and the egg is transformed into an oval embryo. This process of rounding up lasts from two to four hours. The cells of the embryo now develop cilia, and the larva begins to move. At first the movements are feeble, but soon the larva is able to leave the bottom of the aquarium and swim free in the water. Eggs that are laid at five to six o'clock in the morning develop to the free-swimming stage by four in the afternoon. The larva swims with its broad end forward and has a snail or cork-screw motion, which propels it onward. This method of swimming is common to hydroid larvae. When the embryo reaches this stage the cells become very numerous and small. And before the cilia are developed and
movement begins it resembles an unsegmented egg very much, except that instead of being spherical it is now oval. In size it is about the same as the unsegmented egg, if anything rather smaller. The decrease in size must be accounted for by the fact that some of the yolk has been digested; and the larva evidently has not yet acquired any means of receiving food from the external world.

The larva remains in this oval condition for some hours, after which it elongates to form a typical planula. When the embryo is twenty-four hours old it lengthens out and becomes more slender and assumes a general appearance as shown in Figure 23. As it becomes older it grows still longer. Figure 24 shows a larva of thirty hours. It has now the power of contraction; and is sensitive to stimuli. When the cilia are first developed and for some time during the oval condition of the larva it swims near the bottom of the aquarium. But as it grows longer and elongates it rises in the water and swims at or near the surface. The length
of time during which the embryo remains in the free-swimming planula stage is variable; but as a rule by the time it is about forty-eight hours old, it begins to sink toward the bottom of the aquarium, and to swim less rapidly. After the spiral swimming movements are lost, the planula is capable of gliding along the bottom of the dish for some time. Finally the motion ceases altogether and the larva loses its cilia and is ready for attachment. This stage of development is reached under favorable conditions about forty-eight to fifty hours after the eggs have been laid.

The planula is very opaque, and thus it is impossible to make out anything about its internal structure in studying the living forms. Specimens in various stages of development were preserved and sectioned for the study of cellular structure. The description of this structure will be given in connection with the formation of the germ layers.

Brooks describes and figures an ectodermal invagination
at the posterior end of the planula. He says: "In a living planula it is easy to make out the posterior end, an ectodermal invagination, which looks very much like the mouth of an invagination gastrula, but this resemblance is misleading, for the careful study of a similar structure in the planula of Eutima shows that the invagination has no connection with the digestive cavity, but is an ectodermal gland for the attachment of the planula." From my observations I am forced to regard this structure, which he describes, as a variation rather than a normal feature. It seems to be an abnormal occurrence which is found only rarely. Among the many specimens which I studied both in life and from preserved material, such an invagination was met with only on one occasion. Then it was at the anterior end of the planula instead of the posterior. These features are clearly abnormal features of the developing Turritorsis planula.
EXPERIMENTAL.

The very irregular character of the segmenting egg and the loose connection of the blastomeres; and their tendency to separate into more or less definite lobes and protuberances, as has been described in the section on segmentation suggested the problem: What would be the effect of dividing the eggs during the comparatively early stages of cleavage? With this question in mind a few experiments were tried. The eggs were divided during several stages of segmentation. The best method for separating the cells was found to be by placing them on a clean glass plate moistened with sea-water. Then with a finely pointed needle or with a very delicate scalpel the blastomeres could be cut or torn apart without being crushed. After they were divided, they were flooded from the glass plate by water from a pipette into a dish of sea-water and watched in their development. The advantage of separating the eggs on a glass plate is that they are held slightly by surface tension, and no
not rotate as readily while being cut apart. Eggs were divided during different stages of cleavage from two to six hours old. They were then placed under conditions as nearly like those under which the eggs not divided developed as possible. Unfortunately, as these experiments were incidental and incomplete, no eggs were divided during the two-cell stage and their cleavage followed in detail. Some eggs that were laid between five and six in the morning were divided at 10:45 A.M. More than one half of the fragments continued to develop and by six o'clock in the evening had reached the free-swimming stage. They were retarded a little in their development; whole eggs usually arrive at this stage at about four to four-thirty. They were slightly smaller than embryos from whole eggs, but apparently just as active and normal, except in size. By the next morning they had reached the elongated planula stage and were in good condition, swimming at the surface of the water.
At another time some younger eggs were divided. These showed practically the same results in development. The opacity of these embryos made the study of their minute structure impossible during life; and because of scarcity of material none could be preserved to study their histology from sections. However these few incomplete experiments show that fragments of the egg of Turritopsis are capable of developing into apparently entire and normal embryos of slightly smaller size.

Hargitt artificially divided some Pennaria eggs during the first cleavage and figures a number of resulting segmentation stages, which are very similar to those of whole eggs. He says: "As will be seen, each of the resulting halves behaved in a manner indistinguishable from that of normal eggs. These half embryos were followed through the entire process of cleavage and through the later metamorphoses into planula and polyp, and in every respect,
size alone excepted, the process was perfectly normal."

To my knowledge Haeckel was the first to publish the statement that halves of hydromedusa eggs would develop into normal embryos. For some time naturalists in general were inclined to doubt the fact; but since the work of Boveri, Hertwig brothers, Roux, Driesch, Wilson, Morgan, Loeb and others on the fragments of eggs, the development of embryos, abnormal and normal, from the portions of eggs is a question no longer to be doubted.

FORMATION OF THE ECTODERM.

In the development of the egg of Turritopsis the germinal layers are not differentiated by process of eribole, delamination or cellular ingression. During segmentation the blastomeres do not separate and arrange themselves around a segmentation cavity which later is transformed into a blastocoele. Thus instead of having formed a coeloblastula, we find that cleavage results in the formation of a solid
oval embryo destitute of a blastocoele, which may be called 
a morula stage. The cells of the segmenting egg are all 
alike in structure and nearly equal in size; so that they are 
not distinguishable into primitive ectoderm and primitive 
endoderm, which is the case in forms where a definite de-
laration takes place, as is so beautifully shown in 
Diplopterae and Geryonia, and in species where cellular impres-
sion occurs as in Stomatocae and Clytia for example. Fig-
ures 85 to 86 illustrate the uniformity of the cells, and 
the solid character of the egg during segmentation. In 
Figure 27 a space exists between the blastoreres near one 
end of the egg, but this is not to be regarded as a true 
cleavage cavity. The next figure shows three of these false 
cleavage cavities. They occur only occasionally. As 
stated before most of the eggs are entirely solid.

About the tire the irregular mass of segmenting blasto-
eres is transformed into the oval embryo, the cell boun-
daries are lost for a short time and a syncytium is formed. 
This syncytial structure is crowded with yolk granules and
a number of nuclei are scattered through the protoplasm. The nuclei soon become more numerous near the periphery; and then cell walls begin to appear as shown in Figure 33. These cells are to become the ectoderm, which is soon separated from the inner structureless mass by the development of the mesoglea. Now the ectoderm forms a distinct layer, composed of columnar cells all of which are at first similar in structure and lie parallel to each other as shown in Figure 34. The differentiation of the ectoderm cells takes place later.

The formation of the germinal layers in Turritopsis is different from that which has generally been described for the development of Hydrozoa. In the majority of forms previously studied the differentiation took place either by delamination or by cellular impression, unipolar or multivolar. These methods have been well described and figured by Etschri'off for a number of species.

In Aplaura and Thoralopora there is found, according to Etschri'off, a solid so-called "crula stage destitute
of cleavage cavity, the superficial cells of which are converted into the ectodermal layer, while those within represent the endoderm. Here the two layers are formed directly without the formation of a syncytial structure.

In Eudendrium and Ferraria according to Harfitt's description a condition somewhat similar to that of Turritopsis is found. He says: "Indeed in both Eudendrium and Ferraria, not to mention other cases, cleavage would seem to result primarily in the formation of a more or less characteristic syncytium, the subsequent development of the germ layers taking place by a gradual differentiation of the syncytial elements, first and naturally the ectoderm, and later, often very much later, the endoderm."

The syncytial character in Turritopsis is acquired under favorable conditions, when the embryo is about six hours old; at the time that the irregular mass of segmenting cells is metamorphosed into the oval embryo. And I am inclined to think that the formation of the syncytium
and the change of shape of the developing embryo are connected phenomena. The length of time during which this condition lasts is evidently comparatively short, for soon cilia develop and the larva begins to swim. Meanwhile the peripheral region of the syncytium has been transformed into a distinct layer of ectodermal cells, separated from the inner mass of tissue, still structureless in character, by the development of the mesogloea.

From the fact that a syncytium, or blastoderm-like structure is formed, it is impossible to localize any of the blastomeres of the segmenting egg which will form special parts of the future embryo. Ever these cells which are at the surface at the completion of segmentation cannot be regarded as primitive ectoderm, for in the breaking down of the cell boundaries, the formation of the syncytium, and the recasting of the cells it is quite impossible to say what change of the protoplasm may take place.
The formation of the endoderm in *Turritorisia* cannot be adapted to any of the schemes of the development of the Hydro-echinoidea which have been sketched by Mertenshoff. He distinguishes three principal methods for the development of the inner germ layer: First, delamination, a process in which the segmenting blastomeres divide in a plane nearly parallel to the surface; and the inner parts or cells become primitive endoderm, while the outer parts remain as primitive ectoderm. Second, multinuclear ingestion, in which cells migrate into the blastocoele from different regions of the perihernal cell layer, and are transferred into endodermal tissue directly. Of this mode he describes several subordinate types. Third, unincular migration, similar to the preceding except that the primitive endoderm cells are given off at one pole only; at the posterior end of the larva.

In *Turritorisia* the endoderm is derived from the syn-
of tissue left in the centre of the embryo after the ectoderm has been formed and separated off by the development of the mesogloea. The inner germ layer as a rule is formed much later than the ectoderm. Soon after the supporting membrane is developed cell boundaries begin to appear in the syncytium in the interior of the larva. The cells thus formed are primitive endodermal cells, and are crowded together without any definite arrangement for a number of hours. Stages in which the cell walls are rearranging are shown in Figures 34 to 36. When the embryo is about forty-eight to sixty hours old, the time at which attachment takes place, a fissure appears in the middle of the mass of endodermal tissue. This is the beginning of the coelenteric cavity. This separation begins near the anterior part and creeps toward the posterior end. The coelenteron gradually increases in size, and at the same time the endodermal cells begin to be rearranged; and finally become situated parallel to each other with their bases.
against the resecloca and form a definite inner germ layer.

Gerd has observed in Bougainvillia that during the course of cell multiplication the cell boundaries become indistinct and that the peripheral and central nuclei are altogether identical. But this species differs from Turritopsis, according to his description in the formation of the compact scrobula stage, in that it is brought about by a multinucleate migration of cells into the interior of the coeloblastula; while in Turritopsis the scrobula stage results directly from segmentation without any recognizable migration of cells.

The formation of the endoderm in Turritopsis therefore differs from nearly all the methods which have previously been described; and which in the pair conform to one or another of the stereotyped methods as established by Betzschmann. The nearest approach is that described by Harritt for Ludendorum and Pennaria, in which there is also more or less of a syncytium for a time in the differentiation.
During the early cleavage phases the cells multiply entirely by the process of mitosis. But in the later phases, especially when the egg is approaching that stage in which the cell boundaries are lost, there is good evidence that direct cell division is also of frequent occurrence. In this period of development mitosis and anitosis take place simultaneously in the different cells of the segmenting egg. Figure 31a shows a karyokinetic spindle in the prophase; Figure 31b one in the anaphase. The chromosomes are large and prominent; but are too closely crowded together to be counted with accuracy.

The nuclei which divide anitotically vary in size considerably, and have a reticular appearance. Figure 31c shows a large nucleus of this reticular character with the chromatin scattered about in the living arches. Figures
It is to illustrate nuclei in various stages of amitotic division. Frequently in cells where amitosis takes place many of the yolk granules have been digested and consequently are fewer than in cells where digestion is less active. It may be that the more active functions of digestion and the phenomena of direct cell division are associated with each other. Or it may be that the view of Fleming and Ziegler, that amitosis is connected with a high specialization of the cell or is the forerunner of degeneration, applies in this case. This latter conception seems plausible, for we find amitosis to be most abundant shortly before the cell boundaries disappear and the embryo is transformed into the syncytium.

For a number of years it has been known that amitosis is common in follicle cells, digestive epithelial cells, supporting cells, etc.; but generally it was not supposed to take place in early embryonic development. Within the last few years however a number of observers have discovered this
phenomenon in the developmental stages of various forms.

**ATTACHMENT.**

Under favorable conditions when the larva is about fifty hours old it reached that stage of development at which attachment takes place. In preparation for this process the planula settles to the bottom, loses its cilia and consequently its movements cease. The manner of attachment in *Turritopsis* is like that of *Sto-otora* differs from that usually described in hydroid development. Instead of settling down on the anterior end of the planula according to the method which occurs in *Euderrhium*, and which has been regarded as typical and used in descriptions of the embryology of the Hydro-Redusae in text-books, the planula becomes attached on its side by nearly its whole length, and is transformed into a root. The hydranth instead of growing up from the posterior end of the planula as in forms which attach themselves by the anterior end, de-
vulva a byud that is given off from the post, usually about the middle.

Professor Brooks observed the fact that the planula is transformed into a root in Turritopsis, Eutira and Hydractinia; and gives a brief account of the same in his paper on "The life-History of Eutira" (1886). Netschriakhoff describes and figures for Mitrocera the fact that the larva becomes attached by its side and is almost wholly enveloped in the formation of the hydrocrhiza, while the first hydranth grows out of it by a kind of budding (Embryologische Studien, 1886).

In general the attachment of the planula is similar in Turritopsis to the method which is followed by Storctoca, but the former does not commonly produce secondary hydrocrhiza. In Storctoca about the time the hydranth bud appears, or even before, the root branches giving rise usually to one or two secondary roots; in Turritopsis this branching rarely takes place, at least during the first few days of the de-
DEVELOPMENT OF THE HYDRA.
tacular buds is formed some distance below the first circle of tentacles. When the polyp is free twenty to twenty-four hours old, or about forty-two hours after the egg is laid, it is ready to develop the third whorl of tentacles. Thus the tentacles nearest the apex of the hydranth are the oldest and largest. The circles are indefinite, that is the tentacles of a whorl do not all arise from the same level; so that in the advanced hydranth they have rather the appearance of being scattered than arranged in circles. The tentacles when fully developed are stout and filiform; and are capable of such extension and contraction. Figures 37 to 41 illustrate various stages in the early development of the hydranth; the youngest being about fifty hours and the most matured some seventy hours old. Figure 38 shows a form in which the polyp arises from near the end of the hydrocoriza. This is exceptional. A hydranth with the third circle of tentacles is show in Figure 41; the tentacles of the first whorl have become considerably elongated. The hydrocaulus now becomes longer and more slender; and the hydranth assumes a fusci-
form body.

The polyps that I reared from eggs at the age of three days were in the main features like the hydranths of the adult colony found and figured by Professor Brooks, except that they had not yet developed as many tentacles. In his description he says: "The upright stems of the hydra, from 8 mm. to 12 mm. high, bore large terminal hydranths, as well as smaller ones which were scattered irregularly along the stem on short stalks. The long fusiform body of the hydranth carries from eighteen to twenty thick, short, filiform tentacles, which are arranged in three or more indefinite whorls. The reduced buds originate around the stem just below the hydranths, and they are themselves carried on short stems. The perisarc is not annulated, and it forms a loose cylindrical sheath around the main stem, and the short branches which carry the lateral hydranths and the young reducens, while the latter are invested by a
much thinner and more transparent capsule of perisarc. The sheath of the stem is thick and crusted with foreign matter. It terminates abruptly by a sharp collar just below each hydranth. The young hydranths and the medusae are budded off above the collar, but they soon become entirely sheathed in perisarc by the growth of the stem. The male yellowish-red hydranths are very similar to those of Tubularia (Allman) and the hydrozoa is so similar to Dendrocloa Ehrnii recently described by Leismann, that they undoubtedly belong to the same genus."

SUMMARY.

1. The ova of Turritopsis arise in the ectoderm of the manubrium. They grow by the absorption of the primitive ovarier cells; and when mature are densely crowded with large yolk granules.

2. Dehiscence takes place at a definite time, from five to six o'clock in the morning.

3. The egg is spherical and endothecless. It is com-
roset of an outer layer of clearer ectoderm and a central
mass of endoderm which is dense and opaque and filled with
large, dark yellow spheres.

4. Maturation and fertilization take place in the water
after the eggs are deposited. It is impossible to make
out details in the living eggs because of their opacity.

5. Cleavage is total and nearly equal. The first three
divisions are fairly regular; but during the later
segmentation the arrangement of the blastomeres becomes very
irregular and erratic. At the completion of segmentation a
solid morula stage is formed, in which the cell boundaries
are lost for a time giving rise to a syncytium.

6. Parts of eggs which are divided during the cleavage
stages continue to develop and form larvae which are normal
in every respect except size.

7. The ectoderm is formed by the reappearence of cell
walls in the periphery of the syncytium mass; and is separated
from the interior part by the formation of the mesogloea.
The formation of the endoderm follows none of the typical methods described by Etschrikkoff. It arises late in the larval life from the syncytial mass of tissue left in the interior of the embryo after the separation of the ectoderm by mesoglea. When the cells first reappear they are crowded together without any definite arrangement; finally they come to form the distinct endodermal layer.

9. During the late segmentation there is evidence that some of the nuclei divide asitotically.

10. The planula becomes attached on the side by nearly its entire length, and is transformed into a root.

11. The first hydranth develops from a bud which is given off at about the middle of the root soon after attachment.

12. The tentacles develop in indefinite whorls. Each whorl has four tentacles. The oldest are nearest the distal end. In the fully developed hydranth they have the appearance of being scattered rather than being arranged in circles.


1891. Brauer, A. Uber die Entstehung der Geschlechts-produkte und die Entwicklung von Tubularia mesembrantherum. Id.


1883. Claus, C. Organisation und Entwicklung der Medusae.

1881. Lewkes, J. V. Studies of the Jelly-Fishes of Long- 

1842. Forbes, Edward. A Monograph of the British Laked- 

1892. Gerd, W. Zur Frage über die Keimblätterbildung bei der 
Hydromedusa. Zool. Anzeiger. XV.

1897. Gränberg, G. Beiträge zur Kenntnis der Gattung 
Tubulicola. Zool. Jahrb Bd. XI.

1892. Hieber, V. Die Forschung des Fis von Aescuaria 


f. Naturw. Bd. XV.

American Naturalist.

1904. Harpitt, C. W. The Early Development of Pennaria tia-

Jahrb. Bd. XX.


1873. Smallwood, E. Terraria tiarella. American Naturalist, XXIII.


1904. Wilson, F. E. The Development of Penilla. Philos. Trans. CLXIV.


VITA.

Samuel Hitterhouse was born at Pipersford, Pennsylvania, on November second 1873. He received his early education in the District Public Schools. Later he attended the Washington Hall Collegiate Institute and Ursinus Academy. In the fall of 1897 he entered Ursinus College, and received the degree of Bachelor of Arts in 1901. In the autumn of the same year he entered the Johns Hopkins University as a graduate student; his subjects being Zoology, Physiology and Botany.