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ON *TURRITOPSIS NUTRICULA* (McCRADY).

By William Keith Brooks and Samuel Rittenhouse.

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No. 8.— ON *TURRITOPSIS NUTRICULA* (McCRADY).

BY WILLIAM KEITH BROOKS AND SAMUEL RITTENHOUSE.

Part 1. The Metamorphosis of Turritopsis.

By William Keith Brooks.

(The references in Part 1 are to the illustrations in a memoir on the life-history of the Hydromedusae, which was published by the Boston society of natural history in 1886, as volume 3, no. 12, of the Memoirs of the Society.)

In a memoir that was written in September, 1885, and published more than twenty-two years ago, I gave a figure, plate 37, of a hydroid cormus of Turritopsis with medusa buds in many stages of development as well as free medusae at successive stages of growth up to the mature sexual adult. From this memoir I have compiled the following account, in order to point out the more prominent features in the life history (Brooks, '86, p. 391–393). While I have made a few verbal changes and abridgments, the extracts are practically as they were printed in 1886.

"The only colony of the hydroid which I obtained was scraped from the piles of the steamboat wharf at Morehead City, seven or eight feet below low tide mark. The tips of two of its branches are shown in *H*, in Pl. 37. It lived for two weeks in the house, and set free great numbers of hardy medusae which were reared until they had acquired the characteristics of the genus."

The upright stems of the hydra, from 8 mm. to 12 mm. high, bear large terminal hydranths, as well as smaller ones which are 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 medusa-buds, *B*, *B*, 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 medusae, while the latter are closely invested by a much thinner and more transparent capsule of perisarc. The sheath on the stems 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 pale yellowish-red hydranths, are very similar to those of Tubularia (Allman) and the hydroid is so similar to *Dendroclava Dohrnii* recently described by Weismann, that they undoubtedly belong in the same genus.

"The little medusa remains attached to the stem, as shown in Pl. 37, *C*, for some time after the rupture of its capsule of perisarc. At this time it is nearly spherical and covered with large conspicuous ectoderm cells. Its eight short tentacles are thrown backwards in contact with the outer surface of the bell, and their tips are hooked or bent upon themselves in a very characteristic manner, which is shown in the figure. This position of the tentacles renders the bulb at the base, with its ocellus, very prominent.

"The medusa when set free, Pl. 37, figs. *D* and *E*, has eight tentacles, a thin globular bell, and a short simple proboscis. When the animal is in active motion the tentacles are contracted, bent into hooks and thrown back against the umbrella, as shown in fig. *D*." When it is relaxed after a pulsation the bell is nearly globular, but it is lengthened and emarginated at each pulsation, so that it exhibits rhythmical changes of outline. Figure *D* shows a young medusa in the shape which it assumes while swimming, at each period of contraction, while *E* shows a medusa of the same age floating in a relaxed condition. When the medusa is at rest the height of the umbrella is about equal to its diameter, and the shape is that of a spherical segment almost equal to a sphere. The tentacles are capable of extension to a length equal to about twice the diameter of the bell, and when the animal is at rest they are stretched out almost horizontally, and the distal half is bent downward at an acute angle near the middle of the tentacle. The four interradial tentacles, when thus extended, lie nearly in the plane of the velum, while the four perradial tentacles are carried a little lower. This peculiar bending and alternation of the tentacles, which is very characteristic, is well shown in figure *E*, which, like all the other figures, is a careful study from life. Many hydroids carry their tentacles bent so as to form two cycles, and the resemblance to these hydroids, which Turritopsis exhibits, seems to be a larval, or hydroid characteristic, for I have failed to observe anything of the sort in older medusae. The tips of the extended tentacles are slightly clavate, each with a spot of dark orange pigment.

The length of the proboscis of the young medusa is about two thirds the height of the umbrella, and its upper and lower ends are smaller than the middle. The mouth when the medusa is first set free, and for several days afterwards, is simple and circular, and the endoderm of the oral end of the proboscis is thin; but, just below the aboral constriction, it becomes very thick and cartilage-like, and the thickened area arches out into the subumbrella in the course of the radial canals, as is shown in figure 7. This thickening of the endoderm of the proximal ends of the radiating tubes, where they join and open into the stomach, is characteristic of Turritopsis, and, in specimens a week old, (fig. *J*) the peduncle that is thus formed is about equal in length to the proboscis proper, which is suspended at the lower end of a peduncle consisting of four great masses of cartilage-like endoderm cells. These four hollow tubes of cartilage are the endodermal portions of the proximal regions of the radial canals, which meet each other in the central axis. The singular structure which is thus formed is quite unlike anything that has been described in other genera. Some authors have regarded it as an ordinary gastrostyle or gelatinous prolongation of the subumbrella. It is not a gastrostyle but a peduncle formed of endoderm cells. As the young medusa grows, the proximal ends of the radiating tubes are drawn down into the cavity of the umbrella, as is shown in figure *H*, until in specimens two weeks old the stomach is suspended some distance below the sub-umbrella by a transparent mass of large cells meeting in the central axis, and perforated by the cavities of the four tubes. In the adult, figures *I*, *J*, *K*, this body almost entirely fills the upper half of the cavity of the bell.

In a medusa a week old, figure *H*, the four oral lobes or lips are present, and are covered by the stalked bunches of lasso-cells that have been described in the adult by McCrady and others.

At about this time traces of the reproductive organs make their appearance in the walls of the proboscis just below the lower ends of the masses of endoderm cells. The tentacles, at the stage shown in figure H, are still carried in two cycles, the interradials being higher than the perradials. There are only eight, and no more were developed in the medusae which I reared from the hydra, although I captured many specimens in the same stage, and at all the following stages up to maturity.

In specimens from one to two weeks old the lower surface of the very wide velum, figure G, is pushed out to form eight hemispherical pouches, four of them radial and four interradial, in the planes of the eight tentacles. They project so much that they are prominent in profile view, as shown in figure H. May they not be homologous with the pouches that become converted into marginal vesicles in the vesiculated medusae?

"The adult medusa is shown in figs. *I*, *J* and *K*. When it is swimming up from the bottom the tentacles are carried tightly curled up close to the edge of the bell. When it reaches the surface they are suddenly extended on all sides, shown in fig. *K*. They are nearly straight, but their tips are a little bent and sometimes coiled. This attitude is preserved only for a few seconds and the medusa at once begins to sink towards the bottom, while the tentacles coil up at their tips and assume the position shown in fig. *I*. The bell also becomes flattened and nearly hemispherical, and before the animal reaches the bottom of the aquarium it usually assumes the appearance which is shown in fig. *J*. As it nears the bottom it suddenly draws in its tentacles and rises to the surface, and again extends them, as shown in fig. *K*. . . . The figures of the adult medusae, *I*, *J*, *K*, are much less magnified than the others, which are all drawn to the same scale."

The memoir of 1886 also contains an account, (p. 391), of a planula that was reared from the egg of Turritopsis, and of the larval or first hydranth which arises from the planula and forms the basis of a new hydroid cormus. These stages are shown in figures 2 and 3 of plate 42. A record is also made (p. 391) of the fact that the planula does not become converted into a hydranth, but becomes a root, from which the first hydranth is formed as a bud.

Dr. S. Rittenhouse has recently reared a number of planulae of Turritopsis from the egg, and he has verified the observation that the planula becomes a root, from which the first hydranth arises as a bud, and he has traced the further development of the hydranth. When joined to the memoir of 1886, his observations, which are contained in Part 3 of this paper, give us all the prominent facts in the life history of Turritopsis.

PART 2. NOTES ON THE ANATOMY AND SYSTEMATIC AFFINITY OF TURRITOPSIS.

BY WILLIAM KEITH BROOKS AND SAMUEL RITTENHOUSE.

(With plates 30-32. The references in Part 2 and Part 3 are to the illustrations that accompany this paper. Of these, plate 30, figure 4, and plate 31, figure 8, were drawn by W. K. Brooks, and the remaining figures by Samuel Rittenhouse.)

The illustrations of the adult Turritopsis in the memoir of 1886 are too small to show the anatomical details, and they are now supplemented by an enlarged figure of the adult (pl. 30, fig. 4), which was drawn from a living specimen by W. K. Brooks at Beaufort, North Carolina, in 1885; and by the illustrations of sections by Samuel Rittenhouse in plates 30 and 31.

As figure 4 of plate 30 shows, the radiating canal is bent upon itself twice, at right angles, and consists of four sharply defined regions: an ascending region which arises from the stomach, a horizontal region, a descending region which ends in the circular marginal canal, and a blind diverticulum which is a continuation of the ascending region.

Plate 30, figure 1, is a section through the plane of the middle of the reproductive organs in figure 4. It shows that the radial canal arises from the stomach as a groove or channel that consists of prismatic transparent endoderm cells with conspicuous cell walls. As figure 4 shows, the channel arises from the stomach in the plane of the oral ends of the reproductive organs and remains an open groove as far as the plane of the aboral border of these organs, gradually deepening and infolding until it becomes converted into a tube as is shown in plate 30, figure 2. This is a section through a plane that is just above the reproductive organs. The endoderm cells are now chorda cells with conspicuous cell walls and transparent contents. The four radiating tubes meet each other and are flattened by pressure over the areas of contact, where the ectoderm is absent, so that the endoderm of one tube is separated from that of adjacent ones by the supporting layer only. In the center is a square area lined with the ectoderm of the subumbrella, and occupied by the jelly of the umbrella. The convex peripheral portion of each tube is covered with ectoderm. The distribution of the ectoderm will be understood by reference to figure 9 of plate 31, which is part of a cross section in the plane which, in figure 4, is just below that in which the radiating tubes become horizontal. The endoderm cells are here very much elongated, and their transparency and the sharpness of the walls between the cells, and the other chorda-like characters, are most marked at this level. The change from the highly specialized endoderm of the ascending region of the canal to the ordinary endoderm of the descending region takes place in the horizontal region, as plate 30, figure 4, illustrates, in such a way that the aboral surface of the canal is covered with ordinary endoderm where its side walls still consist of the elongated and specialized endoderm. This results in the formation, on the top or aboral surface of each canal, of a groove, which is narrow and shallow at its axial end, while it is very broad and deep peripherally, as figure 4 shows. The floor of the groove consists of ordinary endoderm covered by the ectoderm of the subumbrella, and its cavity is occupied by the jelly of the bell. The sides of the groove are high walls of the modified endoderm covered by ordinary ectoderm. These walls are nearly vertical on their inner surfaces, and rounded on their outer surfaces.

The descending regions of the radiating canals are shown at *a* in plate 30, figures 1 and 2, and more enlarged in plate 31, figure 6. In this figure, *a* is the cavity of the canal; *b* the flattened ectoderm of the exumbrella; *c* the jelly; *d* the endodermal lamella, which consists of flattened and rudimentary endoderm, and joins each radial canal to the adjacent ones; *f* is the ectoderm of the subumbrella, with a well marked layer of longitudinal muscles, and a thick supporting layer, *e*; *g* is the endoderm of the outer or peripheral surface of the canal, consisting of cells that are nearly cubical, and so crowded with small granules that they are nearly opaque in preserved material; *h* is the endoderm of the central or internal side of the canal. The cells are columnar, and they contain numerous granules that are much larger than those in the external endoderm. The endodermal lamella is continuous with the internal endoderm.

Plate 31, figure 7, is a section through the circular marginal canal, *a*. Its wall consists of two sorts of endoderm: flattened and cubical, and long and prismatic; but their relative positions are the reverse of those in the radiating canals, as the cubical endoderm is on the internal side and adjacent to the ectoderm of the subumbrella, and the elongated endoderm adjacent to the exumbrella. This figure illustrates a section through the base of a tentacle, *t*, and shows that the tentacular chorda-like endoderm, which is so common in the tentacles of hydroids and hydroid jellyfishes, is of the same character as that of the radiating canals of Turritopsis. The velum is shown in this figure at *v*; the velar nerve ring at *n 1*; the tentacular nerve ring, which innervates the visual organs, at *n 2*; *f* is the ectoderm of the subumbrella; *b* that of the exumbrella; and *d* the endodermal lamella.

Figures 1, 2, 3, and 5 (pl. 30), are a series of successive sections through the stomach and proboscis. The reproductive organs are shown in cross section in plate 30, figure 1, and in side view in plate 30, figure 4. They are, no doubt, fundamentally radial, but the halves are pushed apart by the channels through which the radiating canals open into the stomach, so that each half joins the half of the adjacent reproductive organ in the interradial plane. There are, in effect, four interradial gonads, although each is to be regarded as the halves of two perradial gonads. Four vertical interradial furrows, shown at *e* in the section, and shown much more plainly in the older medusa that is represented in figure 4 (pl. 30), indicate the planes in which this secondary union is to be regarded as having taken place.

Haeckel recognizes four species of Turritopsis: *T. armata* from the Mediterranean; *T. polynema* from the Atlantic coast of France; *T. nutricula* from the Atlantic coast of North America; and *T. pleurostoma* from the coast of Australia.

A number of medusae, from various parts of the world, which are very similar to Turritopsis, have been described, and placed together in the genus Modeeria. They exhibit most of the distinctive characteristics of Turritopsis, in a much less pronounced condition, and they, no doubt, present these characteristics in their more primitive form. One of them is represented in plate 31, figure 8, which is from drawings made at Nassau in 1886. It closely resembles Turritopsis in habits and form, and the account of the habits and form of Turritopsis in the memoir of 1886 applies to it without any changes. It resembles Turritopsis in the way that the tentacles are carried; in the position of the ocelli, and in the suspension of the proboscis by a stalk of modified, chorda-like, endoderm cells. The radiating canals communicate with the stomach by vertical grooves, as in Turritopsis, and the perradial gonads are divided into halves by these grooves, but not so completely as in Turritopsis. The peduncle consists of the endoderm of the radial canals, as in Turritopsis, but the enlargements are not so great, and they do not meet in the central axis. The lips, which are simple in Turritopsis, are bifurcated.

Haeckel has described, from the Canary Islands, and figured in his "System der medusae," a medusa, *Callitiara polyophthalma*, for which he has established the genus Callitiara. It is very close to Turritopsis, as Haeckel points out, differing from it in little except the presence of a second ocellus on the exumbral side of the bulb of each tentacle, in addition to the one that is situated on the velar side in Turritopsis and its allies. Its endodermal peduncle is less developed than it is in Turritopsis. Its lips are simple as in Turritopsis.

The genus Modeeria was established by Forbes for a medusa, *Modeeria formosa*, with four tentacles, without a peduncle, and with the gonads simple and perradial; and it is so treated by Haeckel in his "System der medusae." It seems, therefore, to be a most inappropriate name for a Turritopsis-like medusa; and it should be restricted to *Modeeria formosa* of Forbes and its natural allies; while the Turritopsis-like medusae, which have no affinity with Modeeria of Forbes, form a natural genus which should be closely associated, in the system of the medusae, with Turritopsis and Callitiara. Haeckel, who puts Turritopsis and Callitiara together in his "System" while he puts the genus Modeeria of Forbes in another family, thus exhibits a sound insight into the question that is here at issue.

We take this occasion to call to the attention of those who may undertake the systematic revision of these medusae to the fact that allies of Turritopsis, which have nothing in common with Modeeria of Forbes, except so far as they are hydroid jelly-fishes, have been wrongly referred to this genus, while they constitute another natural genus.

We suggest, to those who may revise the subject, the propriety of establishing a genus for these medusae, to which the name, Mocradia, might well be given in honor of John McCrady, the discoverer of Turritopsis.

If this suggestion be accepted, three genera of Turritopsis-like medusae should be recognized. The three genera are characterized as follows:

Turritopsis: Tentacles numerous, in two alternating rows in young medusae, in one row in adults, with a single ocellus on the velar side of each tentacular bulb; oral lips four, simple; perradial gonads split on perradii, and fused on interradii; endodermal peduncle nearly solid.

CALLITIARA: Tentacles numerous, in two alternating rows, one row without ocelli, and the other with two ocelli on each tentacular bulb; lips simple; gonads slightly split in perradii; proximal regions of radiating canals with chorda-like endodermal cells.

MCCRADIA gen. nov. (Modeeria in part). Tentacles numerous, in one row, with a single ocellus on velar side of each tentacular bulb; oral lips branched once dichotomously, perradial gonads slightly split in perradii; proximal regions of radial canals with chorda-like endoderm.

PART 3. EMBRYOLOGY OF *Turritopsis nutricula*.

BY SAMUEL RITTENHOUSE

INTRODUCTION.

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 13th. While the medusae could be collected in fairly large numbers, many of them were immature. They lay only a limited number of eggs. Material was preserved and sectioned, however, 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 vescicular character. The nucleolus is conspicuous, and a network of chromatin is scattered throughout the germinal 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 deuteroplasm 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 peripheral zone, in which the protoplasm retains its homogeneous and finely granular character and forms the ectoplasm of the mature egg. Figures 10 to 15 of plate 32 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 deuteroplasm can be formed from figure 15 (pl. 32) which is drawn from a nearly mature ovum. In the fully developed egg the layer of ectoplasm is narrower than 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 it 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 deuteroplasm. That the yolk spheres arise directly through the activity of the cytoplasm, independently of any nuclear or nucleolar function, is doubtful at least. If this were the case we should expect the yolk bodies to arise in other parts of the ovum than around the germinal vesicle. There is no evidence from the study of many eggs that this occurs. 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 of 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 to the cells in other parts of the ectodermal layer of the subumbrella except that they are not as much flattened. 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 seawater for the purpose of counting the number of eggs that she would lay. The next morning at the regular 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 quantities of eggs that are laid by many of the other animals of the ocean, the numbers often reaching many millions, as among some of the Echinodermata 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 5 to 6 A. M. During the two summers that I studied Turritopsis at the seashore, 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 5 o'clock or a few minutes after. Very rarely did it take place as late as 6 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 very prevalent among the medusae in general. In *Stomotoca apicata*, *Stomotoca rugosa*, and a species of Eucheilota, I find that the eggs are deposited also at a fixed hour, namely, 5 to 5.30 A. M. Professor Brooks found that Liriope and Eutima spawn at about 8 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 at about 10 P. M. Mérejkowsky 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 7 o'clock. The pelagic crustacean Lucifer, Professor Brooks observed to deposit its eggs at from 9 to 10 P. M.

Bunting found that by packing Hydractinia in ice and keeping it 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 Gonionema is definitely affected by changes in light. By placing his medusae 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 with regard to either temperature or light, yet the changes of temperature 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 contained, 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 devoid of a membrane when first laid, and none is subsequently formed. In size it is very small and may 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 visible to the naked eye. They measure 0.116 of a millimeter in diameter. They are among the smaller of the medusan eggs. Metschnikoff gives the measurements of the ova of 19 species of medusae. The sizes range from 0.024 mm. to 1.5 mm. *Cunina proboscidea* has the smallest and *Polyxenia albescens* the largest egg of the species included in his list. The egg of Turritopsis is just slightly larger than that of *Rathkea fasciculata* according to the measurement of Metschnikoff.

In the substance of the egg two parts are distinguishable: an outer layer of clearer ectoplasm which consists of viscid formative yolk composed of protoplasm with very fine granules; and a central mass of entoplasm which is dense and opaque and filled with large, dark granules of nutritive yolk. From the fact that the entoplasm 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 entoderm cannot be followed in the living egg. For this reason the egg of Turritopsis is not as suitable for study during life as are the beautifully transparent eggs of Liriope and Eutima 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 seawater 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 a chalky white color, and the egg of *Stomotoca apicata* which is semi-transparent and appears bluish white by reflected light. In color the egg of Turritopsis is yellowish white.

Maturation and Fertilization.

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 sections of the early stages 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 plate 32, figure 16, 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 clear hyaline substance. In the first polar body, which has moved a little distance from the egg, but is still held in connection with it by some means of attachment, the chromatin has come together and forms a single mass in the center 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 even extends beyond the general contour of the 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 spermatozoon or not, could not be decided. But I am inclined to think that the sperm 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 the spermatozoa enter the eggs. Moreover, 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 maturation, 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. 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 Hargitt for *Pennaria 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 monstrosities 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 Turritopsis, 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.

Metschnikoff describes and gives a few figures of a very similar condition of segmentation in *Oceania armata*. He says: "Wenn bei den beschriebenen Medusen verschiedene Abweichungen in dem Zustandekommen des vierten Furchungsstadiums constatirt werden mussten, so konnte man doch bei allen eine gewisse Regelmässigkeit auffinden. Ganz abweichend in dieser Beziehung verhält sich *Oceania armata*, da bei dieser Meduse die kaum mit einander vereinigten Blastomeren durchaus unregelmässig und ordnungslos neben einander liegen.....Das Abweichende in der Embryonalentwickelung der *Oceania armata* hört noch nicht so bald auf. Die Furchung setzt sich in unregelmässigster Weise fort und führt zur Bildung unförmlicher Zellenhaufen, in deren Innern Mann eine Furchungshöhle durchschimmern sieht. Oft nehmen solche Embryonen eine ganz abenteurliche 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 a most irregular manner and lead to the formation of a shapeless and grotesque mass of blastomeres 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 taking place rather rapidly.

Whether these embryos multiply by division, as Metschnikoff states to be the case with *Oceania armata* and to which he attributes 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 blastomeres are separated into two distinct masses held together by a small isthmus of cells. If they do not divide by an internal activity, they must, 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 passes down to the lower pole. Thus the egg is divided meridionally into two cells of approximately equal size. When division is complete the blastomeres do not remain in close union, but move apart so that the two spheres are connected by only small arcs of their circumference. The protoplasmic bridge, which frequently occurs in hydroid eggs at the lower pole just previous to the completion of the two-celled stage, is usually to be seen in the egg of this species; but it is much less conspicuous than is the case in Stomotoca. When it occurs, it is less definite and clearly defined than in Hydractinia, as described and figured by Bunting. Metschnikoff, also, figures a very beautiful example of this protoplasmic connection in the egg of *Nausithoe marginata*. 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 ectosarcal material. Protoplasmic 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 *Pennaria tiarella*" 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 have been 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 films become 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 at the center of the egg next to the furrow of the first cleavage and slowly extends out toward the periphery. When the division is completed the four blastomeres undergo a slight rotation from right to left; and in the center 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 21, plate 32.

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 blastomeres. 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 is, however, 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 move 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 22 and 23 of plate 32. The separation and rotation of the cells of one quartet seem to be constant in their occurrence; but the final arrangement of the blastomeres is not always as regular and definite as that shown in the figure. 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 spread out so 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 readjustment 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 nor definite. They apparently follow no law of symmetry, and may come to lie in any position. Figures 24–26 (pl. 33) show three different forms which the cells of the sixteen-cell stage acquire, and various other arrangements of the blastomeres which could not be figured for want of space, were seen while studying the living eggs. However, the three figures are sufficient to show that the general form of the egg in this stage may be inconstant. In figure 24 of plate 33, it is possible to imagine a direct relationship to a preceding form just a little more irregular than is shown in figure 23 (pl. 32). In a form represented in figure 25 (pl. 33) the descent of the different cells from the individual blastomeres of the eight-cell stage is less easily recognized. Figure 26 (pl. 33) 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 22 (pl. 32). The flat, spread-out position of the cells at once suggests the idea that the egg may have been subjected to pressure. 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 seawater 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 farther. Figures 27–30 of plate 33 show a few of the later stages of comparatively very regular forms. Figure 29 (pl. 33) 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 smaller 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 amoeboid appearance. The term amoeba-like seems most clearly to 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 pseudopodia. 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 are due to an amoeboid property of the cytoplasm of the egg, or to a tendency to multiply by division during cleavage, as was suggested by Metschnikoff for *Oceania armata*, there is not sufficient evidence to decide. It may be possible that both these factors act in determining the shape of the segmenting mass of cells. 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 hydromedusae whose 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 from 5 to 6 o'clock in the morning develop to the free-swimming stage by 4 in the afternoon. The larva swims with its broad end forward, and has a spiral or cockscrew 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. 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 the general appearance shown in figure 32 (pl. 33). As it becomes older it grows still longer. Figure 33 (pl. 33) 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. As it grows older 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 motion ceases altogether and the larva loses its cilia and is ready for attachment. This stage of development is reached under favorable conditions from 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 invaginate 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 structures are clearly abnormal features of the developing Turritopsis 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 seawater. 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 seawater, 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 do 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 as possible like those under which the undivided eggs developed. 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 5 and 6 in the morning were divided at 10.45 A. M. More than one half of the fragments continued to develop and by 6 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 4 to 4.30 P. M. They were slightly smaller than embryos from whole eggs, but apparently just as active and normal. 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 processes were 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 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 the process of epibole, 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 delamination takes place, as is so beautifully shown in Liriope and Geryonia, and in species where cellular ingression occurs as in Stomotoca and Clytia for example. Figures 34 (pl. 33) to 39 (pl. 34) illustrate the uniformity of the cells, and the solid character of the egg during segmentation. In figure 36 (pl. 34) a space exists between the blastomeres 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 time the irregular mass of segmenting blastomeres is metamorphosed into the oval embryo, the cell boundaries are lost for a short time and a syncytium is formed. This syncytial structure is crowded with yolk granules and nuclei are scattered throughout the protoplasm. The nuclei soon become more numerous near the periphery; and then cell walls begin to appear as shown in plate 34, figure 47. These cells are to become the ectoderm, which is soon separated from the inner structureless mass by the development of the mesogloea. 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 48 (pl. 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 hydromedusae. In the majority of forms previously studied the differentiation took place either by delamination or by cellular ingression, unipolar or multipolar. These methods have been well described and figured by Metschnikoff.

In Aglaura and Rhopalonema there is found according to Metschnikoff a solid so called morula 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 Pennaria according to Hargitt's description, a condition somewhat similar to that of Turritopsis is found. He says: "Indeed in both Eudendrium and Pennaria, 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. 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; but meanwhile the peripheral region of the syncytium has been transformed into a distinct layer of ectoderm 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 plasmodium-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. Even those 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 changes of the protoplasm may take place.

Formation of the Endoderm.

The formation of the endoderm in Turritopsis cannot be adapted to any of the schemes of the development of the hydromedusae that have been sketched by Metschnikoff. 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, multipolar ingression, in which cells migrate into the blastocoele from different regions of the peripheral cell layer, and are transformed into endodermal tissue directly. Of this mode he describes several subordinate types. Third, unipolar 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 Turritopsis the endoderm is derived from the syncytial mass of tissue left in the center 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 reappearing are shown in figures 48, 49 (pl. 34) and 50 (pl. 35). 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 grows 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 mesogloea and form a definite inner germ layer.

Gerd has observed in Bougainvillia that during the course of cell multiplication the boundaries of the cells 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 morula stage, in that it is brought about by a multipolar migration of cells into the interior of the coeloblastula;

while in Turritopsis the morula 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 main conform to one or another of the stereotyped methods as established by Metschnikoff. The nearest approach is that briefly described by Hargitt for Eudendrium and Pennaria, in which there is also more or less of a syncytium formed prior to the differentiation of the germ layers.

Cell Multiplication.

During the early cleavage phases the cells multiply entirely by the process of mitosis. 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 amitosis take place simultaneously in the different cells of the segmenting egg. Figure 40 (pl. 34) shows a karyokinetic spindle in the metaphase; and figure 41, (pl. 34) 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 amitotically, vary in size considerably, and have a reticular appearance. Figure 42 of plate 34 shows a large nucleus of this reticular character with the chromatin scattered about in the linin meshwork. Figures 43–46 (pl. 34) 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 Flemming 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 reaches 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 like that in Stomotoca 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 Eudendrium, and which has been regarded as typical and used in descriptions of the embryology of the hydromedusae 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, develops from a bud that is given off from the root, usually at about the middle.

Professor Brooks observed the fact that the planula is transformed into a root in Turritopsis, Eutima, and Hydractinia, and gives a brief account of the same in his paper on "The life history of Eutima" (Brooks, '84). Metschnikoff ('86) describes and figures for Mitrocoma the fact that the larva becomes attached by its side and is almost wholly employed in the formation of the hydrorhiza, while the first hydranth grows out of it by a kind of budding.

In general the attachment of the planula is similar in Turritopsis to the method which is followed by Stomotoca, but the former does not commonly produce secondary hydrorhiza. In Stomotoca at 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 development of the hydranth.

Professor Brooks describes and figures in the planula of Eutima an ectodermal adhesive gland. It occurs after the endoderm and the digestive cavity are formed, and before the appearance of the mouth as an ectodermal invagination at the small end of the planula. In Turritopsis no such special organ of attachment is found. The larva probably becomes fixed by a secretion extruded from the ectoderm cells along the whole length of its body.

Development of the Hydranth.

Shortly after the larva becomes attached, a bud develops, usually at about the center of the root, which is the beginning of the first hydranth. Four small projections appear early around the distal part of the bud; these will later form the first circle of tentacles. At this time no mouth has yet developed. A young polyp in this stage of development is shown in figure 51 of plate 35. The hydranth bud continues to grow taller and after a few hours a second whorl of tentacular buds is formed some distance below the first circle of tentacles. When the polyp is from twenty to twenty-four hours old, or at about seventy-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 hydroid 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 much extension and contraction. Figures 51 to 55 of plate 35 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 53 (pl. 35) shows a form in which the polyp arises from near the end of the hydrorhiza. This is exceptional. A hydranth with the third circle of tentacles is shown in figure 55 (pl. 35); the tentacles of the first whorl have become considerably elongated. The hydrocaulus now becomes longer and more slender and the hydranth assumes a fusiform body.

The polyps that I reared from eggs were at the age of three days like the hydranths of the adult colony found and figured by Professor Brooks in the main features, 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 medusa 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 medusae, while the latter are invested by a much thinner and more transparent capsule of perisarc. The sheath on 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 pale yellowish-red hydranths are very similar to those of Tubularia (Allman) and the hydroid is so similar to *Dendroclava dohrnii* recently described by Weismann, 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 ovarian cells and when mature are densely crowded with large yolk granules.

2. Dehiscence takes place at a definite time, from 5 to 6 o'clock in the morning.

3. The egg is spherical and membraneless. It is composed of an outer layer of clearer ectoplasm and a central mass of endoplasm which is dense and opaque and filled with large, dark yolk 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 reappearance of cell walls in the periphery of the syncytium mass, and is separated from the anterior part by the formation of the mesogloea.

8. The formation of the endoderm follows none of the typical methods described by Metschnikoff. 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 the mesogloea. 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 amitotically.

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 of being arranged in circles.

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EXPLANATION OF PLATES.

PLATE 30.

The anatomy of Turritopsis.

Letters of reference; *a*, peripheral region of radial canal; *b*, central region of radial canal; *c*, groove through which the radial canal communicates with the stomach; *d*, cavity of stomach; *e*, reproductive organ; *f*, ectodermal epithelium of subumbrella.

Fig. 1. Transverse section in the plane of the reproductive organs.
Fig. 2. Transverse section a little above the reproductive organs.
Fig. 3. Transverse section through the proboscis.
Fig. 4. The adult Turritopsis in side view, enlarged about twelve diameters.
Fig. 5. Transverse section through the oral lobes.

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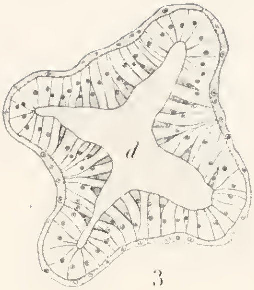

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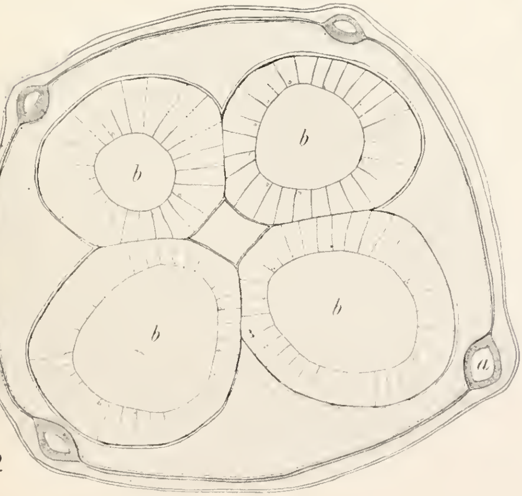

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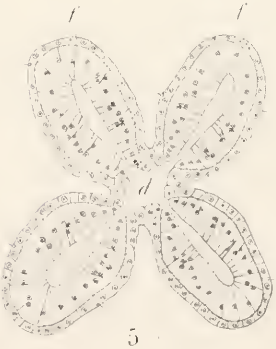

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PLATE 31.

The anatomy of Turritopsis.

Letters of reference; *a*, cavity of radial canal; *b*, ectoderm of exumbrella; *c*, jelly of umbrella; *d*, endodermal lamella; *e*, muscular layer of subumbrella; *f*, ectoderm of subumbrella; *g*, endoderm on external surface of chymiferous tube; *h*, endoderm on internal surface of chymiferous tube; *n 1*, velar nerve-ring; *n 2*, sensory nerve-ring; *t*, tentacle with ocellus; *v*, velum.

Fig. 6. Section through a radial canal close to its junction with the circular canal.

Fig. 7. Section through the circular canal and the base of a tentacle.

Fig. 8. A drawing, made at Nassau, New Providence, in 1886, of a medusa, which has recently been included in the genus, Modeeria. It is here figured in order to show that it has nothing in common with the genus Modeeria of Forbes, and is closely related to Turritopsis.

Fig. 9. A cross section of the ascending region of the radial canal of Turritopsis, above its union with the horizontal region.

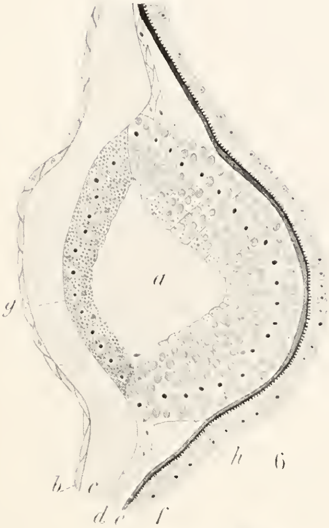

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PLATE 32.

The embryology of Turritcpsis.

Fig. 10–15. Stages in the growth and maturation of the ovarian eggs of Turritopsis.
Fig. 16–18. Stages in the formation of the pole cells.
Fig. 19. A fertilized egg.
Fig. 20. The two-celled stage of segmentation.
Fig. 21. The four-celled stage.
Fig. 22. The eight-celled stage.
Fig. 23. Another view of same.


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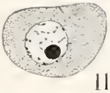
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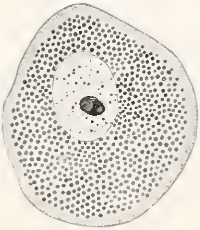
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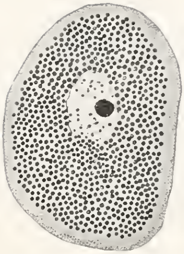
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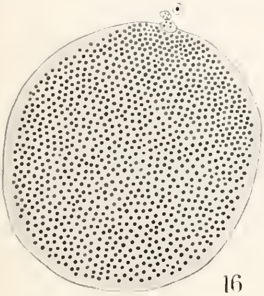
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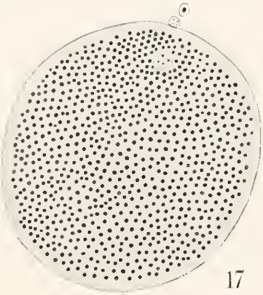
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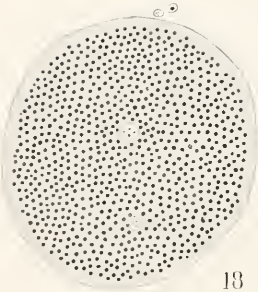
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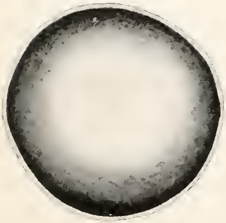
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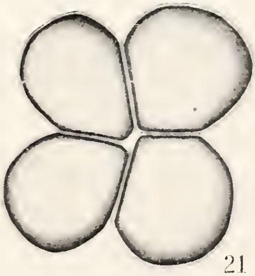
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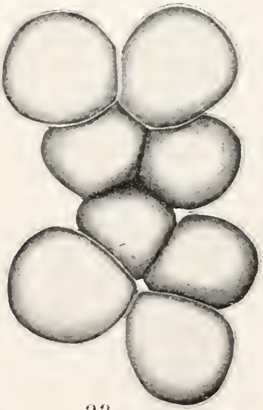
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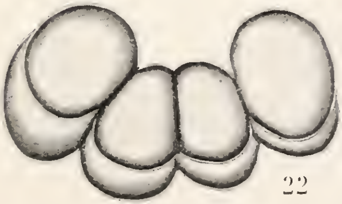
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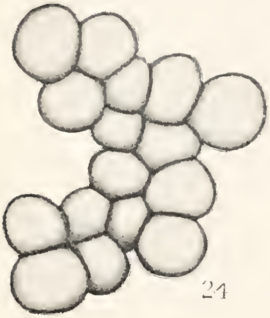
PLATE 33.

The later stages of segmentation, and the planula.

Fig. 24. The sixteen-celled stage.
Fig. 25–26. Two more embryos in the same stage, to illustrate the irregularity of the segmentation.
Fig. 27. A more advanced stage of segmentation.
Fig. 28. An embryo, still more advanced, with an indication of division into two.
Fig. 29. The division into two is still more marked, only a single cell joining them together. When the two portions are separated artificially at this stage a normal planula arises from each, and it is probable that the embryos multiply by fission in a state of nature.
Fig. 30. Another embryo exhibiting division into two parts.
Fig. 31. The young planula.
Fig. 32. An older planula beginning to elongate.
Fig. 33. An older planula, more elongated.
Fig. 34. A section through the embryo shown in figure 28.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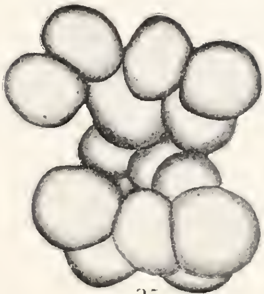
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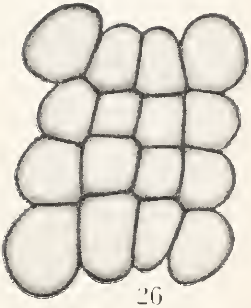
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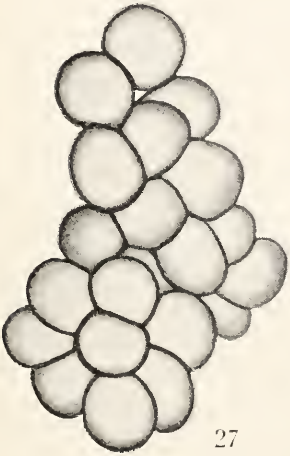
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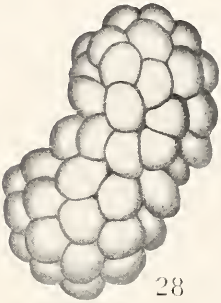
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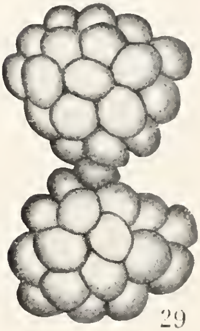
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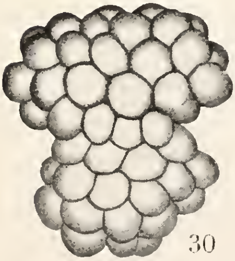
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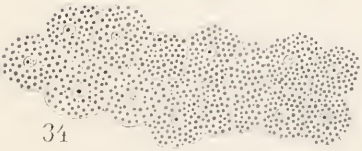
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PLATE 34.

The older stages of the development of the embryo.

Fig. 35–39. Sections of successive stages of development to show the way in which the outlines of the cells gradually disappear until, in the stage that is shown in figure 39, they are converted into a syncytium with no traces of cell boundaries. Mitosis of the cells of the blastoderm in the early stages of segmentation.

Fig. 40–41. Mitosis of the cells of the blastoderm during the early stages of segmentation.

Fig. 42–46. Amitotic division of the cells of the blastoderm during the later stages of segmentation.

Fig. 47. The first traces of the differentiation of the ectoderm.

Fig. 48. The ectoderm is well defined and there are traces of the differentiation of the cells of the endoderm.

Fig. 49. Section of a planula a little older than the one shown in figure 48.


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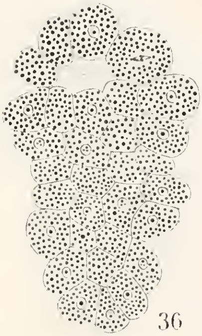
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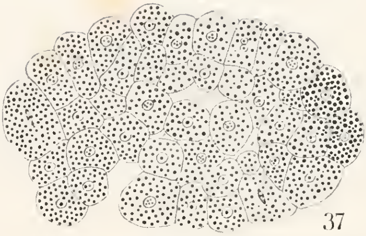
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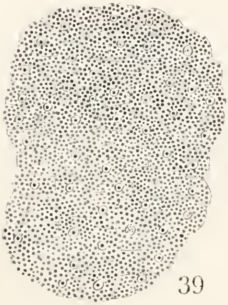
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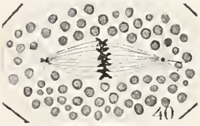
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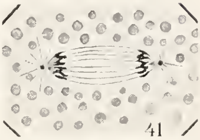
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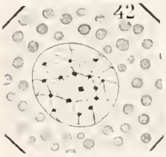
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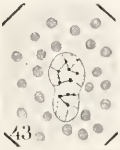
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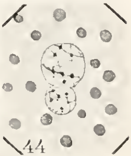
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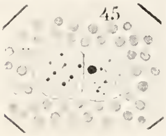
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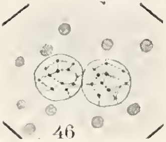
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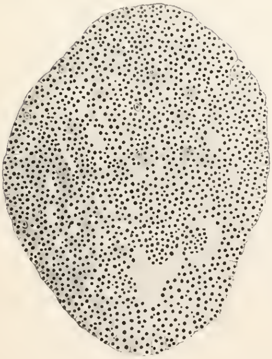
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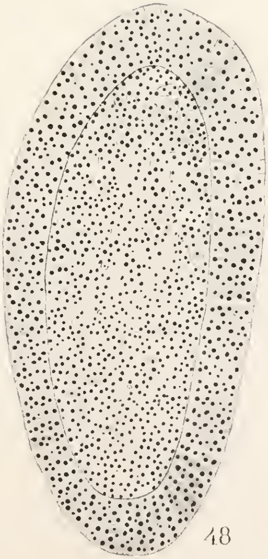
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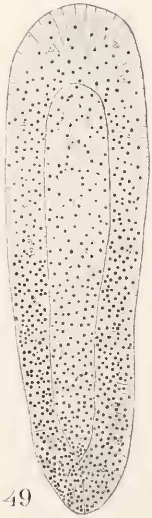
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PLATE 35.

The convertion of the planula into a hydrorhiza; and the origin of the first hydranth as a bud.

Fig. 50. A section of a fully grown planula, with both ectoderm and endoderm well defined.
Fig. 51. The planula has become a hydrorhiza, and the bud that is to become the first hydranth shows traces of the first cycle of tentacles.
Fig. 52. The tentacles of the first cycle are well developed.
Fig. 53. The rudiments of the tentacles of the second cycle are present as buds.
Fig. 54. Two cycles of well developed tentacles are present.
Fig. 55. All three cycles of tentacles are present.

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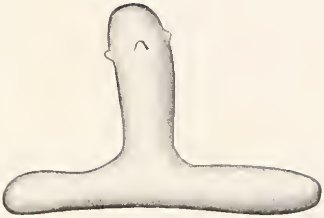

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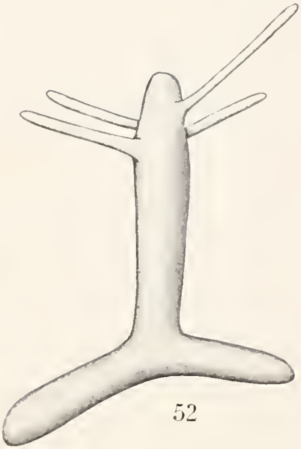

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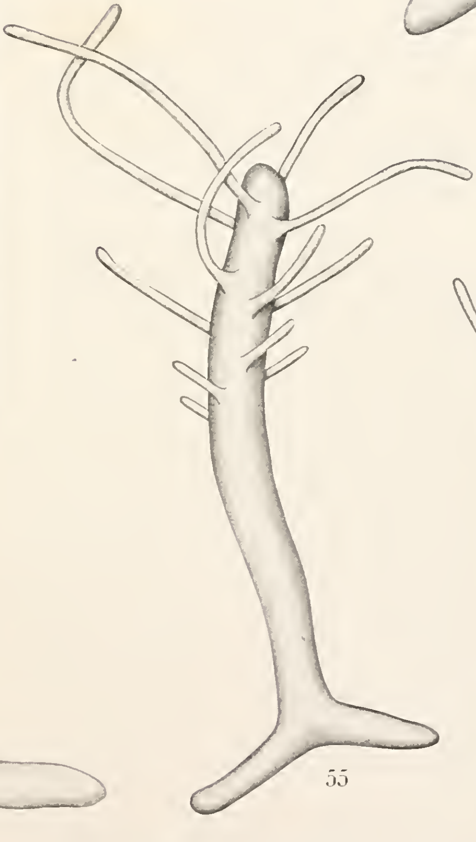

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