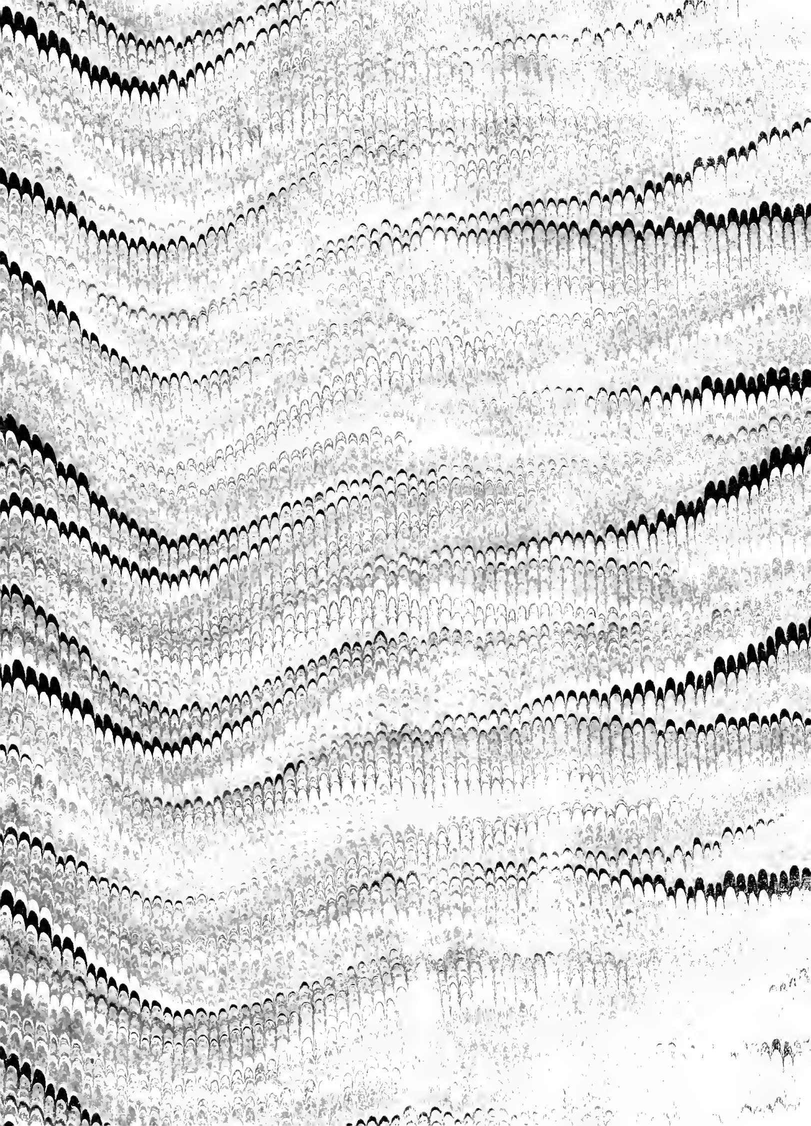

THE

DEVELOPMENT OF

G O N I O N E M A   M U R B A C H I I

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by

Henry Farnham Perkins.

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

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Baltimore
1902.

Contents.

DEVELOPMENT OF *GONIONEMA MURBACHII*.

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Gonionemus: A.Agassiz, 1862. Contrib. Nat. Hist. U.S., IV., p. 350.

From γωνιο angled, and νημα thread- "kneed tentacles".

Gonynema: Haeckel, 1879. System der Medusen.

Gonionemus: Murbach, 1895. Journal Morph., XI., 2

Gonionemus Murbachii: Mayer, 1901. Brooklyn Inst. Sci., Bul., Vol. I., L.

Gonionema: A. Agassiz, mss.

Gonionema Murbachii, Perkins, J.H.Univ. Circ., May, 1902.

INTRODUCTION.

This genus was established by Dr. Alexander Agassiz to include a medusa which he discovered in 1862 in the Gulf of Georgia, Washington Territory. Its most striking character, anatomically, is the peculiar form of the tentacles. which are bent at an angle near the tip, and at the angle bear a sucking organ by means of which the medusa makes itself fast to any favorable object. This peculiarity in the form of the tentacle suggested to Agassiz the name which he

proposed. The form of the word which I use is that which Professor Agassiz offers in correction of the original one, which was in error as to its ending.

For a long time the Gulf of Georgia was the only locality from which this genus was described. In 1894, however, another habitat was discovered far distant, at Woods Hole, Massachusetts, and since then members of the genus have been found at the widely separated localities of the Fiji Islands and Alaska, and a closely allied genus, Olindias, has been described from the coast of Brazil and the Bahamas. This last instance was that in which Mayer described a species (aphrodite) of Gonionema; but as a matter of fact this medusa possesses rather the characters of the Oliniadae, two distinct kinds of tentacles and papilliform gonads being present. These far distant localities will in all probability be connected in the future, during the course of further research, by a chain of other localities.

The history of the Woods Hole Gonionema is interesting. In spite of the fact that the "eel-pond", at the centre of the village of Woods Hole, connected with the outer harbor by a narrow inlet, is from its location easy of access to collectors, and although many students of the group had investigated the waters around Woods Hole summer after

summer for a number of years, *Gonionema* was never found in the Atlantic Ocean until 1894. This season a number of specimens were taken from the "eel-pond", the creature having made an astonishingly sudden appearance upon the scene. It seems incredible that it could have been living in this small body of water for any time previously, or at any rate that a number of individuals had been there. But the medusa at once secured a good "foot-hold" and since the first summer has been very plentiful; its numbers remain undiminished by the wholesale raids of collectors, in spite of the keen anxiety of some of those especially interested in it. During the summer of 1894 when the jelly-fish was first found at Woods Hole Professor W.K.Brooks secured a number of the specimens and made drawings both of live medusae and of sections of preserved material. Some of these drawings, figures 29, 30 and 33 are now published, with Dr. Brooks' generous permission, for the first time.

The first printed account of the Woods Hole *Gonionema*, since recognized as a distinct species, was published in 1895 by Dr. L. Murbach. The species has frequently been mistaken for *G. vertens*. It was not until 1901 that the specific name *Murbachii* was bestowed upon it by Dr. A. G. Meyer (1901). The work which I have done on the life-his-

tory of the species was originally undertaken and has since been prosecuted with Dr. Murbach's kind encouragement, and I have received from him many favors in the way of material and helpful suggestions. The work has been carried on during the past two years at the U.S.Fish Commission laboratory, where I have had the great privilege of working during the summer, and under the direction of Professor W. K. Brooks at the Biological Laboratory of the Johns Hopkins University. I wish to acknowledge my obligations to Dr. Bumpus, Dr. H. M. Smith and Dr. Whitman for courtesies which they have extended to me in my work.

Note on the Ontogeny of the Trachomedusae.

Gonionema, according to Haeckel's classification, falls into his third order, the "Trachomedusae". Haeckel characterized this order as follows: "development, hypogenesis (not metagenesis), but usually with metamorphosis". Subsequent research into the life-history of members of this group has shown that each clause of this statement is open to emendation. In the first place, the "usually" is superfluous, and therefore erroneous. The exceptions which Haeckel supposed to exist and which caused him to say "usually with metamorphosis" have been shown to be no excep-

tions, but cases of somewhat easily misunderstood metamorphosis. Such, for example, was the case of Liriope, which has been studied by Metschnikoff (1874) and Brooks (1886). The larva is a true hydra, although its free swimming mode of life and its superficial aspect caused it to be mistaken. formerly, for a gonosome.

My investigations of a jelly-fish which Haeckel includes in his order "Trachomedusae" show that the first part of Haeckel's statement also requires revision, and that metagenesis does occur in the "Trachomedusae". Although there may be some difference of opinion as to the exact definition of the terms "metagenesis" and "hypogenesis", the following notion of the process of alternation of generations may be safely accepted as that generally held. The production by a larva of offspring unlike itself, and its own ultimate death without undergoing metamorphosis, are frequent accompaniments of the intermediate as of the primary process of multiplication; but they are by no means essential to the process of metagenesis or alternation of generations. Creatures which multiply sexually at one point of their life-history, and at another point nonsexually by budding or fission, are said to have a metagenetic development. In Gonionema (Figs. 3 - 23) a large number of adult individuals are produced from a single egg through

an intermediate process of multiplication; buds which are developed upon the body of the hydra larva drop off and, beginning as planulae, just follow exactly the same course of development as the parent; both parent and offspring later change into fully developed medusae. "Gonionema"has, then, a metagenetic form of development. The mere presence of a hydrula stage in the life-history is of course not enough, according to generally accepted notions of the meaning of terms, to constitute alternation of generations (Murbach, 1895, p. 496).

These emendations of Haeckel's description of the order add evidence to that already put forward (Brooks, 1895, p. 300) and by others that the hard and fast lines drawn by Haeckel and the Hertwigs between the "Trachylinae" and the "Leptolinae", on the ground of anatomical differences or of developmental features, are not borne out by the facts. The Hertwigs (1878) hold that "the marginal sense-organs (Gehörorgane) alone furnish characteristics which enable us in every case to distinguish the 'Trachomedusae' (Trachomedusae and Narcomedusae of Haeckel) from the Campanularian medusae (Vesiculatae) without knowledge of their development". Dr. Brooks has, however, described a species of Laodice which unites in its anatomical features

the characters of both the Leptolinae and Trachylinae, having the ocelli of the former order and the chitinous goangium containing the developing medusa buds, (Agassiz, 1865, p. 125) while Professor Brooks has demonstrated (1895) that it also possesses the true endodermal sense-clubs of the Trachylinae.

It may be that the present record of observations on Gonionema will be of interest as contributing some new points to the present meagre knowledge of the manifold forms and types which are exhibited in the development processes of this great group.

I. GONOSOME.

Gonionema is a very attractive feature of the Woods Hole fauna. Its exquisite glassy umbrella, marked with a cross of yellow or brown by the four radial canals and the gonads, a brilliant row of closely set spots of gleaming phosphorescent green outlining its edge, the fringe of delicate streaming tentacles strung with bead-like clusters of thread cells, are all more or less familiar to many American biologists (Fig. 1). On cloudy days or toward nightfall it is very active, swimming upward to the top of the water and then floating back to the bottom.

In swimming it propels itself upward with rythmic pulsations of the bell-margin, the tentacles shortened and the bell very convex. (Fig. 2) Upon reaching the surface the creature keels over almost instantly, and floats slowly downwards with bell relaxed and inverted, and the tentacles extended far out horizontally, forming a wide expanse of stinging threads, which carry certain destruction to animals even larger than the jelly-fish itself, if they are unwary enough to be caught within their reach. Gonionema continues this peculiar fishing up and down in the water with little respite. Occasionally it fastens itself to the eel grass or other object near the bottom, or stops midway in its course with tentacles extended as in my figure 1, well-nigh invisible, but a deadly foe to small fish or crustaceans, which cross its path.

II. GONADS.

In the mature Gonionema the sexual organs are "frill-like lobes, passing from one side to the other of the chimiferous canal". (Agassiz, 1865). Their form and position is shown in figures 3 and 4. The free edge of the ribbon of tissue is thickened and rounded, and is bent backwards and forwards across the radial canal. The color of

the gonads has been supposed to afford means of descriminating between the sexes, the males differing from the females in the brighter yellow of the gonadial tissue. But this distinction does not hold, and it is necessary to examine the individual medusae with a lens in order to separate the sexes. The ovarian eggs, enclosed in the ectoderm of the gonads of the female, give them a granular appearance as contrasted with the more homogeneous and translucent tissue of the male. When a large number of the jellyfish are separated into two vessels, one containing the males and the other the females, the general color tone is, it is true, brighter and more lively than that of the females. But the specimens in each dish range all the way from light straw color up through orange, ochre, sienna, to dark brown.

III. EMBRYOLOGY.

It is my purpose to give in outline the main points in the early part of the life-history of Gonionema. I have not discovered that this species exhibits any notable peculiarities. I shall lay greater emphasis upon certain features of the later developmental stages, which have more significance in so far as they are less familiar.

A. DEHISCENCE -

The eggs are embedded in the ectodermal tissue of the gonad as in a gelatinous matrix. The round thickened edge of the ribbon contains the riper eggs, but the thinner portion is well packed with maturing ova. Dehiscence takes place by the breaking down of the superficial ectodermal investment and the liberation of the eggs and spermatozoa imbedded in its substance. The contractions of the umbrella in swimming put a strain upon the subumbral walls and help to rupture the epithelium of the gonads. What the cytological change is which precedes the extrusion of the sexual elements or the nature of the causes which effect this change are matters of uncertainty. We know only that these conditions can be artificially induced by means to be mentioned presently.

The process of dehiscence occupies only a very few moments. Most of the eggs which are ready for fertilization are extruded all at once, coming out of the bell-cavity in a cloud at each contraction of the marginal ring. Two or three minutes after the first eggs are extruded only a few belated ones are loosened from the gonads and expelled from the subumbral cavity two or three at a time. Figure 3. is drawn from a sketch made of a medusa in the act

of spawning. The specimen was held inverted in a watch-glass under the microscope. Although not free to swim, it went through the motions, contracting the bell rythmically. In this way the softened ectodermal tissue of the gonad was ruptured, and the eggs expelled. Little round pits are left by the eggs, like bullet-molds. The <u>earliest date</u> at which fertile medusae have been found was the first of July; the latest, the last week of September. The period of maximum sexual activity is from the middle of July to the middle of August.

B. <u>Periodicity</u> - As stated by Murbach (1895) the eggs are normally extruded at about eight o'clock P.M. This is the case during the earlier part of the summer, but later in the season, when the days are shorter, half-past seven, or even shortly after six is the normal time for spawning. Further than this, extrusion of the eggs may be artificially induced in <u>Gonionema</u>. In this respect this form differs markedly from some other marine animals which exhibit equally definite times of spawning. Dr. Murbach found that during the daytime after being shut up for an hour in a dark place the ripe medusae would deposit eggs and sperm. My experiments show that this is true in the

afternoon to a much greater extent than early in the day; before two o'clock in the afternoon the hour in the dark would sometimes bring about the deposition of a very small number of eggs, and if the period of darkening was lengthened to an hour and a half, a slightly larger number of eggs were found in the water. But after two o'clock an hour's shutting away from the light brought about an apparently normal spawning. I found that the stimulus of the withdrawal of light is surprisingly definite in its effects; the condition of the tissues arrives at the point requisite for the release of the eggs almost on the minute. This constancy is not appreciably affected by moderate changes in temperature. A large number of experiments and observations have been made to educe the exact time of stimulation (if we may so speak of an influence which seems to be purely negative) and the results are summarized in the following table. Record was kept of experiments carried on during the whole of the fertile season, partly in one summer, partly in the next. The stimulation-time varies somewhat with the season; the table gives the results obtained during the last week in July, when the eggs were being discharged in the greatest numbers.

Before 2 P.M. small no. of eggs laid after 90 minutes darkening.						
2 - 3 P.M. almost	the normal	number	laid	90	minutes	darkening
3 - 4 "	"	"	"	65	"	"
4 - 5 "	"	"	"	60	"	"
5 - 6 "	"	"	"	60	"	"
6 - 7 "	"	"	"	50	"	"

At 8 P.M. eggs laid normally, without darkening artificially.

As the hour approached the normal time for the deposition of the eggs the precision with which they were discharged became more and more marked. Between four and seven P.M. the time of darkening necessary to produce spawning varies not more than four minutes on either side of the hour.

Some experiments were tried with a view to inhibiting normal deposition of eggs, or at least of hindering it, by keeping the eggs under strong artificial illumination. The results were not conclusive, as the electric lights in the laboratory were not in use until after dusk, when part of the stimulus had already been received. The experiments showed a certain amount of retarding of the process of spawning as a result of strong illumination. It would be inter-

esting to determine whether the use of stronger light begun as soon as daylight commences to wane would result in complete inhibition of the process.

It is evident from the above statements that *Gonionema* is exceedingly sensitive to external conditions. Not all coelenterates are affected in the same degree, some are apparently not affected at all, by changes in illumination. Some medusae lay their eggs always in the early morning, while others of nearly related genera choose the evening or night (Brooks - Life History of the Hydromedusae, p. 399). Experiments carried on by Wilson and Donaldson at Beaufort under Professor Brooks' direction (loc.cit.) showed that in the case of *Renilla* and some sea-anemones, at any rate, changes in light and temperature did not affect the precision with which the regular physiological activities took place. It is well known that a great many marine animals show more or less definiteness in the habit of spawning. Metschnikoff gives a table on page 25 of his Embryological Studies showing the time of spawning of a large number of medusae. In other groups the same tendency is manifest. This phenomenon is probably the result of the working of natural selection, the habit of laying the eggs at a certain definite time having proved of value to the different

species. The fact that in some forms this precision of periodicity is not dependent upon external influences, while in others there is manifest a marked degree of sensitiveness to external influences, seems to me to indicate that the tendency has been arrived at by quite different processes, and may be due to quite different requirements in the various creatures.

But to return to the dehiscence in Gonionema, not all the eggs, by any means, which the ovaries contain are liberated at one time. Individuals have been seen to deposit eggs every night for a week, and while specimens kept in captivity are not very reliable in drawing inferences as to natural processes, this time of sexual activity would be more liable to be shortened than to be prolonged by the unnatural conditions. After the first three or four days on which spawning took place, a small number of ova were left in the gonads, and on the three succeeding evenings these were extruded a few at a time. Late in the summer the specimens taken are usually devoid of sexual products, and the gonads small and shrivelled.

C. Egg-envelope- In freshly laid eggs the polar bodies are only rarely to be found. They are normally given off

and lost in the gonads previous to dehiscence. Before fertilization the eggs float in a cloud through the water, each one surrounded by a very soft thick gelatinous envelope. If the eggs are not fertilized, the surrounding mass of semi-fluid jelly slowly shrinks up, and the increased specific gravity causes the eggs to sink to the bottom. Blister-like vacuoles appear in the substance of the egg, puffing out the envelope, and in the course of several days the protoplasm becomes disintegrated and the egg goes to pieces.

When fertilization takes place, the shrinking of the egg-envelope is more immediate, and greater in degree, so that the egg sinks at once and sticks to the bottom by means of the viscid substance of the surrounding envelope.

METHODS -

The adhesive property above referred to is of great assistance in making preparations of the segmenting eggs, as they may be allowed to settle on glass slides, which are afterwards run up through all the reagents without danger of washing off. For sectioning, the best method of securing the eggs was found to be by stirring about in the water with a camels-hair brush, and preventing them from gluing

themselves down to the bottom of the dish. They would then stick together in masses, and being protected from too much pressure by the gelatinous envelope they were found to segment normally. The bunches of eggs were large enough to see with the unaided eye, and could be easily transferred to the killing fluid, and afterwards stained and cut. The best reagents that were used for killing were corrosive-acetic three per cent. glacial acetic in saturate solution of bichloride of mercury; and the full strength (40 per cent.) solution of formalin. Corrosive-acetic was satisfactory for most purposes, both segmenting eggs and adult medusae being fixed in this solution. They were immersed for from one to ten minutes, according to the bulk of the tissue. Pure (40 %) formalin was used very successfully for the younger stages, giving good cytological fixation of segmenting eggs and of larvae. Fifteen to forty seconds is sufficient to fix the tissues thoroughly. In working with Gonionema, I have experienced none of the difficulty that seems to be met with in other coelenterates in getting uniform results with formalin material. I have used this reagent not only for fixation but for permanent preservation, with the best results. For narcotizing the larvae and adult medusae, I find menthol crystals the most convenient

and rapid chemical to use.

It may be well to mention the method of keeping *Gonionema* alive in the laboratory. Running water is not desirable, and it is of no benefit to either medusae or larvae to change the water frequently, as I learned after much laborious effort to keep the specimens alive in this manner. Balanced aquaria furnish the best environment for these creatures. I succeeded in keeping a large number of larvae in healthy growing condition for six months in aquarium jars in the laboratory. The quantity of water was kept constant by adding fresh water to make up for evaporation. Food was furnished in the form of various protozoans and other microscopic organisms. Oxidation was secured by means of large quantities of diatoms which were reared for the purpose. Cultures were made from the scrapings of eelgrass, etc., and the diatoms which accumulated from them, collecting in clumps on the bottom of the dish, were scraped into the water with the larvae. At the end of January, the polyps which came from eggs laid the preceding August, died without undergoing metamorphosis. Their death was probably due to a lack of food supply sufficient for the requirements of their growing bodies.

D. Segmentation - The egg is spherical, averaging .07 mm.

in diameter. It consists of yellowish rather cloudy protoplasm, sufficiently transparent to permit one to observe the more conspicuous changes which take place in the substance of the living egg. Segmentation is total and equal, of the type which is designated by Metschnikoff as "durchschneidende Furchung". The cleavage-furrow appears at one side of the egg first and cuts through its substance until it reaches the opposite side, dividing it into two hemispheres (Fig. 5). The point at which the furrow starts is that nearest the nucleus, which lies eccentrically in the granular substance of the egg. The first indication of the furrow is a shallow groove which deepens rapidly and at the same time lengthens so as to embrace the egg meridionally. The furrow is finally completed, superficially, a short time before it has entirely separated the egg into two distinct halves. The last point to be cut off corresponds in position almost exactly with the nucleus, but on the opposite side of the egg. This first cleavage is completed one hour after fertilization. The two daughter-nuclei now lie near the plane of fission, and at the same distance from the surface as the original nucleus was. The second furrow normally starts at the same point on the surface as the first, and again divides the egg meridionally, in a plane

at right angles to the first. Sometimes the second furrow starts irregularly, at a point part way around the egg from the origin of the first furrow. One of the hemispheres is thus divided before the other, as in figure 6. Fifty minutes elapse between completion of the first and second furrows. Successive segmentations come in at intervals of 45 to 50 minutes.

With the eight-celled stage rotation of the blastomeres occurs. The four upper cells turn through an angle of 45° upon the lower ones, so that they come to lie in the valleys between the four lower ones instead of being exactly superimposed upon them. Segmentation continues until a hollow blastula (fig. 7) is produced, a layer of thick cells surrounding a small cleavage cavity. The cells are of uniform thickness, and their outer ends give rise to cilia which drive the egg round and round by their motion, within the membrane, sometimes in one direction, sometimes in the other. During this stage the formation of the endoderm takes place. The inner ends of the blastomeres are delaminated, the process going on at an equal rate on all sides, until a uniform layer of endoderm cells lies within the ectodermal layer (Fig. 8). By increase in size of these endodermal cells the cavity of the egg is entirely obliter-

ated. During the subsequent life-history of the larva no cavity exists in the body until several other marked changes have taken place.

IV. THE PLANULA - By the rupture of the egg-membrane the nearly spherical ciliated larva makes its escape and starts upon the stage in which it is a swimming planula (Fig. 9). Its shape soon changes, becoming longer, and more narrow at one pole than the other; this narrower pole is to be the future oral extremity of the larva. The cilia serve to propel the planula in a slow rotating progression through the water, usually not far from the bottom. The larger end is directed forward in swimming. The time at which the planula appears is in the morning, about twelve hours after fertilization of the egg took place. The length of the larva is now between .1 and .15 mm. (Fig. 9). This condition persists for a varying time, usually several days. Towards the end of this time the first indications of a coelenteric cavity appear in the arrangement of the cells at the posterior end of the swimming larva (Fig.10). Their inner margins come to lie in a straight line, following the long axis of the larva (Fig. 10,C). This is better understood when we notice that in changing its shape from the spherical morula to the elongated planula the larva also

underwent a slight rearrangement of its cells. The endoderm was when first formed in the shape of a spherical mass, and its cells were all conical, radiating from the centre to the surface. But as elongation took place in the formation of the planula, the cells were stretched out into a cylinder, and their inner ends overlapped irregularly, as is shown in the anterior end (A) of figure 10. When the coelenteron begins to be developed, the inner ends of these upper endodermal cells change their position somewhat, and, as stated, meet along a continuous line. At the same time a change is to be noticed in the ectodermal cells at the exterior of the future oral pole (Fig. 10, O). The cell-walls at this point become less distinct, and finally a disintegration of the boundaries leaves the tissue an undifferentiated layer of protoplasm. Before separation of the tissue to form the definitive coelenteric cavity, the larva stops swimming, loses its cilia, and settles down upon the bottom. The larger end, which was directed forward in swimming, is downward. Between the free-swimming stage and the sessile hydra stage there frequently, though not always, intervenes a condition which reminds one of a minute planarian in its shape and movements. The planula settles down upon the bottom, and slowly glides along by a

rythmic wave-like progression. This condition seems to take the place of the last part of the ordinary and probably more normal free-swimming stage, and is perhaps due to the unfavorable conditions of the laboratory. This condition is not, however, like the pathological plasmodial forms mentioned below. Its changes in shape are slight, and its manner of movement more of a glide than a protoplasmic flowing. None of the definiteness of structure is lost, and these larvae change into hydras as soon as those which transform directly from the free-swimming planulae. It is then not a phenomenon of degeneration, nor, on the other hand, an essential phase in the life of the creature, but rather an intermediate and probably accidental condition.

V. HYDRA.- As soon as the planula stage has given place to the settled hydra stage, the coelenteron becomes complete. The mouth appears at the tip of the free end, where the tissue has previously showed indications of disintegration, at the end of the axial line formed by the endodermal cells. At first the mouth is visible only when the specimens are killed and cleared or sectioned. Soon, however, it becomes large enough to see in the live animal, by focussing down from above with a high-power lens; it appears as a minute pit in the ectoderm. The coelenteron is more

distinct at the upper end than below, where it disappears into the loosely constituted cell-mass in the interior. The definite cavity of the coelenteron is sometime later in making its appearance. When finally established it is lined with a thick layer of columnar endodermal epithelium. At its bottom it flares out in following the contour of the body-wall, as it appears in figure 14, which shows a late stage, but the same condition of the coelenteron as exists in the newly transformed larva. The figure also shows a thickened core of endoderm which projects upward into the coelenteron as a gastric peduncle (G.P.). This conical mass of cells develops during the later part of the hydra-stage.

A. TENTACLES. -

In the later transformations of the developing Gonionema no definitely determinate periods separate the times of active change. The development-time is variable, depending upon external conditions of food, temperature, etc. In an average larva, however, the first tentacles make their appearance during the third week after the fertilization of the egg, or a week after the commencement of the fixed polyp-stage. Two tentacles appear opposite to

one another at a level about one-quarter of the distance from the upper pole of the hydra (Fig. 11). They are knob-like when they appear, but grow rapidly to a considerable length, the few endodermal cells which form the core of each increasing in number. Figure 11 shows a vertical section of a two-tentacled polyp of the fifth week. The manner of origin of the tentacles will be described in the section on "Origin of Tentacles," under "The Medusa".

The second pair of tentacles (Fig. 13) appear soon after the first, and by their rapid growth soon become as large as the first pair from which they are then no longer distinguishable. Irregularities are common in the appearance of the tentacles of the hydra, as in the adult. It frequently happens that only one of the second pair ever makes its appearance (Fig. 24); or one may be slow in arising, and always remain smaller than the other. On the other hand, an abnormally large number of tentacles is frequently developed; individuals with five or six tentacles are not rare (Fig. 14).

B. Form of Coelenteron. -

The appearance of the tentacles is accompanied by alterations in the form of the coelenteric cavity. The rapid

growth of the cells at thepoint where the tentacles arise, and the out-pushing of the tissue in the process seems to affect the tissue over a considerable area, so that diverticula of the coelenteron and of the mouth extend in the direction of each of the tentacles. A stellate form results, the mouth being in the shape of a cross. This corresponds exactly to the condition in the medusa, especially in young specimens (Fig. 26) in which the twisting which obscures the true relation of parts has not yet taken place. In a three-or five-tentacled hydra the number and arrangement of the oral lobes corresponds with the number and arrangement of the tentacles. Figure 12 represents a polyp with five radial parts, in which one lobe of the mouth is bifurcated. This condition is very similar to that frequently met with in the adult medusae. Hargitt figures a specimen in his "Variations among Hydromedusae" (1901. Pl. III, Fig. 11) which is much like this. The whole aspect of the hypostome of the <u>Gonionema</u> polyp is very similar to that of the manubrium of the young medusa (Fig. 26). The ectoderm at the edges of the mouth becomes thickened and armed with nematocysts, which have by this time appeared, in the manner to be described later, over much of the body of the hydra. Below the mouth, the hypostome becomes nar-

row and tubular, and distinct from the rest of the body, a decided angle separating them at the level of the tentacles.

VI. Habits . - One of the most striking habits of the adult jelly-fish is its prehensile propensity. The adhesive organ at the "knee" of the tentacle is composed of long slender glandular cells, packed into a thick cushion which is enclosed within a strongly muscular rim or collar (Fig. 24). This organ is located on the aboral side of the tentacle(Fig. 25). When at rest the jelly-fish lies on the bottom with inverted bell, and tentacles widely extended horizontally and attached to the bottom by means of the combined vacuum-cup and cement-gland near the tip. How this habit came to be acquired by the adult medusa it is hard to see. But if, as I shall give my reasons for believing, the medusa arises by direct metamorphosis from the hydra, the habits of the hydra would naturally be more or less permanent in the adult. It may be that this particular habit is more likely to be first acquired by the larva than by the adult. The tentacles of the hydra reach a relative length greater than in the case of any other known hydroid polyp, I believe. They frequently stretch out in

the water for a distance three or four times the height of the polyp. Figure 13 shows a hydra with the tentacles fully extended, and their tips touching the ground. This is the habitual attitude. The drooping of the tentacles is evidently caused by their extraordinary length, and is almost as unusual an occurrence among the hydromedusae. At the points where the tips of the tentacles come in contact with the bottom they spread out somewhat, forming a sole-like surface which is closely applied to whatever object the polyp is settled upon (Fig. 13 P.T). This smearing out of the tentacle-tips is like what occurs in live specimens of Hydra held between slide and cover-glass for examination. It is not known that the cells at the tip of the tentacle of <u>Gonionema</u> undergo transformation to form gland-cells and an adhesive organ, but such a change seems at least possible. At any rate, the fact is that the habit of the polyp is much like that of the medusa; both remain when at rest with the mouth expanded, the manubrium stretching upwards, the tentacles widely extended and adhering to the bottom. When an animal swims against one of the tentacles, the reactions are much the same in polyp and adult. The feeding habits of <u>Gonionema</u> have been described at length by Yerkes ("Sensory Reactions in <u>Gonionemus</u>" - 1902). His

account would apply almost as well to the process in the hydra. The tentacle which comes in contact with the prey is contracted with a suddenness and vigor which belies the apparent inertia of the moment before. The animal is seen to be firmly spitted on the microscopic lances of the nematocysts, and it is evident that the first thing that happened when the animal touched the tentacle was the discharge of all the thread-cells in that region. The tentacle in contracting carries the food, protozoan or minute worm or whatever, towards the mouth. The long manubrium then moves slightly, as if in search of the morsel. Finally, the tentacle places the food directly upon the mouth, which proceeds to turn itself over the object and work it downwards until it vanishes into the gastric pouch of the polyp.

VII. DEGENERATION PHENOMENA.

Owing to some peculiar condition in the water of an aquarium jar, the larvae of one lot, when three months old, began to exhibit most singular forms and activity. All appearance of the hydra was lost, ectoderm and endoderm became indistinguishable, and cell outlines dissolved. The larvae in this condition had very much the appearance of amoebae. They slumped down on the bottom of the aquarium,

a shapeless mass, and by protoplasmic flowing changed their shape through an endless variety of forms, and moved slowly from place to place. Thin pseudopodia were sent out along which the substance of the organism flowed, and by the breaking of the connecting band divided into two. The fragments became smaller and smaller until no longer recognizable. These abnormal larvae remained alive for six weeks after which no trace of them remained.

VIII. BUDDING IN THE LARVAE - METAGENSIS. -

Contrary to Haeckel's statement that in the group of jelly-fish which he calls the"Trachomedusae" metagenesis doesnot occur, in Gonionema, which falls into that group, metagensis does take place. By a form of non-sexual multiplication different from any which has previously been described for any member of the Hydromedusae, an intermediate process of reproduction is introduced into the life-history of Gonionema, whereby a large number of adults are produced from a single egg.

Asexual multiplication in the larvae of Scyphomedusae has been known since 1841, when Sars saw and described the formation of buds in a scyphistoma of uncertain identity, but regarded as either an Aurelia or a Cyanea. Since

that time several analogous cases have been made known. The scyphistoma larvae of Cassiopea, for example, were found producing buds in large numbers by Bigelow (1900), who gives a detailed account of the method of budding in his monograph on this Rhizostome. It may be further stated in general, that the non-sexual process of production of buds by the larvae is an important method of multiplication among the Discomedusae. The buds usually develop, after detachment from the parent polyp, into a second generation of scyphistomas, identical in form and fate with the original ones. Buds may arise on the body of the scyphistoma, or upon stolons from its base, and either singly or several at a time. In Cotylorhiza the buds develop so rapidly and remain attached so long that large clusters accumulate about the base of the scyphistoma. According to some authors, Goette, for example, the distal end of the bud in Aurelia and Cyanea is destined to become the oral end of the detached larva, developong mouth and tentacles. Friedmann, on the other hand, says ("Postembryonal dev. of Aurelia aurita", 1902) that in Aurelia he has found the opposite condition, the mouth being invariably develcped at the attached end of the bud. This is the common relation in other forms.

In *Cunina*, which falls into Haeckel's order, the "Narcomedusae", the ciliated, tentacled larva multiplies by buds, produced from an aboral stolan. These buds are not detached until mouth, digestive cavity, and tentacles are well developed. Several are produced simultaneously, and are attached to the parent by the oral extremity. The description of this remarkable process is given by Dr. W. K. Brooks, in "The Life History of the Hydromedusae", (1886).

It is my purpose in this section to give an account of a process of budding in a very different medusae from *Cununa*, and one in which the asexual multiplication takes place quite differently. In *Gonionema* the buds are produced in a manner which reminds one very strongly of the similar process in *Cassiopea*.

In the course of my general study of the embryology and development of *Gonionema* I came upon the budding larvae (Figs. 14 -23). From a lot of eggs obtained at Wood's Hole in August, 1901, a large number of polyps developed and were kept alive in a "balanced" aquarium for several months. This lot was left at Wood's Hole in as nearly natural conditions as possible until the last of November. The water was kept fresh by frequently renewed supplies of diatoms

and ulva, and occasionally changed by carefully adding a quantity taken from the natural habitat of the medusa in the eel pond. A low temperature was maintained. When these larvae were received from Wood's Hole, November 28, they were apparently thriving well. They were settled upon Minot watch glasses which were easily removed from the aquarium-jar without disturbing their contents. These dishes were numbered and the positions of the polyps carefully noted and mapped. Successive examinations showed that the number of polyps was on the increase, and, on December 3, it was seen that one or two of the largest specimens had rounded knob-like bodies upon their surfaces, which were recognized as buds. These specimens were examined as frequently as it was thought safe to remove them from the jar, and camera drawings were made of the growing buds. Observations were made of the different stages in the development of fourteen buds; their phases agreed in all the main particulars.

The first indication of the appearance of a bud upon any individual polyp was seen as a rounded eminence upon the hydrocaulus (Fig. 15, b). It was usually located at a level about half way between the base of the polyp and the

ring of tentacles, as in figure, and interradially, i.e., at the end of a radius which bisects the angle between two tentacles (Fig. 19). Never more than a single bud appeared at one time on any polyp.

All three body-layers of the parent are involved in the formation of a bud (Fig. 16). The cells of both ectoderm and endoderm multiply rapidly in the region of the wall of the polyp where the bud is about to be formed. The endoderm pushes out as a rounded protuberance covered by the ectoderm in a layer of constant thickness. A thin supporting lamella of mesogloea lies between the two. As the bud increases in size it bulges out at its base, around the stalk which connects it with the polyp, and it also develops rapidly at the tip of the free end. In this way it becomes pear-shaped (Fig. 17). As the drawings indicate, the ectoderm is of the same thickness in the bud as in the parent polyp (Fig. 16, ect.); indeed, so nicely regulated is the rate of growth of the two tissue-layers that the thickness of the ectoderm does not change appreciably during the entire growth of the bud, previous to its detachment. The cells of this latter layer are irregular, loosely constituted, and coarsely granular, and their walls are hardly dis-

cernible. No cavity exists in the bud until considerably later. The endoderm of the bud now becomes separated from that of the parent by the constriction of the ectoderm and the cutting off of the core of endoderm which filled it. Its appearance is as represented by figure 18, an isthmus of clear elastic ectodermal tissue (ect.) uniting the bud to the parent. By rapid centrifugal growth the bud becomes sausage-shaped, and as long as the diameter of the polyp. Soon after the bud reaches the stage shown in figure 19 it becomes detached from the polyp. In only one instance was I so fortunate as to see this process taking place. In this individual the bud was drawn out into a long finger-like body, its distal end drooping almost to the ground. Soon the ectodermal isthmus began to stretch out and dwindle in diameter until it was merely a thin stem of transparent protoplasm (Fig. 20). The bud seemed to be reaching out and trying to free itself from the limitations of the connection with the parent. This stretching of the isthmus was brought about by constriction of the tubular ectoderm, as if by circular muscle-fibres. When this stretching had gone on until the isthmus was a quarter as long as the entire bud, (Fig. 20), it began to grow still

thinner at about its middle, and finally, just half an hour after it first began to stretch out, it broke in two and the bud fell away from the parent (Fig. 21). The two ends of the connecting-stalk shrank back into the tissue of the bud and the parent, appearing for a time as drops or points of protoplasm (Fig. 21). After separation from the polyp, this particular bud settled down at once upon the previously free or distal end, and began an independent existence (Fig. 22). Other observations, however, indicate that the usual course of development is slightly at variance with this instance, and that it includes a period of from three to four days, intervening between the detachment of the bud and its settling down as a hydra, during which it has the form of a free-swimming planula. A bud which was growing upon the body-wall one day would be gone the next, and for some time could not be found. Then suddenly it would appear in some previously vacant spot, at a distance from the polyp, perhaps in an entirely different watch-glass on the bottom of the aquarium, with its tentacles just beginning to appear. In one case the bud was drawn and measured, when it seemed to have reached its full size and to be ready to drop off; this was done one evening, and the next morning

the parent polyp had no bud on it. Three days afterward a small creature was found upon a spot which had been certainly unoccupied by any larva up to that time, careful diagrams having been made as stated above. This was a larva like that in figure 23. It was measured, and although changed in shape, as nearly as one could estimate its bulk it corresponded exactly with the bud which had disappeared. Similar experiences were repeated so many times that it seemed unavoidable to consider this a normal phase in the process. This being so, it is an interesting case of reversion in the non-sexually developed larva to a condition earlier in point of ontogenetic order than that in which the parent was when it gave rise to the bud. In any case, the future history of the bud is certain. After settling down upon the bottom, it repeats the changes which occur in the sexually produced polyp. The newly arisen larva (Fig. 22) loses its planula shape, becoming shorter and thicker, especially at the base, on account of the plastic character of the tissues (Fig. 23). It has now secured a firm hold upon the bottom, being so closely applied to it that it can be dislodged with difficulty. The cells at the base increase in thickness until they form a columnar epithelium

(Fig. 20, ect). After the first day a slight pit indicates the point at which the coelenteron is to open externally (Fig. 23,..); this process, as observed in a number of cases, is exactly the same as in the sexually produced polyp. The tentacles also make their appearance in the same manner as described for the hydra which came from the egg.

The length of time required for the complete development of a bud, from its first appearance on the hydrocaulus of the parent as a round knob, until the completion of the formation of the coelenteron and appearance of the tentacles, is from ten to fourteen days; (a) the first period, including as far as the detachment of the bud, five days; (b) free swimming planula, two to four days; (c) from at- attachment to appearance of tentacles, three to five days. These periods refer, of course, to specimens which developed in captivity.

Figure 24 shows a specimen from an entirely different lot of polyps from those which exhibited the budding phenomena shown in figures 14 to 23. This polyp was killed when 23 days old. It may not be a normal individual, but shows a tendency to divide <u>transversely</u> and seems worth calling attention to. The coelenteron has completely di-

vided into two, and the endodermal wall of the pouch has grown in as a solid partition between the two new pouches. The aboral portion of the body is seen to be considerably longer than is usually the case.

IX. TRANSFORMATION OF THE POLYP. -

Up to the present time all efforts to secure specimens of the larval Gonionema in their natural habitat have been well-nigh fruitless. Although the eggs are laid in enormous numbers during four to six weeks of the summer, and even when kept in the laboratory a large proportion develop , it has yet been impossible to find the polyps in the eel-pond where the medusae are so plentiful. Many speculations have been hazarded as to the condition in which the larvae pass the cold months of winter, and no small energy and time have been expended in attempting to get at the secret. And yet I am much more ready to believe that the difficulty has been with our methods of search than that any extraordinary transformation in form or change in habitat should render the success of such search impossible. This seems the more likely from the fact that during the season when the medusae are laying their eggs

most plentifully, and within a few days from the time when an egg is laid it has developed into a fixed polyp with tentacles, and such polyps must be present in great numbers in the mud and on the stones at the bottom of the eel pond, not even then have more than a very few specimens been found, although search has been made by others than myself. It is quite out of the question to suppose that the larvae which develop into the great numbers of medusae which appear year after year in the eel pond have been swept out to sea to undergo their transformations in deep water, because in such case the adults would appear in much wider range of locality, in some of the bays and inlets of the coast where the conditions seem to be almost the same as in the eel-pond. The fact is that only a few stragglers are ever found in the vicinity - as many as would readily be swept out of the shallow water by the tide. Not only these considerations, but all the other indications seem to point to a direct transformation of the polyp to the adult gonosome without leaving the eel-pond. The habit of the polyp of resting with tentacles extended and adhering to the bottom, the feeding reactions, the form of coelenteron, manubrium and oral opening, and the manner of origin of the tenta-

cles, all resemble the corresponding conditions in the adult so closely that it is easy to regard this as the most likely theory. May it not be that the same type of metamorphosis as that which takes place in Liriope (Brooks, 1885) is passed through in this genus as well? In Liriope the coelenteric cavity is transformed into the system of chimiferous tubules by the growth of fusion areas uniting the upper and lower walls of the cavity except where they are to be left separate along the lines of the canals. Fig. 25 is a camera drawing of a twelve-tentacled gonosome of Gonionema, which has very much the appearance of the newly metamorphosed Liriope (Brooks, 1885, Pl. 41; Haeckel, "Die Russelquallen", Pl. 12). The transformations which are necessary to bring about the adult form from the larval are the change in the coelenteron from a pouch to a system of tubes, the centralizing of the diffuse nervous system to form the two nerve-rings, the appearance of new tentacles provided with adhesive disks and of tentacles modified to the form of sense organs, from the expanded tentacular ring, and the growth of the velum. The relative size of the fully developed polyp and the youngest medusa offers no contradiction to the conception of such a process of direct

metamorphosis; if the polyp grows as rapidly in the natural environment of the eel pond as in the laboratory, even allowing a long period of absolute quiesence during the cold weather, the discrepancy in size is easily accounted for.

X. YOUNGEST MEDUSAE. - Arrangement of Tentacles and and Sense-Organs.

During the last of June, 1900, a number of very small specimens of Gonionema were taken in a tow-net at the surface of the eel pond. Several of these had sixteen tentacles, some had twelve, one had only eight. A careful study of these very young and evidently recently metamorphosed gonosomes has brought out some exceedingly interesting points.

Hargitt (1901), in his paper on "Variations among Hydromedusae", discusses the arrangement of tentacles in Gonionema; he approaches the question as a student of variations, and unfortunately lacks the young material from which I have found it possible to educe very definite rules in the arrangement of marginal organs, and their order of appearance. As a natural result Hargitt forms the conclusion that so much irregularity occurs as to render it im-

possible to discover any definite order of appearance or ultimate arrangement in these organs. It is true that the abnormal specimens which he studied most closely do show very little regularity - as would be expected. But in normal individuals quite a remarkable degree of precision is manifest in the position and order of appearance of tentacles and sense-organs, with reference to each other and also to previously arisen organs of the same kind. This is particularly true in the younger stages.

If we examine the eight-tentacled medusae the following points are noticeable: First, the tentacles are evidently of two cycles, in order of appearance. The four at the ends of the radial canals, or the perradials, are equal in size, and larger than the four interradials, which are also equal in size. These tentacles are very similar in structure and appearance to the larval tentacles, and there seems little reason why the four larger perradials may not be the permanent larval tentacles.

Second, the sense-organs are four in number and placed in definite positions, relative to the tentacles. If we look at the bell-margin from the oral side, the newly arisen interradial tentacle in each interradial quadrant

has apparently crowded in between the sense-organ and the perradial tentacle which comes before it, as the hands of a watch go. Text-figure 3 shows this stage, and is made from a camera drawing of the eight-tentacled medusa. And the relation which is here exhibited in the youngest stage of the free-swimming gonosome is the same throughout the growth of the creature: wherever a rudimentary or newly arisen tentacle lies on the bell-margin, it will always, normally, be found to lie just in front of a newly arisen sense-organ and just after a large tentacle, i.e., one of a much earlier cycle.

Much has been written to show that coelenterates, and especially the Hydrozoa, show bilateral symmetry, either in the normal condition or when they depart from the normal form and may be supposed to revert to a more primitive type (Mayer, 1901, e.g.). *Gonionema* shows a very different plan from that of bilateral symmetry. It is rather a certain sort of radial symmetry which has nothing bilateral about it - one in which the radial parts correspond exactly to each other and are superimposable, but none of which is the reflected image of any other. I shall call this relation one of *cyclic symmetry*. With reference to

the order of appearance of the marginal organs I shall speak of cyclic sequence. (These terms were suggested by Professor Morley of Johns Hopkins). New tentacles make their appearance four at a time, or, so to speak, in quartettes; they are 90° apart, so that they occupy identical positions in the four quadrants of the marginal ring. But while the tentacles, and sense-organs as well, appear to arise in fours, the condition in the larvae and in frequent instances among the adults indicates that a paired origin is more primitive and fundamental. It is the universal rule in the early larvae that two tentacles appear opposite to one another (Fig. 11) and later the second pair of the quartette (Fig. 12). And it often happens that in the adult medusae, two members of a quartette, in opposite quadrants, are retarded in making their appearance. In Aurelia, Claus established the theory that while the larval tentacles seem to come in fours after the earliest stage, the first four tentacles appear first two, then two more, as is the case in Gonionema; (v.Claus, 1892). Goette (1887) has examined a great number of specimens of the younger stages of Aurelia, and has come to the same general conclusion as Claus, with regard to the primitive paired

condition and the significance of this in the philogeny. Haeckel (1881), however, regards the appearance of two tentacles in advance of the second two as an accidental and insignificant occurrence, and takes four as the primary number. While this tendency to a paired origin of the tentacles disappears after the earliest stages in Aurelia, Gonionema exhibits this tendency in frequent instances during the whole life of the animal. Its occurrence in the appearance of the sense-organs is of the same significance, because as will be pointed out below, these organs are modified tentacles. Figure 31 shows this condition in the sense-organs, quadrants A and C having 5, while in quadrants B and D only 4 are yet developed. It is true that other variations than these do occur in the appearance of the tentacles and sense-organs of the adult, and in the tentacles of the larva. Polyps with three, five or six tentacles are not uncommon (Figs. 12, 24). It is noticeable that departures from the normal number of tentacles correspond very closely in polyps and adults. This would be expected to be the case from the evidence that the larval tentacles are permanent, and that they determine the position of the four radial canals in the normal medusae,

or of the three, five or six in the specimens which are aberrant. This inference seems a likely one, from the fact that in the adult medusa the tentacles which from their larger size are presumably of the first cycle are always located at the ends of the radial canals. While a few exceptions are found, they frequently occur in cases which have apparently become abnormal by accident subsequent to metamorphosis. The inference is, then, that five-parted adult medusae were five-tentacled larval polyps. And this is borne out by comparison of the relative numbers of each kind of variation among medusae and among polyps. Hargitt (1901) has tabulated the number of medusae that have come under his notice having three or five or six radial canals; and he finds that about five per cent. of all medusae are irregular in this regard, i.e., that they vary from the normal four-parted condition. While I have not had a great number of specimens of the polyps from which to compute averages, my counts show quite a striking similarity to those which are given by Dr. Hargitt for the adults.

Among all the varieties of geometrical figures which appear in the arrangement of parts among the various orders of coelenterates, there is none, so far as I can find, which

is at all comparable with that which appears in the arrangement of tentacles and sense-organs in Gonionema. The only suggestion of such a plan or arrangement as this is given in a paper on the later development in Aurelia, by Friedemann (1902). In the course of the paper the author describes the origin of the eight tentacles which follow the original eight. Four of these appear at once, the other four later. In the appearance of the first four, two possibilities arise, according to Friedmann. Either the four arise in bilaterally symmetrical positions in the four quadrants, the two halves of the tentacle ring being reflected images one of the other, and the new tentacles appearing one on either side of two opposite perradial tentacles (text-figure 1); or else they appear in identical positions in the four quadrants, one appearing next in front of each perradial tentacle, as the hands of a watch move (text-figure 2). Friedmann's figures do not make it clear that he actually found specimens in exactly this state. It appears more probable from his descriptions that he interpreted older stages by this theory. But it may easily be true that in other groups than that to which Gonionema belongs the tentacles originate according to a plan of cyclic

symmetry, or that such a condition may appear in occasional instances, as is perhaps the case in Aurelia. In Gonionema the rule holds with remarkable constancy.

From a study of the successive stages of growing medusae the following table is compiled, to show the relation in time of appearance of tentacles and sense-organs. The numbers in brackets, in the column of sense-organs, indicate half-quartettes, the corresponding pair in each case having been delayed in appearing. Since the sense-organs are only half as numerous as tentacles, they appear with half the rapidity, and are therefore more frequently found in this incomplete condition. That is, if a jelly-fish were killed when the tentacles and sense-organs were in the precise stage indicated by the line A.B, the fifth quartette of sense-organs would be found only half formed - five sense-organs appearing in two opposite quadrants, and only four in the other two. This

	Tentacles	Sense-organs
Larval (Polyp)	2	
	4	(2)
Adult (Medusa)		4
	8	(6)
	12	8
	16	(10)
	20	12
	24	(14)
	28	16
	32	(18)
A - - - -	- - - - - - -	- - - - B
	36	20
	40	(22)
	44	24
	48	

is just the condition which exists in the specimen shown in figure 26. The numbers indicating the sense-organs are put at the intervals between those indicating tentacles, to show that while the eight tentacles between the eight-tentacled and the 16 tentacled stages, for instance, are appearing, the four sense-organs which make up the second quadrant are appearing.

Another rule is followed by the marginal organs in their order of appearance: Each new tentacle arises not only just in front of a sense-organ, but in a definite location with reference to the tentacles already present. And the same thing is true for the sense-organs. It is therefore possible, from a study of successive stages, to predict where each new quartette of tentacles or sense-organs will arise. The diagrams shown in text-figures 3 to 8 are from camera drawings of mounted whole specimens of medusae. If we examine text-figure 4 we see that T (tentacle) III follows T I; and text-figure 5 shows TIV following T II. Thus T III and T IV form a series, arising in corresponding positions in the quadrant relative to the tentacles already present. (By "series" is not meant "cycle", with the idea of simultaneous appearance; the notion is rather

one of relative position, simply.) The next series of four tentacles in each quadrant, T V to T VIII, brings us to the 32 tentacled stage seen in text-figure 6. In this it will be seen that T V follows T I, T VI follows T II, T VII follows T III, T VIII follows T IV, four numbers intervening in each case. The next series comprises T IX to T XVI, which follow the same plan in order of appearance, T IX following T I, etc., eight numbers intervening in each case. While this may seem more like a fanciful exercise of the imagination than an actual condition in nature, the truth is that the larger the number of specimens in which one tests the arrangement of the marginal organs by this rule, the more will one be convinced of the remarkable constant adherence to it. Given a specimen with, say, 28 tentacles, such as that represented in text-figure 7; this is a drawing of a specimen of Olindias, from the Bahamas, a genus which follows the same rule in the order of appearance of the tentacles as that which is being described for Gonionema; the sense-organs are not so numerous in Olindias. In this specimen the most recently arisen tentacle in each quadrant is evidently the one numbered VII, lying just after each perradial tentacle. Then, if the rule which we

have educed applies in this case, we should expect to find the eighth tentacle in each quadrant arising in a corresponding position with relation to the interradial tentacle. And such we find to be the case. Text-figure 8 shows a slightly older specimen of the same species, in which we plainly see the eighth tentacle in each quadrant lying in its appointed place (VIII).

It would be singular indeed if there appeared no exceptions at all to this regular cyclic symmetry. Many variations do occur, but not more than we should expect from the marked tendency to variability in all parts of the medusa. These variations no more obscure the normal definiteness of plan than the occurrences of six- or seven-rayed star-fish obscure the normal pentamerous form in echinoderms. Text-figure 5 shows an irregular condition, the fourth tentacle in each quadrant having arisen aberrantly, following instead of preceding the first sense-organ (1). In figure 31 one of the latest arisen quartette has not put in its appearance (see arrow in quadrant A). In the older specimens, the number of irregularities increases. It seems to me that the bell-margin increases in extent subsequent to and as a consequence of the increase in the number of ten-

tacles, rather than that the tentacles arise, haphazard, wherever there is space enough on the margin to accomodate them (Hargitt, 1901). Certain it is that the most crowded part of the bell-margin at any particular time is that from which the new tentacles arise.

XI. HISTOGENESIS OF MARGINAL ORGANS. -

A. In the Larva.

The similarity in the appearance of the rudiments of a tentacle in polyp and in gonosome make it desirable to describe both in the same section; for that reason the account of the origin of the tentacles in the polyp was reserved for this place. At first the larval tentacle is merely a small rounded knob in external appearance, and internally it is made up of a core of two or three endodermal cells. When the tentacles make their appearance the body wall of the polyp is made up of a double layer of cells, the ectoderm and the endoderm, separated by a thin supporting lamella of mesogloea. These three layers are pushed out somewhat in the growth of the tentacle, the region of greatest activity being in the endodermal layer, where the core of the tentacle is formed by a rapid out-

growth of the cells of the body wall, accompanied by multiplication of these same cells. After some weeks the cavity of the coelenteron becomes drawn out in a diverticulum in the direction of the axis of the tentacle, so that the upper part of the gastric cavity becomes stellate in cross-section. Figure 14 shows this condition in a five months old polyp. This cavity does not reach out into the tentacle itself in any of the specimens which I studied, but may do so before metamorphosis takes place. During the whole of larval life, the tentacle is made up of a core of endodermal cells in a single row (Fig. 27), as is the case in hydra. Figure 11 shows a polyp with the first pair only developed, and the cell-layers are seen as described. The endoderm cells are filled with a loose protoplasmic network (Fig. 27, end), and the nucleus is evident. The condition which is seen in an adult tentacle with several cells of endoderm surrounding the central cavity (Fig. 34) is easily derivable from the larval condition, by longitudinal fission of the endodermal cells repeated until a cross-section of a tentacle at any level cuts several cells (Fig. 34).

B. In the Adult. - The regularity with which the tenta-

cles and sense-organs make their appearance in the adult, as previously described, makes it possible to locate with comparitive certainty the beginnings of one of these organs on the bell-margin. Figure 18 is from a section of a medusa, cut horizontally at the point of origin of one of the tentacles. The figure shows the aspect at the level of the tentacle, somewhat above the velum. Both cell-layers are seen to be concerned in the formation of the new tentacle. The endoderm (End.) is pushed out from the region of the circular canal, and has the shape of a solid plug of tissue composed of a few cells arranged radially about a central axis (T.R.); the nuclei are at the inner ends of the cells. Outside of this endodermal core is the ectoderm (Ect.) which is, in the region of the bell-margin, of the character of gelatinous tissue, stiffened by the presence of a large number of concretions (Con). These concretions and the nuclei of the cells are more numerous at the point where the tentacle is to appear than elsewhere. In the medusa, as in the polyp, the greatest activity in the formation of a new tentacle is manifested by the endoderm. According to Allman (Monograph, 1871), in some hydroids (Campanularia Johnstoni, for example) the first indication of

tentacle formation is the thickening of the ectoderm at the point where the tentacle is to appear. This is contrary to the condition which we have in Gonionema. But to continue our description; along with the growth of the endodermal process which is to be the core of the tentacle the ectoderm also increases rapidly, and constitutes an investment which contains within it numerous nematocysts and concretions which were scattered throughout the ectoderm at the margin of the umbrella. After the tentacle has grown out for a little distance beyond the bell-margin the cells on the upper or aboral surface become modified to form an adhesive organ. (Fig. 27, 28) The cells over a disk-shaped area become elongated until they have the form of a thick pad. The tissue immediately around the pad grows out in a flange so that the organ becomes a vacuum-cup strongly muscular around the edge (Fig. 28).

After the tentacle has grown out to a length of six to eight mm. and has increased in diameter considerably, the cavity of the circular canal is drawn into it. The endodermal cells, arranged radially about the axis, thicken until they are forced away from the centre, and a tubular cavity is left(Fig. 2). As this process takes place first

at the proximal end of the tentacle, within the tissue of the bell-margin (Fig. 34) the cavity of the circular canal is carried out along the axis of the tentacle towards the tip. In this way the tentacle, which was originally imperforate as in the larval condition, has become hollow.

C. The Sense-Organs. - The origin of the sense-organs is very similar to that of the tentacles (Figs. 27 & 30). In fact it seems clear from a study of these processes in Gonionema that the sense-organs must necessarily be regarded as modified tentacles. In the case of these sensory clubs (S.C.) the endodermal tissue of the circular canal (End.) grows down in a plug into the ectodermal tissue of the bell-margin (Ect .) This latter becomes closely applied to the outside of the plug, as a thin investigating epithelium, and it also spreads out in a thin lamella over the inner surface of the capsule which appears in the ectoderm in front of the developing club. Figures 34 and 35 are drawings by Professor Brooks from sections cut transversely across the bell-margin, showing the early stage in the formation of a sense-organ. I have not been able to demonstrate the presence of sensory hairs in the cavity of the capsule. The cells at the tip of the club soon begin to

secrete the solid concretion which later attains a considerable size. The concretion is invested with the thin membranous ectodermal covering. In Gonionema the concretions correspond with the composition which has been given for similar structures in other medusae - a lime carbonate deposit in an organic matrix. Thus it is seen that both tentacle and sense-organ consist of an endodermal core which appears first and grows out from the lining of the circular canal. In each case this core becomes invested with a tunic of ectoderm which remains associated with it.

XIII. NEMATOCYSTS. -

In the hydra stage the earliest appearance of nematocysts was as interstitial cells arising from either tissue-layer. Their growth in Gonionema is much as it is in Cordylophora lacustris, as described by Morgenstern (1901). They are carried out on the tentacles by migration along with the ectodermal layer in which they are set. The extreme attenuation of the tentacle as it is fully extended (Fig. 13) gives an admirable chance to study the construction of the cell-elements, especially the nematocysts. The tentacle appears as a delicate rod of translucent substance

partitioned off at intervals by the transverse walls of the endoderm cells, and studded along its length with numerous glistening bead-like bodies, the nematocysts. Above each of these thread-cells a palpocil projects like a thorn (Fig. 31). The capsule has a slightly unusual form, long and bean-shaped (Fig. 32). Examination with a high power objective, focussed down into the water upon the extended tentacle shows with considerable distinctness a ganglion cell of glistening highly refractive appearance, lying close to each nematocyst (Fig. 32, g.c.). In every case this ganglion cell is situated distal to the thread capsule toward the free end of the tentacle (Fig. 32). A thin strand of nervous tissue runs in each direction from the ganglion cell, towards the nematocyst proximally, towards the free tip of the tentacle distally. It is visible for only a short distance, however, soon vanishing into the ectodermal tissue, and none of its branches or terminations are to be followed. It evidently innervates the nettling capsule, near the base of which it can be seen.

In the <u>gonosome</u> the nematocysts are carried out onto the ectoderm of the growing tentacle <u>in situ</u>, as in the larva. Further growth in the extent of ectoderm is brought

about in two ways: by multiplication of the cells already incorporated in the epithelium of the tentacle, and by immigration of cells from the thick ectodermal pad at the base. The cartilaginous tissue composing this pad is peculiar in character. The cell walls are almost or quite obliterated, and the gelatinous substance contains the concretions already mentioned, which give the tissue something of the nature of a cartilaginous encasement for the nerve ring lying immediately beneath it. These concretions are not very different in appearance from the concretions of the sense-organs - the "otoliths" as they are called, with, however, quite inadequate evidence that they function as auditory organs. They are solid masses of hard substance, partly calcareous and partly organic. They are not made up of concentric lamellae such as give the concretion in the sensory capsule its onion-like layers. In this whole group of medusae the older tentacles are left stranded as it were by the growth of the margin of the umbrella beyond their point of origin. As they are in this way carried up onto the exumbral surface the cartilaginous tissue grows so as to fill the space between the base of the tentacle and the bell-margin, forming a round cushion of hard tissue. In

sections cut through this tentacle pad (Figs. 33 and 34) it is seen that the concretions which lie towards the bell-margin are more dense and homogeneous; that further inwards they are somewhat less solid in appearance, spaces appearing within their outer walls; and that at the side nearest the circular canal there are great numbers of nettling cells in various stages of formation. All gradations are present between the solid concretion and the nettling cell (Fig. 33). It is therefore evident that the two distinct kinds of specialized cell products, the one for the protection of the delicate nerve ring and the other for capturing and paralizing prey, are produced from the same cells and in the same locality; that they are, in fact, homologous. Figure 33 was drawn by Professor Brooks to show this fact in Gonionema. Whether the cartilaginous cells actually change into nettling cells after they have become fully developed is not clear. At the inner margin of the cartilaginous pad the nematocysts lie closely packed together (Fig. 33). From this breeding place the nematocysts work their way out onto the tentacle along which they migrate until they reach a spot where they are needed. In young tentacles, which are still elongating, the nematocysts are car-

ried out with the ectoderm as it becomes applied to the tentacle-base. But after a certain time the tentacle increases only very slowly in length and additional nettling cells are needed to keep up with the increase in diameter. This migration of nematocysts has been seen and described by Murbach in his paper on the nettling organs in Hydroids (Arch. f. Naturg. 1894). After the capsules have become established, the ectodermal covering becomes modified to form the cnidocil (Fig. 32, cn.). The nerve connection is developed at an early stage.

XIV. SEXUAL ORGANS.-

In minute specimens of the adult gonosome the gonads are frequently found in their first stage of development. They appear as outgrowths of the ectodermal covering of the radial canals, at first in the form of rounded lumps projecting downwards from the radial canal into the subumbrella at a point two-fifths of the distance from the top of the bell to the margin. The rudimentary lump of gonadial tissue elongates in both directions from the point at which it started. (Text-figure 8 shows the condition in Olindias, where it is identical at first with that in Go-

nionema). The gonad thus becomes an elongated ridge of tissue which finally reaches to the extremities of the chimiferous tubes, and increases in depth until it hangs down into the subumbrella as a ribbon. Early in its development the ribbon is somewhat sinuous, and as the medusa attains greater diameter the convolutions become more nemerous and farther extended on either side of the radial canal, until ultimately the folds are packed tightly together in a solid band of tissue, which at the time of maturity is extended with sexual elements. The process of formation of the sexual organs is identical in the two sexes; it is impossible to tell whether a given individual is male or female until the sexual products begin to mature.

SUMMARY:-

1. Observations on the development of *Gonionema* indicate that Haeckel's sharp distinctions between jelly-fishes which he groups in his orders "Trachomedusae" and "Leptomedusae" are not justified.

2. Dehiscence occurs in *Gonionema* with precise periodicity, and is definitely affected by changes in light.

3. Segmentation is total and equal; endoderm is formed by delamination of the blastomeres; a solid morula results.

4. A planula stage occurs, and later a hydra stage, in which the polyp develops first two tentacles, then a second pair.

5. Youngest medusae and oldest polyps show marked homologies; direct metamorphosis is suggested.

6. Peculiar pathological phenomena appear, the larva living for weeks in the form of a plasmodium, with amoebiform activities.

7. Alternation of generations occurs. A non-sexual form of multiplication appears during larval life; buds are produced which are detached as planulae and go through the same changes as the parent.

8. The order and arrangement of tentacles in the gonosome follows a definite plan in cyclic sequence, producing a figure which is cyclically (not bilaterally) symmetrical. Tentacles and sense-organs appear at determinate points on the bell-margin.

9. Histogenesis of tentacles and sense-organs shows their homology.

10. The origin of nematocysts from the ectodermal pad at the base of the tentacle, and their homology with cartilaginous concretions, are established.

11. Gonads arise as enlargements by proliferation of the ectodermal subumbral epithelium of the radial canal.

DESCRIPTION OF FIGURES.

Plate I.

Fig. 1. Adult Gonionema in resting attitude; floating after a period of active swimming. 2/1.

" 2. Medusa in act of swimming: bell contracted, tentacles drawn up at the end of a forward impulse. Photograph from life. 1/1.

" 3. One radial canal from ripe male, showing gonad. c. circular canal; r, radial canal. 8/1.

" 4. Gonad of female, during dehiscence. 20/1.

" 5. Egg during first segmentation; cleavage furrow half completed. n, nuclei. 570/1.

" 6. Egg during second segmentation, left hemisphere completely divided, right hemisphere in process of dividing.

" 7. Hollow blastula 7 hours after fertilization. Optical section of live egg.

Fig. 8. Two-layered blastula, endoderm having arisen by delamination.

Plate II.

Fig. 9. Young planula larva. P, posterior end, a, anterior end. 675/1.

" 10. Planula larva; posterior end enlarged; endodermal cells at posterior end arranged along the axis of the larva, marking line of future coelenteron.

" 11. Two-tentacled polyp, in section; four weeks old. Ec, thickened basal ectoderm.

" 12. Polyp, four months old, with five tentacles and five oral lobes, lying in the same planes.

" 13. Polyp, five months old; in typical resting attitude, tentacles expanded 2 mm. P.T., prehensile tip of tentacles, adhering to bottom.

" 14. Five-tentacled polyp, showing form of coelenteron and formation of bud.

Plate III.

Fig. 15. Five months old polyp, with bud just forming.

" 16. The same, bud 8 hours old. Ect, ectoderm.

" 17. " " " one day old, pear-shaped

" 18 " " " three days old. Endoderm isolated from parent by constriction of ectoderm.

" 19. The same bud, four days old, ready to be detached; showing interradial position on parent

" 20. Another individual, bud in process of detachment, showing elongated ectadermal isthmus.

" 21. The same, 15 minutes later. Bud settling on former distal end.

" 22. Detached larva, after three days of free-swimming planula life, just attached. Showing nematocysts.

" 23. The same, four days later; basal ectoderm much thickened, coelenteron developed.

" 24. Larva 23 days old, exhibiting transverse fission of coelenteron and elongated hydrocaulus.

" 25. Young medusa with 12 tentacles and 4 sense-organs;

showing spherical shape and constricted bell-margin

Plate IV.

Fig. 26. 32-tentacled medusa with 14 sense-organs. 7th and 8th tentacles have appeared in each quadrant except quadrant A, where 8th is lacking. 4 sense-organs have appeared in quadrants B and D, 5 in quadrants A and C.

Fig. 27. Horizontal section of bell-margin at level of rudiment of tentacle, T.R. Con. calcarious concretions; N.R. nerve ring; T tentacle; C.C. circular canal.

Fig. 28. Tentacle tip of medusa, showing rings of nematocysts, angle of tentacle, and adhesive organ on aboral side.

Fig. 29. Cross-section of adhesive organ. G.C. gland cells composing cement gland; M.F. muscular flange; 500/1 drawn by W. K. Brooks.

Plate V.

Fig. 30. Radial transverse section of bell, at point of or-

rigin of sense-organ, S.C., showing endodermal origin; Caps, sensory capsule, surrounded by ectoderm; V velum.

Fig. 31. Tentacle tip of larva from above. End, single chain of endodermal cells; Cn, cnidocil; G.C. ganglion cell. 500/1.

Fig. 32. Nematocyst in detail, showing cnidocil, Cn., ganglion cell, G.C., nerve fibres, N.F. running towards distal end. 2000/1.

Fig. 33. Ectodermal pad at base of tentacle. Three areas of cell secretion, C.P. cartilaginous pad, T. tentacle, M. mesogloea; Radial vertical section.

Fig. 34. Transverse section, at bell margin, of base of tentacle, showing tentacle pad, C.P.

VITA.

The writer, Henry Farnham Perkins, was born May 10, 1877, in Burlington, Vermont. He is the son of George Henry Perkins, Ph.D., Yale, 1867, Howard professor of Natural History at the University of Vermont; and of Mary Farnham Perkins, of Galesburg, Illinois, A.B., Knox College, 1869.

The degree of A.B. was received from the University of Vermont in 1898, at the completion of the regular four years' course in classics, literature, mathematics and the sciences. During the four years since October, 1898, the writer has been enrolled as a graduate student in the Johns Hopkins University, in the department of Biology. Physiology and Physics were taken as subordinate subjects. The summers of 1899, 1900, and 1901 were spent in research at the sea-coast of this country and the Bahama Islands. Two papers have been published giving in abstract some results obtained while investigating the development of the Hydromedusa Gonionema Murbachii, the subject of this dissertation. A third paper appeared in the University Circulars on a jelly-fish found at Nassau, N.P.Id., not previously known on this coast.