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CARNEGIE INSTITUTION OF WASHINGTON
ALFRED G. MAYER, DIRECTOR

PAPERS FROM THE TORTUGAS LABORATORY

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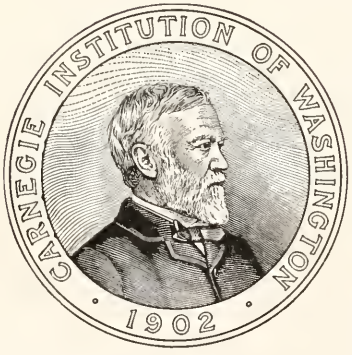
CARNEGIE INSTITUTION OF WASHINGTON

VOLUME I



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I. THE GERMINAL SPOT IN ECHINODERM EGGS

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THE GERMINAL SPOT IN ECHINODERM EGGS.

By H. E. Jordan.

The object of this paper is to report the results of further studies of the prematuration stages of echinoderm eggs. The main problem involved is to determine the relation of the nucleolus to the chromosomes during the growth-period. I have recently shown that in both *Asterias forbesii* and *Hipponoë esculenta* the chromosomes for the first maturation mitosis arise from the nuclear reticulum with some variation of details in the two species.[1] In *Asterias forbesii* the chromosomes subsequent to origin assume a more or less intimate connection with the nucleolus just prior to maturation, and the nucleolus, after passing through a preliminary process of fragmentation, apparently contributes chromatic material to the chromosomes. In *Hipponoë esculenta* the relationship between nucleoli and chromosomes—if indeed one exist at all—is more obscure, but the nucleoli here also disappear about the time of maturation. The ultimate aim of these studies is to obtain some information regarding the function of the germinal spot.

I am now able to report upon two additional species of echinoderms, one again a star-fish (*Echinaster crassispina*) and the other a brittle-star (*Ophiocoma pumila*). The latter appears to agree rather closely with what obtains in *Hipponoë* and with what Wilson[2] reports of some of the sets of eggs of *Toxopneustes variegatus* treated with $MgCl_2$; while *Echinaster* presents a case unique in that the chromosomes here appear to arise as the direct products of nucleolar fragmentation. I regret that my material does not yield stages for the study of either the oögonial history or the maturation mitoses, but the various stages of the growth-period, which are represented in great variety and abundance, give conclusive results in regard to the essential point, *i. e.*, the origin of the chromosomes. I trust the coming summer will yield the stages desired for a more complete study of the oögenesis of these highly interesting forms.

The material at my disposal was collected during a brief stay at the Marine Biological Laboratory of the Carnegie Institution of Washington,

[1] Jordan, H. E., 1908. The relation of the nucleolus to the chromosomes in the primary oöcyte of *Asterias forbesii*. This volume, p. 39.

[2] Wilson, E. B., 1901. A cytological study of artificial parthenogenesis in sea-urchin eggs. Arch. Entwickl., Mech. Bd. 12: 529.

located on Loggerhead Key, Florida. I take this occasion to acknowledge my indebtedness to the above Institution, and particularly to Dr. Alfred G. Mayer, director of the Laboratory, whose cordiality, unstinted aid, and kindness have made this much of the work possible and the collection of the material a real pleasure. Of *Ophiocoma* there was very abundant material on the reef near Dry Tortugas. These eggs were almost ripe at the time of my departure from the key. The sperm were already ripe and very active at this time. Of *Echinaster* only a single specimen was found, and this quite accidentally in the moat about Fort Jefferson. I have since learned that this form is very abundant at the Marquesas Keys and I hope to obtain material from there during the coming summer. This appears to be a most promising object for future study.

The ovarian material was fixed in the sublimate acetic mixture. The sections were cut at 8 micra and stained according to Heidenhain's iron-hematoxylin method and some were counterstained with eosin. The accompanying drawings were made with a Bausch and Lomb $\frac{1}{12}$ oil-immersion lens with a No. 1 ocular, the outlines being obtained by aid of an Abbe camera lucida with the drawing surface 150 mm. below the level of the stage. The details were filled in free hand after study with a Zeiss 2 mm., aperture 1.30 apochromatic lens.

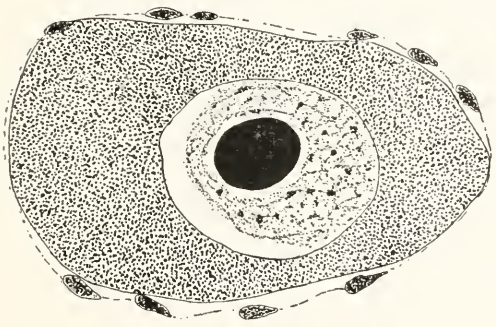
ECHINASTER CRASSISPINA.

The egg of this species at the culmination of the growth-period is very large (fig. 5, *a*). Its nucleus has a diameter of about 300 micra. The cytoplasm is of a beautiful large alveolar type (fig. 7). In the preserved material the nucleoplasm has contracted, leaving a lacuna which is partially filled with a homogeneous coagulum. The nucleoplasm is also homogeneous or very finely granular and non-stainable in basic dyes. Scattered throughout the nucleus are very many (a hundred or more) chromatic masses, most of which have the form of typical tetrads (fig. 5, *b*). Study of abundant transition stages from the ovum at the beginning of the growth-period to the full-grown egg above described reveals the complete and continuous history of the origin and development of the characteristic features of the nucleus and cytoplasm.

The very young egg (figure 1) is already large as compared with the eggs of most echinoderms. It is surrounded by a very thin nucleated membrane of the ovarian stroma. Its cytoplasm is coarsely granular. The nucleus frequently appears shrunken away from one side of its wall, leaving a crescentic lacuna. The nuclear reticulum is delicate, coarse-meshed, and pale-staining, with occasional flakes of chromatic material. The nucleolus is intensely chromatic, homogeneous, and of sharp outline. A slightly later stage shows very decided alterations, both in the cytoplasm and nucleoplasm (fig. 2). Many of the cytoplasmic granules have become greatly enlarged

and appear as yolk-spherules scattered in a granulo-reticular matrix. The nuclear reticulum has become coarser but is still pale-staining. Here and there are scattered chromatin threads of beaded appearance. The nucleolus is breaking up into many intensely chromatic globular bodies.

Successively later stages show a continuation of the above process. Figure 3 illustrates a stage where the nucleolar fragmentation and dispersal has progressed a little farther. The nuclear reticulum is similar to its appearance in the last stage, except that there are fewer of the beaded chromatin threads and, in this particular example, contraction of the nuclear reticulum again produced a peripheral artifact. Examples could have been selected where such contraction artifacts were lacking, but this particular one was chosen to demonstrate by comparison with figures 2 and 4 that the physical effects of the preserving fluid did not essentially modify the progressive changes in the nucleolar history. The cytoplasm now has the appearance of mixed granular and alveolar type. The large yolk-spherules have disappeared, probably by a transformation into fluid, a continuation



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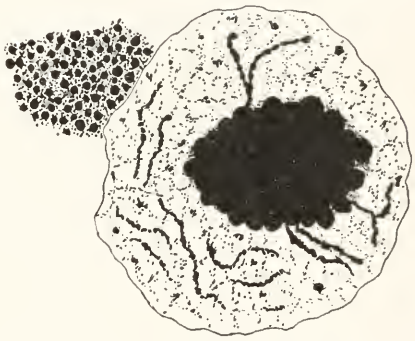

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FIG. 1.—Young ovum of *Echinaster crassispina;* nucleolus large, compact, homogeneous, intensely chromatic and with sharp contour; nuclear reticulum pale and shrunken away from wall at left; cytoplasm dark and coarsely granular. × 1500.

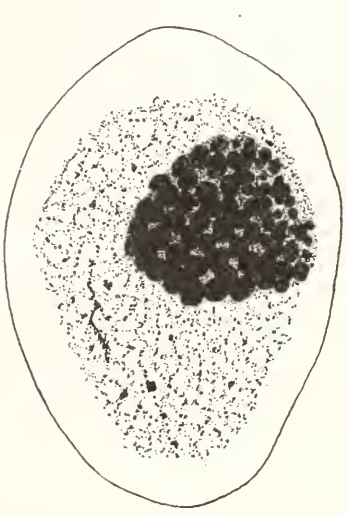
FIG. 2.—Nucleus at slightly later stage. Nucleolus breaking up into globular masses. Nuclear reticulum pale, but through it are scattered chromatic beaded threads. A portion of cytoplasm shown at left. It is still dark and granular, but many of the granules (yolk) have greatly enlarged. × 1500.

of which process for all the yolk-granules, large and small, culminates in the beautiful alveolar cytoplasm of the ripe egg (fig. 7). Figure 4 gives a stage in the dispersal of the nucleolar fragments. The products vary between wide limits, both in size and form, but most may be described either as globes, dumb-bells, or tetrads.

The culmination of the process of dispersal is illustrated in figure 5. Figure 6 shows similar nucleolar fragments from a portion of a single nucleus. Most of these have the form of typical tetrads. The unit of structure here seems to be a globe and the individual mass a four-lobed

body. Single, bilobed, and trilobed bodies can be explained in many instances as portions of tetrads, the latter having been cut in various planes or at different levels by the microtome knife. Several of the beaded chromatic threads have persisted, but they are frequently entirely wanting, always few in number and very variable in morphological characters. They may perhaps represent chains of smaller nucleolar fragments.

I am inclined to believe that these very definite four-lobed chromatic bodies are chromosomes, but I am aware that many objections may be made to such an interpretation. The only decisive test of the matter is lacking until the conduct of these elements is observed at the time of the formation of the maturation spindle. But whether already chromosomes or not, they must at least represent stages in the formation of the definitive



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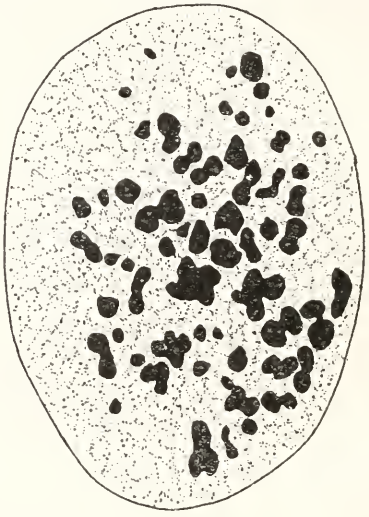

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FIG. 3.—Nucleus at still later stage. Nucleolar fragments separating. Nuclear reticulum, which remains pale-staining, shrunken away from wall. Cytoplasm still dark-staining and now appears as a mixture of fibrillar and alveolar types and contains abundant microsomes. × 1500.

FIG. 4.—Nucleus of later stage, showing chromatin products of nucleus scattered through pale reticulm. × 1500.

chromosomes. However, both cytological study of the eggs and macroscopical examination of the gonads indicates that the eggs are close to maturation and that the chromosomes have already final characters. For most echinoderms thus far reported on, the somatic number of chromosomes is 36. If these bodies are really chromosomes their number (reduced) here must be several times as many. However, according to Boveri, *Echinus microtuberculatus* has only 18 somatic chromosomes, and Tennent[1] reports a

[1] Tennent, D. H., 1907. Further studies on the parthenogenetic development of the star-fish egg. Biol. Bull., Vol. 13, No. 6.

probably similar number for *Asterias vulgaris*. Since the number is known to be less than 36 in some forms it is reasonable to expect that it may be more in other forms. Again, it is possible that the quadripartite masses may represent only parts of final chromosomes. But whatever the value of these bodies in terms of a univalent chromosome it is clear that they originated as the result of the fragmentation of the nucleolus. Nor can the chromosomes originate from elsewhere, for there appear no other chromatic structures anywhere in the history of the growth-period, except the occasional beaded threads which are not invariably present and have the same elongate thread-like appearance in the latest as in the earliest stages of development. These threads are very similar to those occasionally seen in the growing egg of *Asterias forbesii* where they are known not to be chromosomes, but where they probably represent streams of chromatin material in transit to or from the nucleolus.

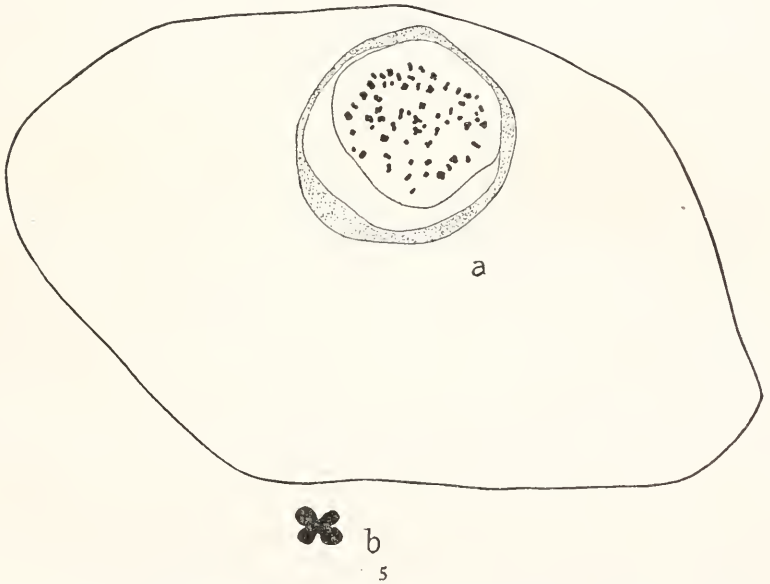

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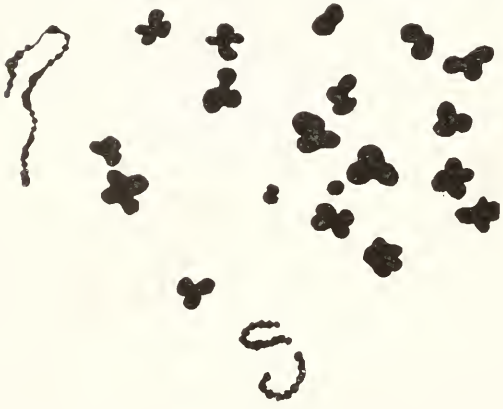
FIG. 5.—*a*, ovum of *Echinaster* at culmination of growth-period. Nucleus shrunken away from wall, leaving a lacuna partly filled by a homogeneous coagulum. Nucleoplasm very finely granular and pale-staining. Throughout it are scattered many chromatic bodies composed of one, two, three, or four globes. Many assume a typical tetrad-shape. *b* shows such a one magnified. × 1500. The cytoplasm is light-staining and typically alveolar. × 160.

Again, it might be objected that these bodies are products of nucleolar degeneration. But in reply it can be said that all the eggs are of similar constitution and that no other portion of the individual eggs gives any evidence of degeneration. Furthermore, it is possible to find hundreds of transition stages between those illustrated in figures 1 and 5, and the change is a progressive one from the very first intimation of fragmentation to the

complete dispersal of the definite and sharply contoured resulting bodies of the full-grown nucleus. In *Asterias forbesii* germinal vesicles were occasionally found in which were scattered as many as fifty or more small chromatic masses. These were interpreted as abnormalities or the result of degeneration. But in this case the bodies were always larger or smaller globes and were found only in the younger eggs. Nor could the nucleolus ever be seen as a whole to break up into such. The striking thing about *Echinaster* is the change of these elements from a globular form to definitely four-lobed bodies.

The case seems to be very clear for *Echinaster crassispina* that here the chromosomes arise exclusively from the nucleolus, the latter not even leaving a plastin remnant behind. The function of the germinal spot here seems to be wholly that of a storehouse of chromatin for the chromosomes, the latter being apparently, at least during the early history of the growth-period, compacted into one solid homogeneous mass or chromatin nucleolus, and their separate individuality merged into one common chromatic whole. It



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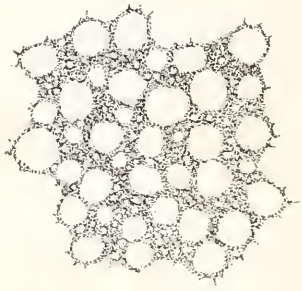

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Fig. 6.—A number of chromatic bodies from portion of a single nucleus; the majority have the form of tetrads and several are arranged in shape of chromatic threads. × 1500.

Fig. 7.—Portion of cytoplasm from an ovum at culmination of growth-period. × 1500.

would be most interesting to observe here the final telophase of the oögonial division, and to study the process of synapsis and the construction of the nucleus and nucleolus of the resting stage of the oöcyte. The eggs are of such goodly size that this part of the investigation seems very promising.

All that the present material yields of definite fact is that the chromosomes arise as the products of a process of fragmentation of the nucleolus. Nor is the nucleolus here a double structure, though in the very early stages there is indication of a plastin ground-substance. But concerning the complete function of the chromatin nucleolus, its relation to a true nucleolus or plasmosome, as also its relation to an accessory chromosome, and concerning

the question of the individuality of the chromosomes, nothing definite can be adduced. The process of chromosome formation here seems to be unique among echinoderms. The only similar case known to me is that described by Wilson in some of the sets of sea-urchin eggs stimulated to develop parthenogenetically by $MgCl_2$, and here the chromatin nucleolus first resolved itself into a chromatic reticulum which subsequently broke up into chromosomes.

OPHIOCOMA PUMILA.

The eggs of this brittle-star are of moderate size, being about that of *Asterias forbesii*. Figure 11 shows an egg near the culmination of the growth-period. Maturation is probably imminent, for the nucleus has moved near the periphery and the nuclear wall is much shriveled. This assumption is confirmed by the fact that male individuals of this same collection carried sperm in ripe condition and very active. The cytoplasm is of the reticular type, with many microsomes and innumerable large dark-staining yolk-spherules. The nucleus has a homogeneous or very finely granular structure and remains unstained in basic dyes. Eosin reveals a delicate network. Scattered through the achromatic nucleoplasm are irregu-

8 9 10

FIG. 8.—Young ovum of *Ophiocoma pumila;* nucleolus homogeneous; intensely chromatic and with definite contour; nuclear reticulum wide-meshed, heavy and very chromatic; cytoplasm reticular, dark and with abundant granules. × 1500.

FIG. 9.—Nucleus at stage near culmination of growth-period; nucleolus intensely chromatic; reticulum massed at one pole and beginning to break up into chromosomes, some of which have a tetrad form. The nucleoplasm appears homogeneous or finely granular and is pale-staining. The cytoplasm is reticular with many large yolk-spherules. × 1500.

FIG. 10.—Nucleus at slightly later stage; wall much shriveled. A portion of the chromatin thread is still breaking up into chromosomes, some of which have the form of tetrads, and frequently one or several are attached to the nucleolus. × 1500.

lar, mossy, deeply-staining chromosomes. They vary much in size and shape, but some appear as tetrad-like bodies (figs. 9–13). The nucleolus has persisted as a compact, homogeneous, intensely chromatic structure with sharp outline and of original bulk. It is partly surrounded by what in many cases appears to be a vacuole. In figure 11 a delicate pale-staining reticulum appears in the vacuole. In many eggs at this stage (or probably of a slightly later stage) the nucleolus seems to disappear by lysis, leaving a pale outline of its original form. I was at first inclined to interpret the apparent

vacuoles as the result of a contraction of the nucleolus due to the action of the preserving fluid, and a subsequent pushing of the hardened nucleolus from its original position by the microtome knife. Further study and comparison with pictures like that presented in figure 13, where the "vacuole" is replaced by a body with a coarse chromatic reticulum, and the observation that the chromatin mass is always to one side, but not entirely outside of the "vacuole," compels the conclusion that one is dealing with a double nucleolus, *i. e.*, with a chromatin nucleolus and a true nucleolus or plasmosome.

It may still be true that the plasmosome serves merely as a plastin ground-substance for the chromatin nucleolus, as in *Asterias forbesii*, and that preserving produced unequal contraction in the chromatin and plastin,

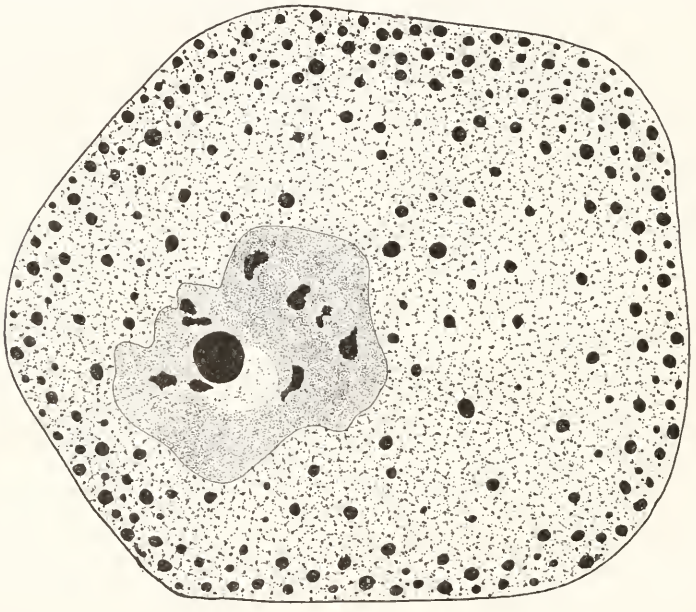

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FIG. 11.—Ovum of *Ophiocoma* at culmination of growth-period; nucleolus still chromatic and with definite contour and partially surrounded by a vacuole through which extends a very pale and delicate reticulum; chromosomes of various shapes and sizes are scattered through the finely granular nucleoplasm. The cytoplasm has a reticular structure and contains very many yolk-spherules and microsomes. × 1500.

and a subsequent shifting, either artificial or natural, brought them into the relation seen in figures 11 and 13. But the fact that the plastin-nucleolus is not always present in the same section with the chromatin nucleolus (and where the latter is of the same size as where a plasmosome is present), as in figures 9, 10, and 12, indicates that there is here a plasmosome and a chromatin nucleolus. That the latter is not of the nature of an accessory chromosome is seen by the fact that it fades out in the later stages prior to

maturation. The vacuole when present is to be interpreted as the result of a resorption of the plasmosome or perhaps plastin ground-substance.

Ophiocoma presents a clear case where the chromosomes are derived exclusively from the nuclear reticulum. At a very early stage in the growth-period the ovum has a coarse granulo-reticular cytoplasm (fig. 8). The germinal vesicle contains a homogeneous, intensely chromatic nucleolus of sharp contour. The nuclear reticulum is heavy, wide-meshed, and very chromatic. Later stages show the segregation of this reticulum at one pole of the nucleus—sometimes about the nucleolus, sometimes at the opposite pole—as a tangled spireme. When insufficiently destained in the iron-alum solution, such stages do not reveal the thread-like character of this structure, but the entire area stains as a solid irregular chromatic mass. Subsequently the thread unravels and segments into chromosomes (fig. 9), some of which soon assume the shape of tetrads. They have an irregular outline and mossy appearance. Frequently one or several are attached to the chromatic nucleolus, and here, as in *Asterias forbesii,* there seems to be a tendency on the part of the thread to become attached to the nucleolus. Figure 9 shows the spireme partially segmented into chromosomes and the remaining thread

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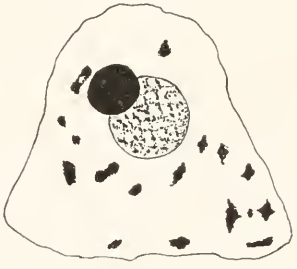

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Fig. 12.—Nucleus at culmination of growth-period showing chromosomes of various shapes and sizes scattered through a pale, homogeneous or finely-granular nucleoplasm. × 1500.

Fig. 13.—Nucleus at final stage of growth showing chromosomes with appearance of tetrads and an intensely chromatic nucleolus connected with a larger spherical body of sharp contour and with a chromatic reticulum corresponding to the vacuole of fig. 11. × 1500.

almost in contact with the nucleolus. In figures 10, 12, and 13 are shown later stages of the same process of chromosome formation, the process being not yet complete in figure 10. Figure 12 shows 18 chromosomes (reduced number) and figure 13 shows 17 chromosomes. The exact number could not be definitely determined, but it is somewhere close to 18.

Here the chromosomes arise very clearly from the nuclear reticulum. The process here agrees essentially with that previously described for *Hipponoë esculenta* (except that *Hipponoë* showed no plasmosome), but differs again from what Wilson described for some of the two sets of *Toxopneustes* eggs artificially fertilized with $MgCl_2$ in that there is here a chromatin nucleolus in addition to the plasmosome common to both forms.

SUMMARY AND CONCLUSION.

In *Echinaster crassispina* the chromosomes are derived exclusively from the nucleolus. In *Ophiocoma pumila* the chromosomes arise exclusively from the nuclear reticulum. The germinal vesicle of both species contains a chromatic nucleolus. There are here two extreme types. *Asterias forbesii* furnishes an intermediate type in that here the chromosomes assume a more or less intimate connection with the nucleolus prior to maturation and receive substance therefrom. Eggs of *Toxopneustes variegatus,* parthenogenetically developed after treatment with $MgCl_2$, according to Wilson yield the two extremes in different sets, there being in one case present a plasmosome and in the other a chromatic nucleolus. It appears that different forms of echinoderms differ in the matter of the origin of the prematuration chromosomes. In some species the chromosomes arise from a chromatin-nucleolus, in others from a chromatic reticulum, and in still others in part from one source and in part from the other. Again, the eggs of different forms appear to differ in that some have only a chromatin-nucleolus, without distinct plastin ground-substance, resting in an achromatic nuclear reticulum (*Echinaster*); others possess both chromatin-nucleolus and plasmosome as well as a chromatic nuclear reticulum (*Ophiocoma*); and still others possess a double nucleolus (chromatin nucleolus and plastin ground-substance), with the chromosome complex gathered in a mass in the achromatic reticulum (*Asterias*).

The chromosomes thus arise inconstantly in different species from any part of the germinal vesicle that contains the chromatin material, and this may be either nucleolus, nuclear reticulum, or both. The function of the germinal spot then appears, in part at least, to be that of a storehouse of material which is to contribute to the formation of the chromosomes. What chromatin is not so employed is resorbed by the cytoplasm, probably returning to the elements from which it was elaborated and serving as a food material. There appears nothing here to support or confirm the theory of the individuality of the chromosomes, but rather much to arouse suspicion regarding the theory. But one may take refuge in the idea of "centers of chromosome activity" as suggested by Davis,[1] and so the chromatin may perhaps be regarded as merely the garb for the determinants of inheritance, and the characters that arise in their manifold variations as the result of a quantitative as well as a qualitative distribution of chromatin.

[1] Davis, B. M., 1905. Studies on the plant cell. Am. Nat., Vol. 39.

II. THE SPERMATOGENESIS OF APLOPUS MAYERI

By H. E. JORDAN
Of the University of Virginia

Plates 1–5

THE SPERMATOGENESIS OF APLOPUS MAYERI.

By H. E. Jordan.

INTRODUCTION.

The object of the present investigation is primarily to trace the history of the accessory chromosome through the various stages in the process of spermatogenesis in the phasmid *Aplopus mayeri*. Incidentally an effort is made to present in as concise a form as appears compatible with completeness the several salient points of similarity and difference between the growth and maturation phenomena as they obtain in *Aplopus* and other Orthoptera previously studied.

The study of the accessory chromosome is approached from the standpoint of its possible relation to the determination of sex as first suggested by McClung (1901), and its support of the hypothesis of the morphological and physiological individuality of the chromosomes as enunciated by Rabl (1885) and later extended by Boveri (1902), Sutton (1902), Montgomery (1904), Baumgartner (1904), and others.

This study purports to be mainly a cytological one. The microscopic anatomy, as also the anatomical relations of the gonads of *Aplopus mayeri*, is essentially similar to that so well described and illustrated by de Sinéty (1901) in the case of *Leptinia attenuata* and *Menexenus obstusespinosus*. Nor shall I here attempt a review of the literature on the subject of the "heterochrosomes" (Montgomery, 1901). This has been very excellently done by several cytologists (Sutton, 1900; McClung, 1902; Montgomery, 1904) and more recently and very completely by Boring (1907).

It seems necessary to state at this point only that the chromosome, for which I have adopted McClung's (1901) terminology, "accessory chromosome," has been described as a closely similar structure in many of the insects, especially the Hemiptera, under the several names of "odd chromosome" (Stevens); "chromatin nucleolus" (Montgomery); "chromosome speciale" (de Sinéty) and "heterotropic chromosome" (Wilson).

MATERIAL AND METHODS.

The material upon which the investigation is based was obtained from Loggerhead Key, Florida, through the kindness of the Carnegie Institution

of Washington and particularly Dr. Alfred G. Mayer, director of the Marine Biological Laboratory at Dry Tortugas.

The testes were fixed in sublimate acetic and Flemming's strong fluid, both methods yielding beautifully preserved specimens. Heidenhain's iron-hematoxylin long method was employed almost exclusively, both with and without counterstaining. The various structures were studied in the light of their morphology rather than their staining reaction and always at the stage of moderate decolorization. However, methyl green and thionin were also employed and confirmed in every detail (except in the case of the ripe spermatozoon, to be noted later) the results of the morphological study in the hematoxylin-stained sections. The sections were cut at 6.67 micra.

OBSERVATIONS.

The testes are paired and consist of two long thin-walled follicles which extend for a considerable distance on either side of the abdomen. Each contains a ventral collecting duct which is continuous with a vas deferens. The follicles are composed of many cysts. Spermatozoa are found in large numbers in the proximal (posterior) end of the collecting duct; immediately surrounding this area is the zone of spermatids; then appear the spermatocytes, and then, when present, the secondary and primary spermatogonia. Usually only the later stages are present in the proximal end of the testis and only the earlier in the distal end.

PRIMARY SPERMATOGONIUM.

The follicular wall consists of large flattened cells with elongate vesicular nuclei. This wall is continuous with the wall of the vas deferens, as well as with that of the numerous cysts. Except at the proximal end the follicular wall appears to be two cells in thickness. The inner cells are large and polyhedral, with very large oval vesicular nuclei, the wall of which is frequently lobed (fig. 2), and with a small amount of cytoplasm. These are the primary spermatogonial cells in the resting stage (fig. 4). At the distal end of the follicle, primary spermatogonia are abundant and the two-layered condition of the wall is not apparent. Nuclei of the cyst-walls are essentially similar to those of the primary spermatogonial and follicular cells. They vary in size between the latter cells, are vesicular, frequently lobed, and contain a delicate reticulum with occasional karyosomes (fig. 3). Neither of these nuclei contain a plasmosome. There are unmistakable signs of amitotic division among the primary spermatogonial cells next the follicular wall resulting in binuclear or polynuclear cells (fig. 1). Less convincing evidence of amitosis appear also among the nuclei of the follicular and cyst-walls. All three types of cells are frequently seen in karyokinesis and the process appears identical in each. The close similarity of nuclear structure and nuclear changes during division among the three types of cells forces the conclusion that they are identical.

Karyokinesis is initiated by an increase of stainable material in the linin network, a subsequent diffusion of the karyosomes and the arrangement of the chromatin into a spireme. This segments into a number of coarse mossy or granular deep-staining threads, which give indication of a longitudinal split (fig. 5). Presently the split is consummated, and the segments now assume the form of slender bipartite rods of varying length (fig. 6). By the time the chromosomes have entered the equatorial plate at the end of prophase, they have attained greater bulk, more definite contour, and greater affinity for basic dyes. The chomosomes are of various shapes, several are large and typically U-shaped, and the number is 35 (figs. 7 and 8). During metakinesis (figs. 9 and 10) the chromosomes separate, probably along the line of the longitudinal split seen in the prophase, similar variations in size obtain as in the prophase, and the halves are drawn to their respective poles, thus producing two secondary spermatogonia. Around these two cells a membrane appears—the persisting cell-wall of the mother primary spermatogonial cell—forming a two-celled spermatocyst (fig. 11).

During metakinesis the only kinoplasmic structures which are clearly visible are the spindle fibers. In the late telophase a very conspicuous mid-body appears, composed of a row of minute, deep-staining granules. Figure 13 shows a two-celled spermatocyst, one of the cells of which is in telophase. I have been able in my material to definitely determine upon at least three generations of secondary spermatogonia. There are probably several times as many, the early orders being so closely similar as to make identification uncertain. Sutton, in the case of *Brachystola magna,* was able to distinguish seven or eight orders of secondary spermatogonia. There are also several—probably many—orders of primary spermatogonia in all essential respects identical. It is possible to distinguish the primary from the secondary spermatogonia by the fact that the former have vesicular nuclei, often lobed, and a relatively small amount of chromatin, as well as by the fact that they are disposed irregularly and not in cysts, as are the older generations. From a telophase in which the daughter-chromosomes pass through a pale-staining granular stage arises the resting stage of the first order of the secondary spermatogonia.

SECONDARY SPERMATOGONIUM.

These cells in the various orders are in all respects similar until the final order is attained. It should be added here that the succession of events throughout the entire spermatogenesis could be definitely determined by the fact that cysts could always be found containing transition stages to and from the typical phase for that particular cyst, and these transition stages overlapped in the several cysts to such an extent as to render clear the order of succession. In the resting stage of the first order of secondary spermato-

gonia a deep-staining chromatin nucleolus (accessory chromosome) appears in the almost achromatic nuclear reticulum, of sharp contour and usually closely applied to the nuclear wall (fig. 13). Thus the accessory chromosome first appears as a definite characteristic nuclear structure in the resting stage of the first order of secondary spermatogonia. It answers to the various morphological and microchemical tests for a chromosome. There was nothing corresponding to this body in the resting stage of the primary spermatogonia, nor yet in the late telophase of the final mitosis. Since the number of chromosomes of the later spermatogonial cells remains the same as that of the primary spermatogonia (35), the accessory represents probably a specifically modified metabolic phase of an ordinary chromosome that had passed into the reticular stage in the telophase and returned to the compact stage much in advance of its fellows. At the next mitosis it passes without visible change into the equatorial plate with the chromosomes that arise from the segmented spireme of the prophase, and its presence there does not alter the constant count of 35 chromosomes for the unreduced number.

During the prophase of the ensuing mitosis the chromatin passes through the fine, coarse, and segmented spireme stages (figs. 14 and 15). Frequently the accessory chromosome gives indication of a bipartite structure presaging its later division in metakinesis. The long, mossy, deep-staining segments of the prophase shorten, condense and split longitudinally into pairs of short, slender rods, among which the accessory chromosome has become unrecognizable. These pairs of rods unite and are assembled in the equatorial plate as very chromatic rod-shaped bodies of variable size. Usually one among the number is typically **U**-shaped (fig. 17). The chromosome count is constantly 35 (figs. 17 and 18). Occasionally an equatorial plate showing as many as five **U**-shaped chromosomes similar in size and shape (fig. 20) is found. Figure 21 shows four spindles with the chromosomes at various stages of metakinesis. There is no mark or sign by which the accessory chromosome can be recognized at this stage. In the ensuing telophase stages (figs. 22 and 23) one pair of chromosomes always lags somewhat behind its fellows. This pair of chromosomes corresponds to those designated by de Sinéty during this same stage as the "chromosomes spéciales." However, while there is always one lagging pair, there may be several (fig. 23), and this fact renders the precise determination in all cases of the accessory chromosome impossible.

Figure 24 shows a twin spindle with the chromosomes at telophase. Again there is a lagging pair. This figure is undoubtedly the result of an amitotic nuclear division in an early primary spermatogonial mitosis resulting in a binucleate cell. The daughter-cells of such a doubly-endowed mother-cell probably give rise to the primary spermatocytes with double the number of chromosomes (36 in this case, 2×17 ordinary $+ 2$ accessory chro-

mosomes = 36) which are occasionally met with, and these in turn to giant spermatids and giant spermatozoa, which are also of frequent occurrence. In the telophase of this mitosis a mid-body again appears, similar to that of the previous division. At this stage the accessory chromosome is again unrecognizable, having probably assumed a brief diffuse form, and is thus lost among the ordinary chromosomes that are passing into the reticular stage of the resting nucleus of the final order of secondary spermatogonia (fig. 25).

In the resting stage of the last order of secondary spermatogonia a chromatin nucleolus (accessory chromosome) again appears in the almost achromatic nuclear reticulum (fig. 26). Its contour is less sharp than in the earlier spermatogonial stages, but it assumes its characteristic position near or closely applied to the nuclear wall. In late stages of the prophase it again appears bipartite. The chromatin again passes through the fine, coarse, and segmented spireme stages (figs. 27, 28, 29, 30 and 31), and compact chromosomes of comparatively small size and less variable form (35 in number) are drawn into the equatorial plate (figs. 32 and 33). A pair of chromosomes (daughter-chromosomes, products of a premature division) are frequently observed to enter the spindle perpendicular to its fibers (figs. 34 and 35). This unique chromosome is probably the accessory, since the latter had partially split already in the prophase.

During metakinesis the dumb-bell-shaped chromosomes become more and more elongate until at anaphase (fig. 36) the connecting chromatic fiber is broken and the chromosomes pass into telophase again with one or several pairs lagging behind (fig. 37). Late telophase stages are shown in figures 38, 39, and 40, the latter as well as the subsequent stages again showing a very conspicuous mid-body. Figures 41 and 42 show the chromosome complex at still later telophase, and the final stages succeed each other in the order given in figures 43, 44, and 45. This time the accessory chromosome does not pass through a reticular stage as previously (or perhaps such a phase is really assumed, but is of extreme brevity), for it is recognizable as such among the granular pale-staining, indefinitely contoured chromosomes of the final stage of telophase. A brief resting stage now ensues, during which the accessory chromosome is very conspicuous in the delicate achromatic nuclear reticulum, of sharp contour and closely applied to the nuclear wall (fig. 46).

PRIMARY SPERMATOCYTE.

The resting stage appears to be very transitory, for almost immediately decided alterations begin to transpire in the nucleus. The reticulum gains chromatin and occasional small karyosomes may appear (fig. 47). In the next stage, which is of comparatively long duration, the reticulum has arranged itself into a close-meshed lattice-work of wide threads. The accessory chromosome has an oval shape, intense staining capacity, and is closely

applied to the nuclear wall (fig. 48). Successive stages show the reticulum arranging itself into the form of a long, continuous (?) thread, more chromatic than during the immediately preceding stages. Attached to one end is the much-elongate, club-shaped accessory chromosome (fig. 49). Figure 50 shows two daughter-nuclei at this stage still connected by a cytoplasmic bridge of the mother-cell. Here the nuclear reticulum is still more or less of a lattice-work character, but the threads are thinner and more chromatic than in figure 48 and the accessory is already decidedly club-shaped. Successively later stages reveal a segmentation of the continuous thread and the arrangement of these segments into loops at one pole of the nucleus (figs. 51, 52, and 53). The final stage probably represents the synizesis stage of McClung. While these changes are taking place in the general reticulum, the accessory chromosome lengthens into a heavy rod-like structure, always with the pointed end attached to the chromatic spireme. Presently it begins to split longitudinally (fig. 52), and at synizesis it has also assumed the form of a loop, intensely chromatic, however, in contrast to the lighter-staining loops at the pole. The loops now begin to straighten out (figs. 54 and 56) and unite at their free ends into pairs, forming two-armed larger pointed loops (figs. 54 and 55). These loops are in length almost equal to the diameter of the nucleus, whereas the loops of synizesis were only of the length of the radius. This is the synapsis stage.

The point of synapsis is frequently very definite and after the consummation of the process is marked by a more intensely staining area. In synapsis the longitudinal split of the accessory is again closed up, and this structure becomes again a compact, deep-staining, more or less club-shaped body closely applied to the nuclear wall and now apparently unconnected with any of the chromosomal loops. It is as though the univalent accessory chromosome had segmented for the purpose of again uniting the products in unison with the synapsis of the ordinary chromosomes. If synapsis, then, means the fusion of pairs of chromosomes to produce bivalents, and since the accessory has no mate, the reduced number of chromosomes should theoretically be 18, and this is just what one finds in the equatorial plates of the ensuing mitoses (figs. 74, 75, 76, and 77). Figures 57 and 58 represent later stages in synapsis and are probably identical with the "bouquet stage" of Eisen. In figure 59 is shown a late postsynaptic stage which is very similar to the early presynaptic stage (fig. 48), except that the chromatin thread is not disposed in a regular lattice-work fashion and the reticulum is more highly chromatic. A very brief resting-stage is again interpolated after post-synapsis (figs. 60 and 61) just as before presynapsis, but almost immediately signs of the subsequent prophase appear.

In figure 60 the accessory chromosome is ring-shaped (representing a hollow sphere), an appearance frequently met with in methyl green preparations. In fig. 61 the accessory is very chromatic and applied to the nuclear

wall; in figure 62 it begins to assume a bipartite form (a preparation for its division in the telophase of the first maturation division). At this stage the sparse cytoplasm of the primary spermatocyte frequently contains several deeply-staining spherical bodies (fig. 63). These probably represent basichromatin rejected during the last preceding mitosis or the succeeding synapsis stage, for such bodies are occasionally seen in process of transit from the nucleus to the cytoplasm.

Preparatory to the first maturation mitosis the nuclear reticulum passes through the fine, coarse, and segmented spireme stages (figs. 62, 63, and 64). These segments are at first delicate and stain intensely. Subsequently they lose their staining capacity and assume a mossy or granular form. At this stage a longitudinal split appears in the segments (figs. 65 and 66). This is followed by a transverse split and typical tetrads are now formed, including **U** and ring-shaped forms (figs. 67, 68, 69, 70, and 71). During these changes among the ordinary chromosomes the accessory has retained its compact form and intense staining capacity, though it appears in various shapes (figs. 64, 66, 67, 68, 69, and 70). The splits in the tetrads close up again and the chromosomes pass into the late prophase as compact, deep-staining bodies, among which the accessory is only occasionally to be recognized (fig. 73). In equatorial plates of the prophase spindle the accessory chromosome can frequently be identified by its characteristic **U**-shape or larger size (figs. 74, 75, 76, and 77). The number of chromosomes here is 18. During metakinesis the bivalent chromosomes divide into two elongate products of various sizes and shapes, most conspicuous among which are long and short rods, cones, clubs, wide, shallow, **U**-shaped bodies, and elements with the shape of short golf sticks (figs. 78, 79, 80, 81, 82, 83, and 84). Usually a chromatin-connecting fiber remains until the early anaphase (fig. 83). The accessory meanwhile has passed undivided to one pole and in advance of the ordinary chromosomes and generally retains a **U**-shape (fig. 90). Occasionally, however, it becomes a double structure (figs. 81, 87, 88, 89, and 92), the result of a premature fission in anticipation of its division in the secondary spermatocyte mitosis. Among the ordinary chromosomes also some frequently appear double in the late anaphase and telophase, thus also evidencing a premature division.

During metakinesis a longitudinal split frequently appears in the elements of the dividing chromosomes (fig. 78). Thus the bivalent chromosome again assumes the tetrad condition of various forms (fig. 119). Upon the question as to which of these splits represents the longitudinal split of the early prophase and which the subsequently formed transverse split hinges the decision as to whether the first maturation division is a reducing or an equation division. In *Scolopendra,* Blachman (1903) finds a similar sequence of events in regard to the chromosomes during the early prophase, and on the basis of his own observations and the fact that "in all the inves-

tigations with which I am acquainted it has been reported that the longitudinal cleavage is first to be made evident in the prophase" believes that "it is only logical to conclude that this division is completed by the first spermatocyte mitosis." Upon the question of the sequence of the two spermatocyte divisions in Arthropoda the various workers are about equally divided. McClung, in the case of some Orthoptera studied, believes that the equation division comes first. Von Rath (1895), Henking (1890), Paulmier (1899), and Montgomery (1898, 1900, and 1901) arrived at the opposite conclusion in the case of various arthropods. Since the same result is obtained for the spermatozoa in either event, this point is really not of the vital importance it was formerly believed to possess. Nevertheless, it will be noted that during the later prophase, when the longitudinal and transverse splits again close up and compact chromosomes are formed, the long axis of these chromosomes does not appear to become the short axis and *vice versa,* but the earlier proportions and relations are approximately adhered to; therefore, since the first maturation division takes place transversely to the long axis of the chromosome, it appears that the first division of this mitosis is along the line of the second split marked out in the early prophases. If the generally accepted interpretation of synapsis is correct, *i. e.,* that it represents an end-to-end (telosynapsis) union of two chromosomes (paternal and maternal, Montgomery, 1901), and, furthermore, if these chromosomes do indeed separate during maturation along the line of their previous fusion, then the tetrad figures of the early prophase represent bivalent chromosomes divided first longitudinally or in the plane of the long axis (fig. 67), followed by a transverse split, *i. e.,* along the line of previous fusion and perpendicular to the long axis. Accordingly, the first division of the long chromosomes at metaphase must represent the transverse or second split of the early prophase and is a reducing division, since it separates whole chromosomes. Figure 119 shows several of the characteristic dividing figures (tetrads) of metakinesis. In the light of these figures and the probable relations of the splits here shown to those of the earlier prophase chromosomes, I believe that the first maturation division is transverse and reductional and the second is longitudinal and equational (including also the accessory). De Sinéty interprets both divisions as longitudinal in *Leptinia* and *Meuexenus.* Due to the persistence of connecting linin threads, Stevens (1905) was able to demonstrate very conclusively that in *Stenopelmatus* (California sand-cricket) the first division is longitudinal and equational.

The chromosomes vary considerably in size and shape. Figure 80 shows a typical spindle with variously shaped chromosomes. Spindles at metaphase invariably show two pairs of reversed ⅂-shaped chromosomes, three pairs of long rod-shaped chromosomes, and the remainder are of the short rod or dumb-bell-shaped types. Correspondences of size between the biva-

lent chromosomes of the equatorial plates of the first maturation mitosis and pairs of chromosomes of the spermatogonial stages can be found, but these are hardly of sufficient precision to seem convincing in support of the theory of the individuality of the chromosomes or to add anything confirmatory of the selective character of synapsis.

The accessory chromosome in *Aplopus,* it will have been noticed, passes undivided to one of the poles of the first maturation spindle. In the late telophase and during the stages when the daughter-nuclei are formed and the ordinary chromosomes pass into the nuclear reticulum, the accessory becomes more or less bipartite, but always retains its sharp contour and deep-staining reaction and its usual position in close connection with the nuclear wall (figs. 92, 93, 94, 95, and 96). The accessory chromosome still retains these distinguishing characteristics throughout the resting stage (fig. 97), which is interpolated between the two maturation mitoses, as well as during the early prophase of the ensuing division. At this stage the cytoplasm of the secondary spermatocyte also frequently contains several larger or smaller masses of eliminated chromatin. Obviously only one-half of the secondary spermatocytes resulting from the previous division can have the accessory chromosome. Study of many sections shows without a doubt that only about one-half contain the chromatic body (or any body that reacts to a selective chromatin stain) which we have identified as the accessory chromosome. Figures 101 and 102 show two secondary spermatocytes side by side, one with the accessory chromosome and the other without it.

According to the several investigators, there is variation among the Arthropoda in regard to the time when the accessory chromosome divides, *i. e.,* in the first or second mitoses. McClung (1900 and 1902*b*), in the case of several Orthoptera, has traced the accessory back into the spermatogonial rest-stages, and finds that it subsequently divides only in the *first* spermatocyte division. Baumgartner (1904) in *Gryllus domesticus,* Stevens (1905) in *Stenopelmatus* and *Blatella germanica,* and Otte (1906) in *Locusta viridissima* find that this chromosome divides in the second division instead of the first. Moore and Robinson (1905) claim that the accessory in *Periplaneta americana* is only a plasmosome that dissolves before each division and is reconstructed after it. This can not be the case in *Aplopus.* No indication of a true nucleolus (plasmosome) can be demonstrated by any of the several staining methods employed in any of the cells in the line of the spermatogenesis.

SECONDARY SPERMATOCYTE.

The prophase stages of the second maturation division present nothing extraordinary. The succession of events is similar to that of the spermatogonial division and an ordinary homeotypic mitosis, with the exception of the presence of the accessory chromosome. The latter never passes into

the reticular stage and assumes its usual position close to the nuclear wall (figs. 103 and 104). Occasionally it is double (fig. 105). As the ordinary chromosomes take on the compact form and intense staining capacity, the accessory becomes unrecognizable among them (figs. 107, 108, and 109) in the equatorial plates. The latter give a chromosome count of 18 and 17, representing daughter-plates from primary spermatocytes, with and without the accessory chromosome, respectively. Plates with 18 chromosomes show one large **U**-shaped body (fig. 110). This, however, while usually peripheral, is never greatly eccentric, neither here nor in the equatorial plate of the first mitosis, as frequently represented in other insect forms. Plates giving a count of 17 lack the **U**-shaped chromosome (figs. 111 and 112). The chromosomes at metaphase have the same characteristic dumb-bell shape as they had in anaphase of the previous division. While most of the chromosomes are already separating in metakinesis, a pair is just entering the spindle with their long axes perpendicular to the fibers (fig. 115). This pair occasionally has its distal ends apparently fused at this stage (fig. 113). It represents the fission products of the accessory chromosome which has undergone an equation division similar to that of the ordinary chromosomes of this mitosis. This pair is seen to lag behind in the anaphase and even in the late telophase (figs. 116, 117, and 118), Obviously only two out of every four spermatids can have the accessory chromosome, and actual count of spermatids in several cysts corroborates this theoretical conclusion. Figure 114 shows four contiguous secondary spermatocytes in the equatorial-plate stage—probably daughter-cells of two adjacent primary spermatocytes—giving a chromosome count of 17 and 18 alternately. The two cells with 18 chromosomes show an odd large **U**-shaped element, the accessory chromosome. In the final stages of the telophase the ordinary chromosomes again pass into the nuclear reticulum, but the accessory chromosome remains intact as a more or less dumb-bell-shaped body (fig. 120).

SPERMATID AND SPERMATOZOON.

Figure 121 shows three spermatids, two of which contain the accessory chromosome. The latter may assume various shapes in the spermatid (figs. 122, 123, and 124) and is usually again closely applied to the nuclear wall. In the younger spermatids (fig. 121) the chromatin, which is very sparse in amount, is arranged in clumps, mostly close to the nuclear wall. During the later stages of metamorphosis into a spermatozoon the nuclear contents assume a chromatic reticular character. Small karyosomes are abundantly present, and the accessory chromosome now assumes a spheroidal shape and more or less central position (fig. 125). Occasionally dark-staining granules of eliminated basichromatin are seen in the cytoplasm (fig. 126).

The spermatid begins to lengthen its cytoplasmic body into a blunt tail (fig. 125). Presently a very delicate axial filament begins to grow out

into this cytoplasmic fin. It is attached to the nuclear wall by a distinct chromatic granule, probably a centrosome. Up to this point no structure could be definitely decided upon as a centrosome in any of the mitoses, and it is only rarely that even an indication of an aster can be observed. However, the spindle fibers are always distinct and come to a definite point at the poles. Were the centrosome not here pointed out by the attached axial filament it would very probably escape notice among the various minute chromatic granules of the peripheral zone of the nucleus. The nucleus at this stage frequently shows a polar cap of a material that stains intensely in iron hematoxylin. This structure is not stained with methyl green or thionin and probably represents the head-cap or acrosome of the adult spermatozoon (figs. 136 and 137).

The axial filament now enlarges proximally (fig. 127). While this structure elongates distally and sends a slender thread into the long cytoplasmic tail, it differentiates into a proximal stout neck definitely marked off from the distal filament, and represents the future middle-piece of the spermatozoon. Successive stages with and without the accessory chromosome are shown in figures 128, 129, 130, and 131. The mass of cytoplasm surroundng the axial fiber subsequently becomes the cytoplasmic fin of the tail, spirally arranged about the filament (fig. 136).

Thus far in the metamorphosis the nucleus has remained approximately spherical and is surrounded by a thin cytoplasmic envelope. Presently the cytoplasm disappears, the nucleus becomes oval in shape, and the proximal end of the middle piece widens and flares so as to form a concavity to receive the nuclear convexity. The head-cap is conspicuous and the accessory chromosome retains its compact spherical shape. Later stages show processes of disintegration by fragmentation and karyolysis (figs. 133 and 134) and its final disappearance (fig. 135). The spermatozoon undergoes still further changes of form, until in its final stage the nucleus is comparatively small and the middle-piece large. The latter has typically a cigar-shape and the nucleus is approximately spherical, with a depressed cone-shaped head-cap (fig. 136).

The mature spermatozoon presents a very strange phenomenon in regard to its staining reaction to recognized selective chromatin stains. Figure 136 shows a spermatozoon stained in iron hematoxylin; figure 137 shows a spermatozoon of similar age stained with methyl green (or thionin). It will be observed that the chromatic portions are exactly reversed as interpreted by the two stains. The portion picked out by the methyl green corresponds with the nucleus (head) of other spermatozoa, and since this is a very selective chromatin stain, it probably definitely marks the true limits of the nucleus. The reaction obtained with the iron-hematoxylin stain, however, yields a very happy result in that it permits the observation of the disintegrating accessory chromosome. Figures 138 and 139 show

a giant spermatid (with the accessory chromosome, of double size in this case) in process of metamorphosis, and a fully formed giant spermatozoon.

Giant spermatozoa have been frequently observed among the insects. Wilcox (1895) found them extensively and made a study of them in *Cicada tibicen* and *Caloptenus femur-rubum.* With Wilcox, I believe that they are non-functional also in *Aplopus mayeri,* and that "they are excluded from the developmental series and really come to nought."

I have noted above that the primary spermatogonial cell frequently divides amitotically. This may occur several times, giving rise to a multinucleate cell. Contrary to what Wilcox has found in *Cicada tibicen,* where the giant spermatozoon "arises directly from spermatogonia without cell-division, by a metamorphosis of the nucleus," figure 24 shows that the binucleate cell resulting from an amitotic nuclear division may subsequently divide karyokinetically. Such a cell would give rise to spermatocytes of 36 chromosomes (which have been observed) and eventually to giant spermatozoa. I have not observed spermatocytes with 72 chromosomes, but such may very well arise as a result of two successive amitotic nuclear divisions.

Frequently spermatids are seen with two or even several tails. This phenomenon is due probably to an accidental or abnormal division of the centrosome, from each product of which an axial filament grows out. Adult spermatozoa thus deformed are only seldom seen; they probably early undergo degeneration.

THEORETICAL CONSIDERATIONS.

INDIVIDUALITY OF CHROMOSOMES.

Among the ordinary chromosomes morphological individuality can not be convincingly demonstrated. This is due to the fact that between every mitosis, both spermatogonial and spermatocytic, as well as previous and subsequent to synapsis, a brief resting stage is interpolated when the chromosomes are merged into the nuclear reticulum. Correspondence of size can readily be found between the chromosomes of the equatorial plates of the primary and secondary spermatocytes, as also between these and pairs of chromosomes of the spermatogonial mitoses, but I do not consider the correspondence sufficiently close or striking to contribute reliable evidence in favor of the above hypothesis; nor do I believe it possible to find very strong evidence from this source in cases where we are dealing with so large a number of chromosomes. What evidence there is, however, points in the proper direction, as will be noticed by comparing figures 17, 74, and 114.

The evidence yielded by the accessory chromosome, however, is definitely corroborative of this hypothesis. When once fully differentiated in the later orders of the secondary spermatogonia it retains thereafter a persistently definite shape, size, and location in the nucleus, and never passes into a reticular stage. Even when assembled among the ordinary chromosomes of

an equatorial plate it is usually recognizable by its larger size or U-shaped form. The accessory chromosome, once having appeared in the spermatogonial cell, preserves its identity and morphological individuality unimpaired until it disappears in the ripening spermatozoon; and though it is never seen isolated within a separate vesicle, as described by Sutton (1900) for *Brachystola magna* (while each pair of spermatogonial chromosomes also become inclosed in a separate compartment of the nucleus) and Baumgartner (1904) for *Gryllus domesticus*, it nevertheless doubtless preserves also a strict physiological individuality, since it never unites with the ordinary chromosomes (except when connected for a brief period with the presynaptic thread) and ultimately passes to one-half of the spermatozoa, thus probably altering the physiological activity of those possessing it as compared with those lacking it in providing for the former a sex-determining factor.

DETERMINATION OF SEX.

In *Aplopus* a dimorphism of spermatozoa has been demonstrated, consisting in the presence of an accessory chromosome in one-half of the spermatozoa and its absence in the other half. McClung (1900) first suggested the possible causal connection between the dimorphism of sex and the observed dimorphism of spermatozoa. His conclusions were drawn from observations on some of the insects and the fact that sex appears to be the one character that divides the individuals of a species into two approximately equal groups.

Sections of ovarian material presented several favorable opportunities for making chromosome counts in somatic mitoses in the female. Equatorial plates of follicular cells of the developing ovum in mitosis yielded a chromosome complex, very distinct, and well separated. Though the number of such plates was not as large as could have been desired for absolute certainty, I am convinced that the somatic number of chromosomes in the female is 36 (fig. 19). Any number of very favorable equatorial plates of spermatogonial cells in mitosis give a chromosome count of 35 (fig. 17). Figure 114 and others give a clear demonstration of a dimorphism of secondary spermatocytes consisting in the presence of a large U-shaped chromosome in one-half the cells when the number of chromosomes is 18. Cells lacking this odd element have a chromosome count of only 17.

The reduced number of chromosomes in the mature egg must be 18, and an egg fertilized by one or the other type of spermatozoon will develop into an organism with 36 or 35 somatic chromosomes. Obviously the former (female) contains the accessory chromosome, and the latter (male) lacks it. From the chromosome standpoint the presence of an additional chromosome (the accessory chromosome) distinguishes the female cell from the male; hence the accessory chromosome appears to have some connection with the sex the organism is to acquire.

But as Stevens (1905) points out, it remains a question whether the accessory chromosome is really a sex chromosome in the sense that it determines sex or merely represents sex-characters. Bateson (1907) suggests that the accessory body may be merely associated with the cause of sex. Wilson (1906) suggests that the heterochromosomes (therefore accessory chromosome) may merely transmit sex-characters, sex being determined by cytoplasmic conditions external to the chromosomes; or again, that the accessory may be a sex-determinant only by virtue of a difference in activity or amount of chromatin. In view of its apparent function as a sex-determinant (whether of sex-condition or sex-characters), it hardly lends itself to the interpretation suggested by Paulmier and Montgomery to the effect that it is a degenerating chromosome—"such [heterochromosomes] as are in the process of disappearance in the evolution of a higher to a lower chromosome number" (Montgomery). Nevertheless, Wilson's further suggestion that the accessory of Orthoptera is the homologue of the large member of the idiochromosome group in certain Hemiptera, and that its missing mate is the homologue of the small idiochromosome—the accessory thus perhaps representing the residue of a pair of idiochromosomes after the loss of a pair of microchromosomes—is very helpful in formulating a working hypothesis in regard to the accessory chromosome considered as a sex-determinant.

Expressed in Wilson's (1906) formula for sex-determination, the facts in *Aplopus mayeri* are as follows:

A. Egg (18 chrom.) + Spermatozoon (18 chrom.) = female (36 chrom.)
B. Egg (18 chrom.) + Spermatozoon (17 chrom.) = male (35 chrom.)

Castle (1903) developed a theory of sex in which he applied a modification of Mendel's principle of segregation to sex-phenomena. This has recently been more fully elaborated and applied to the case of the accessory chromosome by Wilson (1906). Castle's theory involves several assumptions: (*a*) the fact of two kinds of eggs (male and female), as also of two kinds of spermatozoa, which have been actually many times observed; and (*b*) selective fertilization or infertility of gametic unions of like sex-chromosomes, *i. e.,* an egg with a female determinant must be fertilized by a spermatozoon with a male determinant, and *vice versa.* Castle further believes that there are no individuals pure in regard to sex, but that only hybrids are produced. Observation also seems to show the dominance of the female over the male determinant.

If the accessory is actually a sex-determinant, and as such represents the homologue of the large idiochromosome as suggested by Wilson, then, since an egg fertilized by a spermatozoon lacking the accessory chromosome produces a male, the egg itself must contain the factor that determines maleness, and the missing chromosome must be the female determinant. Consequently, since an egg fertilized by a spermatozoon containing the acces-

sory produces a female, the egg must contain the character that determines femaleness and the accessory chromosome must be a male sex determinant, which, however, is recessive to the dominant female determinant in the egg. Extending the above formulæ (following Wilson) to express these assumptions, they become—

A. ♀ Egg (18 chrom.) + (♂) Spermatozoon (18 chrom.) = ♀ (♂) female (36 chrom.)
B. ♂ Egg (18 chrom.) + (o) Spermatozoon (17 chrom.) = (♂) (o) male (35 chrom.)

The facts in *Aplopus mayeri* admit of interpretation according to Castle's theory of sex-production, and contribute to the cumulative evidence in favor of the hypothesis that there exists a causal relation between the accessory chromosome and sex-phenomena.

SUMMARY.

(*a*) Primary spermatogonia divide both mitotically and amitotically. In the latter instance cell-division is frequently not consummated and a bi or multi-nuclear cell results. A binuclear cell has been observed subsequently to divide karyokinetically, thus giving rise to primary spermatocytes with double the number of chromosomes ($2 \times 17 + 2$ accessory chromosomes = 36 chromosomes), which may develop into giant spermatozoa. Primary spermatogonia have neither accessory chromosome nor plasmosome.

(*b*) In the first order of the secondary spermatogonia the accessory chromosome appears in the resting-stage. During the late telophase of ensuing spermatogonial divisions the accessory is lost (probably assuming a brief reticular phase) until a spermatogonium of the last order is attained, when the accessory persists as a unique structure characterized by its definite form, staining reaction, position in the nucleus, and its behavior during synapsis and the maturation mitoses.

(*c*) During synapsis the accessory chromosome lengthens into a club-shaped structure attached by its lesser end to the presynaptic thread, undergoes partial longitudinal division, closes up again during the height of synapsis, and returns again to its previous characteristic form and location in the nucleus of the growing primary spermatocyte.

(*d*) Brief resting-stages are interpolated between the telophase of the final spermatogonial mitosis and synapsis and between the latter stage and the prophase of the first maturation division. The latter resting stage corresponds to a portion of the growth-period of the primary spermatocyte.

(*e*) The somatic number of chromosomes for the female *Aplopus* is 36; the spermatogonial number is 35; and the number for the primary spermatocytes is 18. One of these chromosomes is characteristically large U-shaped and situated at the periphery of the chromosome complex and is the accessory chromosome.

(*f*) The accessory chromosome passes undivided to one of the poles during the primary spermatocyte division. This mitosis is the reductional

division separating whole chromosomes which had united to form bivalents in synapsis. This division results in dimorphic secondary spermatocytes, one group possessing, the other lacking, the accessory chromosome.

(*g*) The second maturation division is equational, effecting a longitudinal division of univalent chromosomes. The accessory also divides equationally in the cells containing this element and lags somewhat behind the ordinary chromosomes.

(*h*) A dimorphism of spermatozoa results; the accessory chromosome possessed by one-half probably represents a sex-determinant.

(*i*) Nothing appears in the phenomena of synapsis or reduction as regards the ordinary chromosomes to suggest anything contradictory to the theory that synapsis signifies the final phase of fertilization and the union of maternal and paternal chromosomes, nor yet to contravene the theory of the individuality of the chromosomes; but no clear evidence appears in support of either hypothesis.

(*j*) The history of the accessory chromosome gives evidence that it at least possesses a strict morphological and probably also a physiological individuality.

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DESCRIPTION OF PLATES.

All figures were drawn from camera-lucida outlines made with a B. and L. 1/12-inch oil-immersion objective and a 1-inch B. and L. ocular. The length of the tube for all drawings was 160 mm., and the drawing-surface of board was 255 mm. below the level of the stage. The initial magnification thus obtained was 1,750 diameters. All drawings have been reduced one-third in reproduction.

Plate 1.

Fig. 1. Primary spermatogonium with two nuclei. Nucleus at right contains a large karyosome. Binucleate condition is due to amitotic division, which is frequent among these cells. The cell is attached to the attenuated follicular wall, which contains broad, elongate, flattened nuclei.

Fig. 2. Resting-stage of primary spermatogonium, showing chromatin arranged in clumps connected by delicate linin threads. Quantity of cytoplasm small. Contour of nucleus appears somewhat lobed. Primary spermatogonial cells have no nucleoli.

Fig. 3. Nucleus of cyst membrane of a spermatocyst, similar to that of the resting primary spermatogonia. Division is usually mitotic, but frequently amitotic.

Fig. 4. Resting-stage of primary spermatogonium with fine-meshed nuclear reticulum containing one large and numerous small karyosomes. Amount of cytoplasm very scant.

Fig. 5. Late prophase. The chromosomes are in the stage of a partially segmented and longitudinally split mossy spireme.

Fig. 6. Still later prophase. The chromosomes are disposed as very slender split rods, some of which are greatly elongate.

Fig. 7, 8. Equatorial plates of primary spermatogonial mitosis; 35 chromosomes. (The larger number of elements—40 and 37 respectively—due to cross-section of limbs of U-shaped chromosomes.)

Fig. 9. Late metaphase.

Fig. 10. Anaphase.

Fig. 11. Late telophase; chromosomes in form of long, mossy, pale-staining threads. Mid-body appears as a very conspicuous structure of fine, deep-staining granules. The daughter-cells are inclosed by cyst-wall of mother-cell.

Fig. 12. Prophase and late telophase of secondary spermatogonium. The investing wall is that of the mother primary spermatogonial cell in form of a cyst membrane.

Fig. 13. Resting-stage of secondary spermatocyte. Nuclear reticulum only very slightly chromatic. A distinct chromatin nucleolus ("chromosome nucleolus"—"accessory chromosome") is present.

Fig. 14. Early prophase. The nuclear reticulum contains numerous karyosomes and a chromatin nucleolus which evidences a bipartite structure.

Fig. 15. Late prophase; spireme has segmented into a number of mossy lightly-staining chromosomes. The accessory chromosome has retained its sharp contour and deep-staining capacity.

Fig. 16. Still later prophase; chromosomes in form of delicate split rods.

Figs. 17, 18. Equatorial plates of secondary spermatogonia with 35 chromosomes. Accessory chromosome indistinguishable from the ordinary chromosomes.

Fig. 19. Equatorial plate of a dividing cell of follicle of a young egg, showing 36 chromosomes.

Fig. 20. Equatorial plate of secondary spermatogonium, showing five of the chromosomes U-shaped.

Fig. 21. Cyst showing three cells at metaphase and a fourth partially at early anaphase.

Figs. 22, 23. Late telophase; the pair of lagging chromosomes at left may be the accessory ("special chromosome"—de Sinéty) chromosomes.

Fig. 24. Telophase of a binucleate cell. Ensuing divisions give rise to spermatocytes with double the usual number of chromosomes (*i. e.*, 2×17 ordinary $+ 2$ accessory chromosomes) and eventually to giant spermatids and spermatozoa. The binucleate condition is probably due to an early amitotic division of the nucleus.

PLATE 2.

FIG. 25. Very late telophase; mid-body prominent.
FIG. 26. Resting-stage of the final order of secondary spermatogonia, showing a chromatin nucleolus ("accessory chromosome").
FIGS. 27, 28, 29, 30, 31. Successive stages in the prophase of ensuing mitosis; accessory chromosome in figure 30 distinctly bipartite.
FIGS. 32, 33. Equatorial plates each with 35 chromosomes.
FIG. 34. Two spindles at metaphase, showing accessory chromosome splitting at the right of lower spindle.
FIG. 35. Early anaphase of similar cell.
FIG. 36. Later anaphase.
FIG. 37. Telophase stage, showing two pairs of lagging chromosomes.
FIGS. 38, 39, 40, 41, 42. Successive stages in the telophase of final secondary spermatogonial division.
FIGS. 43, 44, 45. Still later steps in the telophase of similar division; mid-body conspicuous in each spindle; figure 44 shows persistence of accessory chromosome as a body of sharp contour and deep-staining capacity among the pale-staining, irregularly shaped ordinary chromosomes.
FIG. 46. Nucleus of primary spermatocyte in resting-stage. Reticulum delicate and only very slightly chromatic. Accessory chromosome very close to nuclear wall. This phase is of very brief duration, as also the following one.
FIG. 47. Early growth-period of primary spermatocyte; nuclear reticulum more highly chromatic and with several karyosomes.
FIG. 48. Late growth-period; chromatin in form of close-meshed network of broad, mossy, lightly-staining threads. Accessory chromosome closely attached to nuclear wall. Amount of cytoplasm very small.
FIGS. 49, 50, 51, 52. Successive stages in the presynaptic phase of the growth-period. The accessory chromosome has become an elongate, club-shaped structure attached at its narrower end to the presynaptic chromatin thread; the latter in the form of a close-meshed lattice-work. Figure 50 shows two such cells still connected by cytoplasm of mother-cell. In figure 52 the accessory chromosome is beginning to split longitudinally.

PLATE 3.

FIGS. 53, 54, 55, 56, 57, 58. Stages of synapsis. X = synaptic point. Accessory chromosome is closely applied to nuclear wall; frequently it is split as in figure 57. Figures 57 and 58 may be identical with the "bouquet stage" of Eisen.
FIG. 59. Postsynaptic stage. Chromatin in form of an irregularly loosely-meshed network of broad, mossy threads, which give indication of a longitudinal split. The accessory chromosome is close to the wall; the cytoplasm is still scant.
FIG. 60. Resting-stage of postsynaptic period (stained in methyl green). The accessory chromosome has the appearance of a hollow sphere.
FIG. 61. Resting-stage (stained in iron hematoxylin). Nuclear reticulum very delicate and very slightly chromatic. Accessory chromosome solid and attached to nuclear wall.
FIG. 62. Resting-stage in which the reticulum is more chromatic; contains karyosomes and has a bipartite accessory chromosome.
FIG. 63. Early prophase of heterotypic mitosis; nuclear reticulum consists of coarser and more highly chromatic threads; accessory chromosome in form of a deeply chromatic sphere some distance from nuclear wall. The sparse cytoplasm contains several small masses of eliminated chromatin.
FIG. 64. Later prophase; accessory chromosome clearly double.
FIG. 65. Still later prophase; pale-staining, mossy spireme partially segmented and with indication of a longitudinal split.
FIGS. 66, 67, 68, 69, 70, 71. Stages in the prophase of the heterotypic division. The accessory chromosome (*a*) assumes various shapes, but always retains its definite contour and intense staining capacity. Several of the ordinary chromosomes, which stain only slightly at this stage, are in the form of tetrads.
FIGS. 72, 73. Later prophases; all the chromosomes have similarly sharp contours and intense staining capacity. Figure 73 has a ring-shaped tetrad and dumb-bell-shaped accessory chromosomes (*a*).

FIGS. 74, 75, 76, 77. Equatorial plates of first maturation mitosis, each with 18 chromosomes; among these, the accessory can be recognized as the large U-shaped or irregularly oblong body.

FIG. 78. Early anaphase of first maturation mitosis, showing very elongate daughter chromosomes with indication of a longitudinal split. The accessory has assumed a position in advance of the ordinary chromosomes near one pole and appears double.

FIGS. 79, 80, 81, 82, 83, 84. Spindles of primary spermatocyte mitoses with some of the chromosomes at late metaphase and some at early anaphase. These figures show the various typical forms of the accessory chromosome always in advance of the ordinary chromosomes; also the various forms of the latter in the heterotypic division. This is a reducing division separating entire chromosomes.

PLATE 4.

FIGS. 85, 86, 87, 88. Anaphase stages in the first maturation mitosis. U-shaped accessory chromosome attached to pole by single thread. In late anaphase stages the accessory chromosome becomes longitudinally split; the consummation of the split represents a separation at the bend of the U.

FIGS. 89, 90, 91. Late telophase; the mass of ordinary chromosomes has moved by the side of and beyond the accessory.

FIGS. 92, 93, 94, 95, 96. Successive stages in the late telophase of the first maturation mitosis; the accessory chromosome appears distinctly double and never passes into a reticular phase with the ordinary chromosomes at this stage.

FIG. 97. Resting-stage interpolated between first and second maturation mitoses; accessory chromosome double and closely applied to nuclear wall.

FIG. 98. Early prophase of second maturation mitosis; accessory chromosome still double. The narrow rim of cytoplasm contains several masses of eliminated chromatin.

FIGS. 99, 100. Later prophase of secondary spermatocyte division; both cells have the double accessory chromosome.

FIGS. 101, 102. Daughter-cells of the heterotypic mitosis in prophase for the second division. Only one of these cells contains an accessory; only half of the secondary spermatocytes have an accessory chromosome.

FIGS. 103, 104, 105. Three secondary spermatocytes in prophase of final maturation division, all of which contain the accessory chromosome. The segments of the pale-staining mossy spireme give indication of a longitudinal split. Cytoplasm is very scant.

FIGS. 106, 107, 108. Later stages in the prophase of the second maturation mitosis, of which figure 107 contains an accessory chromosome (*a*).

FIG. 109. Metaphase of second maturation division.

FIG. 110. Equatorial plate with 18 chromosomes. The large U-shaped chromosome is the accessory.

FIGS. 111, 112. Equatorial plates with 17 chromosomes; the accessory lacking.

PLATE 5.

FIG. 114. Four contiguous cells (pairs of daughter-cells from two primary spermatocyte mother-cells) showing the chromosomes in the equatorial plate. The number of chromosomes alternates from 18 to 17 among the four plates. The first and third contain a large U-shaped odd chromosome, the accessory.

FIGS. 113, 115. Early anaphases of second maturation mitosis, both showing the accessory chromosome entering the spindle perpendicular to the mantle fibers.

FIGS. 116, 117. Late anaphase and telophase, respectively, showing the lagging of the division products of the accessory chromosome.

FIG. 118. Late telophase; accessory chromosome projecting beyond the main mass of ordinary chromosomes.

FIG. 119. Various tetrad figures showing forms assumed by the ordinary chromosomes in the prophase and early metaphase of the heterotypic mitosis.

FIG. 120. Two spermatids, both with daughter accessory chromosomes; the ordinary chromosomes are in the irregular, granular, lightly-staining condition of the late telophase. The cell to the left shows the mid-body at its lower pole.

FIG. 121. Three spermatids, two with accessory chromosome. Only one-half of the spermatids can have an accessory.

FIGS. 122, 123, 124. Three spermatids, showing the various shapes and locations of the accessory chromosome.

FIGS. 125, 126, 127. Successive stages in the metamorphosis of the spermatid to form the spermatozoon; the accessory chromosome shown in form of a central intensely chromatic sphere. These same cells show a polar cap of chromatic material (this seen only in iron-hematoxylin preparations). The second contains a mass of eliminated chromatin in its cytoplasm and karyosomes in the nucleus. A short, slender chromatic filament has grown out from the centrosome into the cytoplasm.

FIGS. 128, 129, 130, 131. Successive stages in the later development of the spermatozoon. Only one-half contain a chromatin nucleolus (accessory chromosome). The cytoplasm has elongated into a tail through which extends the slender chromatic filament much enlarged at the nucleo-proximal end to form a middle-piece or neck.

FIGS. 132, 133, 134, 135. Successive stages in the final development of the spermatozoon, showing the progressive disintegration of the chromatin nucleolus (accessory chromosome—still U-shaped in figure 133).

FIG. 136. Mature spermatozoon stained in iron hematoxylin; showing chromatic archoplasmic cap, vesicular head (nucleus), chromatic neck, and axial filament with spiral cytoplasmic fin.

FIG. 137. Mature spermatozoon stained in methyl green (thionin yields similar result) showing an achromatic archoplasmic cap, a chromatic head, and an achromatic cigar-shaped neck.

FIGS. 138, 139. Two of the final stages in the formation of a giant spermatozoon.


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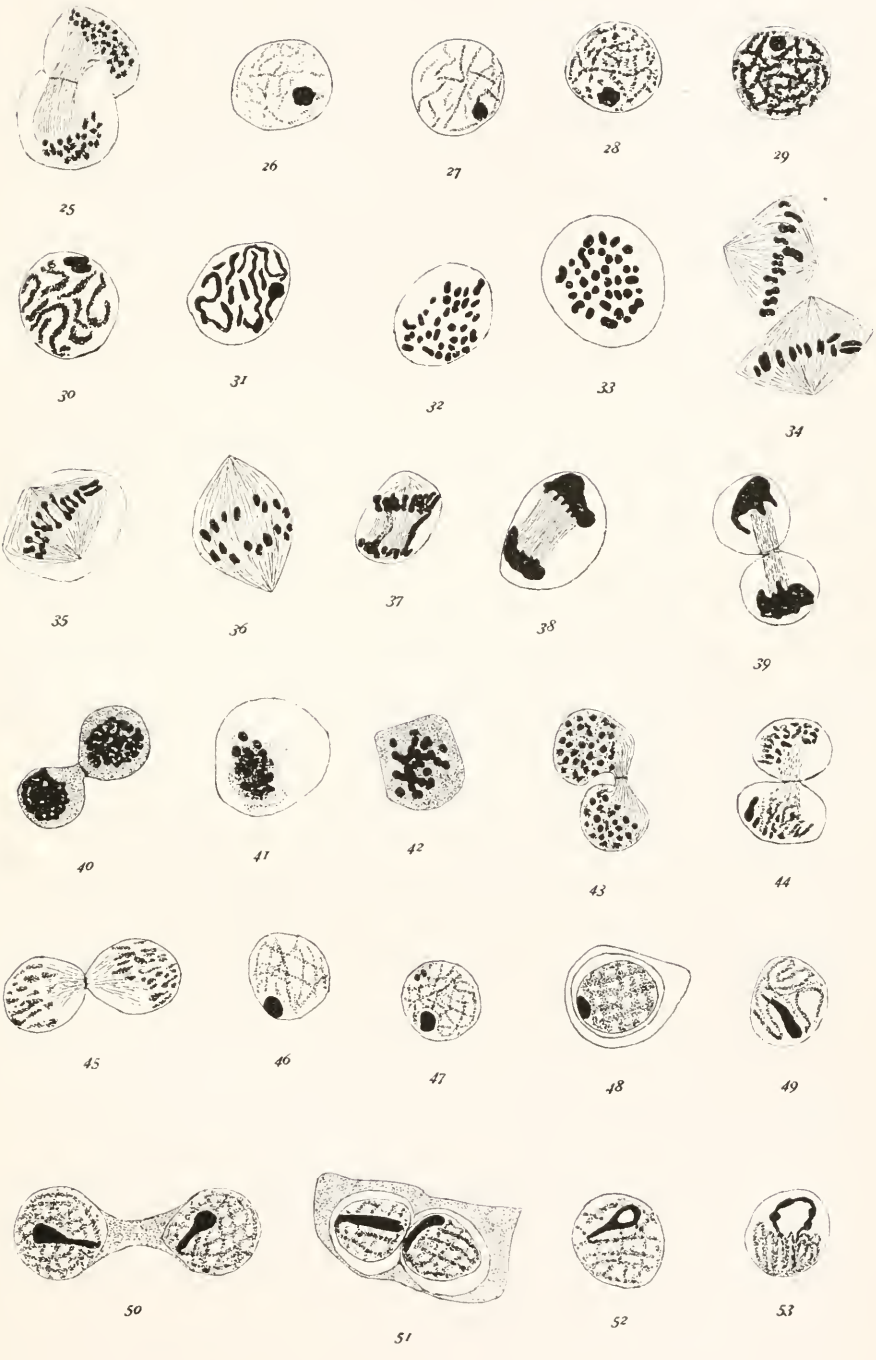
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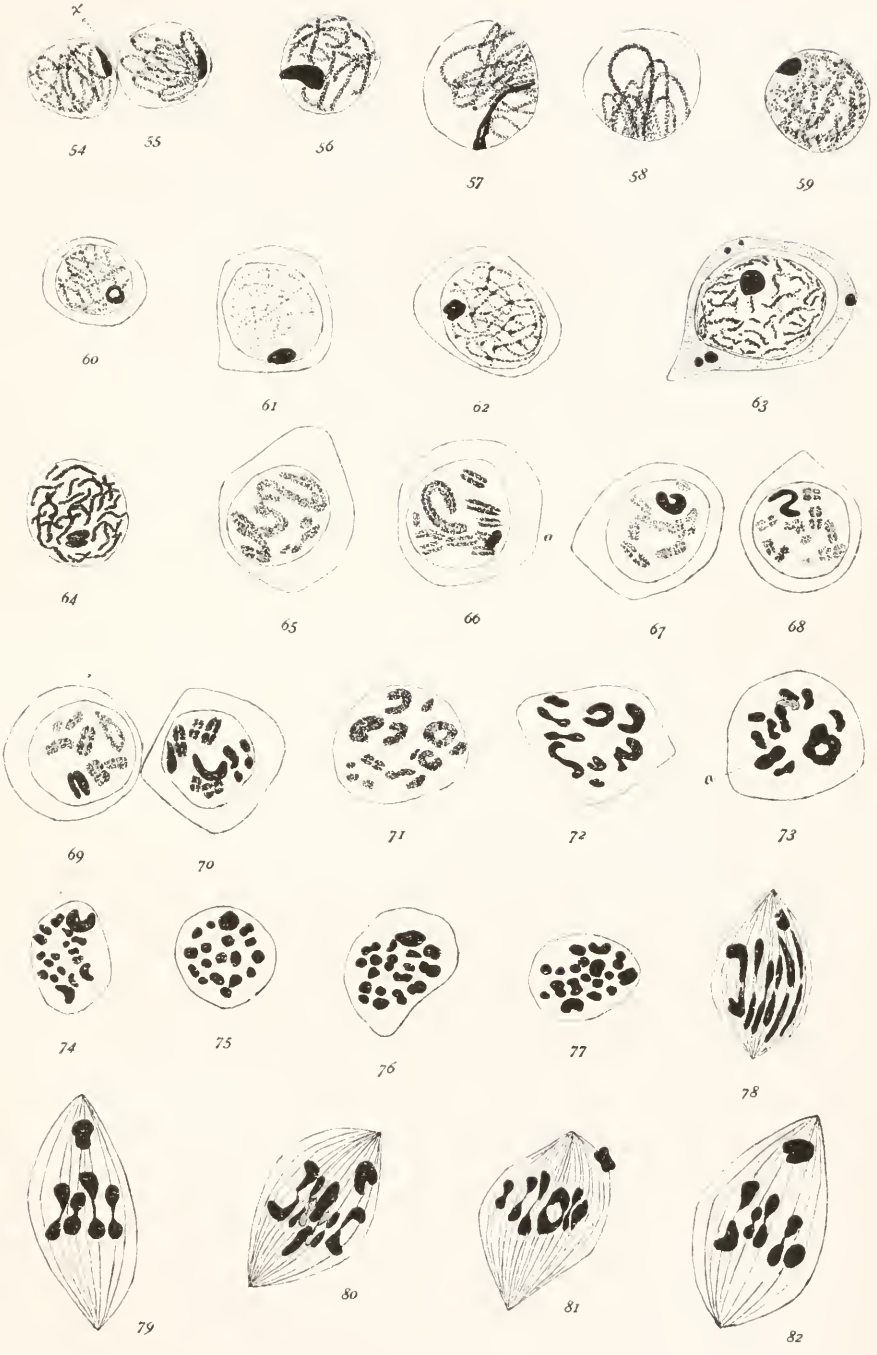
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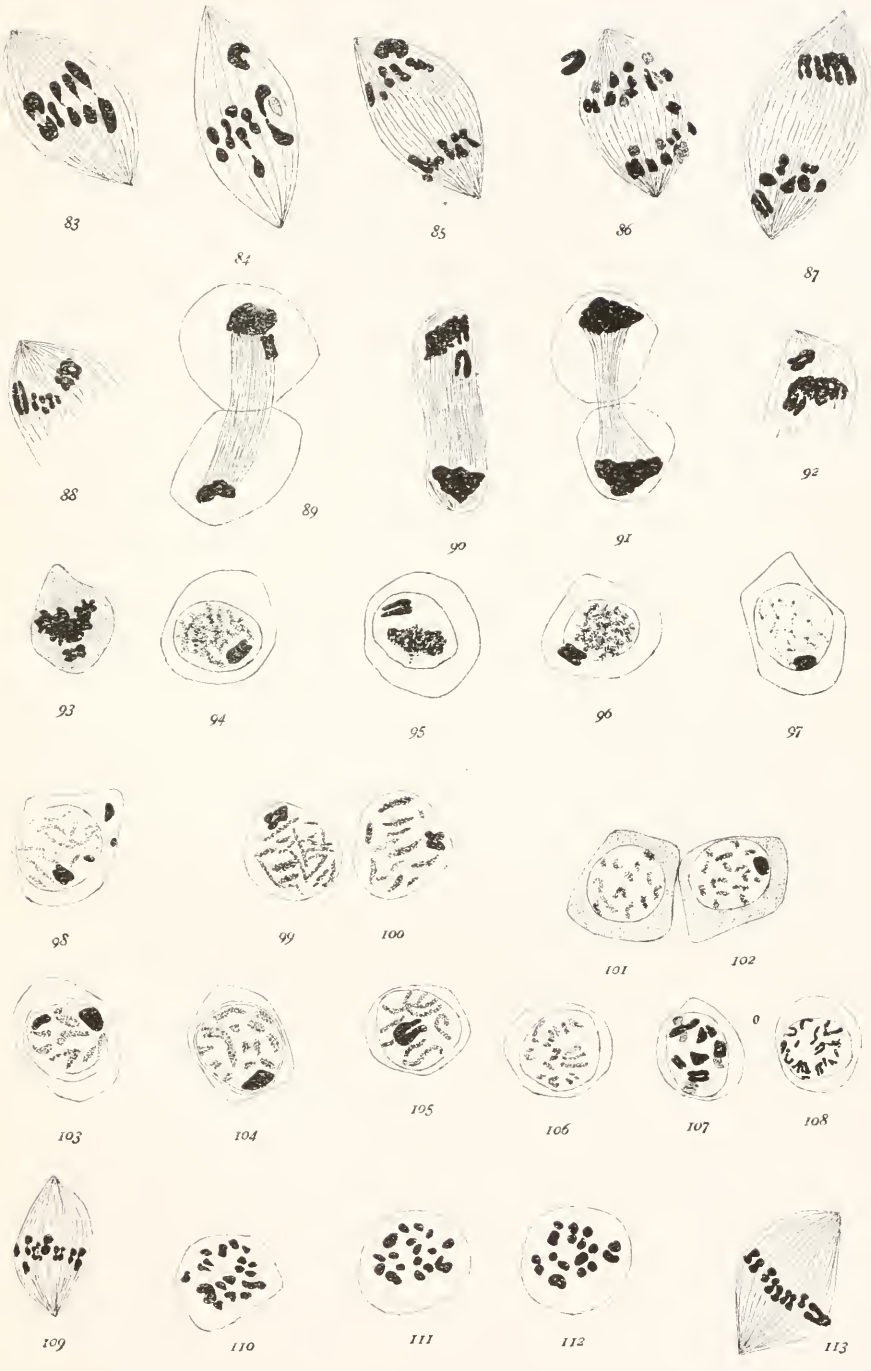
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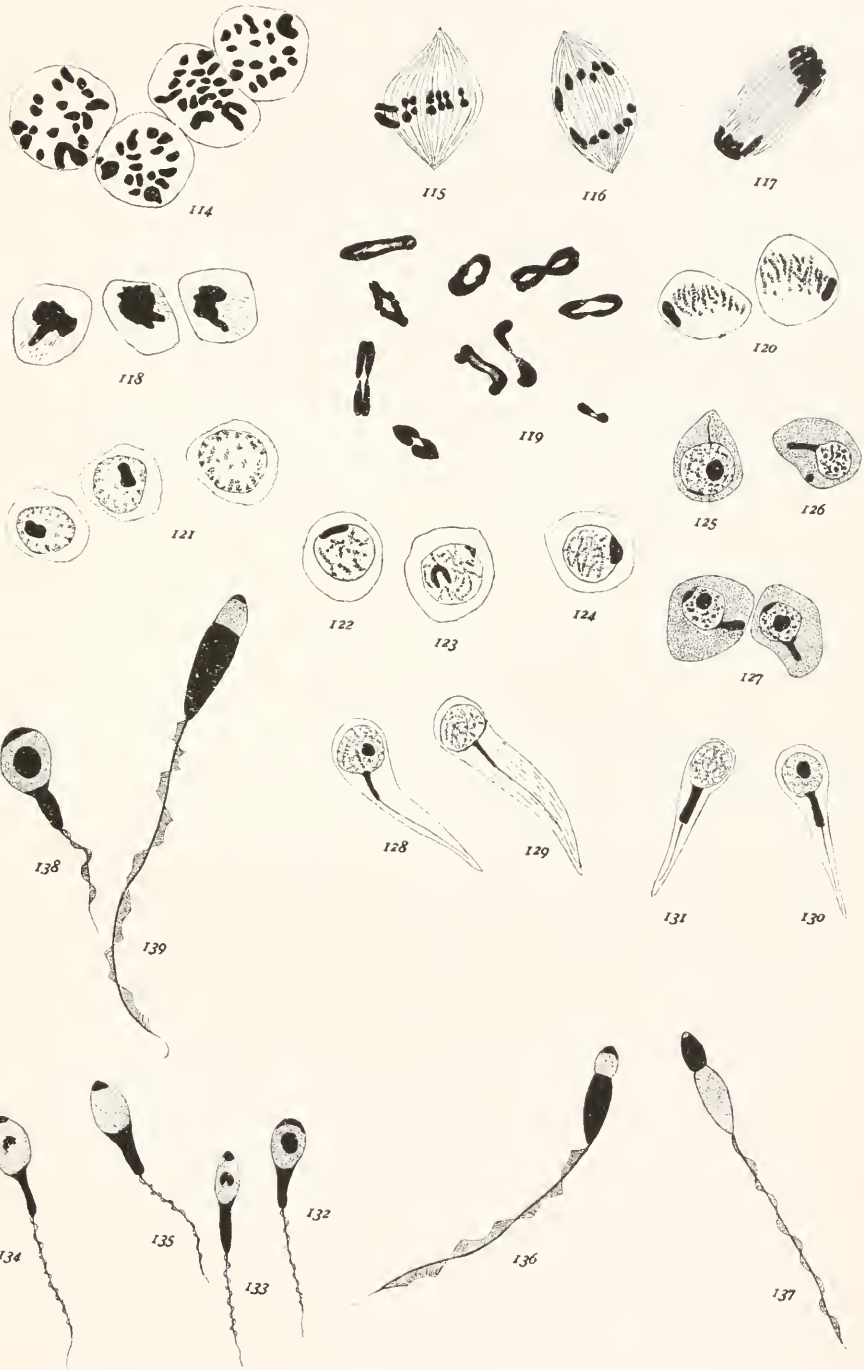
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III. THE RELATION OF THE NUCLEOLUS TO THE CHROMOSOMES IN THE PRIMARY OOCYTE OF ASTERIAS FORBESII

By H. E. JORDAN
Of the University of Virginia

Plates 1–7

THE RELATION OF THE NUCLEOLUS TO THE CHROMOSOMES IN THE PRIMARY OÖCYTE OF ASTERIAS FORBESII.[1]

By H. E. Jordan.

INTRODUCTION.

The following investigation was undertaken at the suggestion of Prof. E. B. Wilson, to whom I am very greatly indebted for help during the progress of the work. Its primary object is to contribute to the subject of the relation between nucleolus and chromosomes during maturation. Hartman (1902) and Guenther (1903) maintain that in certain echinoderms the chromosomes of the first maturation spindle are derived from the nucleolus. If this view is correct for all of the forms, the oöcyte development in favorable cases ought to reveal the entrance of the chromosomes into the nucleolus. Accordingly, I have attempted to trace the history of both chromosomes and nucleolus from the last series of oögonia through the growth-period and maturation process of the oöcyte of a species of starfish, *Asterias forbesii.* My results show conclusively that in this echinoderm form, at least, the chromosomes do not arise out of the nucleolus at maturation. The nearest approach to such a state of affairs is where a close superficial attachment gives the appearance of a nucleolar origin. Incidentally are involved the questions of the function of the nucleolus, the significance of the nucleolar vacuoles, and a consideration of the mechanism of maturation. Observations were made on the living material and some study was devoted to preserved material during a two months' stay at the U. S. Fish Commission station at Woods Hole in the summer of 1906. I wish here to acknowledge many courtesies extended to me by the director, Dr. F. B. Sumner, and I desire also to express my appreciation of the splendid facilities for research offered by the laboratory. Further study was given to the preserved material in the Histological Laboratory of Princeton University during the winter of 1906–07, under the direction and with the kindly help of Prof. Ulric Dahlgren.

Through the kindness of the Carnegie Institution of Washington I have been enabled to extend the investigation to a comparative study with *Hip-*

[1] Thesis presented to the faculty of Princeton University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

ponoë esculenta, a sea-urchin very common in the shallow waters of the reefs in the vicinity of Dry Tortugas, Florida. I am under obligations to Dr. Alfred G. Mayer, Director of the Laboratory at the above place, for daily favors and many helpful suggestions.

METHODS.

As is now well known, when the immature ova of star-fish are shaken out of the ovary into sea-water they immediately begin to form the polar bodies. In ovaries opened during July and August I found from 50 to 70 per cent of the oöcytes at the stage of development where they could be thus induced to mature. The eggs were placed in water which was kept by frequent changes as nearly as possible within the limits of normal ocean temperature. In every case the development was allowed to proceed to the early segmentation stages, so as to assure a large percentage (about 60 per cent) of normal stages among the eggs previously preserved. In some cases sperm was added to the sea-water, but produced no noticeable effect, beyond the formation of a fertilization membrane, until after the second polar body was formed.

Ovarian material was fixed in Gilson's fluid, sublimate acetic, picro-acetic, and picro-aceto-sulphuric mixtures, and Bouin's solution. The sublimate acetic and picro-aceto-sulphuric mixtures proved most serviceable in that the coagulation product was less coarse and the relations of nucleolus, nucleus, and cytoplasm continued intimate and their internal structure undisturbed.

The stains employed were Heidenhain's iron hematoxylin followed by orange G, Delafield's hematoxylin with Congo-red counter-stain, borax carmine with orange G counter-stain, Auerbach's stain, and Flemming's triple stain. Heidenhain's iron hematoxylin with orange G yielded by far the most satisfactory results. Auerbach's stain was very serviceable in that it differentiated beautifully between definite chromatin and plastin. Flemming's triple stain proved very unreliable and generally unsatisfactory on account of its varying reaction. Still other combinations of stains were employed, as will be noted in subsequent descriptions.

The maturing eggs were fixed at intervals of 10 minutes through a period of 2 hours. Several series were put up at intervals of 5 minutes through a period of 1 hour; and still others at intervals of 30 minutes through a period of 3 hours. The results from four complete and several partial series are wholly in accord with each other. Besides the above-mentioned fixing reagents used for the ovarian material, Flemming's strong solution was also employed. In the case of the free ova, also, the sublimate acetic and picro-aceto-sulphuric mixtures proved most satisfactory in that they caused less disturbances within the cell substances. Flemming's solution, as also Gilson's fluid, preserved beautifully the mitotic figures, but

rather poorly the cell-cytoplasm. Similar combinations of stains were used with the eggs as with the ovaries and yielded similar results.

EARLY OVARIAN STAGES—SYNIZESIS.

Among masses of oögonia it is difficult to distinguish definite cell-borders. The cells have comparatively large very chromatic nucleoli, centrally situated and enmeshed in a very delicate achromatic (linin) nuclear reticulum (fig. 1). The diameter of the nucleus is approximately 2.5 microns and that of the nucleolus one-third as much. What cytoplasm could be seen between the cells stained straw-color with orange G. I have been unable to discover a single instance of mitosis among the oögonia either in very young or mature individuals. A stage of slightly larger size (figs. 2, 3) has a faintly chromatic nuclear network and a slightly more definite cell-border. With iron hematoxylin and orange G the nucleolus again stains deep black, while the granular cytoplasm stains blue. This is a typical oöcyte of the first order just entering upon its growth-period.

Among these earliest reproductive cells are frequently seen dark-staining (black with iron hematoxylin and green with Auerbach's stain) bodies of about the size of the nuclei of the smallest oöcytes. They are probably degenerating oögonia, now performing the function of "nurse cells." They lie scattered singly or in clumps among the oögonia. Occasionally there is faintly visible around some of these bodies a slight cytoplasmic rim. These chromatic bodies are probably similar to the "nutritive nuclei" described by Griffin in *Zirphæa*.

Very conspicuous among these youngest apparently resting oöcytes are such as are in more or less close synizesis (McClung) (figs. 6, 7, 8, 9). Usually these stages appear in pairs or quartettes. Such pairs indicate that they are the daughter-cells of primordial germ-cells undergoing synchronous development. The oöcytes in synizesis are slightly larger than the youngest oöcytes above described. The minute size of the cells makes it difficult to determine with certainty upon definite transition stages leading to the contraction stage. It is clearly evident that the cells are gaining in volume and I have accordingly adopted as my best criterion of development the size of the nucleus. Proceeding on this basis, I have discovered the following characteristics of the transition stages: The cytoplasm continues granular and basic in staining reaction. The nucleolus remains intensely chromatic and usually assumes an eccentric position. The nuclear network gains in chromatin and becomes progressively more conspicuous, both as to its chromatin and linin elements. The chromatin is largely gathered in clumps along the nuclear membrane (fig. 4). Presently the entire network becomes chromatic and in a slightly later stage assumes the form of a spireme (fig. 5). The threads (probably two in number) then shorten and thicken and contract in a tangled knot about the nucleolus and gradually close up in complete synizesis (fig. 10, size of nucleus 5.0 microns).

After synizesis the oöcyte begins a period of very rapid increase in size as well as a very rapid increase and alteration of its chromatic substance. Comparison of the youngest with the full-grown oöcyte shows that during the growth-period the nucleus increases in volume about 8,000 times (20 diameters). Oöcytes showing the first stages in the disentangling of the spireme from synizesis are abundant. All of them show the nucleolus intact and still highly chromatic (figs. 11, 12, 13, 14, 15). Later stages show that the stout primary thread becomes double (figs. 19, 20, 21, 22, 26). With iron hematoxylin and orange G the moieties stain intensely black throughout. The threads have regular swellings (chromomeres) along their entire extent, giving the appearance of a string of beads. With Auerbach's stain the picture differs somewhat. Only the "beads" stain green, while the remainder of the threads and network stain red. There is evidence here, I believe, in support of the view now very generally held by investigators that linin (paranuclein, O. Hertwig) and chromatin are closely related chemical substances and of equally important physiological value.

The double thread begins to divide transversely into a number (18?) of segments, as occasional specimens clearly show (figs. 20, 22, 23). Sometimes the spireme appears to segment while yet single (figs. 16, 17, 18). The pairs of beaded rods scattered through the nuclear reticulum undergo various transformations. They shorten, grow stouter, and often appear to unite at their ends, giving a ring form (fig. 25). Whatever shape they assume—ring, rod, sphere, or irregular mass of chromatin (figs. 24, 25, 26, 27)—they very generally have a mossy or feathery appearance at this stage. This is probably due to a transfer of chromatin from the chromosomes to the nucleolus through the nuclear reticulum. The reticulum stains more intensely at this stage with basic dyes and at its culmination the nucleolus has greatly enlarged. Subsequent stages show the chromosomes reappearing with bilobed forms and sharp contour and reduced size (figs. 28, 29). Ultimately the chromosomes become grouped in a mass (sometimes several masses) where they persist as much-reduced bilobed bodies (figs. 30, 33, 34, 41, 42) until they are taken into the first polar spindle at maturation.

The later stages in the development of the oöcytes above outlined are by no means sharply defined. Transition from one to the other is more or less variable as to characteristic forms and the time of its appearance. This is probably due to the fact that the amount of chromatin in the germinal vesicle of different oöcytes of the same age (as reckoned by size of nucleus) varies somewhat. Apparently the process of chromosome formation and chromatin absorption and elimination is hastened in some eggs and retarded in others.

The extreme minuteness of the cells and the total absence of division figures preclude all observations on synapsis, and consequently deny the sole

trustworthy clue as to the manner of the reduction of the chromosomes. The slight evidence that synizesis and the maturation divisions give seems to indicate that *Asterias forbesii* agrees with the parasynaptic type of reduction.

Up to the period when the oöcyte has reached about half its full-grown size the cytoplasm is wide-meshed and coarsely granular, the granules usually lodging at the intersections of the meshwork. With the iron hematoxylin and orange G combination of stains, the cytoplasm stains dark blue or in cases where the staining action of the orange G is prolonged the resulting stain is dark brown. In subsequent stages the cytoplasm is always stained a lighter or darker orange color. This difference in color reaction to similar staining combinations between the cytoplasm of the growing and full-grown oöcyte is very striking and due, I believe, to the presence of a great amount of yolk or to the fore-products of chromatin formation in process of transportation to the nucleus and nucleolus. There is a progressive and approximately proportional increase in volume of nucleolus, nucleus and cell-body through the growth-period.

Auerbach's stain reveals a similar difference in staining reaction between the cytoplasm of the growing and that of the full-grown oöcyte. In the latter the cytoplasm has a deep-red (fuchsin) color, while that of the smaller oöcytes has a grayish or bluish-red color, showing undoubtedly the influence of the methyl green constituent of the stain in its reaction to the forming chromatin. Similar observations have been reported by Griffin in the case of the egg of *Zirphæa*.

The ovaries upon which the above observations are made were gathered during July and August. A study of ovaries gathered during the last week in December confirmed in every respect the description given above of ovarian material. In both cases the sparsity of transition stages and the large number of apparently full-grown oöcytes is very striking. It appears that *Asterias* has no special periods of reproductive activity, as is the case with most of the Metazoa, but produces ripe eggs perpetually.

THE FULL-GROWN PRIMARY OÖCYTE.

The full-grown primary oöcyte of spherical outline has a diameter of about 100 microns. The nucleus of such an oöcyte has a diameter of from one-half to somewhat less than one-half the diameter of the cell (50–40 microns). The nucleolus varies in size from one-fourth to one-fifth the diameter of the nucleus. The nucleolus (or germinal spot) invariably takes an eccentric position as regards the germinal vesicle (figs. 37, 38, 39). A generalization to this effect for many kinds of eggs was made by Montgomery in 1898. The eccentricity of the nucleolus showed no regularity in respect to any particular side of the nucleus or even to the wall of the alveolus (figs. 38, 39). The nucleus also often holds an eccentric position as regards the egg-cell, though not infrequently it is located centrally (figs.

34, 56). At the periphery of the egg is a coarsely granular area one or several granules in depth (figs. 38, 39). With iron hematoxylin and orange G the granules stain deep black like chromatin or yolk granules. They are undoubtedly the latter. After a fertilization membrane has been formed these peripheral granules have disappeared, nor are they again to be seen in the blastomeres of the segmentation stages. It is probable that this granular layer contributed to the formation of the fertilization membrane and so disappeared when this was fully separated off. If this view is correct the fertilization membrane is the egg-membrane thickened by the contribution from the yolk-granules and as such separated from the ovum.

Particularly after fixation with sublimate acetic and picro-aceto-sulphuric is the close similarity between the cytoplasm in the living and fixed condition (but for increased definiteness in the latter case, amounting to an identity) very striking. It exhibits a structure of larger and smaller alveoli about whose walls are ranged in single line the minute granules or microsomes as already described by Wilson and here and there throughout the network large granules, probably yolk, similar in size to the peripheral granules. The faithfulness of preservation as observed in the cytoreticulum leaves little room for doubt that also what is seen in regard to the nuclear reticulum, the maturation mitoses, and particularly the dissolution of the nucleolus, are true representations of what actually occurred in the living egg. Atypical stages (fig. 56) can, therefore, in no case be regarded as artifacts due to faulty fixation, but must be interpreted as the result of abnormal development or degeneration processes.

The living egg shows a nucleus with an extremely delicate meshwork spun through a homogeneous ground-substance. Owing to the presence of a large amount of nuclear sap, the fixed nucleus differs greatly from that of the living egg. The reticulum now appears coarse and wide-meshed (figs. 29, 38, 39). It forms a dense basket-work about the nucleolus. After staining with iron hematoxylin and orange G, when the hematoxylin is greatly withdrawn the reticulum is uniformly yellow; when the stain is only slightly extracted chromatic swellings appear along the reticulum, giving it a beaded structure, and larger dark-staining masses (karyosomes) are seen at the intersections of the meshes (fig. 39). Auerbach's stain shows no such difference, but leaves the entire network uniformly red. Thus again it becomes evident that in the linin of the reticulum are areas which in degree of metamorphosis or condensation represent a transition stage between linin and chromatin.

The nucleolus yielded different appearances according to the stains that were employed and the length of their application. With the iron hematoxylin and orange G combination the oöcyte at the height of the growth-period exhibits a nucleolus extremely tenacious of the hematoxylin stain. An amount of extraction which renders the cytoplasm and nuclear retic-

ulum scarcely visible leaves the nucleolus as an intensely black homogeneous sphere, showing no trace of the vacuoles that are at other stages so conspicuous. Auerbach's stain applied at this stage also reveals a dark-green homogeneous nucleolus. That vacuoles are really present is seen from a study of living eggs and becomes manifest also when other stains are employed. The only reasonable conclusion seems to be that the entire nucleolus, vacuoles and ground-substance, are filled or impregnated with chromatin all at approximately the same stage of elaboration.

The same stains applied at earlier and later stages show vacuoles. Borax carmine stains the entire oöcyte red, the cytoplasm, nuclear reticulum, and nucleolus showing progressively deeper shades. The nucleolus under this stain always appears abundantly vacuolated. Orange G stains the nucleolus yellow and reveals a vacuolated structure; combined with iron hematoxylin the main portion of the nucleolus stains black, while the vacuoles remain yellow. With Auerbach's stain the main body stains dark green and the vacuoles red. A combination of orange G and Lyons' blue yields an interesting result. The nucleolus stains yellow and appears vacuolated, while the cytoplasm and nuclear reticulum stain blue. In these sections also the nucleolus shows a very distinct dark-blue wall. This result would seem to indicate that the nucleolar wall is derived from the nuclear reticulum.

Occasionally one meets stages likes the one shown in figure 38, stained with iron hematoxylin and orange G. Here one sees a dark-stained (chromatin) mass separating from a yellow-stained (plastin) mass of similar shape and size. The early maturation stages yield abundant evidence, as will appear in the descriptions which follow, that the nucleolus consists of a plastin ground-substance, throughout which, partly in the form of spherules (vacuoles) and partly as a fluid imbibed by the plastin itself, the chromatin is scattered in varying degrees of elaboration and condensation. Hartmann (1902) likewise describes a double structure of the nucleolus in *Asterias glacialis,* as also Guenther (1903) in *Psammechinus microtuberculatus.* Chubb (1906) states that in *Antedon* the "nucleolar material consists of two substances—the one acidophile and extending throughout the nucleolus, the other deeply basophile and borne by the acidophile ground-substance, to which its presence imparts a considerably firmer consistency."

This gives a clue for the interpretation of the varying appearances when different stains are employed—the nucleoli are formed by the union of a plastin ground-substance with a more or less fluid chromatin content. We have thus a mixed nucleolus. There is here another fact to support the view of a very intimate relation between linin (plastin) and chromatin. There is evidence also to show, as I shall describe later, that chromatin is capable of manufacturing its own plastin. The red vacuoles seen after Auerbach's stain and the yellow vacuoles after iron hematoxylin and orange G are thus the appearance of the plastin ground-substance, now visible be-

cause of the loss of chromatin from the spherules. The yellow nucleolus seen after staining with orange G and Lyon's blue must be explained, I believe, on the assumption that here only the plastin element of the nucleolus took a stain, the chromatin element showing no affinity for either of the stains employed. That the nucleolus should select one cytoplasmic (acid) stain and the remainder of the oöcyte another, remains inexplicable. Nor does it appear whether the selection is a chemical or a physical phenomenon.

Chromatin appears to be transported in a highly fluid form through the nuclear reticulum, where some of it becomes condensed as karyosomes, and some carried to the nucleolus, where it is lodged in the form of spherules. Here the chromatin undergoes further elaboration and condensation and is thus imbibed by the plastin, leaving vacuoles more or less emptied of fluid chromatin. Thus the fact that in all living eggs the nucleoli appear vacuolated is explained by the reasonable assumption that plastin and fluid chromatin in the shape of spherules have different indices of refraction, due to a difference in degree of condensation and possibly of chemical composition. When all the spherules are filled and all the chromatin is approximately at the same stage of elaboration (as at the culmination of the growth-period) the nucleolus stains homogeneously black with iron hematoxylin. When some of the spherules have lost their contained chromatin through extraction by the plastin, real vacuoles appear which seem colored according as the underlying plastin is stained. Whenever the plastin of the nucleolus becomes freed of the chromatin elements it always stains similar to the linin of the nuclear reticulum.

Occasionally one finds full-grown oöcytes which contain besides one large nucleolus several smaller accessory nucleoli scattered through the nuclear reticulum (fig. 56). I have counted as many as fifty of these in oöcytes in which the chief nucleolus was of almost normal size. Most of these were chromatic and stained similar to the chief nucleolus. Evidently such oöcytes have a superabundance of chromatin. I have never seen any such oöcytes mature. Cases of such eggs observed by me are too rare to permit the generalization that an unusually great amount of chromatin is detrimental to maturation and normal development. There is evidently a slight variation in the amount of chromatin contained in different oöcytes of the same individual, but a limit is probably reached beyond which a greater amount is abnormal. These cases, however, give certain evidence that the chromatin may manufacture plastin, or at least compel the morphological arrangement and chemical modification of plastin (linin) to be used as ground-substance, for it was frequently possible to see that these accessory nucleoli had each a plastin ground-substance, and occasionally one was seen with vacuoles similar to those of the chief nucleolus. Again, when the female pronucleus is formed it always contains a plastin nucleolus (plasmosome) (figs. 82, 83, 84). Very rarely this was seen to be chromatic (fig. 81), which may mean

either that the plastin elaborated chromatin or more likely that chromatin extracted from the nuclear reticulum took lodgment there. The male pronucleus exhibits a similar structure (fig. 85). In both pronuclei the plasmosome is lost shortly prior to fusion (fig. 86). All the evidence thus points to a very intimate physical and chemical relation between the linin, plasmosome, and chromatin nucleolus. I am inclined to the belief, in view of my results, that these three substances simply represent different stages in the process of elaboration of the same fundamental substance.

In every full-grown oöcyte are found usually one, sometimes several, masses of chromatin granules (figs. 31, 32, 33, 40). Generally these are in close proximity to the nucleolus (fig. 41), but frequently also removed at varying distances (fig. 38). Almost invariably this mass of granules is on that side of the nucleus nearest the periphery of the oöcyte (figs. 37, 49). In favorable cases I have been able to determine in it the characteristic dumb-bell shape of the definite chromosomes, only somewhat reduced in size. Again, in favorable sections I have been able to count approximately 18 such individual bilobed bodies (figs. 40, 43). There is no doubt that these are the chromosomes which have persisted with identity unimpaired all through the growth-period and are now taking their position in proximity to the nucleolus and periphery of the cell preparatory to maturation. Occasionally the chromosome group or strand lies closely connected with the nucleolus in the reticulum immediately surrounding it (figs. 37, 39, 41). This gives the appearance of a union with the nucleolus. Conklin (1902) describes very similar conditions in the maturing egg of *Crepidula.* It is doubtful if the chromosomes ever penetrate within the nucleolus, but they do often come into very close connection externally. In this state one can often see a portion of the mass extending beyond the border of the nucleolus, simulating an extrusion from the latter. Mathews (1895) mentions the presence of such a mass of granules in *Asterias forbesii,* but beyond saying that it gives rise to the chromosomes of the first maturation spindle gives no further details. Bryce (1901) ventures the suggestion concerning a similar mass of granules in *Echinus* that it may possibly represent synapsis. Tennent (1906), on the other hand, suggests on the basis of experiments on eggs of *Asterias forbesii* subjected to CO_2 treatment and subsequently fertilized that "a conjugation or synapsis of egg chromosomes and sperm chromosomes takes place immediately before the formation of the equatorial plate of the first segmentation spindle" (p. 539). Judging from analogy with other forms where synapsis has been definitely observed, and on the strength of appearances in the youngest oöcytes which seem to agree with the descriptions of early postsynaptic processes in some of these forms, I believe that in *Asterias* also true synapsis occurs some time during the telophase of the last oögonial division. The results above reported, I believe, justify the interpretation of this mass of chromatin granules as the persist-

ence of the postsynaptic chromosomes (sometimes still partially arranged in a thread). Thus the chromosomes have remained throughout the growth period in small individual bulk (always retaining their morphological identity) and in a compact mass. Their proximity to the nucleolus is to be explained in connection with the maturation phenomena.

Occasionally I have seen very close to the nuclear wall a small dumb-bell-shaped body (figs, 40, *a,* 49) (sometimes the body consists of three or even four globes)—somewhat larger than the ordinary bivalent chromosomes. Mathews (1895) describes very definitely the origin of the centrosome from within the nuclear membrane. I have tried to identify this problematical body with the centrosome of Mathews. This I am unable to do for several reasons. Bryce (1903) also describes very similar bodies in *Echinus esculentus,* but says that he has not been able to convince himself "that they are more than accidents of staining and fixing" (p. 491). Hartmann (1902) makes no definite mention of such structure in *Asterias glacialis,* probably including this as well as the above-mentioned masses of granules under the "clumps of chromatin" and "accessory nucleoli." However, eggs in which such distribution of chromatin occurs Hartmann classifies as "abnormal," stating further that in normal eggs all "genuine chromatin and plastin were combined in a single nucleolus." Nor does Guenther (1903) mention this body in *Psammechinus microtuberculatus* or *Holothuria tubulosa.* I tried also to identify it with portion of the chromosome mass, but unsatisfactorily. Though I can make no positive statement concerning it, inclining to the opinion that it represents several or perhaps a single large bivalent chromosome, since it is not invariably present with certainty, I am convinced that it can not be the centrosome. For reasons soon to be given, I hold to an extra-nuclear origin of the centrosome. Furthermore, this problematical body is always at least double, while the centrosome arises as a single structure. Again, its size is several times larger than the largest centrosome I have seen during the maturation process. I have not been able to follow satisfactorily the fate of these bodies amid the general mingling and concentration of chromosomes and nuclear fragments and their exit from the nucleus at one point during the initial stages of maturation.

MATURATION.

NUCLEAR AND CYTOPLASMIC ALTERATIONS.

Study of sectioned material confirms in every respect my observations on the living eggs. In batches of eggs fixed from 5 to 10 minutes after deposition in sea-water the initial stages of maturation are already visible. The first indication is a puckering of the nuclear wall on the side nearest the periphery of the egg (figs. 38, 49, 50). Dissolution or rupture of the wall occurs at this point after an interval of from 15 to 20 minutes (time always reckoned from time that eggs are placed in sea-water) allowing an inter-

change of nuclear and cytoplasmic contents (figs. 53, 54). The puckering and subsequent dissolution of the nuclear wall extend progressively over the entire circumference until after an interval of about 30 minutes, or by the time the first polar spindle is fully formed (though still tangential to surface of egg), the nuclear wall has completely disappeared (figs. 52, 55, 62). Mathews claims to have been able to trace the origin of the centrosomes from a small granule (dividing into two before passing out of the germinal vesicle) within the nuclear membrane, and figures, the centrosomes passing through the ruptured wall. I am unable, as I described above, to identify any chromatic bodies (or such as were differentiated by the stains employed) with a possible centrosome. I am unable to trace any of the several bodies seen in many nuclei in passage through the nuclear wall. Nor is it possible to identify any of the many small dark-staining bodies outside of the nuclear wall with centrosomes. If there really be such, they are indistinguishable from the yolk-granules abundantly scattered through the cytoplasm. It is only where an archoplasmic sphere appears about such bodies that they become recognizable as centrosomes. As such I have seen them arise in the narrow strip of cytoplasm between the nucleus and periphery of egg while the nuclear wall was still, as far as the microscope revealed, wholly intact (fig. 50). This is conclusive proof, I think, against their intranuclear origin, though it still remains possible that the centrosomes may arise from the outer layer of the nuclear wall. I believe it more probable, however, that here also, as described by Griffin for *Thallasema* and by other investigators for various forms, the centrosomes arise in the cytoplasm. Hartmann (1902), without making any mention as to their origin in *Asterias glacialis,* shows illustrations where two fully formed centrosomes with surrounding centrospheres and rays are present, identical with my own in *Asterias forbesii.*

Bryce (1903) thinks that in *Echinus esculentus* the centrosomes arise in the mass of cytoplasm which projects into the nucleus at the time of the rupture of the nuclear wall, thus also indicating cytoplasmic origin for these structures. Their origin in this location may be due to the fact that "the wall of the vesicle is always very close to the surface of the egg, leaving no room for such a formation, and the aster seems to form within the process" (p. 188). The astral and spindle fibers, however, he believes to be spun from the nuclear reticulum. In *Asterias forbesii* there is no such difinite mass of cytoplasm projecting into the nucleus, nor are there any facts to support the view that the asters and spindle fibers arise from the nuclear reticulum.

The centrosome in *Asterias forbesii* is at first a single structure of the form of a central granule (staining black with iron hematoxylin) within a surrounding sphere (centrosphere) of orange-staining (with orange G) archoplasm. There is unmistakable evidence that this single body divides

into two. Even shortly before this division astral rays are seen to take form round the single centrosphere. These are undoubtedly formed from the cytoplasmic reticulum, as Wilson has shown in the sea-urchin's egg. The rays abut the nuclear wall and indent it slightly. The archoplasmic mass now begins to elongate. Presently it assumes a dumb-bell shape, a bridge of delicate fibers showing between the two globes. Each of the globes now contains a centrosome (centriole of Boveri) surrounded by a centrosphere and astral rays. The two centrosomes separate more and more, moving over the surface of the nucleus, their astral radiations elongate and press upon the nuclear wall, indenting it deeper and deeper until it finally ruptures, allowing thus an intermingling of cytoplasm and nuclear content. Rupture seems to be due to pressure plus some solvent influence of the rays. It is apparent that no part of the achromatic structure of the first polar spindle came from within the nucleus, for the wall in some cases is still intact when the two centrosomes and asters (from which the spindle is spun) are already formed (fig. 50). While the centrospheres enlarge and the astral rays lengthen, the latter also increase perceptibly in thickness. This is particularly true of the traction fibers (Zugfasern), to which the chromosomes become attached and by which they are drawn into the central spindle (figs. 51, 52, 61, 62). The spindle is spun between the two centrosomes by the union of the astral rays very much in the manner described by Child (1896) in *Arenicola marina*. The outermost rays and those that do not unite or blend to form the fibers of the central spindle interdigitate with each other in the median plane (figs. 52, 64). The astral rays merge at their distal ends into the general cytoreticulum. When the first polar spindle is fully formed the centrospheres have a reticular or alveolar structure, and occasionally two centrosomes are seen (fig. 66).

NUCLEOLAR CHANGES.

When maturation is imminent the nucleolus is usually in a position on that side of the nucleus nearest the cell wall (fig. 37, 50). In this position many of the astral rays of the first polar spindle are extended directly upon it (figs. 53, 58, 59). The fact that the nucleolus as well as the mass of chromosomes and the occasional problematical body are all concentrated into this narrow space, combined with the further fact that the nucleolus begins to fragment and the chromosomes to scatter among these fragments, while the astral rays are extended among them indiscriminately, makes it difficult ordinarily to trace the fate of these several structures. However, exceptionally favorable conditions permit of observations which leave no doubt as to the correct interpretation of the more complicated processes. Particularly favorable for such study is the condition where the chromosome mass and nucleolus at the beginning of maturation are widely separated (figs. 32, 38, 43, 46, 47, 48, 49).

Very evident is the fact that the nucleolus undergoes dissolution. The first stage of this dissolution is a fragmentation into larger and smaller masses (figs. 46, 47, 48, 53, 58). Less evident is the ultimate fate of the nucleolar fragments. Even before the rupture of the nuclear wall the initial stages of the nucleolar dissolution begin (figs. 43, 50). It is usually consummated by the time that the first polar spindle has revolved into the radial position, all traces of either the plastin or chromatin elements of the nucleolus having been lost. Occasionally, however, a small chromatic nucleolus, "metanucleus," persists for a considerably longer time, even until the first polar body is formed (figs. 55, 60, 61, 62). It is ultimately also resorbed by the cytoplasm of the egg and is never seen until the first segmentation, when it is passed to one of the blastomeres, as described by Wheeler (1895) for *Myzostoma glabrum* (here persisting to 8-cell stage).

The dissolution of the nuclear wall and the fragmentation of the nucleolus are synchronous processes. Undoubtedly both these processes contribute to the decided change that the nuclear reticulum now undergoes. Immediately prior to maturation the nuclear reticulum was achromatic and wide-meshed (figs. 32, 35, 38). By the time the nuclear wall has partially disappeared the network becomes markedly close-meshed and chromatic (figs. 51, 52, 53, 54, 55). The meshwork takes on a characteristic beaded structure. Such nuclear residuum (always closer meshed and deeper staining in iron hematoxylin than the surrounding cytoplasm) is clearly seen to persist until the time the first polar body is fully formed (figs. 52, 63). It accompanies the polar spindle in its progress to the periphery of the egg. The major mass lies about the central pole, closely surrounding it and forming a mantle about the spindle to about the middle, and parts of it are seen even as far peripheralward as the distal pole of the spindle (fig. 67). Conklin (1905) in *Cynthia* and *Ciona,* and Lillie (1906) in *Chætopterus,* have described this "residual substance" of the nucleus in detail and have succeeded in tracing it through the early ontogenetic stages following fertilization. I was unable satisfactorily to trace the "residual substance" in the eggs of *Asterias forbesii* beyond the stage when the second polar spindle was being formed (figs. 70, 71). It appears that at this stage it becomes assimilated with the cytoplasm, probably contributing thereto the chromatin that it received from the nucleolus at the time of its dissolution and so playing the rôle of a "formative stuff."

The nucleolar fragmentation and dissolution may occur in several different ways. Usually the nucleolus breaks up into several larger masses and from these the chromatin gradually escapes in the shape of granules (viscid drops) leaving the several large masses of plastin ground-substance (figs. 50, 52, 53, 54). Sometimes all the chromatin leaves the plastin nucleolus in a mass (fig. 38) and subsequently breaks up in the nucleus, the plastin being gradually resorbed by the protoplasm. Sometimes the plastin ground-sub-

stance is vacuolated and frequently it is very finely alveolar or apparently homogeneous (figs. 38, 48). Sometimes the chromatin condenses in the center of this plastin mass, leaving numerous vacuoles behind and subsequently fragments leave the main mass. Sometimes the chromatin breaks up into numerous small spherical bodies scattered over the plastin. Again, small masses of chromatin leave the nucleolus one after another, each leaving a vacuole behind (here the chromatin was partly held as viscid drops in the form of spherules in the plastin) until all the chromatin is extracted from the plastin. All of the above processes of nucleolar dissolution are apparently normal. There appears to be absolutely no uniformity in the manner in which the nucleolus dissolves. The important thing seems to be that the nucleolus should break up at maturation and be prepared for appropriation and assimilation by different parts of the egg, the manner of its dissolution having no significance. In all cases a plastin remnant is left behind by the chromatin, which appears to have no further function, but is straightway resorbed by the cytoplasm (figs. 52, 54). The chromatin, on the other hand, is distributed partly to the chromosomes, partly to the nuclear reticulum, and occasionally persists in part as a "metanucleus."

THE ORIGIN OF THE CHROMOSOMES.

Concerning the origin of the chromosomes of the first maturation spindle, varying opinions are held by different investigators, even regarding very closely allied animal species. Some maintain that the chromosomes arise exclusively from the nucleolus; others hold that the nucleolus contributes nothing to their formation; and a third class holds that they arise in part from the nucleolus and in part from the nuclear reticulum. A brief survey of recent expressions of opinion on this point is desirable here, particularly because I believe that my results indicate that the divergence of opinion is not as great as at first appears, and that a nucleolar origin of chromosomes does not really involve the question of the individuality of the chromosomes, as some investigators have recently held in several cases where the chromosomes seemed to arise from the nucleolus.

Among botanists a similar difference of opinion has arisen. Wager, in his recent paper on the nucleolus in the root-tip cells of *Phaseolus,* gives an excellent review of the literature on the nucleolus in the plant-cell. He concludes that the nucleolus in many of the higher plants is really a portion of the nuclear network and that it contributes some material at least to the formation of the chromosomes.

Manifestly where the nucleolus is a simple plastin body the question as to its relation to the chromosomes does not arise. In amphibia, where many chromatin nucleoli are present, this point has been much discussed. O. Schultze (1887) states that in the ova of *Rana* and *Triton* the nucleolar substance takes part in the formation of the chromosomes. Born (1894) is

of the contrary opinion. Carnoy and Lebrun (1897–1899) state their conclusion concerning amphibia that the chromosomes are derived from the nucleoli. Their figures are very convincing on this point. Macallum (1891) believes that the chromosomes have a double origin in amphibia. Jordan (1893), basing his opinion on observations made on the ova of the newt, believes that the nucleolar particles do not contribute to the formation of the chromosomes. Lubosch (1902), in the case of the ova of *Triton,* states: "Es ist sicher, dass die in diesen Stadium (maturation) vorkommeden Chromosomen zum Theil nukleolären Ursprung haben" (p. 250), thus agreeing substantially with Schultze.

Holl (1893), investigating the ovum of the mouse, finds that the central granules of the nucleolus wander out and so become chromosomes. Sobotta (1895), on the other hand, holds that the chromosomes are not derived from the nucleoli only, but from the whole chromatic substance of the nucleus.

According to K. Foote and E. E. Strobel (1905), the chromosomes of the first maturation spindle in the annelid *Allobophora fœtida,* "are formed by a gradual segregation of the chromatin, which is dispersed through the germinal vesicle," the nucleolus meanwhile persisting in its original form and size. The chromosomes are thus not formed at the expense of the nucleolus. Similar conclusions were reached by Korschelt (1895) in the case of the annelid *Ophryotrocha,* and by Wheeler (1897) for *Myzostoma,* and by Griffin (1899) for *Thalassema.* There seems to be almost complete agreement among investigators that in annelids the chromosomes are not derived from the nucleolus. Coe (1899) inclines to this opinion also in the case of *Cerebratulus,* as also Gathy (1900) for *Tubifex* and Van Beneden (1883) for *Ascaris megalocephala.* However, in the case of *Chætopterus,* according to the figures of F. Lillie (1906), the chromosomes find at least a partial source of origin in the nucleolus; also Vejdovsky (1888), who studied the ova of *Rhynchelmis,* and Blockman (1882), who investigated *Neritina,* incline to a nucleolar origin of the chromosomes. Both Halkin (1901) and Goldschmidt (1902), as a result of their study of the ova of the trematode *Polystomum integerrimum,* hold that the chromosomes are derived exclusively from the nucleolus.

Results of recent observations on echinoderm eggs point to at least a partial nucleolar origin of the chromosomes in the various forms. R. Hertwig (1896) studied the unfecundated eggs of the sea-urchin and the starfish poisoned with strychnine. He states that the nucleolus vanishes within the nucleus as the chromosomes appear, and he holds the opinion that the chromosomes receive a portion of their substance—"notwendiger Ergänzungs material"—from the disappearing nucleolus. E. B. Wilson (1901) finds two widely different types of chromosome formation in the eggs of the sea-urchin (*Toxopneustes variegatus*) artificially fertilized by Loeb's magnesium chloride method. The two types, however, did not coexist in the

same series of eggs. In one type the chromosomes arose from the nuclear reticulum (here the nucleolus was a plasmosome), in the other from a chromatin nucleolus. *Asterias* presents no condition which can be reconciled with either of the types described and figured by Wilson.

Max Hartmann (1902) concludes for *Asterias glacialis* that "during the growth-period of the ovarian eggs there occur 'vegetative nuclear alterations,' the distribution of chromatin substance in the nucleus, and accumulation of the same in the nucleolus. At the end of this period all the chromatin and plastin is combined in the nucleolus, and out of this there arise at the time of the shedding of eggs into the water with the appearance of an astral structure and dissolution of the germinal vesicle, *the chromosomes of first maturation division.*"

K. Guenther (1903) reports the following in the case of *Psamechinus microtuberculatus* and *Holothuria tubulosa:* "Der Nucleolus stellt einen vom Kerngerüst ausgeschiedenen Tropfen, in den das Chromatin hineindringt um sich in ihn zu sondern und für seine Theilung zu ordnen," p. 23. He holds that the chromatin of the germinal vesicle is collected and stored in the nucleolus during the growth-period of the oöcyte, that it undergoes there possibly some physical and chemical changes, and so wanders forth again at the time of maturation to give rise to the chromosomes of the first division. He remarks further that the "Chromatinfaden (hat) bei seiner Auswanderung eine kleine Vacuole zurückgelassen, und wenn nun bei diese der Kernsaft eintritt, so ist am Raum nicht verloren."

T. H. Bryce (1903) states that in *Echinus esculentus* "the chromatin substance is at first confined to the nucleolus, and later leaves it to form the chromatin basis of the nuclear network as a whole and therefore also of the future chromosomes," but adds that the nature of his material makes it impossible for him to deny or affirm the direct origin of the chromosomes from the nucleolus.

My own observations on the eggs of *Asterias forbesii* establish beyond a doubt, I believe, the fact that while the chromosomes often *appear* to arise from within the nucleolus, as described by Hartmann (1902) and Guenther (1903) in certain echinoderms, they never really penetrate beyond the surface of the nucleolus, at least to the extent that their individuality is lost and their substance merged into the common chromatin substance of the nucleolus. Study of the different stages throughout the growth-period shows that the chromosomes always retain their identity, though they are greatly decreased in size, and they mass together into a clump which often attaches itself closely to the nucleolus, from whence the chromosomes pass into the spindle during early stages of maturation. This phenomenon of chromosome disposition is very similar to what Conklin (1903) has described in *Crepidula* and Lillie (1906) in *Chætopterus.* According to the former, "the chromosomes, which are at first widely scattered through the nucleus,

gather together more closely and often lie immediately around and upon the nucleolus. In some cases it looks as if these chromosomes were being formed out of the substance of the nucleolus . . . and though it is possible that they may later receive substance from the dissolving nucleolus, it is impossible to suppose that they are fragments of the nucleolus." The latter states in the case of *Chætopterus* that "the chromosomes begin to separate from the surface of the nucleolus as soon as the wall of the germinal vesicle is ruptured, and the nucleolus (in consequence?) appears shrunken and vacuolated" (p. 176).

In *Asterias forbesii* I found many eggs in which the relation between the chromosomes and nucleolus was far less intimate than that described above in the case of *Crepidula* and *Chætopterus*. Indeed, numerous transition stages were found between such in which the nucleolus and chromosomes were at opposite poles of the nucleus and such in which the chromosome mass was closely applied to the nucleolar surface. Figures 38, 41, 42, 43, show such transition stages. In figure 46 the fragmenting nucleolus was at the pole nearest to the aster and its rays were seen really to touch the chromatin mass. The chromosome group was near the opposite pole of the nucleus. No chromosomes or chromatin masses could be found among the astral rays. This is additional proof that no chromosome could come from the nucleolus alone. In another case, where the nucleolus was breaking up at the opposite pole of the nucleus, while the chromosome group was situated next the aster, some chromosomes were seen among the rays (figs. 47, 48). These could not under the circumstances have come from the nucleolus. In these cases the chromosomes are always very small.

Where the separation of the nucleolus and chromosome mass is of less distance there is very clearly evident a tendency for the two to get into more intimate connection. Frequently a chromatin thread is seen to pass out from the nucleolus (figs. 30, 31, 32, 36). Delicate threads of chromatin and small chromatin masses are also seen to pass from the nucleolus into the nuclear reticulum (figs. 35, 38, 49) to which they probably give its darker staining capacity, which is seen to progressively increase at this time. Where the distance between the nuceolus and chromosome mass just previous to maturation is slight there is usually a chromatin cross-connection between the two. It appears very probable that the normal condition is a very close connection between nucleolus and chromosomes (figs. 37, 41). Perhaps if the eggs had been allowed to be shed and mature normally, a very close connection between nucleolus and chromosomes would in all cases have been established before the chromosomes were drawn into the spindle. It is possible that in these artificially matured eggs sufficient time had not been given for the two to draw together. That eggs in which such close connection is not attained may still mature normally is proved by many

instances where the chromosomes thus related to the nucleolus do still pass into and help complete the first maturation spindle. The point of importance seems to be that some connection, however remote, be established whereby chromatin may be transferred from the nucleolus to the chromosomes. Such cross-connections of chromatin are very frequent and conspicuous. There remains little doubt that the arrangement signifies a transfer of matter from the nucleolus to the chromosomes. Moreover, the chromosomes, in cases where such connections are made, always increase in size before they pass to the spindle. Where the chromosomes are in such intimate relation with the nucleolus that they detach themselves from it at maturation (figs. 51, 52, 58, 59) they are frequently seen to draw out the chromatin after them, thus giving the appearance of coming from out of the nucleolus (figs. 52, 54). The entire body of the nucleolus is broken up at this time, and each particle, with its plastin ground-substance and some of the fragments, is drawn toward the spindle, only, however, to contribute their substance to the growing chromosomes or to be eventually resorbed by the cytoplasm. What chromatin is not thus disposed of for the time being may be retained as a small spherical nucleolus lying in the residual substance of the nucleus until about the time that the second polar spindle is formed (fig. 60). It seems important to emphasize again that in *Asterias forbesii* the chromosomes are not derived from out of the nucleolus, but that a close connection is generally established between the two, for the purpose of bringing about a transfer of chromatin material, the chromosomes in consequence increasing in size during the early maturation stage.

THE POLAR SPINDLE AND POLAR BODIES.

After an interval of from 30 to 45 minutes the first polar spindle is formed. The transfer of the chromosomes from the nucleus to the complete spindle is effected in from 20 to 30 minutes. The process appears to be as follows: While the asters are still close together their rays have penetrated into the nucleus and some of these (Zugfasern) have become attached to the chromosomes. Which is the active factor in effecting a connection I have been unable to determine. It seems likely, however, that the astral rays seek the chromosomes (largely a fortuitous matter, except that the two are normally always in the same vicinity of the nucleus) rather than the reverse. As the chromosomes are becoming attached to the rays, the asters gradually move apart, progressively decreasing the angle at which the rays spring from the centrospheres and so drawing the chromosomes into the spindle, whose rays are now approximately horizontal to the periphery of the egg and parallel to each other. The astral rays become arranged parallel to each other and continuous from one centrosphere to the other by blending of those from separate asters to form the central spindle. Some of the spindle fibers are clearly stouter than others, and it seems very probable that here

also, as Child holds in the case of *Arenicola marina*, several fibers may have united into one. Some of the astral rays interdigitate above the equatorial plate. All seem to merge into the cytoreticulum. Frequent cross-connections are seen. The central ends of the rays are seen to penetrate for some distance into the centrosphere, but they could never be traced as far as the central granules (centrosome). It is very probable that the rays are centrally continuous with the reticulum (very delicate, and sometimes alveolar in structure) of the centrosphere. The spindle now begins to rotate and ultimately assumes a radial position. This rotation is effected very rapidly. In 10 to 15 minutes more the first polar body is fully formed. Thus it requires about 1 hour for the first polar body to form, which agrees with what was observed in the case of living eggs. To recapitulate, the single aster appears about 10 minutes after transference of the eggs to sea-water. At twenty minutes there are two asters some distance apart, with rays extending through the ruptured nuclear wall and becoming attached to the growing chromosomes. At 45 minutes the first polar spindle is fully formed and in process of rotation. The last 20 or 30 minutes represent the prophase of maturation. The spindle moves rapidly into a radial position, metaphase and anaphase are passed through very quickly, and after 60 minutes the first polar body is fully formed.

In the radial position, the spindle is at first comparatively slender and sharply pointed at the ends (figs. 66, 67). The central sphere often contains two centrosomes (fig. 66). The spindle moves bodily toward the periphery and as it approaches the cell-wall it becomes stouter, somewhat barrel-shaped, slightly shorter, and its ends less pointed (fig. 69). This change of shape and size is undoubtedly due to the resistance met with by the spindle in its passage through the cytoplasm. The outwardly pointing rays of the distal pole disappear, the horizontal rays are at first bent inward, and all eventually disappear into the cytoreticulum as the outer pole of the spindle is forced out of the egg to form the first polar body (fig. 70). The rays of the central aster have also meanwhile become shorter and less definite, while the centrosphere has become more reticular, somewhat flattened, very much less definite, and in the later stages of the first polar spindle is scarcely to be recognized. In the late anaphase the spindle exhibits a distinct mid-body (Zwischenkorper) in the form of swellings or small granules in the equatorial plane of the spindle. This marks the line of division between the first polar body and the central pole of the spindle. Traces of spindle fibers are at first visible in the first polar body, but these gradually disappear, and the chromosomes (about 18 double dumb-bell-shaped bodies, often assuming the form of "tetrads") are seen to lie in a homogeneous or finely granular light-staining substance (figs. 70, 71). In my study of the living eggs I found a single instance of a division of the first polar body. Study of sectioned material failed to contribute a duplicate of this solitary example.

Cases of undoubted abortive attempts (fig. 74) at a division were seen, but never a consummation of such process. The isolated case, therefore, must stand as an exception to the ordinary process of maturation.

The second polar body is constricted off in about 30 minutes after the first is formed. The process of spindle formation must here be very rapid. As far as I know, the process of spindle formation between the two centrosomes of the central pole of the first spindle has never been described in echinoderms. Miss Hogue figures a second maturation spindle in tangential position and believes she has evidence that the spindle is always so formed and subsequently revolves into radial position (p. 525). Mathews (1895) states that "the outer centrosome of the second polar spindle is formed at the 'Zwischenkörper' of the first" (p. 334). All the evidence that my study of this stage yields tends to corroborate the correctness of Mathews's observation. Figures 70 and 71 seem to show transition stages of such process. The evidence from these figures, coupled with the fact that the actual formation and growth of the second spindle at the pole of the first has never been seen, renders it very plausible that the second spindle is formed as described by Mathews. Furthermore, the central portion of the first spindle is never seen to disappear, and after the second spindle is fully formed two centrosomes are still often seen in the centrosphere. It is strikingly characteristic of the second spindle that it usually bends to one side of the first polar body, the second being then extruded to one side of the first (fig. 74). Such oblique position of the second polar spindle seems to add evidence in favor of a rotation from a tangential position, but facts like those just recorded render the normally tangential origin of the second polar spindle very improbable. The second polar spindle is somewhat shorter and slighter in bulk than the first (figs. 71, 72, 73). It again shows very conspicuously a mid-body composed of granules on the spindle fibers in the equatorial plane (fig. 73). The second polar body is constricted off along the line of the mid-body (fig. 74). It is slightly smaller than the first polar body and contains 18 single small dumb-bell-shaped chromosomes. In other respects it is similar to the first polar body. The chromosomes soon lose their dumb-bell shape and become short, stout rods or spherical masses. At this stage the chromosomes of the first polar body have assumed similar shapes or occasionally have become massed into two larger clumps connected by a strand of chromatin (figs. 73, 74). The eighteen dumb-bell-shaped chromosomes remaining in the egg after the second polar body is formed undergo similar transformations as those in the polar bodies, becoming short, stout, cubical bodies or small spheres (fig. 75). The centrosomes have disappeared from view and distinct trace of the centrosphere is lost. The astral rays still persist and are often seen to accompany the female pronucleus up to the time of fertilization, so that the egg-nucleus seems to be provided with an aster of its own, as also reported by Tennent (1906) in

some cases. The female pronucleus is formed from the fusion of five or six vesicles, the product of transformation of the eighteen chromosomes remaining in the egg after maturation.

At no stage in the maturation process are all of the chromosomes at the same stage of transformation. Almost every spindle shows several chromosomes lagging far behind (figs. 64, 67, 68, 73). This explains why the counting of the chromosomes in polar view becomes difficult, not to mention their minute size. The most favorable sections for counts are such as pass longitudinally through the poles of a prophase figure. Since the chromosomes are scattered through the equatorial plane, sections of from 7 to 10 microns often include the major part of all the chromosomes. Such sections almost invariably show 18 chromosomes in prophase (fig. 64). Similar sections through the anaphase stages again invariably show 18 chromosomes. The number could never be made less than 18; frequently in the anaphase of the first division 20 (occasionally even 24) V-shaped and bilobed chromosomes were counted, but these counts could usually be satisfactorily reduced to the usual number by taking into account the fact that some had prematurely split in preparation for the second mitosis. Polar views of either the prophase or anaphase stage also never showed less than 18 chromosomes. This exact number could frequently be counted (figs. 76, 77, 80), but in such sections even more frequently than in longitudinal sections of the spindle the number counted was 20 (fig. 79), 22, 23 (fig. 78), or even as high as 24 chromosomes. It is very evident that premature splittings of bivalent or univalent chromosomes—the normal process in the first maturation division—would raise the count in such sections and show wide and illusive variations in number. The best evidence shows that the reduced number of chromosomes is 18.

THE REDUCTION OF THE CHROMOSOMES.

The maturation phenomena of *Asterias forbesii* agree with those reported by Bryce in *Echinus* and likewise present a simple case of double longitudinal division. My results are at variance with those reported by Tennent (1905) for this same species. Tennent describes the second maturation division as transverse, separating bilobed chromosomes into chromosomes of globular shape. I have conclusive evidence (figs. 73, 74) that the chromosomes resulting from the second polar mitosis are also for some time after their separation bilobed (dumb-bell-shaped) bodies and only subsequently become stubby or globular. The fact that the eggs observed by Tennent had undergone previous treatment with CO_2 in sea-water, which seems to have greatly retarded the maturation process (40 minutes according to Tennent), may account for the discrepancy in appearance of the chromosomes. It is possible that in the CO_2 treated eggs the bilobed chromosomes had become globular proportionately earlier—in consequence of a much slower transit toward the spindle poles—than in eggs under normal conditions.

The fact that the early segmentation stages give a chromosome count in the prophase of approximately 36 indicates that the 18 bilobed chromosomes of the first maturation prophase are mostly, if not entirely, bivalents. Moreover, their double character is occasionally plainly evident (figs. 64, 65). However, since synapsis could not be observed, no conclusive evidence appears as to the valency of the chromosomes, nor as to whether the chromosomes coupled endwise or sidewise, nor in which direction the condensation occurred to give rise to the bilobed form; hence no positive statement is justified as to whether the reduction is quantitative or qualitative. If the chromosomes fused side by side in synapsis and the bivalents were so transformed into a bilobed body that each globe can be represented by *AB*, then the double longitudinal division effects a true reduction, and the resulting chromosomes are *A*'s and *B*'s. If one globe represents *A* and the other *B*, then the resulting chromosomes are *AB*'s. Similar possibilities result from a double longitudinal division if the synapsis was endwise.

FUNCTION OF THE NUCLEOLUS.

As to the function of the nucleolus in the germinal vesicle, besides giving rise to the chromosomes, various opinions are held by different writers, some ascribing to it an incidental rôle in conjunction with chromosome formation, others a very definite rôle exclusive thereof. I shall not undertake to give a full discussion of so complicated and difficult a subject. I desire merely to call attention to a few of the most divergent views in regard to this enigmatical cell-constituent and harmonize my own observation with one or the other of these.

Pfitzner (1883), basing his opinion on his investigation of the ectodermal cells of *Hydra*, makes the generalization that the nucleolus has merely a passive function in mitosis: "die einer aufgespeicherten Nahrungs-materials zur Neubildung von Chromatin." He terms the nucleolar substance "prochromatin," since he finds that in mitosis it changes into chromatin. Schneider (1901) thinks that the large nucleoli of echinoderm eggs are but reserve masses of chromatin. The experiments with artificially fertilized echinoderm eggs by R. Hertwig (1895) and Wilson (1901) seem to confirm the validity of Schneider's view, at least under certain conditions. Rhumbler (1893) says: "Mir scheinen die Binnenkorper (nucleoli) Reservestoffe darzustellen, die für Zeit aufgespeichert werden, wo die Theilung eine grosse Zunahme des Vererbungs Apparates bez. des Idioplasm im Sinne Weismann's erfordert, wo aber diese Stoffe nicht rasch genug durch die Zellmembran hindurch Nahrung finden können."

Häcker (1895) states that in *Æquorea* and in various annelids and echinoderms "the nucleolus is cast out bodily into the cytoplasm, afterwards lying there for some time as a 'metanucleus' before degenerating. In these cases the chromosomes are formed independently of the nucleoli . . . it seems

quite certain that the nucleoli do not contribute to the formation of the chromosomes and that their substance represents passive material, which is of no further direct use." On the ground that in *Echinus* he found the large vacuole of the nucleolus contractile, he regarded the latter as an excretory organ collecting the by-products of nuclear activity.

E. B. Wilson (1896), agreeing with the conclusion of Häcker, states his opinion "that the nucleoli of the germ-cells are accumulations of by-products of the nuclear action, derived from the chromatin either by direct transformation of its substance or as chemical cleavage products, or secretions."

Certain observers, notably Flemming, O. and R. Hertwig, and Carnoy, regard the nucleoli as storehouses of material—paranuclein and plastin—which plays an active rôle in nuclear activity in contributing to the formation of chromosomes during division. Strasburger (1895) considers the nucleoli storehouses of active material which he calls "kinoplasm," and which he thinks gives rise to the achromatic part of the division figure, Hautschicht, membrane, and cilia.

Montgomery (1899), in his masterly work, "Comparative Cytological studies, etc.," gives a very complete review of the literature on the nucleolus. As the result of his own observation he is led to consider the nucleoli of egg-cells and somatic cells in the Metazoa as homologous cell organs. He regards the nucleoli "as extranuclear in origin, and not a secretion or excretion of the nuclei . . . consisting of a substance or different substances, taken into the nucleus from the cell-body." He thinks it probable that "these substances stand in some relation to the nutritive process of the nucleus."

According to Fick (1899) the nucleolus is simply a storehouse or laboratory of nuclein. Bryce (1903) combines the views of Strasburger and Fick in regard to the nucleolus in *Echinus esculentus*.

My study of the nucleolus (germinal spot) in the egg of *Asterias forbesii*, both in the living and fixed condition, yields no evidence in support of either Häcker's "Kernsecret-theorie" or Strasburger's "kinoplasmic" theory. Nor do my results accord with the view of Bryce in so far as he adds the "kinoplasmic" to the "storehouse" theory, to explain the nucleolar function in *Echinus*. The evidence above given is conclusive, I believe, that in *Asterias* the nucleolus is a storehouse of reserve chromatin. This is demonstrated to be true by the fact that just previous to maturation connections are established by virtue of which chromatin material passes from the nucleolus to the chromosomes and in consequence of which the latter grow in size. In view of the fact that the chromosomes never really enter the nucleolus, it is very doubtful whether any "idioplasm," as Rhumbler believes, is stored in the nucleolus. I believe that all the hereditary elements are persistently held by the chromosomes whatever their various size and form throughout the growth-period of the eggs, and that these merely receive

nutritive material from the nucleolus. That only a small portion of the chromatin material of the nucleolus is contributed to the growth of the chromosomes is very clear, but whether the extra portion of matter is similar in chemical nature to that which goes into the chromosomes, and whether this material changes its chemical nature on entering the chromosomes, I am unable to determine, staining reaction giving no indication of such a change. It is possible that some of the chromatin-like material stored in the nucleolus is waste material and the product of metabolic process of the growing egg. The fact that this residue, as well as the plastin ground-substance, are both ultimately resorbed by the cytoplasm gives some color to this latter view. However, it seems more probable that all the material (chromatin as well as plastin) is of closely similar chemical composition and has similar nutritive value, whether it passes into the chromosomes or cytoplasm of the mature egg. It seems more reasonable that excretion products consequent upon cell metabolism should be voided continually, instead of being stored in the same structure in common with the undoubted nutritive material. Moreover, I have no evidence to show any direct genetic relation between the material of the disappearing nucleolus and the achromatic structure of the egg.

The nature of my material has made it impossible for me to trace the origin of the nucleolus from its earliest stages, but the fact that for a time it appears to grow by additions of material from without the nucleus (as indicated by the varying staining reaction of the cytoplasm during the growth-period of the oöcyte) adds support to Montgomery's view that the nucleoli of all cells are of extranuclear origin. The nucleolus increases in size still more by the addition of the chromatin surrendered by the post-synaptic spireme as it segments and condenses into chromosomes. What the chemical alterations which accompany this local change and morphological transformation of the chromatin are it is idle to conjecture.

If my interpretation of the vacuoles which are seen in the nucleoli of the living egg is correct, the chromatin at first enters the nucleolus in the shape of spherules or fluid drops of chromatin. This fluid chromatin is continually imbibed by the plastin ground-substance, which increases in amount as the chromatin content increases, being probably manufactured by the chromatin and, as also the linin, representing probably merely a different phase of the same substance. During this local change the chromatin seems to alter its physical composition from a fluid to a more or less viscid constituency. The vacuoles that appear in the sectioned oöcytes, and which take a stain similar to that of the underlying plastin, thus represent the remains of the spherules of fluid chromatin after this has been incorporated into the main mass of the nucleolus. Fusion of several such spherules will leave large vacuoles in the nucleolus. When all of the spherules are entirely filled with chromatin, sections of such nucleoli stained with specific chromatin stains (such as iron hematoxylin) appear homogeneous. Stains for which

chromatin has no affinity reveal only the plastin portion of the nucleolus and as a much vacuolated structure.

All the data at my command as a result of the study of the egg of *Asterias forbesii* support the view that the nucleolus is a storehouse of reserve nutritive material, combining also the function of "nuclein laboratory" (Fick) in the sense that chemical alterations may transpire in the material while thus stored in the nucleolus.

COMPARISON WITH HIPPONOË ESCULENTA.

Material for a comparative study of the present problem in echinoderms was collected during a four weeks' stay at the Marine Biological Laboratory of the Carnegie Institution of Washington at Dry Tortugas, Florida. It was my intention to extend the investigation over many different forms. Among at least ten different genera sufficiently abundant and apparently equally favorable for similar study, *Hipponoë esculenta* alone had ripe eggs at the time I left the island on June 13. From the appearance of the gonads at that time it seemed probable that most of these forms would not ripen their eggs for several months.

Hipponoë esculenta was apparently at the height of its breeding season during May and the early part of June. Due to the smaller size of the egg, and particularly to the fact that the oöcytes mature in the ovary, this form is much less favorable for a study of the maturation phenomena than *Asterias,* where the process takes place after spawning and can be readily observed and controlled in the free egg. Unsuccessful attempts were made to induce the immature oöcyte of *Hipponoë* in sea-water to form the polar bodies by agitation, the addition of sperm, and the addition of various salts and acids. However, the addition of a drop of HCl to a dish of sea-water containing mature eggs caused a small percentage to develop through the early cleavage stages. Controls of eggs from the same batch and in the same water without the HCl showed no segmentation stages.

As in *Asterias,* there is in the ovary of *Hipponoë* a striking lack of transition stages between the oögonia and the full-grown primary oöcyte, giving evidence of the great brevity of the growth-process. The infrequency of the oöcytes in maturation shows that this process also is consummated very quickly. Batches of eggs taken from the ovary of even the smallest specimen yielded at the highest only about 10 per cent of immature ova. In the larger specimens all the ova were fully matured. A section through the ovary of the latter revealed a layer of oögonial cells, a few immature full-grown oöcytes, and a large number of mature ova. The female pronucleus is comparatively larger than that of *Asterias,* and it is characteristically devoid of a nucleolus (fig. 95). Toto mounts of eggs show two small polar bodies. Sections of the first polar body reveal an attempt at a second division (fig. 94).

The primary oöcyte near the end of the growth-period has a large eccentrically located nucleus. The nuclear network is wholly achromatic and enmeshes several chromatin nucleoli. The number of nucleoli at this stage (fig. 87) is never less than two and more frequently six or more, all without vacuoles. As development proceeds, the oöcytes enlarge slightly in size. There appears now a progressive increase in the amount of chromatin (fig. 88). The nuclear network becomes more closely meshed and the threads become stout and intensely chromatic (fig. 89). A single nucleolus only remains. A slightly later stage (fig. 90) shows the same process merely carried to a greater degree. The nuclear reticulum, furthermore, appears arranged in the form of a stout spireme. The single nucleolus still persists.

In a succeding stage (fig. 91) the nucleolus has entirely disappeared. The chromatin network has become arranged in a tangled knot and the astral rays of the first polar spindle are pushing upon the fading nuclear wall. The single(?) spireme breaks up into a number of chromosomes (fig. 92), which are drawn as bilobed bodies into the equatorial plane of the first polar spindle (fig. 93). The achromatic parts of the spindle are very coarse and distinct. As in *Asterias,* here, also, the first maturation spindle is somewhat larger than the second. There is no indication of the persistence of the central aster of the second polar spindle about the female pronucleus, as was noted in the case of *Asterias.* Due to the very minute size of the chromosomes, both in the maturation and cleavage divisions, the exact number could not be counted, nor could the manner of the reduction be definitely determined. The reduced number of the chromosomes lies somewhere between 16 and 20. Some evidence appears here also to indicate that the reduction is accomplished by a double longitudinal division of the original bivalent chromosomes. Figure 93 shows the elements of a postsynaptic chromosome drawing apart, with each element at the beginning of a longitudinal fission. The chromosomes in the anaphase of the second polar spindle are still bilobed and so support the hypothesis of a second longitudinal division.

In the case of *Hipponoë esculenta* it is clear that the chromosomes originated proximately from the nuclear reticulum of the full-grown primary oöcyte. It is equally clear that ultimately they received much (possibly all) of the chromatin from the disappearing nucleoli. Hipponoë thus agrees with *Asterias* in the essential point that the chromosomes do not originate from within the nucleoli, but that the latter contribute chromatin (in *Hipponoë* much; in *Asterias* little) to them prior to their entrance into the first polar spindle. A marked difference in the process between these two forms is in regard to the time when the chromosomes are first formed. In *Asterias* they appear very early in the growth-period and persist as individual bodies until maturation. In *Hipponoë* the chromosomes arise from the segmenting chromatin spireme, which makes its appearance only toward the close of the growth-period. There is a general similarity of the

main processes, but a slight variation in details, together with a non-correspondence in the time of occurrence of the successive stages.

On the question of the individuality of the chromosomes *Hipponoë* yields no positive results. Even in the late growth-period there is no indication of chromosomes as such. And it is clear that at least a large part of their chromatin is either contributed by the disappearing nucleoli or elaborated by the cell protoplasm. *Hipponoë* supports the conclusions drawn in regard to the function of the nucleolus in the case of *Asterias,* that it serves in part at least as a storehouse of nutritive material contributed to the chromosomes prior to maturation.

SUMMARY OF RESULTS ON ASTERIAS FORBESII.

1. Synizesis occurs in the oöcyte of the first order at the very beginning of the growth-period (size of nucleus 5 microns).

2. The growth-period is passed through rapidly. The single spireme of the contraction phase becomes double and segments into a number (18?) of irregularly shaped chromosomes. These decrease in size and collect in one or several masses of minute bilobed bodies in close proximity to or upon the nucleolus.

3. During the latter half of the growth-period all the chromatin, with the exception of what is held by the chromosomes, becomes stored in the enlarging nucleolus, the linin meshwork of the nucleus being left entirely achromatic shortly prior to maturation.

4. The nucleolus consists of a plastin ground-substance infiltrated and covered over with chromatin. In the living condition of the oöcyte the nucleolus appears vacuolated. The "vacuoles" are spherules of fluid chromatin, and where these are filled, properly stained sections reveal a homogeneous structure of the nucleolus. Linin, plastin ground-substance, and chromatin appear to represent closely related substances, possibly different phases of elaboration of the same fundamental material.

5. The chromosomes do *not* arise out of the nucleolus. The latter contributes nutritive substance to them, by virtue of which they increase slightly in size before entering the first maturation spindle.

6. The number of chromosomes in the prophase of the first polar mitosis is 18. They vary somewhat in size (one is considerably larger than the rest), all have a characteristic dumb-bell-shaped appearance, and some are clearly double (bivalent).

7. The two maturation divisions effect a double longitudinal fission of the original bilobed chromosomes. The reduced number of chromosomes is again 18.

8. Observations on *Hipponoë esculenta* agree in essential points with those made on *Asterias forbesii* and support the conclusions regarding the origin of the chromosomes, the function of the nucleolus, and the reduction phenomena.

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EXPLANATION OF PLATES.

Plate 1.

Fig. 1. Sectional view of mass of oögonia; cell-boundaries are indistinct. × 2100.
Fig. 2. Primary oöcyte at the very beginning of the growth-period. Cell-outline indicated by dotted line. × 2100.
Fig. 3. Slightly later stage, showing decided increase in size of nucleus. × 2100.
Fig. 4. Still later stage, showing increase in amount of chromatin and the characteristic distribution of the same in masses along the nuclear membrane. × 2100.
Fig. 5. Oöcyte entering upon the contraction phase (synizesis, McClung). The spireme appears to arrange itself in two parallel strands. × 2100.
Figs. 6, 7, 8, 9. Nuclei with chromatin threads in more or less complete synizesis. Linin network has become indistinguishable. × 2100.
Fig. 10. Oöcyte showing the spireme in complete synizesis. × 2100.
Figs. 11, 12, 13. Oöcytes showing the first stages in the disentangling of the spireme from the contraction phase. Linin network faintly visible. × 2100.
Figs. 14, 15. Nuclei showing still later stages in the disentangling of the spireme from synizesis. Nucleoli clearly visible. × 2100.
Figs. 16, 17, 18. Oöcytes in which appear the first indications of a transverse segmentation of the apparently continuous spireme. × 2100.
Figs. 19, 20, 21. Oöcytes showing a distinctly beaded character of the now discontinuous spireme. Occasional segments clearly indicate that the spireme has become double. × 2100.
Figs. 22, 23. Oöcytes with chromosomes in form of beaded threads. A portion of the spireme appears still unsegmented and double. × 2100.
Fig. 24. Oöcyte in which some of the chromosomes have become condensed into irregular masses while others remain as beaded threads. × 1320.
Fig. 25. Oöcyte at slightly later stage, showing all the chromosomes in the form of condensed irregular chromatic masses. There are indications of ring, V, and dumb-bell shapes. Some of the chromosomes appear paired; all have a mossy or feathery outline under high magnification. × 2100.

Plate 2.

Figs. 26, 27. Young primary oöcytes, showing chromosomes in various stages of transformation from beaded threads to compact bilobed bodies. Many of the chromosomes appear disposed in pairs. × 1320.
Fig. 28. Oöcyte at a somewhat later stage. Attachment to ovarian stroma shown; also the enveloping connective tissue membrane. Chromosomes have dumb-bell and globular shape. Linin network conspicuous. Cytoplasm densely granular and deeply staining with basic dyes. × 1320.
Fig. 29. Primary oöcyte at late stage in growth-period. Chromosomes of irregular shapes scattered through the slightly chromatic linin network. Nucleolus very large and homogeneous. × 700.
Fig. 30. Oöcyte at stage just before maturation. The chromosomes, gathered into a single mass, are connected with the nucleolus by a double chromatic thread. × 700.
Figs. 31, 32. Oöcytes at the stage just prior to maturation. The chromosome mass is in position next the cell periphery. The nucleolus and chromosome mass are connected by a chromatic thread. × 700.
Figs. 33, 34. Oöcytes in which the chromosomes are collected in a mass close to the nucleolus. The puckering of the nuclear wall shows that maturation is imminent. The linin network still achromatic. Chromatin appears to leave the nucleolus in the form of drops. × 700.
Figs. 35, 36. Oöcytes showing a connection in the form of a beaded chromatin thread between nucleolus and chromosomes. × 700.

FIG. 37. Oöcyte showing the passage of chromatin from nucleolus to adjacent chromosome group just prior to maturation. × 700.

FIG. 38. Nucleus in which the chromosome mass and nucleolus are at opposite poles. The chromosomes are in position to meet the polar spindle, which is about to form. The chromatin of the nucleolus is seen leaving the vacuolated plastin ground-substance (plasmosome) en masse and moving toward the chromosomes, at the same time contributing chromatin to the achromatic nuclear network. The peripheral layer of yolk granules is also shown. × 1320.

FIG. 39. Primary oöcyte at culmination of growth-period and some time prior to maturation. The eccentric nucleolus is homogeneous, with some of the chromosomes closely attached. The nuclear network is wide-meshed, beaded, and slightly chromatic, and with occasional karyosomes. The peripheral layer of the egg is filled with large yolk-granules. × 1320.

PLATE 3.

FIG. 40. Nucleus with chromosomes gathered into three groups (*a*, *b*, and *c*). These are enlarged at *a*, *b*, and *c*, × 2100. The majority of the chromosomes are bilobed. Their number is approximately 18. × 700.

FIG. 41. Oöcyte in which the chromosome mass lies very close to the nucleolus. × 440.

FIG. 42. Oöcyte showing the beginning of the establishment of a chromatin connection between nucleolus and chromosome group. × 440.

FIG. 43. Oöcyte in which the chromosome mass is less compact. Nucleolus is breaking up and establishing connection with the chromosomes. × 700.

FIG. 44. The above mass of chromosomes magnified × 2100. About 15 chromosomes may be counted.

FIG. 45. Cross-section of central pole of first maturation spindle after first polar body has been constricted off (this seen in next section), showing 20 chromosomes, the excess of 2 chromosomes above the usual number of 18, due to a premature longitudinal splitting of two of the elements. Compare with figure 44. × 2100.

FIG. 46. Maturing oöcyte in which spindle is forming and the astral rays have penetrated far into the nucleus. Both chromosome group and fragmenting nucleolus near distal pole of nucleus. There are no chromosomes among the rays in this or any of the adjacent sections. × 700.

FIG. 47. Oöcyte in initial stages of maturation, showing the nucleolus in transit toward the polar spindle, leaving a vacuole (the remains of the resorbed plastin ground-substance) and chromatin particles behind. The chromosome group has already separated and some of its elements have passed among the astral rays. × 700.

FIG. 48. Composite figure of two consecutive sections. Nucleolus seen to break up at one pole of the nucleus, leaving a plastin remnant. Spindle forming at opposite pole with several chromosomes already drawn toward it. × 700.

PLATE 4.

FIG. 49. Primary oöcyte showing nucleolus and chromosomes at opposite poles of nucleus. Maturation is imminent and the chromosomes are in proper position to be drawn into the spindle soon to appear. Chromatin in form of beaded threads is seen to pass from nucleolus toward chromosomes. × 700.

FIG. 50. Two centrospheres with centrosome and aster pushing into nucleus. Nuclear wall still intact. Nucleolus in process of dissolution. Two chromosomes are seen emerging from the mass. × 1320.

FIG. 51. Composite figure of three consecutive sections, showing one aster (*a*), a group of chromosomes scattering (several are detaching themselves from nucleolar mass of chromatin), and a spherical chromatin mass (*b*), and two additional spherical nucleolar masses (*c*). × 700.

FIG. 52. Chromosomes are entering the polar spindle. Last one in process of being detached from mass of disorganizing nucleolus. Nuclear network very close-meshed and highly chromatic. × 1320.

FIG. 53. Nuclear wall is ruptured. First polar spindle is being formed. Nucleolus is breaking up. Chromosomes are becoming attached to astral rays. Nuclear network is becoming close-meshed and more chromatic. × 1320.

Fig. 54. Entire nucleolar mass has moved toward asters, leaving a vacuole and chromatin particles behind. Chromosomes are being detached from nucleolus at upper border, and chromatin drops are passing out at lower border. × 1320.

Fig. 55. Chromosomes are entering the polar spindle. Nucleolus is fragmenting. "Metanucleus" persisting at left. Nuclear network is becoming close-meshed and chromatic. × 1320.

Fig. 56. Oöcyte in which chief nucleolus has given rise to many accessory nucleoli. These left chief nucleolus as drops (witness spherical form) and evidently manufactured their own ground-substance, from which the chromatin has in some cases become subsequently extracted. × 440.

Fig. 57. Cross-section of first maturation spindle near one of the poles, showing the chromosomes in process of transit into spindle (note size differences) and fragments of nucleolus. At right several chromosomes (?) arranged in manner of thread. These have not yet attained full size and final form. × 2100.

Fig. 58. One of the asters of the first polar spindle is shown. Its rays are directed upon the fragmenting nucleolus. Several chromosomes and plastin remnants are clearly distinguishable. × 1320.

Fig. 59. First polar spindle is forming. Chromosomes are detaching themselves from the irregular nucleolus. Nuclear membrane has entirely disappeared. Extent of residual substance indicated by dotted line. × 1320.

Fig. 60. Sectional view of median plane of first polar spindle. Chromosomes are in position on the spindle fibers. "Metanucleus" with outer chromatic ring persists in the residual substance of the nucleus. × 700.

Plate 5.

Fig. 61. Oblique transverse section of spindle, showing the chromosomes in process of transit into the spindle by means of the astral rays. "Metanucleus" persists in the residual substance of the nucleus. × 1320.

Fig. 62. Chromosomes are being drawn into the spindle by means of the astral rays, to which they become attached. Two spherical chromatin masses ("metanuclei"), each in a vacuole, persist in the close-meshed, beaded chromatic residual substance. × 1320.

Fig. 63. Longitudinal section of first maturation spindle, showing the chromosomes entering the spindle (several have already become attached to the spindle and begun the first division), a plastin remnant, the residue of the fragmenting nucleolus, and the extent of the residual substance. × 1320.

Fig. 64. Median longitudinal section of the first maturation spindle tangential to surface of egg and in prophase. Centrosomes here very distinct and surrounded by finely alveolar centrospheres. Chromosomes not yet fully in spindle show beginning of longitudinal fission. × 2100.

Fig. 65. Tangential section of first polar spindle. Chromosomes not yet all on spindle; their bivalvent and bilobed character is plainly shown. The V-figures show the beginning of the first longitudinal fission. × 2100.

Fig. 66. First polar spindle with two centrosomes in central centrosphere. Rays of distal aster begin to disappear. Chromosomes in metaphase. One chromosome seen en face shows beginning of second longitudinal fission. × 2100.

Fig. 67. First polar spindle in early anaphase. Chromosomes mostly dumb-bell-shaped. To the right is a large undivided chromosome. × 2100.

Figs. 68, 69. Two consecutive longitudinal sections of first polar spindle in anaphase. Spindle has become blunt and barrel-shaped. 68 shows at the right a figure representing the consummation of the first longitudinal fission giving rise to four globes in serial arrangement. Above and below this are pairs of smaller bilobed chromosomes representing the completion of the second longitudinal fission, preparatory to the second polar mitosis. In 69 the chromosomes for the most part are stubby or globular masses of chromatin. In the combined sections 20 chromosomes may be counted at each pole, representing, however, probably only 18 first maturation products. × 2100.

Fig. 70. First polar body completed. Second polar spindle is being formed, its distal pole arising in the region of the mid-body. Residual substance of nucleus still persists. Several of the chromosomes appear to be double (the result of a premature second longitudinal fission in the anaphase of the first mitosis). × 2100.

Fig. 71. Prophase of second polar mitosis. Several of the chromosomes are arranged radially on the spindle. Residual substance still persists. × 2100.
Fig. 72. Metaphase of second polar mitosis. The bilobed products of the premature fission of the anaphase of the first mitosis are simply drawn apart to opposite poles. × 2100.
Fig. 73. Late anaphase of the second polar mitosis. Many chromosomes still bilobed. Two chromosomes are seen to lag far behind. Mid-body again very conspicuous. × 2100.
Fig. 74. Telophase of second mitosis. Second polar body constricted off with mostly bilobed chromosomes. Those remaining in egg also bilobed or globular. The arrangement of the chromosomes in the first polar body indicates an abortive attempt at a division. × 2100.
Fig. 75. Female pronucleus forming as a vesicle containing the chromosomes, and surrounded by rays of central pole of second maturation spindle. × 2100.

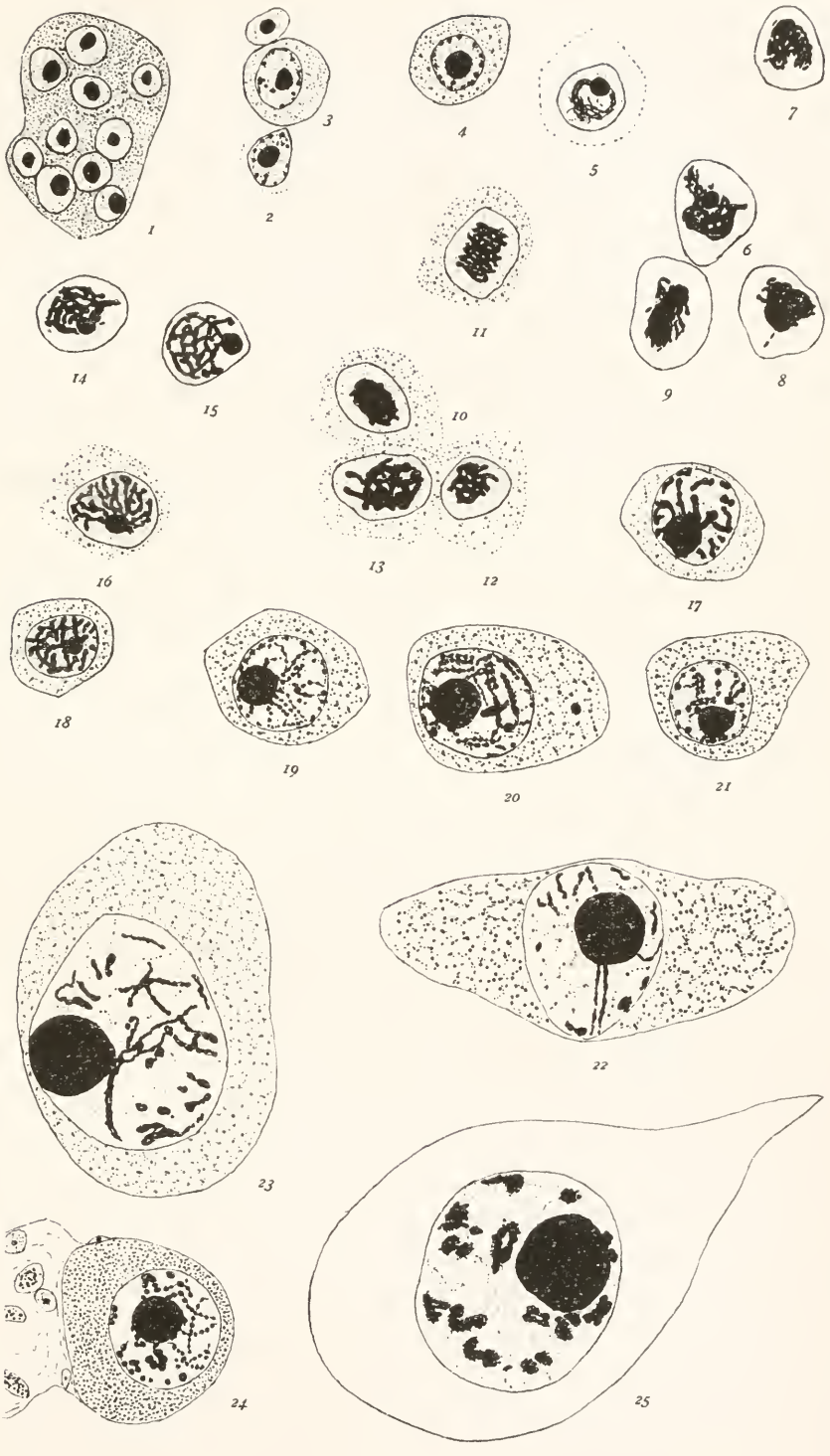
Plate 6.

Figs. 76, 77. Cross-sections of prophase figures of first maturation mitosis with 18 chromosomes. × 1320.
Fig. 78. Two consecutive sections of a metaphase figure of first maturation spindle. Note size differences among the chromosomes. × 2100.
Fig. 79. Cross-section of prophase figure of first maturation spindle with 20 chromosomes. × 2100.
Fig. 80. Cross-section of distal pole of first maturation spindle at anaphase showing 18 chromosomes. × 1320.
Fig. 81. Female pronucleus with chromatin nucleolus and stout deep-staining network. × 1320.
Figs. 82, 83, 84. Different types of female pronuclei, with plasmosomes showing variation in chromatin distribution over the linin network. × 1320.
Fig. 85. Fertilized ovum showing the male and female pronuclei, both with plastin nucleoli (plasmosomes). × 700.
Fig. 86. Section through the pronuclei in process of fusion to form the segmentation nucleus. Nucleoli have disappeared. × 2100.

Plate 7.

Hipponoë esculenta.

Fig. 87. Two oöcytes near the culmination of the growth-period, each with several chromatin nucleoli and but slightly chromatic nuclear reticulum. × 440.
Fig. 88. Primary oöcyte at slightly later stage; nuclear reticulum more highly chromatic. × 440.
Figs. 89, 90. Primary oöcytes at still later stages of development, showing but a single nucleolus and stout, compact, and very chromatic nuclear network. × 440.
Fig. 91. Oöcyte at beginning of maturation. Nuclear wall is disappearing. The nuclear reticulum in form of compact spireme. First polar spindle in process of origin. Nucleolus has vanished. × 440.
Fig. 92. Early prophase of first maturation division. Chromosomes still in form of loose, stout spireme and surrounded by residual substance of nucleus. × 440.
Fig. 93. First polar spindle at early metaphase. × 2100.
Fig. 94. Second polar spindle at anaphase. × 2100.
Fig. 95. Mature ovum with female pronucleus. × 440.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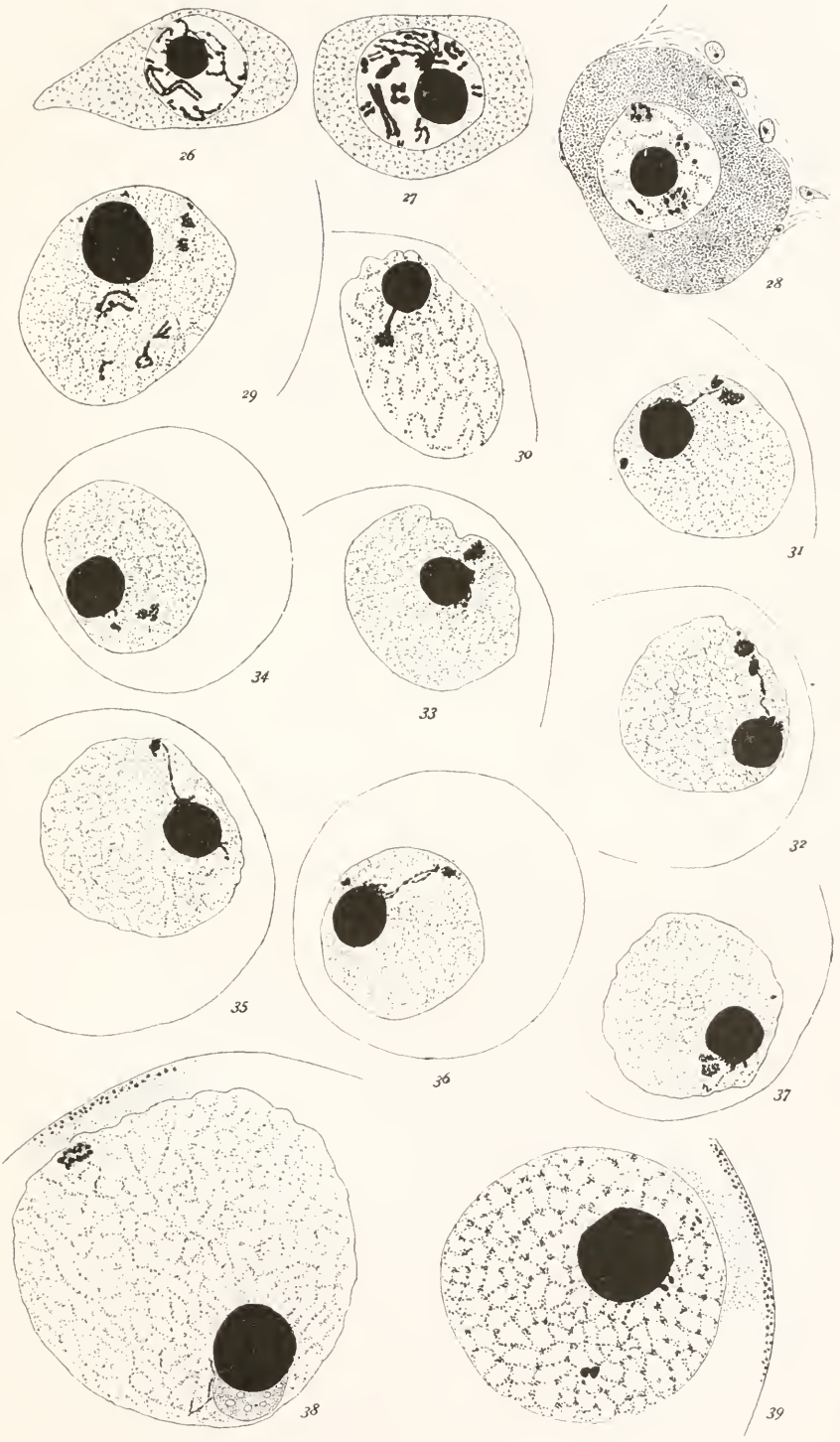
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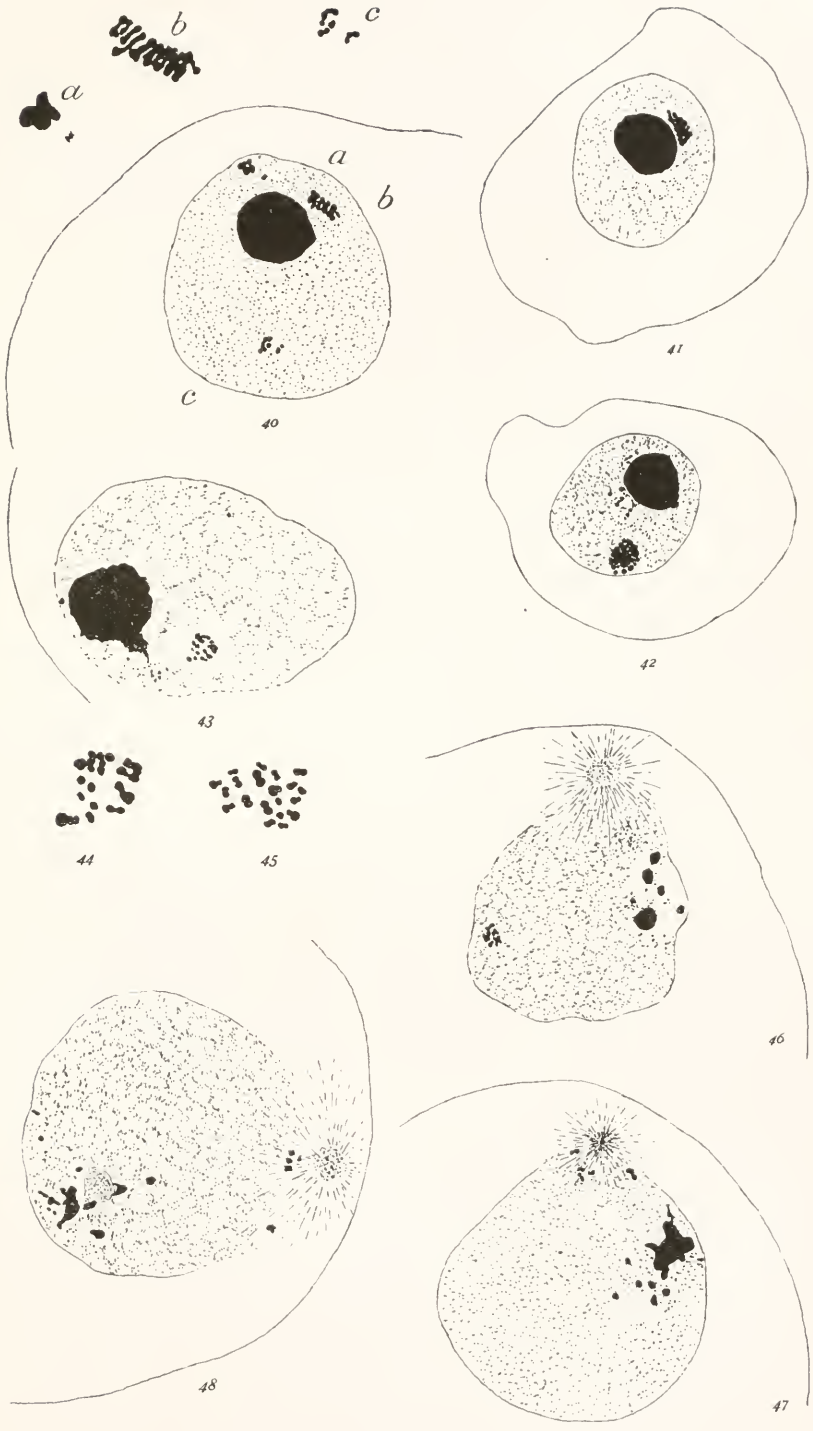
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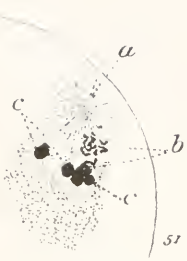

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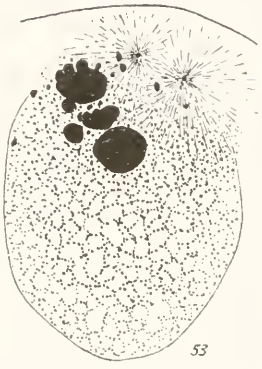

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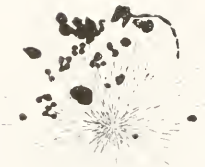

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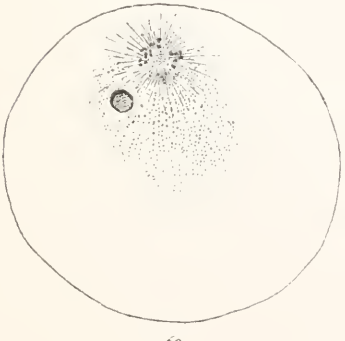

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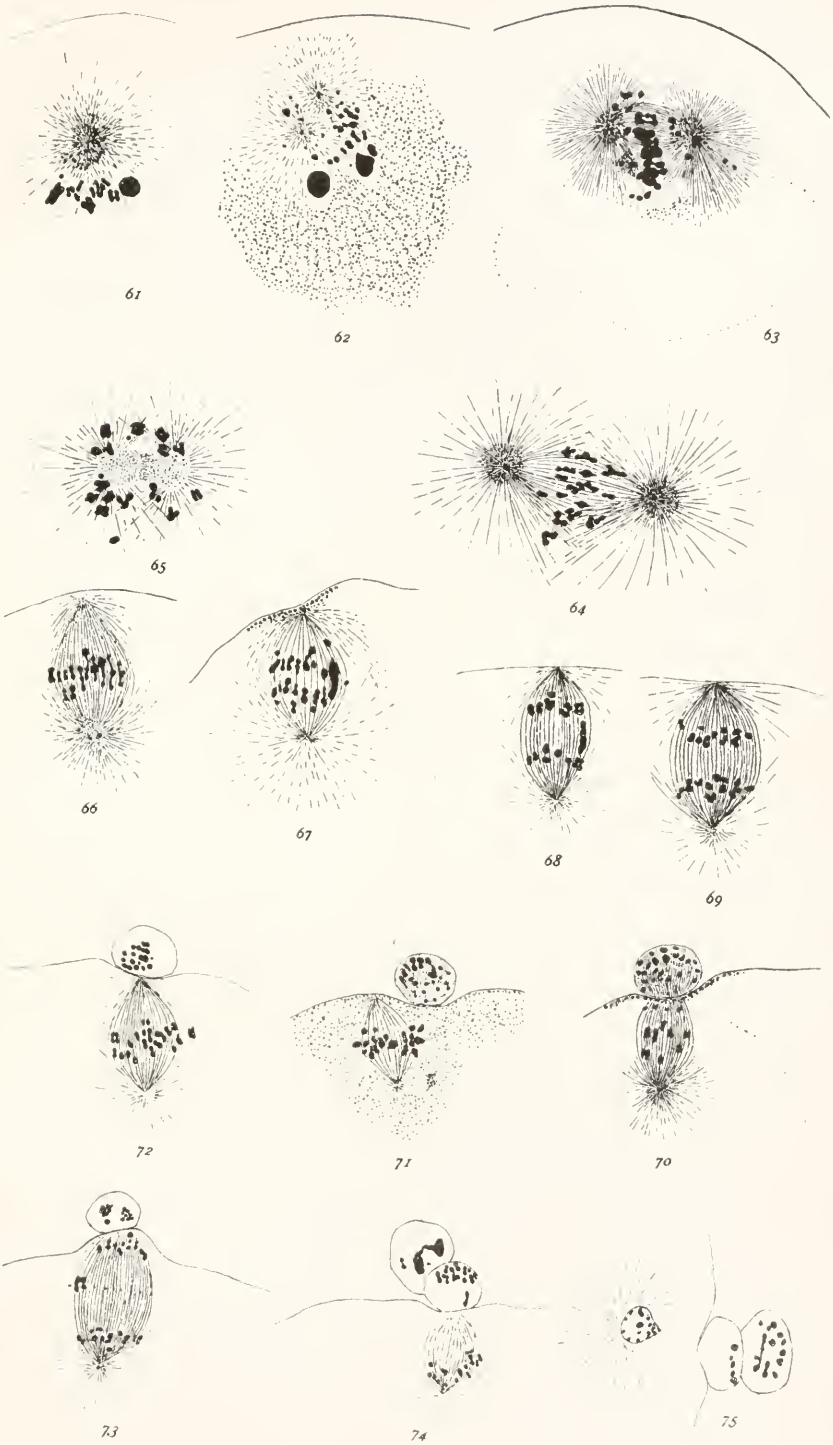
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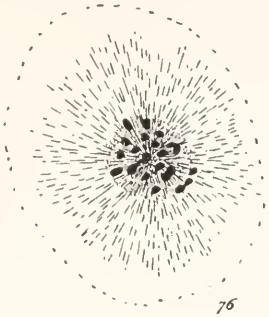

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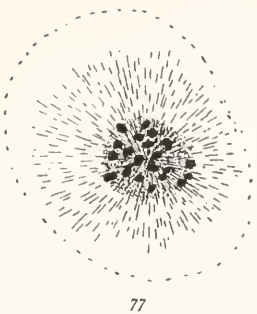

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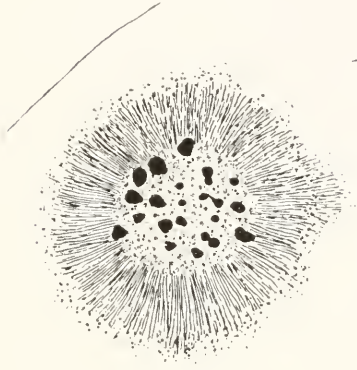

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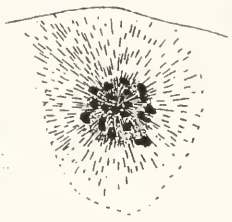

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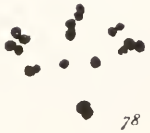

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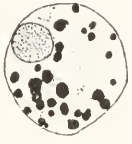

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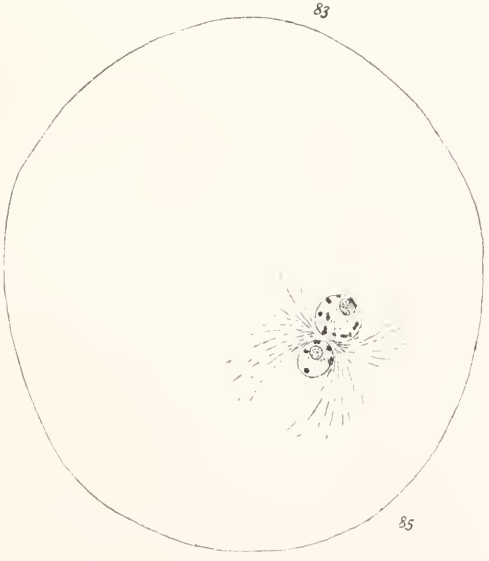

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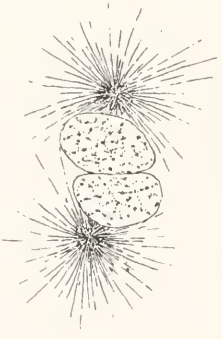

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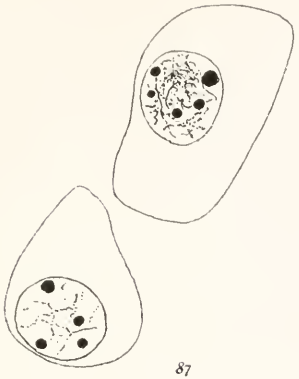

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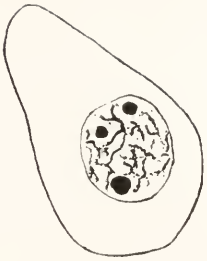

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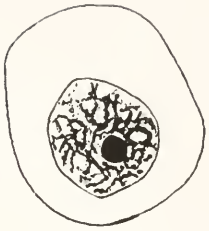

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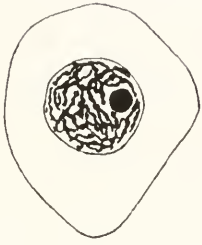

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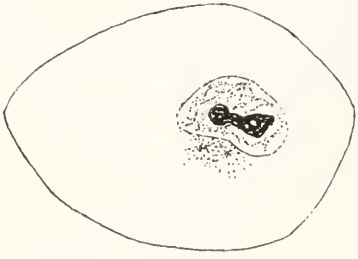

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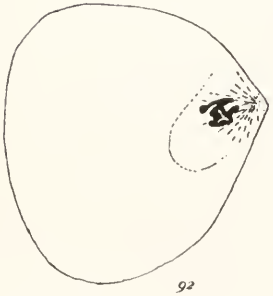

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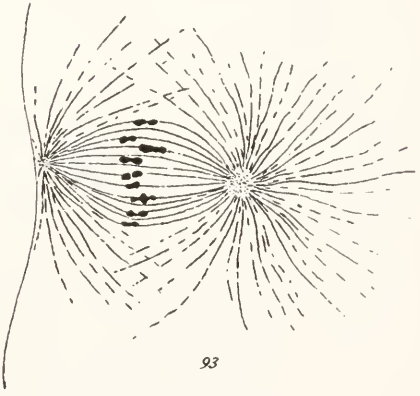

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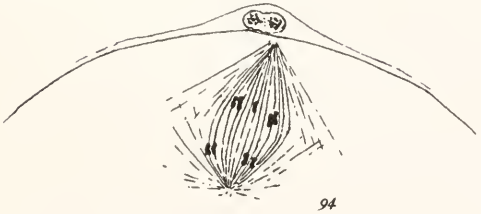

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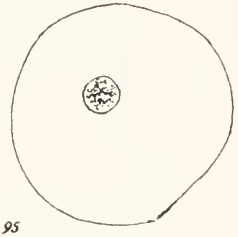

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IV. THE PELAGIC TUNICATA OF THE GULF STREAM

PART II. SALPA FLORIDANA (APSTEIN)

PART III. THE SUBGENUS CYCLOSALPA

PART IV. ON OIKOPLEURA TORTUGENSIS, N. SP. A NEW APPENDICULARIAN FROM THE TORTUGAS WITH NOTES ON ITS EMBRYOLOGY

Plates 1 to 8 and 3 text figures

William Keith Brooks.

A sentiment of mournful interest must ever associate itself with the following papers by Professor William Keith Brooks, for they are the last that can fall from his able pen. He died on November 12, 1908, in the sixtieth year of his age.

Science must mourn him as a profound philosopher, the discoverer of many truths in morphology, and a teacher whose pupils are the greatest of American biologists to-day. The practical world will recollect him as the father of the science of oyster culture in America.

Good as these things be, deeper and above them all, there lives in our hearts a love for this kindly man of culture, the modest, hopeful teacher, who was free from all trace of pedantry.

The spirit of his simple faith in research he has passed on to those whose lives were enriched by knowing him, and who now follow where he led in the study of his science.

A. G. M.

THE PELAGIC TUNICATA OF THE GULF STREAM.

PART II—SALPA FLORIDANA (APSTEIN).

By William Keith Brooks.

(Plate 1, figs. 1, 2, 3, 4, 5, 6; plate 2, figs. 7 and 9.)

This rare *Salpa,* which is little known, has been noted, by Traustedt, as *S. dolichosoma-virgula.* (Ergebnisse der Plankton-Expedition der Humboldt-Stiftung: Edited by Victor Hensen. II, A. Die Thaliacea der Plankton-Expedition: Systematische Bearbeitung; von M. P. A. Traustedt, 1892.)

It has been more thoroughly described and figured by Apstein, who shows that it is very different from *S. dolichosoma,* and gives it the name *S. floridana.* (Ergebnisse der Plankton-Expedition der Humboldt-Stiftung: Edited by Victor Hensen. II, B. Die Thaliacea der Plankton-Expedition: Vertheilung der Salpen, von Dr. Carl Apstein, 1894.)

Apstein gives figures of both the solitary and the aggregated form, but neither stage is drawn from an adult, the figures of the solitary form being drawn from an embryo and those of the aggregated form from a detached member of a young colony. His account contains many minor inaccuracies, as it is based upon these immature specimens, which seem to have been badly preserved and ill-suited for study.

Mature specimens of both stages were found, in May, 1906, on the surface in the vicinity of the Marine Biological Laboratory of the Carnegie Institution of Washington at Tortugas, Florida; and an opportunity was thus afforded to study and sketch them while alive, and thus to make additions to, and some slight corrections of, the account of the species which Apstein gives.

It is in the shape of the colony of the aggregated form, and in the number and arrangement of its muscles, and those of the solitary form, that my observations are most in conflict with Apstein's account. He says there are ten muscles in the solitary form, while I find no less than sixteen definite and characteristic muscles, without counting the slender ones around the mouth and the cloacal aperture. This discrepancy is due, in part, to the fact that he sometimes regards as one a muscle that is single on one surface of the body, dorsal or ventral as the case may be, when it is represented by two or more on the opposite surface. The hoop-like muscles of *Salpa* are not like blood-vessels or nerves that have their origin in a central

Note.—Professor Brooks's long illness rendered it impossible for him to revise the proof of the following papers, and should any short-comings be discovered in them, such may be attributed to the fact that they have not had the benefit of his personal supervision.

organ, for neither end of the muscle can be regarded as the origin. Apstein's method of enumerating the muscles makes his account difficult to follow with a specimen, and often leads him into inconsistency. Clearness seems to demand that a muscle that is single on one surface and represented by two or more on the other surface should be described as several muscles, and Apstein sometimes follows this rule, while upon other occasions he departs from it.

I have found it very difficult to make comparisons between the muscles of different species without a more minute system of enumeration than the diagnosis of species seems to require, and my chief reason for the method that I here employ is to facilitate the description of homologies among the muscles.

THE SOLITARY SALPA FLORIDANA.

(Plate 1, figs. 1, 2, 3, 4; plate 2, fig. 7.)

In the figures, the muscles that are on the surface of the body that is nearest the observer are designated by Arabic numerals, while those that are seen on the far side through the transparent body are designated by Roman numerals. Plate 1, figure 1, is a dorsal view of the adult, magnified 16.5 diameters. Plate 1, figure 2, is a ventral view of the same specimen. Plate 1, figure 3, shows the digestive organs of the same specimen in ventral view. Plate 1, figure 4, is a ventral view of an embryo at the stage that is described by Apstein, magnified 30 diameters. Plate 2, figure 7, is a side view of a younger embryo magnified 100 diameters.

On each side of the body of the solitary form there is an organ that Apstein calls a glandular lateral organ. It is a luminous organ like those of *S. pinnata*. It makes its appearance in the young embryo (plate 2, figure 7, *lum.*) in the plane of the muscle that is numbered 9 in my figures, and it lengthens at each end as development progresses. In the older embryos (plate 1, figure 4) it occupies the intermuscular spaces 7–8, 8–9, and 9–10, and even reaches beyond 8 and 10, as in plate 1, figures 1 and 2. It is in the body-cavity, and not in the muscles.

The solitary *S. floridana*, from which plate 1, figures 1, 2, and 3 were drawn, is about 10 mm. long, and the average length is about 12 mm., as Apstein says. The living animal is cylindrical, and Apstein is, no doubt, right in attributing the flatness of his specimens to pressure against the bottle in which they had been preserved.

THE MUSCLES OF THE SOLITARY SALPA FLORIDANA.

The homology between the muscles of *S. floridana* and those of the other cyclosalpas is so exact that the equivalent of each muscle can be recognized in the other species without difficulty, and I shall give no detailed account of them in this place, as the reader may refer to the general account of the muscles of the cyclosalpas in Part III of this memoir.

The following characteristics in respect to the muscles are distinctive of *S. floridana:* Muscles 4, 5, and 7 meet near the middle line of the dorsal surface to form a common trunk, which does not cross the middle line nor meet its fellow of the opposite side. Muscles 6, 7, 8, 9, and 10 meet on the middle line of the ventral surface to form a common trunk which crosses the middle line and unites with its fellow of the opposite side. Muscle 11 is independent of other muscles, and its halves are separated from each other, both dorsally and ventrally. Muscle 12 crosses the middle line of the ventral surface, but its ends are independent near the middle line of the dorsal surface. Muscle 13 is complete both dorsally and ventrally and free from union with other muscles. It crosses, but does not unite with, muscle 14. Muscle 14 arises on the middle line of the ventral surface, from the middle of muscle 12, in a short longitudinal stem, which quickly divides into a pair of slender muscles, which, running outwards and backwards, bend around on to the dorsal surface and unite with muscle 15. Muscle 15 is a slender muscle which crosses the middle line of the ventral surface and unites, on the dorsal surface, with muscle 14 to form a single dorsal muscle.

The method of muscle-enumeration that I have employed is the one that is best suited for discussing the homologies of the muscles in different species, and I give a table to show the difference between Apstein's enumeration and my own.

Table to contrast Apstein's enumeration of the muscles of the solitary Salpa floridana with author's enumeration.

Brooks.	Apstein.
Muscle 1	1 on dorsal surface of figures 3 and 4; part of 2–3 on ventral surface of figure 3.
Muscle 2	Part of 2 on dorsal surface of figure 4; part of 2–4 on dorsal surface of figure 3.
Muscle 3	2 on dorsal surface of figure 4; 1 on ventral surface of figure 3.
Muscle 4	Part of 2 in figure 4; part of 2–3 in figure 3.
Muscle 5	3 in figure 4; part of 2–4 on dorsal surface of figure 3; part of 2–3 on ventral surface of figure 3.
Muscle 6	Part of 2–3 on ventral surface of figure 3.
Muscle 7	4 in figure 4; 2–4 on dorsal surface of figure 3; 4–5 on ventral surface of figure 3.
Muscle 8	5 in figure 4; 5 on dorsal surface of figure 3; 4–5 on ventral surface of figure 3.
Muscle 9	Muscle 6 in all figures.
Muscle 10	Muscle 7 in all figures.
Muscle 11	Muscle 8 in all figures.
Muscle 12	Muscle 9 in all figures.
Muscle 13	Muscle 10 on dorsal surface in figures 3 and 4.
Muscle 14	Muscle 10 on ventral surface of figure 3.
Muscle 15	Not designated nor represented correctly.
Muscle 16	Not figured nor noted.

In plate 1, figure 4, I give a ventral view of an old embryo 4.5 mm. long, magnified 30 diameters. It is in the same stage of development as the one on which Apstein bases his account of the species. The muscles are essentially like those of the adult, except that muscles 12, 13 and 14, are rela-

tively thicker and more easy to trace, and the specimen shows clearly that muscles 13 and 14 are not united where they cross each other. The younger embryo that is shown, in side view, in plate 2, figure 7, shows the muscle 16 that joins muscle 1 to muscle 5 on the side of the body. This muscle is difficult to see in a dorsal or ventral view. The left-hand one of the adult is shown, at 16, on the left side of figure 1 and the right-hand one is shown on the left side (right side inverted) of figure 2.

Since the muscles of *S. floridana* are homologous with, but much more specialized than, those of *S. pinnata,* it seems natural to conclude that it is a modified descendant of an ancestral form that resembled *S. pinnata,* and this conclusion is strengthened by the comparative study of other organs.

Apstein says that there is no other *Salpa* in which the muscles are joined to each other in as great a degree as they are in *S. floridana,* but a reference to Traustedt's figure of *S. hexagona* will show that all the muscles are thus united in this species. (Traustedt, Spolia Atlantica, Tab. 1, fig. 14.)

THE DIGESTIVE ORGANS AND STOLON OF THE SOLITARY SALPA FLORIDANA.

(Plate 1, fig. 3.)

The opening by which the pharynx communicates with the œsophagus is a large, funnel-shaped aperture, on the right side of the ventral end of the "gill." It diminishes in size very rapidly, and opens into the anterior end of the stomach by an opening, *c,* which is very small as compared with the pharyngeal end. The stomach is joined, on the left, by a large blind pouch, the so-called liver. The aperture by which the stomach communicates with the intestine, *i,* is at the junction of the blind pouch with the stomach. The intestine, *i,* is long, and it runs upwards and forwards to open at the anus, *a,* into the median atrium or cloaca, near its dorsal surface and a little posterior to the ganglion. The intestine lies in that part of the body-cavity that is included in the so-called "gill."

The stolon is twisted into a right-hand spiral, bending to the left from the fixed growing end of the median line and then bending to the right, so that the free end points to the right and is on the right of the middle line. In this respect the stolon of this species is very different from that of *S. pinnata,* and like that of ordinary salpas, such as *S. democratica.*

THE AGGREGATED FORM OF SALPA FLORIDANA.

(Plate 1, figs. 5 and 6; plate 2, fig. 9.)

The colony of the aggregated form of *S. floridana* (plate 2, figure 9) is a circular rosette of four, five, or six or more individuals. In my collection there are some with four, some with five, and one with six, and none with more than six, although six may not be the maximum. The number is very much smaller than it is in other Cyclosalpas, as is shown by comparing figure 9 with the colony of *S. pinnata* shown in plate 2, figure 8. The

colony is about half the size of that of *S. pinnata.* The one from which figure 9 was drawn is 12 mm. in diameter, and our largest specimens, with advanced embryos are about 20 mm. in diameter, which is, probably, near the maximum for *S. floridana.* The colony of *S. pinnata* from which plate 2, figure 8, was drawn is 34 mm. in diameter, and the maximum is about 40 mm.

The members of the colony of *S. floridana* are joined together by slender processes, with lance-shaped ends which meet in the center in a star. The long axes of the animals are in the radii of the colony, while they are at right angles to it in *S. pinnata.* The animals are barrel-shaped, with their mouths central and their cloacal apertures distal—an arrangement that is the reverse of that of *Pyrosoma,* to which the colony presents a superficial resemblance. In the preserved specimen from which figure 9 was drawn, and in all my preserved specimens, the colony resembles a flat wheel, but in the living specimens the dorsal surface is slightly concave and the ventral surface slightly convex, these being features in common with *S. pinnata,* as plate 2, figure 8, shows. In the living colony the long axes of the members of the community are slightly inclined, with the oral end a little nearer the central axis than the aboral end, so that the living colony is not as distinctly wheel-shaped as the preserved specimen from which figure 9 was drawn.

The muscular contractions of the aggregated *S. floridana* are vigorous, and the course of the gill differs according to the phase of muscular contraction of the animal when it was killed. The animals shown in figures 6 and 9 contracted as they were dropped into the killing fluid, and the gill is twisted into a right-hand spiral, which is constant in all the members of this community. The one that is shown in plate 1, figure 5, was killed with its muscles relaxed and its gill is straight, as it is in all the members of the colony from which this specimen was taken. Since neither phase of contraction is any more normal than the other, the gill of the aggregated *S. floridana* must be characterized as straight when the muscles are relaxed, and spiral when they are contracted.

THE DIGESTIVE ORGANS OF THE AGGREGATED SALPA FLORIDANA.

The digestive organs of the aggregated *S. floridana* are very different from those of the solitary form, as Apstein points out. In the solitary form the intestine is long, straight, and it runs, inside the gill, from the ventral stomach to the dorsal anus, which is a little posterior to the ganglion. In the aggregated form (plate 1, figure 5) the intestine runs backwards, and, bending upon itself, runs dorsalwards to the anus, which is on the left side of its gastric end, as Apstein shows it in his figure 2, while he represents it on the right in his figure 1.

Apstein says the endostyle of the aggregated form extends in a straight

line from its anterior end to the region of the digestive organs, but my older and better preserved specimens show a sharp upward bend at the anterior end (plate 1, figure 5).

THE MUSCLES OF THE AGGREGATED FORM OF SALPA FLORIDANA.

(Plate 1, figs. 5 and 6; plate 2, fig. 9.)

Figure 6 shows the muscles in ventral view; figure 9 in dorsal view, and figure 5 in side view. In describing the muscles I have used the method of designating them which is most convenient for comparing them with those of other species, and for studying their homologies. Apstein's account and figures of these muscles is so unsatisfactory that I shall not attempt to compare my own account with his.

In the solitary *S. floridana* all the muscles except 1, 2, 13, 14, and 15 are incomplete dorsally, and do not cross the dorsal middle line, while all except 11 are complete ventrally and cross the middle line. In the aggregated form all the muscles cross the middle line of the dorsal surface, while only 6, 7, 8, 9, and 10 cross the middle line of the ventral surface. The muscles at the anterior end of the body, which I have numbered from 1 to 6, can be identified without difficulty with those of the solitary form, which I have designated by the same numbers in figures 1 and 2. The muscles back of 6 are highly specialized, and it is difficult to homologize them with equivalents in the solitary form without comparative study of the other species of *Cyclosalpa*. I find reason, which is given in Part III of this memoir, on the subgenus *Cyclosalpa*, for a very exact homology between the muscles, and the reader may refer to this discussion.

The following are the most conspicuous and important differences between the muscles of the solitary form and those of the aggregated form: In the aggregated form, muscle 3 is prolonged ventrally into that part of the body-cavity which is included in the process that joins the aggregated form to the other members of the community, and it there unites with muscle 7; muscles 4 and 5 unite dorsally to form a common muscle *A*, which crosses the middle line of the dorsal surface and becomes continuous with its fellow of the opposite side. The muscle *A* has no equivalent in the solitary form. Muscle 7 is continued ventrally into the organ of attachment, where it unites with muscle 3; while muscle 7 unites dorsally with a common muscle formed by the union of muscles 8 and 9, and thus gives rise to the muscle *B*, which crosses the middle line of the dorsal surface to become continuous with its fellow of the opposite side; muscles 6 and 8 unite with each other ventrally, and cross the middle line of the ventral surface; muscles 8 and 9 unite dorsally to form a common muscle, which is joined by muscle 7 to form muscle *B;* muscle *C* is a short oblique muscle which joins muscle 10 to the muscle that is formed by the union of muscle 8 with muscle 9.

PART III—THE SUBGENUS CYCLOSALPA.

By William Keith Brooks.

The subgenus *Cyclosalpa,* including *S. pinnata, S. affinis, S. virgula,* and *S. floridana,* is well marked, and distinguished from other salpas by many structural characteristics. The subgenus owes its name to the shape of the colony of the aggregated form, which is a wheel or rosette, as is shown in figures 8 and 9, plate 2.

Three of the four known species of *Cyclosalpa—S. pinnata, S. affinis* and *S. floridana*—have in the solitary form, peculiar luminous organs. No luminous organs are described in *S. virgula,* and I have had no opportunity to study this species. It is not impossible that these organs will be found to be present in this as in the other cyclosalpas. In *S. pinnata* the luminous organs are present in the aggregated form as well as in the solitary form. In the other species they seem to be restricted to the solitary form.

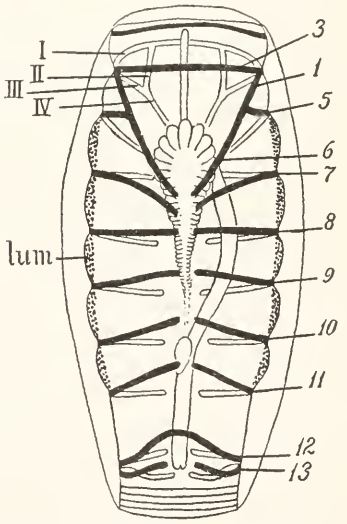


Fig. 1.—The solitary *Salpa pinnata* in ventral view.

One of the most notable of the common characteristics of the cyclosalpas is the intestine of the solitary form. This is long and runs upward and forward from the ventral stomach to open into the cloaca or median atrium on the dorsal side of the body a little posterior to the ganglion. It lies in that part of the body-cavity which is included in the so-called gill.

Plate xxxv of my memoir on "The Genus *Salpa*" is a median longitudinal section of an advanced embryo of *S. pinnata,* showing the gill, *g,* between the pharynx, which is colored red, and the cloaca, which is colored green. The intestine, *p,* is shown in its place in the cavity of the gill, while the anus, *p',* opens into the cloaca. The relation of the intestine to the gill is also shown in *S. pinnata* in figures 1, 2, and 3 of this memoir. Figure 7 of plate 2 of this memoir is a side view of an advanced embryo of *S. floridana,* showing that the relation of the intestine to the gill is the same as it is in the other cyclosalpas.

There is much confusion, among writers on the embryology of *Salpa,* as to the nature and homologies of the so-called "gill," which is in no way comparable to the gill-clefts of *Doliolum* and *Pyrosoma* and other Tunicata. If *Salpa* has gill-slits, it has only one on each side, and this is enormous, putting the pharynx into free communication with the cloaca or atrium. The so-called "gill" is homologous with the dorsal lamella of other tunicates. It is a rod-like organ, extending obliquely upward and forward from the middle line of the ventral surface in the region of the œsophagus to the middle line of the dorsal surface near and a little posterior to the ganglion, thus separating the pharynx from the atrium on the middle line, while the lateral portions of the pharynx are in free communication with the lateral regions of the atrium. There is, in *Salpa,* no recognizable boundary between the lateral atria and the median atrium, and it has seemed best, for this reason, to most writers on *Salpa* to call the whole atrial system the cloaca. The "gill" is nearly circular in cross-section; its anterior or pharyngeal surface consists of the ciliated pharyngeal epithelium of the dorsal lamella, while its side walls and posterior or cloacal surface consist of the ectodermal epithelium of the cloaca. The "gill" is hollow, lined

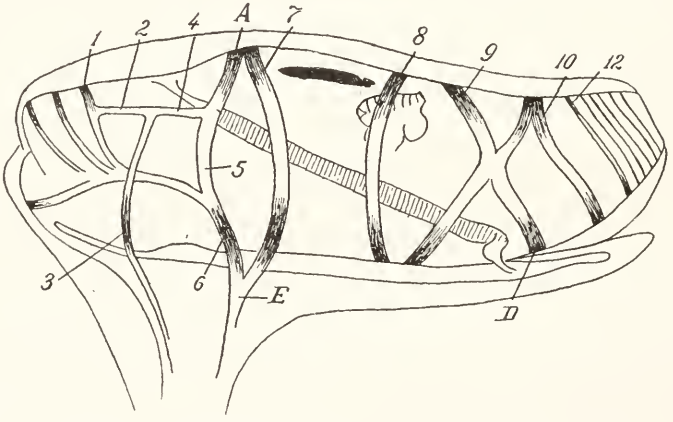


FIG. 2.—The aggregated *Salpa pinnata* in side view.

with mesoderm, and its chamber is part of the body-cavity. It is in this chamber that the intestine of the solitary *Cyclosalpa* lies.

While the solitary cyclosalpas agree in this respect, the aggregated ones differ greatly in the form and position of the intestine. In the aggregated *S. pinnata* the intestine is long, as it is in the solitary form, and the anus is far forward, but the intestine is not in the gill, and it lies on the middle line of the ventral surface, in the body-cavity under the endostyle, as is shown in text-figure 1.

In all the other aggregated cyclosalpas the intestine is very different from that of the solitary ones, and forms a loop with the gastric end and the anal

end close together, and the anus on the left of the stomach. The loop is loose, and the intestine **U**-shaped in *S. affinis* (text-figure 3) and in *S. virgula* (Apstein, Die Thaliacea der Plankton-Expedition, fig. 1); while it is most compact in *S. floridana* (plate 1, figures 1, 2, and 3). In this latter species it resembles the compact nucleus of the ordinary salpas. The comparative anatomy of the intestine is therefore consistent with the view that S. *pinnata* is the most primitive and *S. floridana* the most specialized among the cyclosalpas.

The Stolon.—In *S. pinnata* and *S. affinis* the stolon is straight, and on the middle line of the ventral surface, under the endostyle, with its free end anterior. I know of no satisfactory account of it in *S. virgula*. In *S. floridana* (plate 1, figures 1, 2, and 3) the stolon is twisted into a right-hand spiral, as it is in most of the ordinary salpas, such as *S. democratica*. In *S. floridana* it first bends to the left, from the growing end, and then, bending upon itself, turns to the right, so that the free and oldest end is on the right of the middle line, and also on the right of the growing end. The position of the stolon in the various species of *Cyclosalpa* is consistent with the view that *S. pinnata* is most primitive and *S. floridana* most specialized.

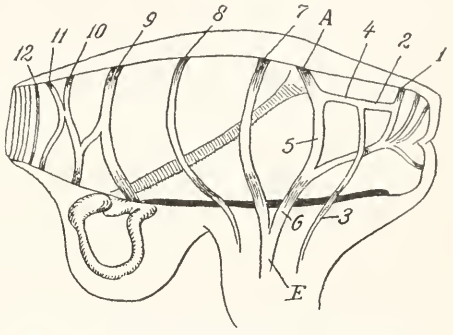


FIG. 3.—Aggregated *Salpa affinis* in side view.

Most, if not all, the species of *Cyclosalpa* have luminous organs, either in the solitary form alone, or in both the solitary and the aggregated form. The light that is emitted by these organs in *S. pinnata* is so intense that it glows brilliantly under the noonday sun of the Tropics. The organs are similar in structure, but not in position, to those of *Pyrosoma*. In *Cyclosalpa* they are in pairs on the sides of the body, a little nearer to the dorsal than to the ventral surface. In the solitary *S. pinnata* they extend over the five intermuscular spaces 5–7, 7–8, 8–9, 9–10, and 10–11. In the aggregated *S. pinnata* they lie in the intermuscular space 7–8. In the solitary *S. floridana* they occupy the intermuscular spaces 7–8, 8–9, and 9–10, and extend a little beyond 7 and 10.

THE HOMOLOGY BETWEEN THE MUSCLES OF THE VARIOUS SPECIES OF CYCLOSALPA.

The text-books still continue to give currency to the opinion that the muscles of *Salpa* are always incomplete, while those of *Doliolum* are always complete rings, and that this is an important and absolute distinction be-

tween *Doliolum* and *Salpa,* showing that the two are not closely related. In relation to this opinion, I give two quotations from my memoir on "The Genus Salpa" (Baltimore, 1893: the Johns Hopkins Press). My purpose in calling attention to the subject in 1893 was to show that there is no basis for the opinion that the salpas and the doliolums are so different in the character of their muscles that they can not be included in a single group nor have had a common origin. Since the text-books still convey the impression that the difference is fundamental and of great importance, it has seemed best to call renewed attention to the subject.

(Page 9.) This error has been most persistent, and it has been made the basis of the fundamental classification of the whole *Salpa*-family, for which Claus has proposed the name Desmomyaria, and Herdman the name Hemimyaria, as distinguished from the Doliolidæ, for which Gegenbaur has proposed the family name Cyclomyaria. Even if this distinction between *Salpa* and *Doliolum* were absolute, the selection of a characteristic so inconstant as the form of the locomotor muscles as a basis for fundamental classification would be most unwise, and it is quite untenable if it is not absolute. As a matter of fact, some of the muscle-bands of *Doliolum* are incomplete, and in at least one species of *Salpa* some are complete. In the first generation or "amme" of *Doliolum* the seventh body-muscle is incomplete dorsally, and in the animal that arises from a median bud—the Pflegetheir—it is incomplete ventrally, while the Ernahrungstheire, or products of the lateral buds, depart very widely from the cyclomyarian type. So far as I am aware, Traustedt is the only modern writer on *Salpa* who has used his eyes and described the muscle-bands as complete circles, as they really are. In his description of this species (Spolia Atlantica. Bidrag til Kundsscab von Salperne. Mem. Acad. Royal, Copenhagen, 6, 2, II, 8, Ger. by M. P. A. Traustedt) and also in his description of the variety *flagellifera,* p. 369, he states the facts correctly; but while his draughtsman, Cordts, has figured *S. democratica* correctly, plate II, figures 25 and 26, he has followed tradition in his figure of *Salpa flagellifera,* plate I, figure 12, rather than nature and Traustedt, so hard it is to combat established error.

(Page 128.) I have already shown, p. 9, that the contrast in the muscle-bands upon which the groups Cyclomyaria and Hemimyaria are based has no existence. In all the doliolums some of the muscle-bands are imperfect rings; in most species of *Salpa* the oral and atrial muscles are perfect rings, and, in the most common and best-known species of *Salpa,* the solitary *S. democratica,* most of the body-muscles are as perfectly closed, dorsally and ventrally, as the rings of *Doliolum. Anchinia,* at least, is only by courtesy a Cyclomyarian, for it has no circular muscles except the oral and atrial sphincters, as the figure of the sexual animal given by Kowalevsky and Barrios clearly shows. (Journ. Anat. Phys., XIX, 1883.) The groups Cyclomyaria and Desmomyaria are then purely artificial and without scientific value.

In the extracts that I have quoted from my memoir of 1893 I had no thought of denying the well-known fact that the various species of *Salpa* form a natural group and the doliolums and their allies another. The context of the two passages that I have quoted shows that my purpose was to show the error of the opinion that these two groups are widely separated and belong in different phyla in the Tunicata, an opinion that is formulated by Uljanin, in his memoir on *Doliolum,* in the statement that he regards *Salpa* as standing alone among the tunicates, and that its resemblance to

Doliolum is superficial and due to secondary adaptation, an opinion which seems to be shared by most of the writers of text-books.

THE MUSCLES OF THE SOLITARY CYCLOSALPA.

The homology between the muscles of the various species of *Cyclosalpa* becomes very conspicuous as soon as careful and accurate drawings are compared, for it is very exact and complete, and the equivalent of each muscle in any one species can be identified without difficulty in all the others. For this purpose more careful drawings of the muscles and a more minute system of designating them than are found in most of the descriptive literature are necessary.

I give, in text-figure 1, a ventral view of the adult solitary *S. pinnata,* and, in plate X, figure 13, a side view of the embryo of the same, with the system of lettering that is used for *S. floridana* in plate 1, figures 1 and 2. The muscles 6, 7, 8, 9, 10, and 11 are incomplete, both dorsally and ventrally, and they are independent and not united into groups, as they are in *S. floridana,* but, in other respects, the description of the muscles of *S. floridana* on pp. 76–78 applies to *S. pinnata,* as it does also to *S. affinis,* and, probably, to *S. virgula,* although I have not had an opportunity to study this species, and know of no good figure of the solitary form.

The muscles of *S. floridana* are shown in dorsal view in plate 1, figure 1, in ventral view in figure 2, and side view in figure 7. In all of the figures the muscles that are on the near side are designated by Arabic numerals, and those on the far side, that are seen through the transparent body, are designated by Roman numerals.

Muscle 1 crosses the middle line of the dorsal surface of the body, just posterior to the slender oral muscles, and, bending around on to the ventral surface, runs backwards, and, uniting with muscle 5, forms muscle 6.

Muscle 2 is a dorsal longitudinal muscle, which connects muscle 1 with muscle 4.

Muscle 3 crosses the middle line of the ventral surface posterior to the anterior end of the endostyle and the ciliated band, and, bending around on to the dorsal surface, unites with muscle 2 to form muscle 4.

Muscle 4 is a dorsal longitudinal muscle that is formed by the union of muscle 2 and muscle 3.

Muscle 5, arising near the middle line of the dorsal surface, runs outwards and forwards, and, bending on to the ventral surface, and running inwards and backwards, unites with muscle 1 to form muscle 6.

Muscle 6 is a ventral muscle that is formed by the union of muscle 1 with muscle 5.

Muscle 7 is nearly transverse dorsally, while its ventral half runs inwards and backwards.

Muscle 8 is transverse, both dorsally and ventrally.

Muscle 9 is nearly transverse dorsally, whereas its ventral half runs inwards and forwards.

Muscle 10 is like muscle 9, except that its ventral half is still more inclined forwards.

Muscle 11 is like muscle 9 and muscle 10, except that its ventral half is still more inclined forwards.

Muscle 12 crosses the middle line of the ventral surface, while its dorsal portions do not meet on the middle line.

Muscles 11, 13, 14, and 15: I am not able to trace an exact homology between the muscles posterior to muscle 12 in the various species of *Cyclosalpa,* although this failure may be due to a lack of detail in this region in the figures.

Muscle 16, which occurs also in *S. pinnata,* is shown in *S. floridana* in figure 7, is a longitudinal muscle that connects muscle 1 with muscle 5. It is not visible in a symmetrical dorsal or ventral view, as it is on the side of the body, midway between the dorsal and the ventral surface. It is shown in the adult *S. floridana* on the left side of figure 1, which is not a perfectly dorsal view.

The four known species of *Cyclosalpa* fall into two sets as regards the muscles of the solitary form. In *Salpa pinnata* and *S. affinis* the muscles are not united into groups or bundles, while they are so united in *S. virgula,* and *S. floridana.*

In *S. floridana* (plate 1, figure 1) muscles 4, 5, and 7 of each side are united near the middle line of the dorsal surface into a single muscle, which does not cross the middle line nor unite with its fellow of the opposite side. In the ventral surface of the same species (figure 2) muscles 6, 7, 8, 9, and 10 unite to form a common trunk, which crosses the middle line of the ventral surface to become continuous with its fellow of the opposite side.

Since the muscles of the solitary cyclosalpas are homologous, and those of *S. pinnata* and *S. affinis* simple, or not united into groups, while those of *S. floridana* and *S. virgula* are more specialized, it seems natural to regard *S. pinnata* and *S. affinis* as more primitive. The study of the digestive organs corroborates this view and seems to indicate that all the cyclosalpas are derived from a *pinnata*-like ancestral form.

In the solitary *S. pinnata* muscles 7, 8, 9, 10, and 11 are incomplete both dorsally and ventrally, and they are not joined into bundles. In *S. affinis* muscles 8, 9, 10, and 11 are complete dorsally, but not united into bundles. In *S. floridana* they are incomplete dorsally, but 4, 5, and 7 are united into a bundle on the dorsal surface, while muscles 6, 7, 8, 9, and 10 are complete ventrally and united into a bundle.

THE MUSCLES OF THE AGGREGATED CYCLOSALPA.

(Plate 1, figs. 5 and 6; plate 2, figs. 8 and 9.)

In the aggregated forms of the various species of *Cyclosalpa,* the muscles at the anterior end of the body, which I have numbered from 1 to 8, are so much alike that they can be identified without difficulty, both with each other and with the muscles of the solitary *Cyclosalpa.* There is much greater specialization in the muscles in the posterior region of the body, which I have numbered from 7 to 12. Here it is difficult to trace exact homologies among the aggregated forms of the various species, or between the aggregated form and the solitary form, although it is probable that the muscles of all the cyclosalpas, solitary and aggregated, are homologous.

In all the aggregated forms there is a muscle that is very characteristic. It is marked *C* in figure 5, *S. floridana,* and is shown unlettered in text-figure 2, *S. pinnata,* and text-figure 3, *S. affinis.* It extends obliquely upwards and backwards from one of the encircling muscles to the next. As it appears to be equivalent in all the species, I assume that the muscles that it joins are also equivalent, and I have designated them by the same numbers, 9 and 10. In all the aggregated salpas the stalks that bind the members into a community contain prolongations of the body-cavity into which two or more of the muscles are prolonged, as is shown in text-figures 2 and 3, and these appear to be homologous in the different species.

The muscles of the aggregated *S. floridana* are shown in plate 1, figures 5 and 6, and plate 2, figure 9; those of the aggregated *S. pinnata* in plate 2, figure 8, and in text-figure 2; those of *S. affinis* in text-figure 3.

Muscle 1 crosses the middle line of the dorsal surface, just posterior to the slender oral muscles, and, bending around on to the ventral surface, runs backwards, and, uniting with muscle 5, forms muscle 6, resembling the corresponding muscle of the solitary form in all respects.

Muscle 2 is a longitudinal muscle which connects muscle 1 with muscle 4, as in the solitary form.

Muscle 3 is prolonged ventrally into the attaching process, and, running dorsally, posterior to the anterior end of the endostyle, unites with muscle 2 to form muscle 4, as in the solitary form.

Muscle 4 is a dorsal longitudinal muscle that is formed by the union of muscle 2 and muscle 3, as in the solitary form, and it unites with muscle 5, as in the solitary *S. floridana,* and gives rise to a muscle, *A,* which crosses the middle line to unite with its fellow.

Muscle 5 unites with muscle 1 to form muscle 6, as in the solitary form, but it unites dorsally with muscle 4 to form muscle *A.*

Muscle 6 is formed by the union of muscle 1 with muscle 5, as in the solitary form. In *S. affinis* and *S. pinnata* it unites ventrally with muscle 7 and prolonged into the attaching process, where it unites with muscle 7 to form muscle *E.* In *S. floridana* it crosses, but does not unite with,

muscle 7, while it does unite with muscle 8 and with its fellow of the opposite side, on the ventral middle line.

Muscle 7 is in the organ of attachment in *S. pinnata, S. affinis,* and *S. floridana,* and probably in *S. virgula.* In *S. pinnata* and *S. affinis* it is united ventrally to muscle 6, but it is not so united in *S. floridana.* Dorsally it crosses the middle line close to, but not united with, muscle *A* in *S. pinnata* and *S. affinis,* while in *S. floridana* it unites dorsally with muscle 9 to form the median dorsal muscle *B.* It is no doubt homologous in all the aggregated cyclosalpas, and also homologous with muscle 7 of the solitary cyclosalpas.

Muscle 8 is a complete and independent ring in *S. pinnata;* prolonged ventrally into the organ of attachment in *S. affinis;* united with muscle 6 on the ventral middle and with muscle 9 laterally in *S. floridana,* it is homologous with muscle 8 of the solitary forms.

I am not able to identify the muscles posterior to 8 in the various aggregated forms with each other or with those of the solitary forms with any confidence, as the various species are much specialized in this region.

In *S. pinnata* the muscle that I have marked 9 crosses the middle line of the dorsal surface close to, but not united with, muscle 8. In *S. affinis* it crosses the dorsal middle line independently of and at a distance from muscle 8. In *S. floridana* it crosses the middle line of the ventral surface, and unites dorsally with muscle 8, and then with muscle 7 to form muscle *B,* which crosses the dorsal middle line close to but independently of muscle *A.*

Muscle 10 is a circular muscle that seems to be homologous in *S. pinnata, S. affinis,* and *S. floridana,* and it is joined to muscle 9 by the oblique muscle *C.*

The results of the study of the muscles of the various species of *Cyclosalpa* in the solitary and the aggregated form may be summarized as follows: The muscles are homologous, and in most cases the homology can be traced without difficulty, while it is more obscure in other cases; it is most easy to trace in anterior ends of the bodies, while the specialization is greater, and the homology more obscure, in the posterior region of the body. There is a very complete series which connects the simplest and most primitive form—the solitary *S. pinnata*—with the most specialized form—the aggregated *S. floridana.*

PART IV — ON OIKOPLEURA TORTUGENSIS, A NEW APPENDICULARIAN FROM THE TORTUGAS, FLORIDA, WITH NOTES ON ITS EMBRYOLOGY.

By William Keith Brooks and Carl Kellner.

(Plates 3–8.)

INTRODUCTORY.

A large appendicularian (plate 3, fig. 4) in its house is found in abundance in the vicinity of the Marine Biological Laboratory of the Carnegie Institution of Washington at Tortugas, Florida. It is, no doubt, widely distributed along the coast of Florida, as it has been found by Dr. Mayer and Professor Brooks at Miami, and by Mr. Kellner in the Tortugas. The specimens are from 5 to 8 mm. long, and occur in great swarms at the depth of from 5 to 8 fathoms. They belong to the genus *Oikopleura* and to a species that seems to be new, although its differences from *O. longicauda* and *O. intermedia* of Lohmann are slight. The house (plate 3, figs. 4 and 5) is large, about 20 mm. in diameter, and nearly spherical. In its internal structure it resembles the houses that have been described in other species of the genus.

Most of the houses contain small appendicularias of various sizes, but all are well advanced and in the appendicularia-stage. It is possible that these are the young of the animal in whose house they are found, but it is also possible that they have been drawn into the house by the current of water and that they are not, of necessity, the young of the species which forms the house.

On the tails of some of the specimens (plate 7, fig. 13) are the eggs and early stages in the development of an appendicularian which may be *Oikopleura,* although it is possible that they belong to some other species. The eggs (plate 7, fig. 16) are inclosed in thick capsules of follicle-cells. Some are loosely attached to the tails, while others (plate 7, fig. 19) have, at one end, a process of modified follicle-cells that penetrates the tail like a root and firmly attaches the egg. Two embryos, at two successive stages of early development (plate 7, figs. 20 and 15) were found. They are deeply rooted in the tail by a process that penetrates the blood-sinus of the adult, and the embryos are parasites. On the ventral surface of the older embryo (plate 7, fig. 14) the two spiracles, opening to the exterior at one end and into the respiratory pouches of the pharynx at the other, prove that the embryos are appendicularians, although we have not been

able to make out much of their development from two imperfectly preserved specimens of such minute size. The so-called gland-cells that have been figured and described by Lohmann on the tail of *Oikopleura* are no doubt eggs or embryos.

All of the houses that we examined contained small elongated gregarina-like parasites (plate 3, fig. 6). On the tails of some of the specimens are rhizopods of the genus *Gromia* rooted in the tails by a network of pseudopodia, and with the body covered by a thin transparent shell (plate 4, fig. 12). This rhizopod is described on page 93.

Oikopleura tortugensis sp. nov. (Kellner).

(Plates 3 and 4, figs. 1, 2, 3, 4, 5, 8, 9, 10, 11.)

DIAGNOSIS OF THE SPECIES.

Both slightly elongated. Dorsal outline convex in profile view (fig. 1). Ventral surface between anus, *a*, and mouth, *f*, strongly convex. Mouth nearly dorsal. Whole surface of body, dorsal or anterior to a line that joins the anus to the dorsal end of the reproductive organ, covered with large cells (plates 3 and 4, figs. 1, 2 and 3). In region of oikoplast there are four membranoplasts, *c, c,* one on each side of the middle line of the dorsal surface, and one on each side of the body dorsal to the rectum and anterior to the anterior end of stomach.

Endostyle nearly vertical, anterior end near mouth, posterior end much anterior to the spiracles.

Digestive organs (plates 5 and 6, figs. 7, 8, 9, 10).—Stomach large; with two lobes. The œsophagus opens into left lobe at some distance from its posterior end, which terminates in a dorsal conical protuberance. Right lobe is continued into the intestine, which becomes much enlarged near the anus.

Reproductive organs (plate 5, figs. 7, 8) convex posteriorly, anteriorly consisting of two concave lateral portions that partially cover the posterior ends of the lobes of the stomach, and a thicker central portion that lies between these lobes.

Length of body of our largest specimen, 2.5 mm.

Tail (plate 4, fig. 11) broad, with well-developed muscles; end of tail broad, rounded, with no terminal fold.

House (plate 4, figs. 4 and 5) spherical, delicate, transparent, with two openings, *p, p,* through which water enters, and an unpaired opening, *q,* through which it is discharged and through which the animal leaves it when it is abandoned.

Distribution.—Abundant near the Dry Tortugas and in Gulf Stream near Cape Florida.

Remarks.—The differences between this species and *O. longicauda* and

O. intermedia, as described by Lohmann (Appendicularien der Plankton-Expedition, von Dr. H. Lohmann. Ergebnisse der Plankton-Expedition der Humboldt-Stiftung. Bd. II. C. c. Edited by Victor Hensen, 1894) are slight. The tail is like that of *O. longicauda,* lacking the fin-like lobe that is described and figured in *O. intermedia* by Lohmann. In other respects it is like *O. intermedia* resembling it, and differing from *O. longicauda,* in the following respects: The digestive organs are less compact than in *O. longicauda* and like those of *O. intermedia.* The œsophagus joins the left lobe of the stomach much anterior to the curved process that forms the posterior end of the left lobe, as in *O. intermedia* (see plate 5, figs. 7 and 8), and not close to it, as in *O. longicauda.* The endostyle is near the mouth and nearly vertical, as in *O. intermedia,* and it is separated from the region of the gills by a wide interval, while it is more nearly horizontal and farther back in *O. longicauda.* The thin membranous veil (Schleier) that Lohmann describes and figures in *O. longicauda,* overhanging the dorsal surface of the posterior end of the body, to which it is attached near the reproductive organ, is not present in any of our specimens, nor does Lohmann mention it in *O. intermedia.*

NOTES ON EMBRYOLOGY.

In plate 7, figure 13, part of the tail of *Oikopleura tortugensis* is shown magnified 43 diameters. Attached to it are three eggs, *r, r, r,* of which one is shown, more magnified in figure 16, and an embryo, *s,* which is shown, more enlarged, in figures 19 and 20. The eggs are attached to the tail by a process that penetrates into the tissues. In figure 16 one of the eggs is shown, magnified 200 diameters. The opaque egg, *r,* is inclosed in a follicle, *z,* of elongated cells with flat outer ends. At the bottom of the figure the fastening process or root, *x,* is shown. It is formed of follicle-cells, and shows indications of an axial cavity. No test-cells can be made out between the yolk and the follicle, the minute, badly preserved specimens not being favorable for observation. All of the specimens were fixed with picro-acetic fixative and carefully preserved with changes of alcohol; but as they were preserved for the identification of the species, with no thought of eggs or embryos, they were not isolated, but were handled wholesale as they were collected. While we found several eggs, we found only two embryos in our collection. While they are much better preserved than the eggs, they are too few to afford much information about the details of the life-history. One of them, shown at *s* in figure 13, is shown, magnified 300 diameters, in plates 7 and 8, figures 17, 18, 19, 20. An older one is shown in ventral view in plate 7, figure 14, in dorsal view in figure 15, and in sections in plate 8, figures 21, 22, 23, 24 and 25. The structure of the older embryo has features of resemblance to an adult appendicularian, and as it is, therefore, more intelligible than the younger one, it will be described first.

It is shown in dorsal view, magnified 300 diameters in plate 7, figure 15, and in ventral view in figure 14. It is deeply rooted in the tail of the adult by a process from the anterior, or oral, end of its body. The process is hollow, as is shown in the sections drawn in figures 24 and 25 of plate 8, but there is no trace of an opening at its tip, and it no doubt absorbs its food in a liquid state. As the sections show, its wall consists of a single layer of large cells, with here and there a cell or a group of cells on its outer surface. These outer cells seem to be blood corpuscles of the adult. In the body-cavity of the embryo there is a row of four big cells in the axis that passes through the root. One of them is shown in section in plate 8, figure 23. They are no doubt concerned with the nutrition of the embryo, making the food that is taken up by the root available.

On the middle line of the dorsal surface, near the attached end of the body, there is an opening, shown at *f* in plate 8, figure 23, which we regard as the mouth. It communicates with a thin-walled, V-shaped chamber, *ad*, which we regard as the oral or stomodæal chamber of the pharynx. At each end it opens into one of the large, thick-walled, ciliated branchial pouches of the pharynx, which are shown at *t*, *t*, in plate 7, figures 14 and 15, in the entire embryo, and in section at *t*, *t*, in plate 8, figures 21, 22, and 23. In side view of the adult, figure 1, the gill is shown, at *l*, as a thin-walled tube, opening at its inner end into the pharynx through the internal spiracle, which is shown to be ciliated in plate 4, figure 3, at *k* and *l;* and opening to the exterior through the external spiracle, which is on the ventral surface near the middle line. In all these respects the embryo that is shown in plate 7, figures 14 and 15, is so much like the adult as to show, beyond question, that it is the embryo of an appendicularian. The ciliated pharyngeal pouches are shown in figure 15 in dorsal view and in figure 14 in ventral view, at *t, t*. The gills are shown, *u, u,* in ventral view, communicating with the pharyngeal pouches at the posterior ends, which are above in the figure and opening to the exterior, near the middle line, through openings that are much more elongated than they are in the adult.

In the sections in plate 8, figure 23 is most anterior, and it cuts the mouth, *f*, and the oral region of the pharynx, *ad*, showing its communication with the pharyngeal pouches, *t*, *t*, which are shown in figure 22, communicating, at *y*, with the gills, *u*, *u*, through the internal or pharyngeal spiracles; while figure 21 shows the external spiracles at *ac*.

The large, thick-walled chamber shown at *v* in plate 8, figures 22 and 23, may be the stomach, bilobed at its tip. In plate 8, figures 17 and 18, which are optical sections of the younger embryo shown in plate 7, figures 19 and 20, it is shown, at *v*, communicating with the branchial pouches, *t, t*, of the pharynx.

The tissue that occupies the space between the three chambers in figures 21 and 22, and which is badly preserved, may perhaps be the notochord and nervous system.

ON A NEW SPECIES OF GROMIA (G. APPENDICULARIÆ).

On the tails of some of our specimens of *Oikopleura* unicellular parasites were found. One of them is shown in plate 4, figure 12. They are rooted in the substance of the tail by a tuft of pseudopodia that do not anastomose, and the exposed body is covered by a thin, transparent, stiff capsule. The organism is unquestionably a *Gromia,* and we propose for it the specific name, *Gromia appendiculariæ.*

EXPLANATION OF REFERENCE LETTERS ON PLATES.

a. Anus.
b. Endostyle.
c. Membranoblasts.
d. Œsophagus.
e. Pharynx.
f. Mouth.
g. Stomach.
h. Right lobe of stomach.
i. Left lobe of stomach.
j. The large cells that cover more than half the body.
k. Right gill.
l. Left gill.
m. Reproductive organ.
n. Gelatinous mass.
o. Parasites.
p. Openings through which the currents of water enter the house.
q. Opening through which the water is discharged, and the animal escapes when it leaves the house.
r. Egg.
s. Embryo.
t. Branchial pouches of pharynx.
u. Spiracles.
v. Stomach (?) of embryo.
w. Chorda (?) of embryo.
x. Root-like process from the body of the embryo, embedded in the tail of the adult.
y. Internal or branchial spiracle, through which the branchial pouch of the pharynx opens.
z. Follicle cells.
ab. Part of tail of adult.
ac. External opening of spiracle.
ad. The median oral division of the pharynx, showing its communication with the two branchial pouches, *t*. *t*.

EXPLANATION OF PLATES.

Plate 1.

Fig. 1. *Salpa florida*, solitary form, dorsal view of adult.
Fig. 2. *Salpa florida*, solitary form, ventral view of adult.
Fig. 3. *Salpa florida*, solitary form, digestive organs of adult.
Fig. 4. *Salpa florida*, solitary form, ventral view of young 4.5 mm. long.
Fig. 5. *Salpa floridana*, aggregated form, side view.
Fig. 6. *Salpa floridana*, aggregated form, ventral view.

Plate 2.

Fig. 7. *Salpa floridana*, solitary form, side view of very young embryo.
Fig. 8. *Salpa pinnata*, aggregated form.
Fig. 9. *Salpa floridana*, aggregated form, dorsal view of colony.

Plates 3 to 8.

All figures on plates 3 to 8 refer to *Oikopleura tortugensis* nov. sp.

Plate 3.

Fig. 1. Side view of body of *Oikopleura tortugensis*.
Figs. 4 and 5. Views of the house.
Fig. 6. Gregarina-like parasites in the house.

Plate 4.

Fig. 2. Ventral view of body of adult.
Fig. 3. Dorsal view.
Fig. 11. Side view of entire adult animal.
Fig. 12. Rhizopod *Gromia* rooted to the tail of *Oikopleura*.

Plates 5 and 6.

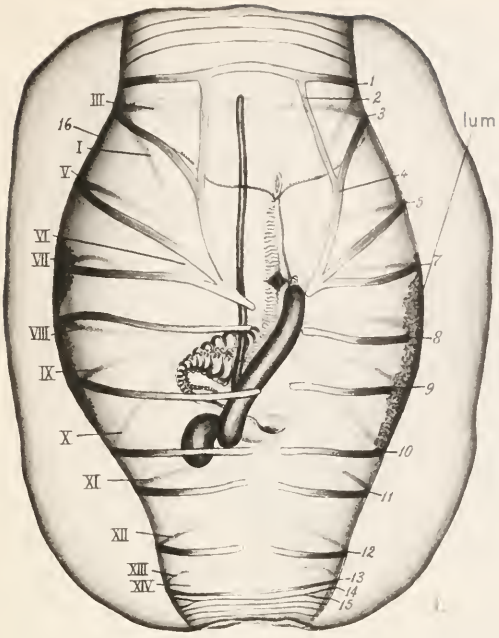
Figs. 7, 8, 9, 10. Digestive and reproductive organs of adult.

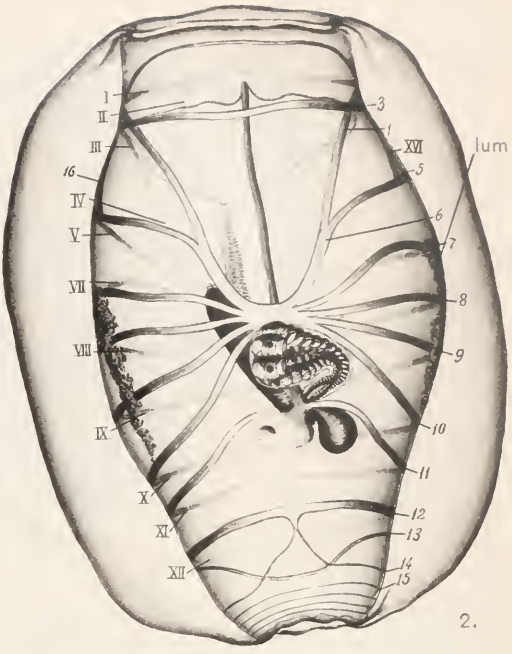
Plate 7.

Fig. 13. Part of tail, magnified 13 diameters, showing eggs and embryos rooted as parasites upon it.
Fig. 14. Ventral view of embryo magnified 300 diameters.
Fig. 15. Dorsal view of the embryo shown in figure 14.
Fig. 16. Egg within its follicle, attached to the tail.
Figs. 19 and 20. Views of an embryo younger than the one shown in figures 14 and 15.

Plate 8.

Figs. 17 and 18. Views of the embryo shown in figures 19 and 20, plate 7.
Figs. 21 to 25. Sections of the embryo shown in figures 14 and 15, plate 7. Figure 23 most anterior section. Figures 24 and 25, sections of the root of attachment imbedded within the tissues of the tail of *Oikopleura tortugensis*.





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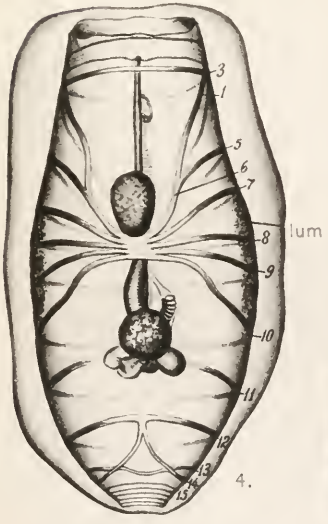

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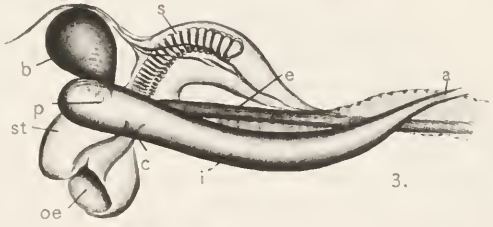

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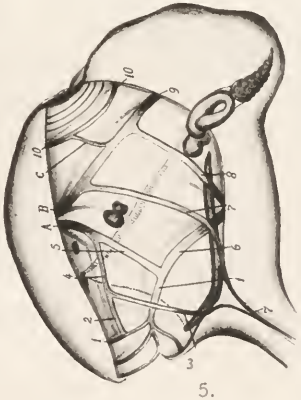

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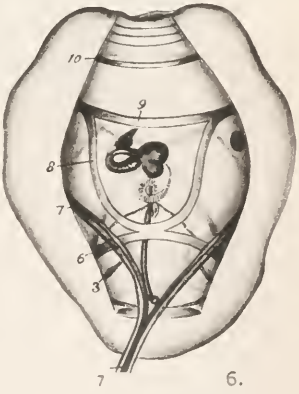

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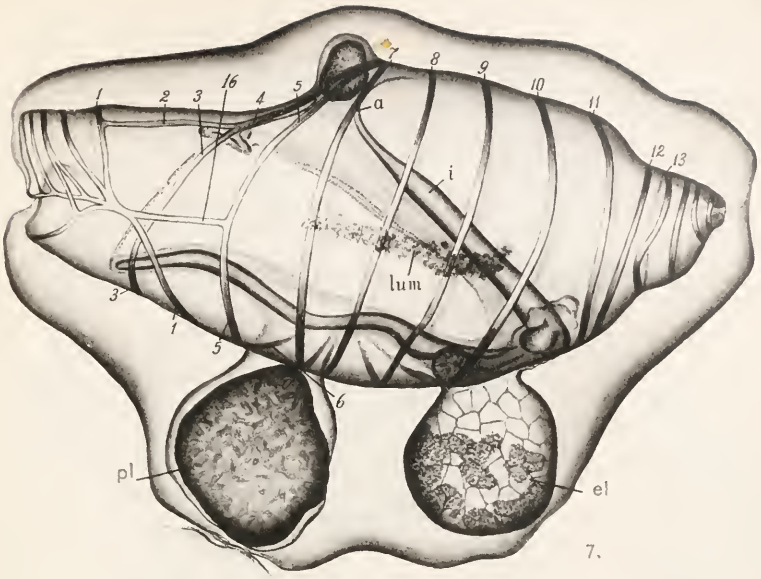

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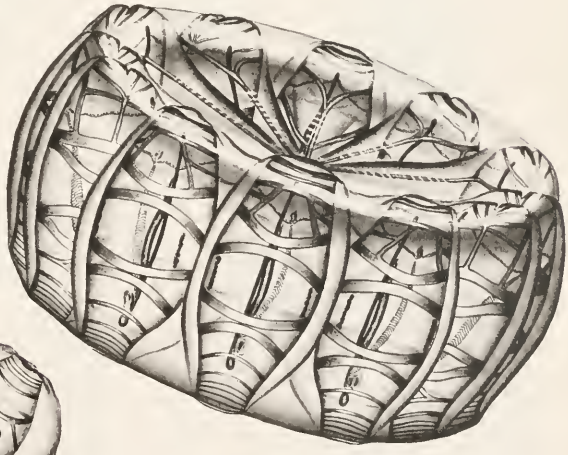

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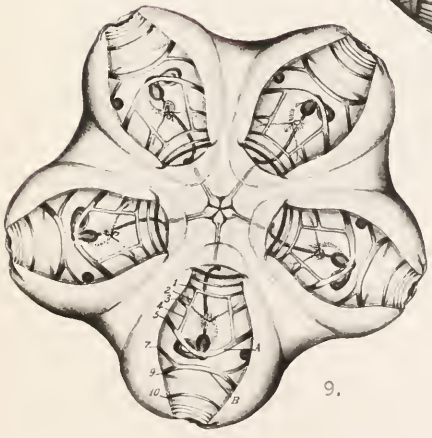

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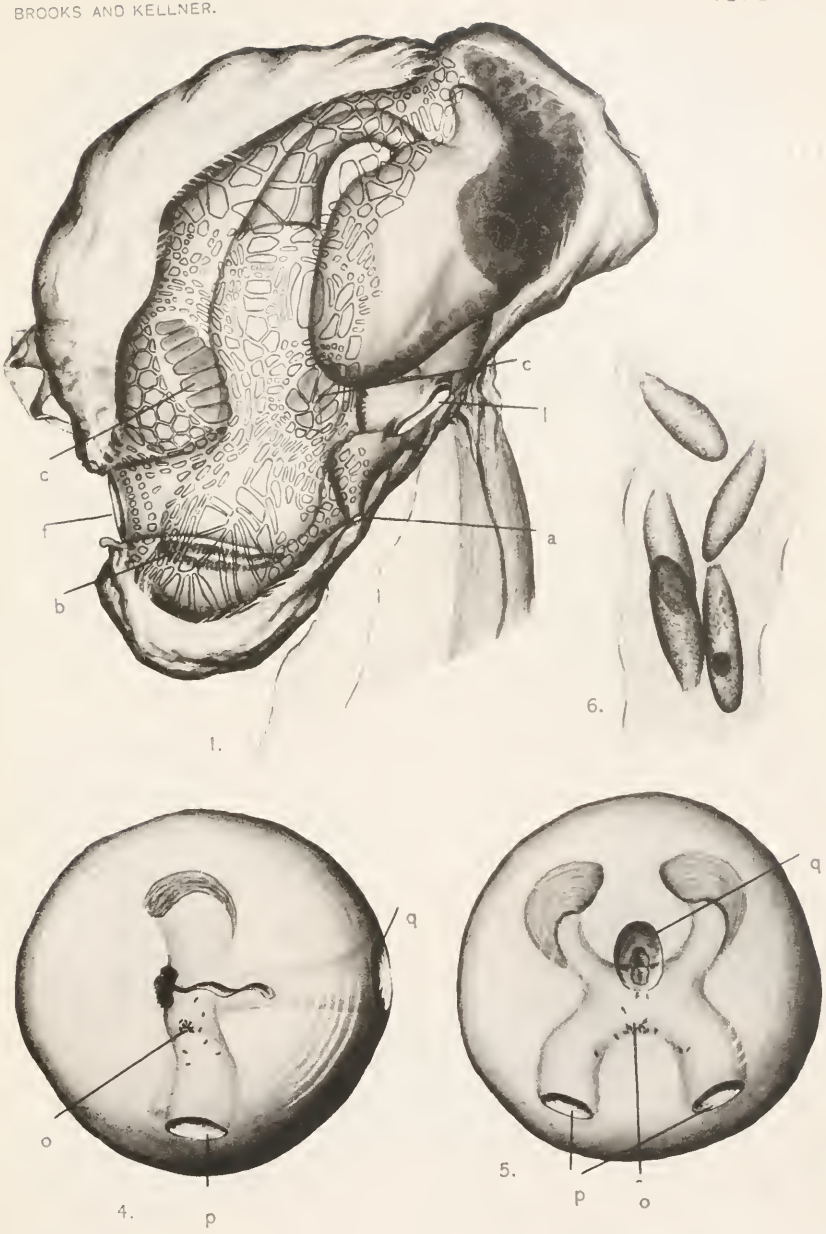

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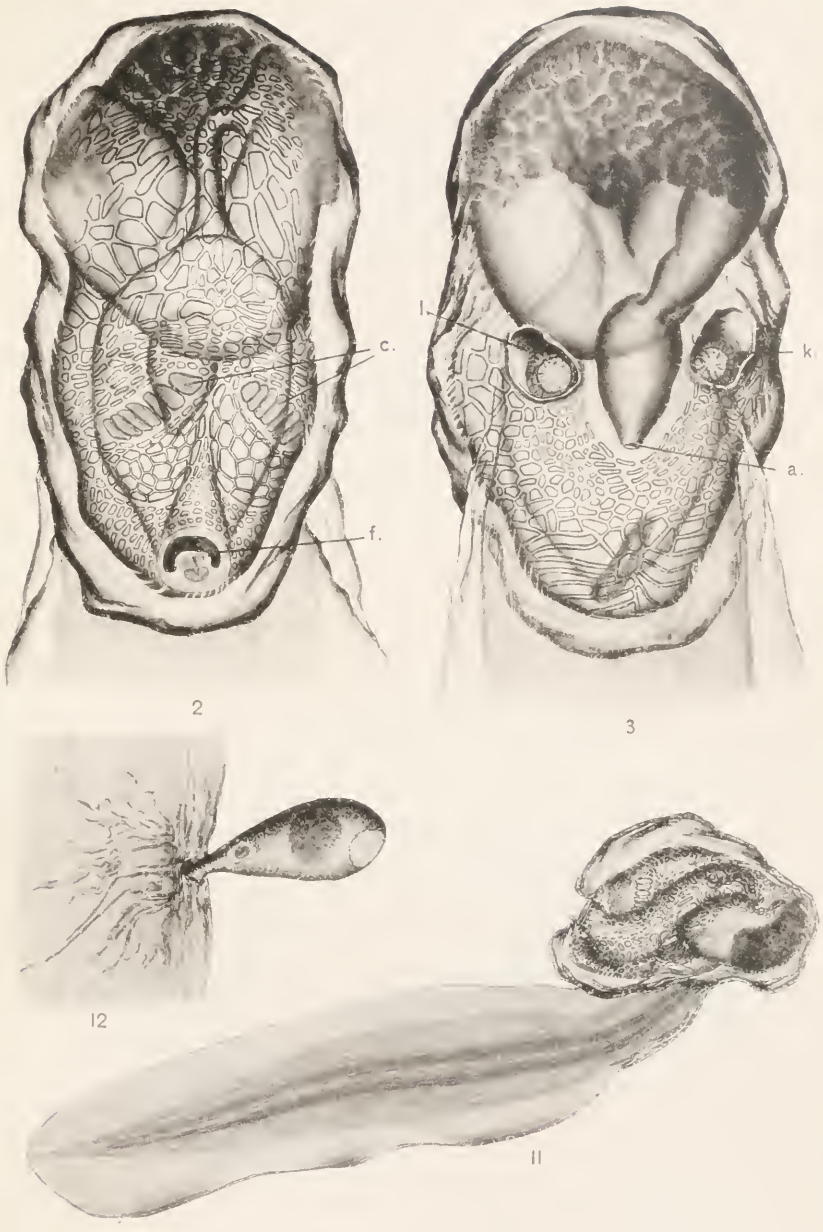
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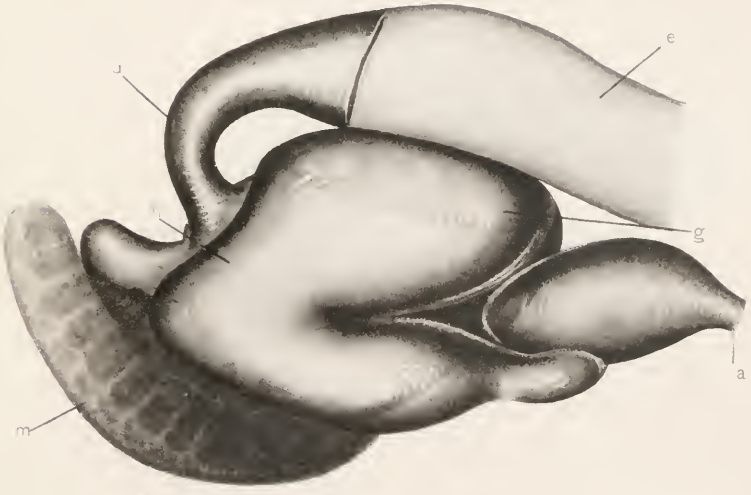
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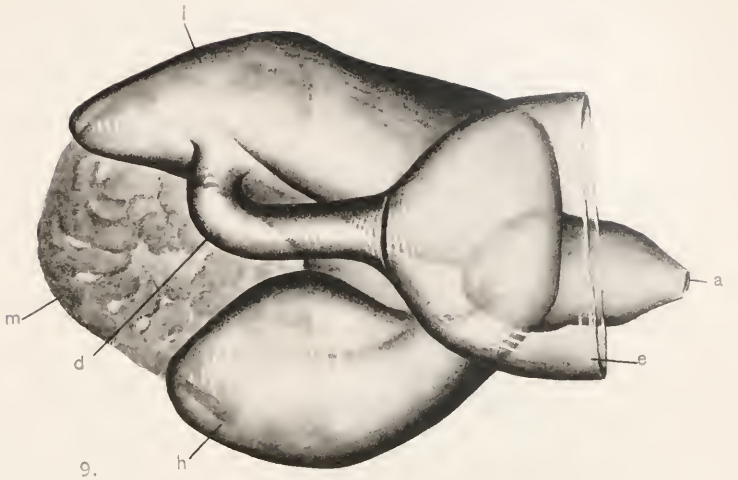

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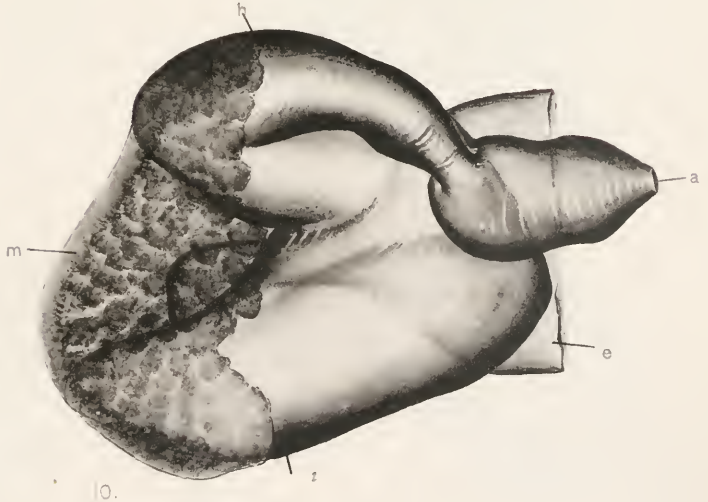

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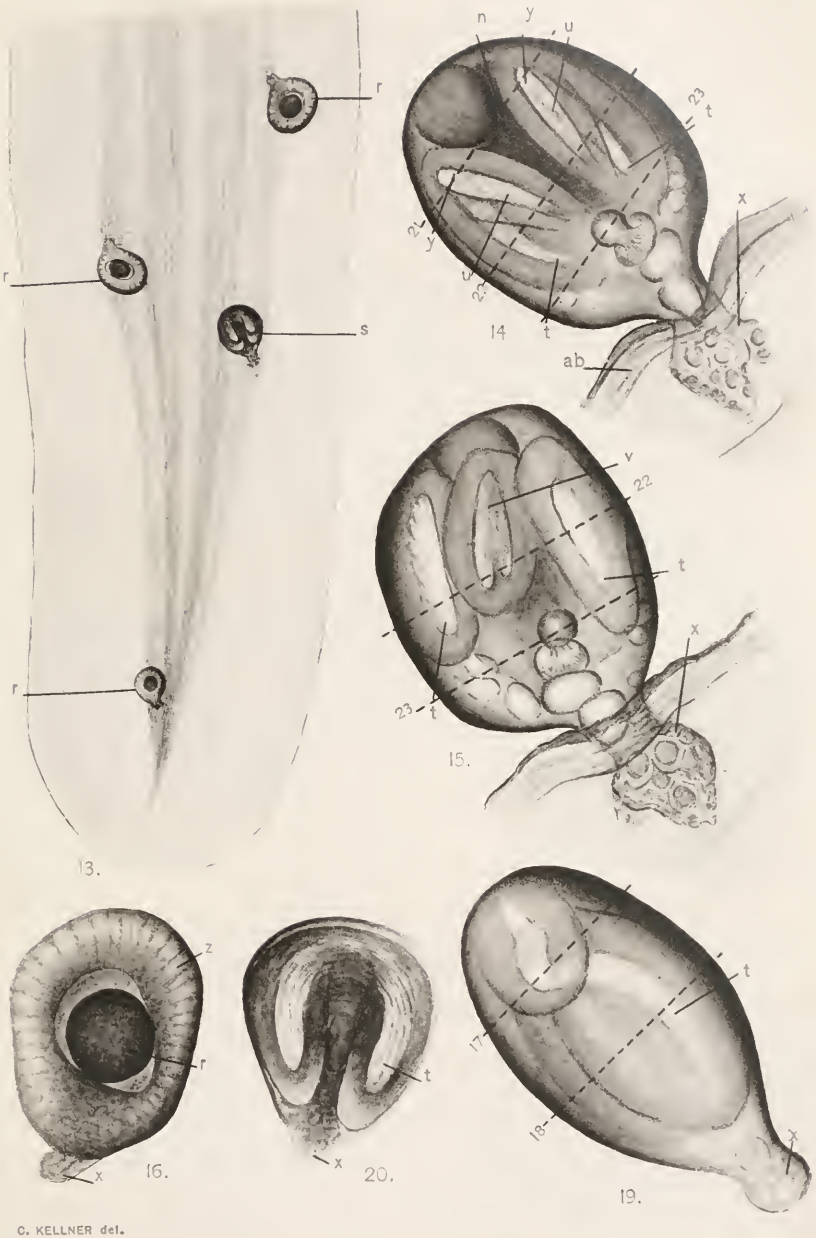

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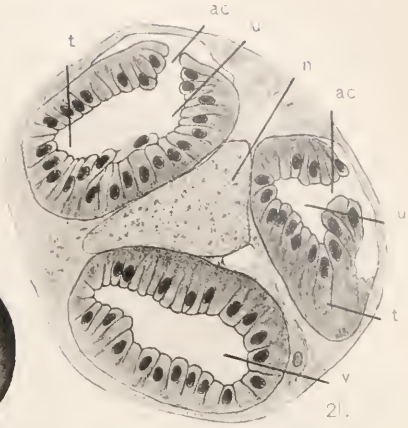

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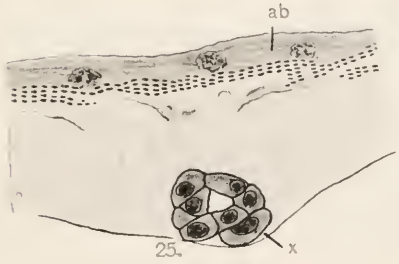

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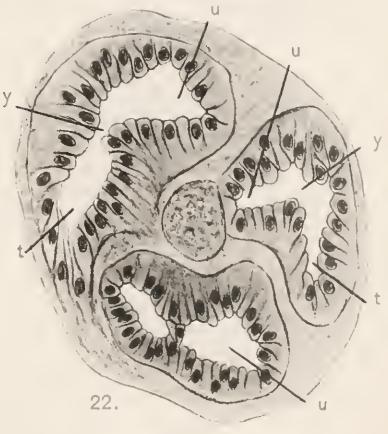

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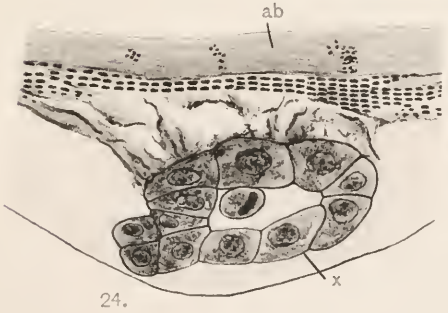

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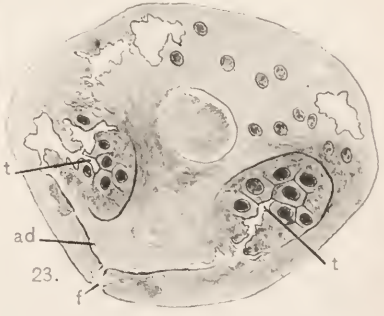

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V. THE ORIGIN OF THE LUNG OF AMPULLARIA

By WILLIAM KEITH BROOKS and BARTJIS McGLONE

Plates 1–7

THE ORIGIN OF THE LUNG OF AMPULLARIA.

By W. K. Brooks and Bartgis McGlone.

INTRODUCTORY.

One of the authors was able to visit and partially explore the Everglades of Florida, in March, 1906, through the courtesy of the Director of the Marine Biological Laboratory of the Carnegie Institution of Washington, Dr. Alfred G. Mayer, in whose company the expedition was made. As we pushed our way through the tall reeds and grasses that cover the shallow water of the Everglades, we found great numbers of small eggs attached to the stems, above the surface of the water, but close to it (fig. 8, plate 1).

The eggs are arranged in vertical rows and inclosed in calcareous shells, resembling, in this respect, the eggs of the terrestrial pulmonate gasteropods. We also found, in the water, the prosobranchiate gasteropod, *Ampullaria*, in great abundance, and when some of the older eggs were opened, they were found to contain young ampullarias. The Paludinidæ, which are closely related to the Ampullaridæ, are aquatic, viviparous, and breathe by gills; and their structure seems to indicate that they are true prosobranchs, descended from, and closely related to, the marine prosobranchs. *Ampullaria* has gills, is partially aquatic, and seems to be a true prosobranch; but as it has a lung, and is able to breathe air and live out of the water, and as it also lays, in the air, eggs with calcareous shells like those of the terrestrial pulmonates, the question whether it, and the Paludinidæ, are primarily pulmonates with secondary resemblances to the prosobranchs, or primarily prosobranchs with secondary resemblances to the pulmonates, suggests itself. As the embryonic history of the organs of respiration may be expected to throw light upon this question, a quantity of eggs was collected and taken to the Marine Laboratory in the Tortugas. There the eggs were opened, the embryos removed, sketched and studied, and then hardened and preserved for more thorough examination.

Sections show that the lung is a member of the series of gill-filaments, and it must be regarded as a modified filament, or more than one. It is, therefore, a secondary acquisition which is not derived from the lung of the pulmonates.

Both lung and gill arise very early, and simultaneously, in the embryonic

history. In a young embryo, soon after the mantle is formed, a ridge or thickening of the epithelium of its inner surface indicates the region where the gill-filaments, the osphradium, and the lung are to arise. The osphradium is developed from one end of this ridge, the gill-filaments from the other, and between the two the ridge becomes infolded into the substance of the mantle to give rise to the lung, which may be regarded as a modified and invaginated gill-filament. The similarity of the lung to that of the pulmonates is nothing more than a new illustration of resemblance between organs that have been acquired independently under like physiological conditions.

DESCRIPTIVE.

MATERIAL AND METHODS.

The investigation that is here described is based upon the study of the young stages in the development of an *Ampullaria* which occurs abundantly in the Everglades of Florida. It is identified as *Ampullaria depressa* Say by Professor Wm. H. Dall. The shell of the adult is shown of the natural size in plate 1, figures 1 and 2; and at two early stages in figures 3, 4, 5, and 6. Of these, 3 and 4 show the shell, enlarged, at the time when the young mollusk leaves the egg, while that of a young embryo is shown, much more enlarged, in 5 and 6. The eggs are laid, two or three inches above the level of the water, on the stems of the reeds and grasses, in vertical rows that are usually regular, as is shown in figure 8. Each is attached separately by a tenacious cement or glue. They are inclosed in white, chalky, calcareous shells. As the yolk is pink and, at first, fills the shell, while it is shrunken and partially replaced by air in the older eggs, these undergo a change of color during development, the younger ones being pink, while the older ones are white. It is clear that the attempt to distinguish species by separating ampullarias with pink eggs from those with white ones rests upon a misconception.

It is well known that *Ampullaria* has both gills and lung, and is adapted for both aquatic and aerial respiration. The lung is a large, elliptical, thin-walled pouch in the mantle, with an opening that is on the left side, above the left siphon, and immediately posterior to the osphradium. It is well shown in figures 5, 6, 7, and 8 of plate 1 of the Atlas of the Mollusca of the Voyage de l'Astrolabe.

The series of embryos that afforded the material for this research is complete, so far as the history of the respiratory organs is in question. In the younger ones the shell is a thin, transparent flat cap, and there are no traces of gills nor of lung; and the collection includes an abundance of embryos at each successive stage up to the time of hatching.

The young mollusks were kept alive in captivity for four weeks or more after hatching, but they did not undergo any observable change.

Those that were kept immersed drowned before the end of the second day, but those that were kept in damp air or were permitted to climb out of the water, remained in good health. The adults survive an immersion of a month or more without injury, but they leave the water occasionally when permitted to do so.

The embryos that have been studied were washed out of the broken shells by a stream of water, and they were fixed in picro-acetic acid or in formalin and preserved in 70 per cent alcohol. After the shells were removed by dilute nitric acid (8 to 15 drops in 100 c.cm. of alcohol) they were cut into sections in three planes, transverse, horizontal, and sagittal, a transverse section being one that is perpendicular to the long or principal axis of the extended embryo; horizontal, one that is parallel to the principal axis and at right angles to the median plane of morphological symmetry; sagittal, parallel to the principal axis and to the median plane of symmetry.

Haidenhain's hematoxylin was used for staining on the slide, with eosin as counterstain. Excellent results were obtained by shortening time. For instance, the alum bath was used for one or two hours, and the hematoxylin for only half an hour. Embryos that were to be mounted whole were stained in acid borax carmine and cleared with clove oil.

THE GENERAL ANATOMY OF THE EMBRYO.

In order to make the account of the development of the organs of respiration intelligible, it should be preceded by an outline sketch of the general anatomy of the embryos.

The anatomy of the embryo, at the time when the first traces of the organs of respiration are found, will be understood from plate 1, figure 7, and plate 3, figure 11. Plate 1, figure 7, is a surface view of a young embryo, and plate 3, figure 11, a reconstruction of a series of oblique sagittal sections of a slightly older one. Both are of the same length, 1.2 mm.

The anterior region consists of a great head-vesicle, *hv*, and the foot, *f*. In the ventral anterior region of the vesicle is a lipped opening, the mouth, *m*. Above this, and encircling the vesicle, is a ciliated ridge, the velum, *v*. The foot, *f*, is longer than broad, and its lower surface is flattened. On its posterior border are a few large, clear cells, apparently calcareous, three of which are shown in plate 1, figure 7. In young embryos they are large and numerous, but they disappear as growth progresses, and none are to be found at the stage shown in plate 3, fig. 11. On the dorsal surface of the posterior region of the foot the ectoderm becomes thickened and secretes a calcareous operculum, *o*. The posterior region of the body, with the mantle, mantle-chamber, and shell, is shown in front view in plate 1, figure 7, and in section in plate 3, figure 11. It resembles a bowl in shape, with the inner concave surface facing to the right. The convex left-hand surface is composed of the thickened ectoderm that secretes the shell, *sh*.

It is surrounded by a folded ridge, *mf*, the margin of the shell-gland. In plate 3, figure 11, the shell covers the whole of the posterior end of the body, and the raised edges of the shell-gland are pushed far apart. The growth of the shell brings about changes in the position of the mantle-chamber, in that of the mantle itself, and in that of other organs, as will be noted later.

From the mouth the œsophagus, *oe*, runs upward, within the head-vesicle, to the dorsal anterior region, where it opens into a huge chamber, the primitive stomach, *pr. st.*, which fills most of the head-vesicle. The sac of the radula (*r*, fig. 11) arises from the posterior (ventral) border of the œsophagus near the mouth, and extends under the ventral surface of the primitive stomach. A pair of salivary glands that are not shown in the figure arises from the œsophagus, near the mouth, as a pair of pouches or outgrowths. The primitive stomach narrows posteriorly and gives rise to the intestine, which, arising on the left, bends to the right, and opens into the cavity of the mantle, through the anus, *a*, which is at first median and ventral, but moves upward on to the right side of the mantle as development advances. Near the anus there is, in young embryos, a rosette-shaped cluster of large transparent cells like those that have been mentioned on the foot. They persist until about the time of hatching. The buccal and cerebral ganglia arise as compact groups of spindle-cells below and above the radula, while a similar group of cells arises in the posterior part of the foot and becomes the pedal ganglia.

The heart is shown in plate 3, figure 11, near the bottom of the figure; although the opening through which the auricle, *au.*, communicates with the ventricle, *ven*, does not lie in the plane of this section. The heart is inclosed in a spacious pericardium, *per*, with a thin wall of spindle-shaped mesoderm-cells. The aortic sinus, which arises as a split in the mesoderm, is shown at *a. s.* in figure 11, which cuts it in the region of the foot and again close to the pericardium. The right kidney, which is functional in the adult, is shown in figure 11 at *k*. It, as well as the abortive left kidney, arises as an evagination from the pericardium, while its duct, which is far to the right of the plane of the section, arises as an invagination of the inner surface of the mantle where this becomes continuous, on the extreme right, with the body-wall.

THE DEVELOPMENT OF THE ORGANS OF RESPIRATION.

In the adult these are on or under the inner surface of the mantle, and consist of the lung, the right gill, and the osphradium; and they stand in intimate anatomical relation to the heart and the renal organ. The lung is a large, elliptical, thin-walled pouch which opens into the chamber of the mantle through an aperture that is protected by valves. The opening is on the left side of the mantle above the left siphon and posterior to the osphradium—a small, oblong, laminated organ, about 3 mm. long. The gill ex-

tends along the right boundary of the lung, from which it is incompletely separated by a fold or ridge on the body (plate 5, fig. 15; also plate 57, fig. 6, Atlas of the Mollusca of the Voyage de l'Astrolabe). Anteriorly the gill diverges from the lung and ends, together with it, on the extreme left, dorsal to the heart, into the auricle, of which the veins of both organs enter through a common trunk.

The earliest stage in the development of the organs of respiration is shown, in surface view, in plate 1, figure 7, at *resp. rud.;* and, in section, in plate 2, figure 9. It makes its appearance as two parallel ridges, *g. r* and *o. r*, separated by a furrow, *l. r*. As the section shows, the ridges arise as thickenings of the epithelium of the mantle. One of them, *g. r*, is the rudiment of the gill, and the other (*o. r*) the rudiment of the osphradium; while the furrow, *l. r*, is the first trace of the lung, which is ciliated. The substance of the mantle, between the inner layer of epithelium which gives rise to the organs of respiration and the outer layer which forms the shell-gland, *sg*, is loosely filled with mesoderm, which is derived from the ectodermal epithelium by migration. At the stage that is shown in the figure the mesoderm is so arranged as to leave an unoccupied space, the pulmonary sinus, *p. s.*

A section of a stage that is somewhat older is shown in figure 10. Comparison with figure 9 shows that the gill-filament, *g. r.*, grows through the thickening and multiplication of the epithelial cells together with the multiplication of the underlying mesoderm. In the region of the lung, *l. r.*, the thickening and multiplication of the epithelium are of such character that they lead to an infolding instead of an outpushing, as is illustrated by the figure. An older stage, with traces of three gill-filaments, is shown in plate 3, figure 11. This is a reconstruction from a series of oblique sagittal sections of an embryo about 1.2 mm. long, with a shell that is nearly hemispherical, *sh*.

The lung, *l. r.*, is now deeply infolded and there are three gill-folds, *r. g. f.* The pulmonary sinus, *p. s*, is now a well-defined chamber under the respiratory organs, and it now opens into the auricle, *au*, by an aperture that is not shown in the figure. The gill-filaments are filled with mesoderm in which there is as yet no indications of a cavity. The mantle, mantle-chamber, and respiratory organs, at this stage, are shown, more enlarged, in plate 4, figure 12. The gill-filaments are filled with a compact mass of mesoderm, in the plane of the section, although this is looser to the right of the section and the interspaces communicate with the pulmonary sinus, *p. s.* The lung, *l. r.*, bends to the left as far as the osphradium, which is now nearly round.

The chamber of the mantle of an older ambryo, 1.4 mm. long, is shown in plate 5, figure 14, in order to illustrate the relation of the respiratory organs to other structures; while these organs are shown, more enlarged, in plate 4, figure 13, to illustrate the details. They are from an embryo

with a shell that nearly half covers it, with knob-like tentacles with eyes, and other approximations to the structure of the adult. The gill runs obliquely from left to right, and its filaments are visible through the shell in an entire embryo.

Figure 14 is a transverse section through the posterior half of this embryo. On the right, near the lower point of the mantle-chamber (near the top of the figure), is the rectum, *rec;* a cylindrical tube lined with columnar epithelium. Above the rectum (to the right of it in the figure) is a well-defined group of spindle-shaped mesoderm cells, the kidney, *k.* On the right (bottom of the figure) is the thin-walled pericardium, *per.* Above (to the right of) this is the pulmonary sinus, *p. s.* The gill-filaments, *g. f,* now project freely into the chamber of the mantle, *m. c,* the newer ones arising on the left (below in the figure) between the older filaments and the lung, *l. g,* which is a deep groove running forwards (downwards in the figure). The gills and lung are shown, more enlarged, in plate 4, figure 13. The gill-filaments are outfoldings of the columnar epithelium of the mantle, and they are loosely filled with a tissue of spindle-shaped mesoderm cells, between which are spaces that communicate with the pulmonary sinus, *p. s.* The lung-groove is lined with elongated ectoderm cells covered, on its internal surface, by a cap of mesoderm cells which arise from the ectoderm, as the illustration shows.

The chamber of the mantle, with the lung, *l.,* the gill, *g. f,* and the adjacent organs, are shown, at four successive stages of advanced development, in plate 6, figure 17; plate 6, figure 16; plate 7, figure 18, and plate 5, figure 15; the last figure showing the respiratory organs at the time of hatching. In this series, figure 16 is from an embryo 1.8 mm. long. The shell is shown, at this stage, in plate 1, figures 5 and 6, with one complete turn in the spiral.

The section shown in figure 17 is one that, if continued, would pass through the stomach, the liver, the operculum, and the part of the foot that is ventral to the operculum. The part of the section that is figured shows, besides the respiratory organs, the rectum, *rec;* the kidney, *k;* the osphradium, *osph,* and part of the pericardium, *per.* The lung, *l.,* is a deep pit, with an opening into the chamber of the mantle and is constricted by a projecting fold, *r,* of the respiratory region. While figure 16 is from an older embryo, it shows the anatomical relations that would be shown in another section of the stage shown in figure 17, and the two figures may be regarded as showing the lung, *l.,* in two sections of a single specimen. In figure 16 the cavity of the lung, *l.,* occupies nearly the whole thickness of the mantle, so that its lining of epithelium is separated from the epithelium of the shell-gland only by a thin layer of mesoderm. In this figure the intestine, *int,* is shown as a fold in the wall of the stomach. Figure 18 is from an embryo about 2.1 mm. long, with a shell like that

which is shown in plate 1, figures 3 and 4. The lung is deeply infolded and the section cuts nine gill-filaments, *g. f.*

Plate 5, figure 15, is from a young *Ampullaria*, at the time of hatching, with its organs of respiration in their definitive adult condition. The lung, *l*, is now a spacious chamber occupying most of the thickness of the mantle and opening into its chamber by a small, closable aperture.

At about this time the gill and the lung become separated from each other by a valvular fold (*part*) which grows out from the body into the chamber of the mantle.

Summary.—The gills, the lung, and the osphradium of *Ampullaria* arise simultaneously, or nearly so, in the embryo; and they are developed out of a ridge or thickening of the epithelium of the mantle. They must, therefore, be regarded as a series of homologous organs specialized among themselves in different directions, and not as independent acquisitions. There is no reason to think that there is any ancestral connection or relationship between the lung of *Ampullaria* and that of the pulmonates, although the embryonic history of the lung of *Ampullaria* shows that the origin of the lung of the pulmonates through the modification of a gill, or part of a gill, is not impossible. The lung of *Ampullaria* becomes functional before the gill does; for the newly hatched young die very quickly if they are prevented from leaving the water, while the adults survive a long immersion.

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DESCRIPTION OF PLATES.

Letters of Reference.

an. Anus.
a. s. Aortic sinus.
au. Auricle.
f. Foot.
g. r. Rudimentary gill.
r. g. f. Rudimentary gill filament.
g. f. Gill filament.
r. Fold that forms part of floor of lung.
h. v. Head vesicle.
k. Kidney.
l. r. Rudimentary lung.
l. g. Lung groove.
l. Lung.
m. Mouth.
m. c. Cavity of mantle.
m. f. Edge of shell gland.
n. Nerve tissue.
oe. Œsophagus.
o. Operculum.
o. r. Rudimentary osphradium.
osph. Osphradium.
part. Partition of cavity of mantle.
per. Pericardium.
p. s. Pulmonary sinus.
p. v. Pulmonary vein.
r. s. Radula sac.
rec. Rectum.
resp. rud. Rudiment of organs of respiration.
sh. Shell.
sh. g. Shell gland.
p. st. Primitive stomach.
st. Stomach.
v. Velum.
ven. Ventricle.

EXPLANATION OF PLATES.

PLATES 1 TO 7.

All of the figures are of *Ampullaria depressa* Say.

PLATE 1.

FIGS. 1 and 2. Adult, natural size.
FIGS. 3 and 4. Enlarged view of shell at time of hatching.
FIGS. 5 and 6. Enlarged view of shell of unhatched embryo 1.8 mm. long.
FIG. 7. Surface view of an unhatched embryo 1.2 mm. long.
FIG. 8. Eggs attached to grass.

PLATE 2.

FIGS. 9 and 10. Sections of the organs of respiration in their earliest stages. Figure 10 shows a stage somewhat older than that illustrated in figure 9.

PLATE 3.

FIG. 11. Reconstruction from a series of oblique sagittal sections of an embryo in the stage shown in figure 7, plate 1.

PLATE 4.

FIG. 12. Sagittal section of mantle-chamber and respiratory organs in an embryo 1.2 mm. long.
FIG. 13. Transverse section of the mantle-chamber of an embryo 1.4 mm. long.

PLATE 5.

FIG. 14. Transverse section of the mantle chamber of an embryo 1.4 mm. long.
FIG. 15. Section of the respiratory organs at the time of hatching.

PLATE 6.

FIGS. 16 and 17. Section of mantle-chamber showing lung, gill, etc. Figure 16 shows a stage wherein the embryo is 1.8 mm. long and is older than that shown in figure 17.

PLATE 7.

FIG. 18. Section of an unhatched embryo 2.1 mm. long.

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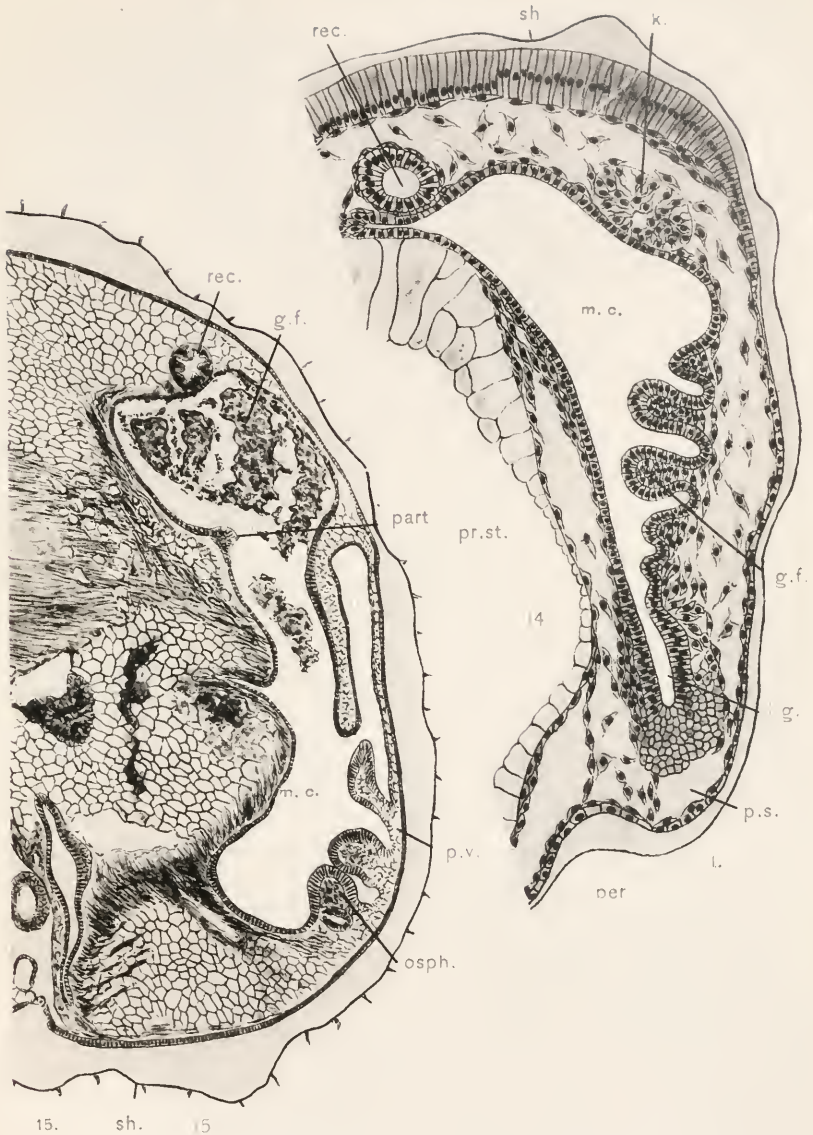

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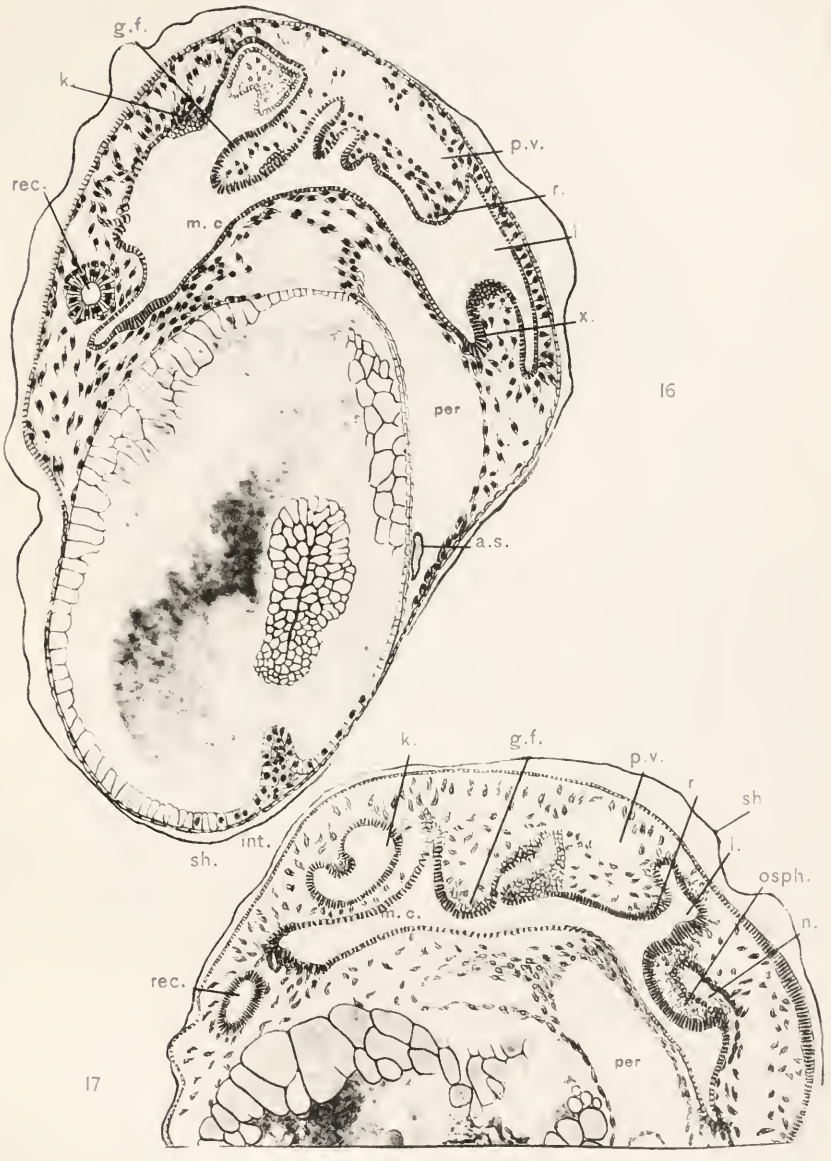

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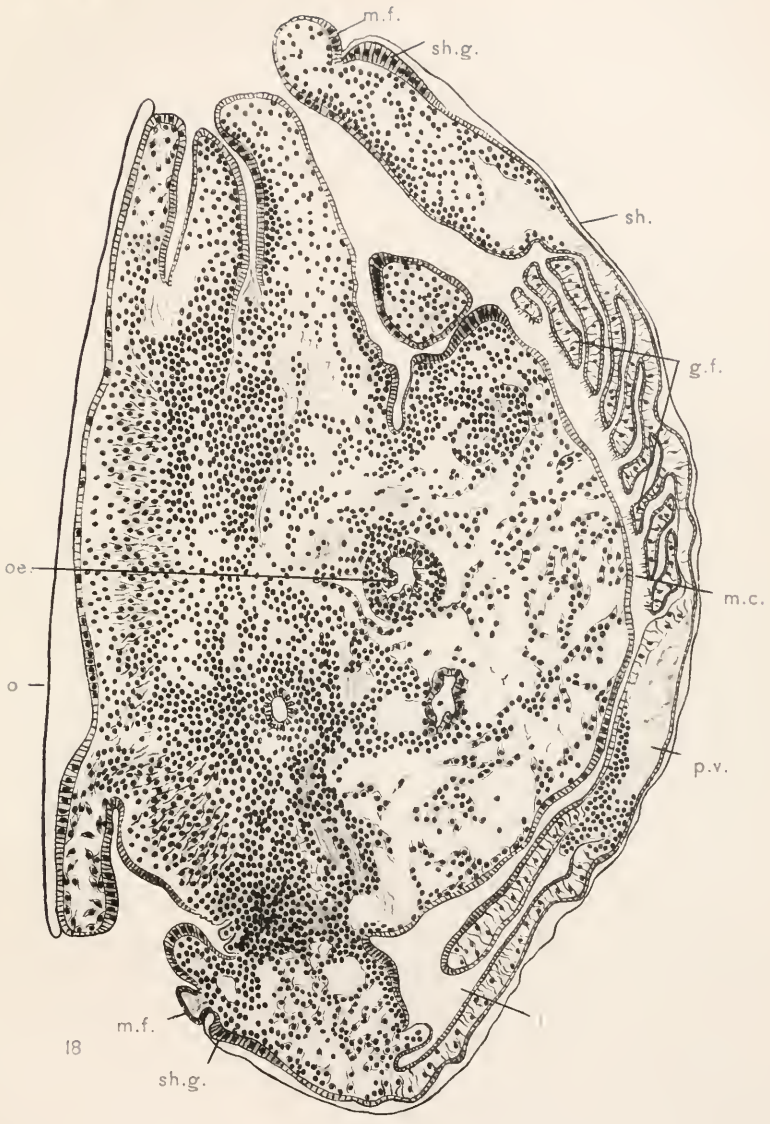

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VI. THE ANNUAL BREEDING-SWARM OF THE ATLANTIC PALOLO

By ALFRED GOLDSBOROUGH MAYER

Director of the Department of Marine Biology,
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1 plate

THE ANNUAL BREEDING-SWARM OF THE ATLANTIC PALOLO.

By Alfred Goldsborough Mayer.

The habits of the "Atlantic palolo" are quite similar to those of the palolo worm of Samoa and the Fiji Islands. The worms are, however, specifically different, the Atlantic palolo being *Eunice fucata* Ehlers, and the Pacific worm *E. viridis* Gray. Moreover, the annual breeding-swarm of the Pacific palolo comes upon or near the day of the last quarter of the moon in October and November, whereas the Atlantic palolo swarms within three days of the day of the last quarter of the moon between June 29 and July 28. The annual swarming of the Atlantic palolo has been observed only at Tortugas, Florida, and although the worm is abundant in the Bahamas and in other parts of the West Indies, it has not been observed to swarm elsewhere than at Tortugas. This may be due to lack of observation, but in 1903 I looked for the swarm in Nassau Harbor, Bahamas, on every morning between July 10 and 24, inclusive, but no swarm occurred, although in the same year, at Tortugas, Florida, Mr. George R. Billbury observed a dense swarm on July 17, this being the day of the last quarter of the moon.

The Atlantic palolo worm lives within crevices in dead, corroded coral, or in limestone beach-rock which has become honey-combed by the burrows of marine animals. It inhabits only rocks which lie below low-tide level, but will live within reefs which are at least 6 fathoms below the surface.

Large worms are apt to be found only in large coral rocks, and the worm is usually coiled backward upon itself, or twisted within its tortuous cavity. Mature worms are about 250 mm. long, and the sexual products are confined to the 150 posterior segments, which, when swollen by the contained eggs or sperm, are thicker than the slender middle part of the worm's body. A mature male worm is represented in natural size in figure 1.

Before sunrise on the morning of the day of the annual breeding-swarm, the worm crawls out backwards from its burrow until all of the sexual segments and a portion of the slender middle part of its body have been protruded. A vigorous helical, corkscrew-like twisting movement then comes over the sexual segments. Viewed from the head end of the worm this screw-like twisting is in the direction of the movement of the hands of a watch. This rolling motion is confined exclusively to the posterior sexual

end of the worm, and ceases abruptly at the point marked *a* in figure 1, which is the segment separating the narrow middle part of the worm from the swollen sexual part of its body.

The sexual segments are thus twisted off at the point *a*, and on being set free they swim vertically upward to the surface, where the posterior end of the worm continues to progress rapidly along, moving backward, as is shown in figure 2.

The male sexual ends are salmon red or dull pink, while the females are greenish-gray or drab, so that they can readily be distinguished at a glance.

If while the sexual end is swimming we cut it into pieces, each separate length continues to swim backward with its characteristic rolling movement. This shows that the stimulus which produces the twisting movement is not localized, but is developed throughout the sexual end of the worm.

The worms continue to swim in all directions over the surface, and show no tendency to congregate in masses, each worm pursuing its own course without regard to its fellows of either sex. I have seen them in such abundance over the surface above the coral reefs at Tortugas that hardly a square foot of the surface was free of a worm.

When the sun is about to rise, and the first faint rays of light fall over the ocean, the worms begin to contract violently, so that the sexual products are cast out through rents and tears in the dermo-muscular wall, and the torn and shriveled cuticula sinks down to die upon the bottom (figure 3). Light is not the sole, but only a contributory, cause of this muscular spasm of contraction, for it will take place even in swimming worms which have been removed to a dark-room, although in this case the "bursting" of the worm may be delayed for an hour or more after all of its fellows in nature have cast forth their genital products and disappeared. Moreover, some few of the worms of the swarm discharge their genital products before the rising of the sun. Any mechanical shock will bring about an instant bursting of the worm, the females being far more sensitive than the males. It is comparatively easy to stupefy the males by slowly adding alcohol to the water and killing them without their bursting; but this is more difficult of accomplishment in the case of the females.

After casting off its posterior sexual segments, the anterior part of the worm crawls back into its burrow and regenerates a new sexual end. Only the mature worms cast off their posterior ends; the immature worms take no part in the swarming reaction.

This swarming of the Atlantic palolo has been observed for nine years at Tortugas, Florida. The principal swarm commonly occurs within three days of the day of the last quarter of the June 29 to July 28 moon, although smaller swarms may occur upon one or two days preceding or succeeding the day of the densest swarm. When the last quarter of the moon falls late in July there may be a response to the *first* quarter as well as to the last

quarter. The following record gives the *date* of the principal swarm in heavy type, while the dates upon which only a few worms were observed swarming are shown in ordinary type.

Record of the swarming of the Atlantic palolo, 1898 to 1908.

Year.	Dates upon which Atlantic palolo swarmed.	Date of moon's last quarter.	Year.	Dates upon which Atlantic palolo swarmed.	Date of moon's last quarter.
1898	July **9**, 10	July 10	[1]1903	July **17**.....	July 17
1899	**1**, 2..	June 29	1905	9, 10, 21, **22**, 23, 24 ..	24
[1]1900	**19**	July 18	1906	**11, 12**, 13.....	13
1902	24, **25**, 28...	27	1907	**2, 3**.....	2
			1908	**10**, 19.....	19

[1] In 1900 and 1903, the dates of the principal swarm were observed by Mr. George R. Billbury, and I know nothing of the less conspicuous swarms of those years. In other years the swarms were observed by the author. No observations were made in 1901 and 1904.

The most interesting fact revealed by the above table is that in 1905, when the last quarter of the moon came late in July, about 200 worms were observed swarming on July 9, and a few fresh-laid eggs were found on the morning of July 10. The *first quarter* of the moon fell on July 9, 1905, and it is evident that the worm may respond to the *first* as well as to the *last* quarter of the moon. In 1908 the maximum swarm came on July 10, and the *first quarter* fell on July 6. A fair swarm also came on July 19, the day of the moon's last quarter; and these were the only swarms of the year. This is the more interesting in view of the observations of Osawa and Izuka that the Japanese palolo, *Ceratocephale osawai*, swarms in the Tokyo River at the time of the *new and the full* moon.

For the past five years I have been carrying out experiments designed to determine the nature of the stimulus to which the Atlantic palolo responds when it swarms. If at any time before the date of the normal swarm we crack open the rock within which a full-grown worm is living, the mechanical shock will often cause the worm to crawl partially or wholly backward out of its burrow. The worm is then very apt to break itself into lengths, and the sexual end often swims through the water with the corkscrew movement characteristic of the normal swarm. The worm may even constrict its muscles and cast out its genital products; but this is never done with such completeness as in the normal swarm, and even if the eggs be cast out within 24 hours of the date of normal swarm, they do not mature and no embryos develop.

Any appreciable impurity in the water, or the lack of sufficient circulation, will prevent the worms from swarming at the normal time. If we are to obtain reliable results, the water in which the worms are living must be free from an excess of carbon dioxide or other products of putrefaction, and this fact renders the experiments difficult of execution. In partially stagnant water the worms may live very well, but they will not take part in the

breeding-swarm, and if the lack of proper circulation is still greater the worms may still live, but their sexual products atrophy, and they do not react to the stimulus which calls forth the breeding-swarm in worms living under normal conditions.

Worms living in rocks which are placed in a floating live-car will still swarm, even though they be in a "tideless sea." Thus rocks containing full-grown worms were placed in a scow-shaped live-car 2 meters long, 1 meter wide, and 1 meter deep. This scow was allowed to float half full of water upon the sea, being buoyed up by barrels. Ample circulation was secured by holes in the sides and bottom, and a white canvas awning served to shield off the rays of the intense noon-day sun of the tropics. This scow was entirely open, so that moonlight fell freely upon it. In 1905 and in 1908, the rocks containing the worms were allowed to remain in the floating live-car for 30 days previous to the date of the normal swarm, but in 1907 they were placed in the floating scow only three days before the normal day of swarming; but in all three experiments *some* of the worms swarmed normally within three days of the day of the last quarter of the July moon. Altogether, 4 out of 11 mature worms swarmed normally in these tideless live-cars, but 7 of them did *not* cast off their posterior ends, but remained passive in their burrows.

In nature *all* of the mature worms swarm at the annual breeding-time, and this partial failure of the worms to swarm may indicate that the changing pressure due to rise and fall of tide over the reefs is a contributory but *not a necessary* component of the stimulus which calls forth the breeding-swarm. It is more probable, however, that confinement within the wood-inclosed space of the live-car and the lack of perfect circulation of water acted as a partial preventive of the swarming, and that the reaction is wholly independent of the rise and fall of the tide. In any event, it is evident that the worms *can* swarm normally in a tideless sea, and that rise and fall of tide is not a necessary or sole cause of the swarming.

On the other hand, the worms have never swarmed when moonlight was prevented from falling upon the rocks within which they lived. In order to test this, I had floating scows similar to those used in the previously described experiment, but they were provided with light-tight wooden covers, so that they could be closed at sunset every evening and opened soon after sunrise every morning, thus preventing the moonlight from falling upon the rocks. Altogether I had at least 22 mature worms in the rocks within these darkened live-cars, and in 1907 the moonlight was excluded for 5 days, in 1906 for 14 days, in 1905 for 30 days, and in 1908 for 2 days before the date of the swarm; but none of these worms showed any indication of swarming, and it appears that they could not respond, owing to the absence of light. It is probable, therefore, that the worms can not swarm unless moonlight falls upon the rocks. In nature the worms will swarm in

overcast or cloudy weather, so that even diffuse moonlight appears to be capable of calling forth the breeding-swarm.

In the Atlantic palolo the annual breeding-season is only of 1 to 6 days' duration, and the males outnumber the females in the ratio of about 3 to 2, whereas in *Nereis,* where the breeding-season is fully 100 days long, the males greatly outnumber the females. It is evident that a shortening of the breeding-season would cause a greater concentration of breeding individuals, and would therefore permit of a relative *decrease* in the number of *males* and a corresponding *increase* in the number of *females;* for whenever a female swarms it is important for the preservation of the species that there should be a male near her to fertilize her eggs. If the breeding-season be of long duration, the males must greatly outnumber the females to secure this fortuitous proximity, but if all of the females swarm within a few days very much fewer males will suffice to accomplish this purpose.

We have advanced beyond the period in the history of biology when one had but to discover an advantage to determine a cause; but that some such cause may have contributed to shorten the breeding-season in such animals as the Atlantic, Pacific, and Japanese palolo worms is shown by the fact that more eggs are fertilized when males are near the female than when they are far away. For example, I took a female Atlantic palolo from the midst of the swarm and placed her in sea-water 200 meters away from the nearest swarming males. Of the eggs which were laid by this female in the water removed from the place of the swarm only an occasional one developed, whereas practically every egg developed in the sea-water where males were near.

The polar bodies are given forth as soon as the eggs are cast out from the female, and fertilization occurs in the water; but the egg does not mature if it be cast out at any time other than that of the normal breeding-swarm. When about 10 to 15 hours old the larvæ are nearly all negatively phototactic either in diffuse light or in sunlight. When about 28 hours old, however, they mainly become positively phototactic and remain thus, even after the eighth day, when they will have ceased to swim through the water and have sunken to the bottom. Within 24 hours after sinking to the bottom, however, they become indifferent to light in so far as their movement is concerned.

The segmentation closely resembles that of *Nereis* and the larva is telotrochal.

Further accounts of the Atlantic palolo will be found in Bulletin of the Museum of Comparative Zoology at Harvard College, 1900, vol. 36, pp. 1–14, plates 1–3, and in the Science Bulletin of the Museum of the Brooklyn Institute of Arts and Sciences, 1902, vol. 1, No. 3, pp. 93–103, 1 plate.

The Japanese palolo is treated of in detail by A. Izuka, 1903, Journal

of College of Science, University of Tokyo, vol. 17, article 2, 37 pp., 2 plates.

The Pacific palolo has been treated of by numerous writers, the most important modern accounts being those of B. Friedlander, 1898, Biolog. Centralblatt, Bd. 18, pp. 337–357, 2 figs.; of Collin, 1899, in Krämer's Bau der Korallenriffe, pp. 164–174, and of W. McM. Woodworth, 1903, American Naturalist, vol. 37, pp. 875–881, 1 fig. and 1907, Bulletin Museum Comparative Zoology at Harvard College, vol. 51, pp. 1–22, 3 plates.

Lysidice oele, the "wawo" of Amboina, Malay Archipelago swarms on the second and third nights after the full moon of March and April. It is described by R. Horst, 1905 (Over Wawo (*Lysidice oele* n. sp.) Rumphius Gedenkboek, Kolon. Mus. Haarlem, pp. 105–108).

EXPLANATION OF THE PLATE.

[The drawings are from life by the author.]

FIG. 1. Mature male with posterior sexual segments still attached. They are destined to break away at the point *a*.

FIG. 2. Female sexual segments swimming through the water, showing rolling, twisting movement of worm as it progresses backward.

FIG. 3. Torn and shrunken sexual segments sinking to bottom to die after having discharged the sexual products.

FIG. 4. Enlarged photograph of an immature male worm, 1.5 times the natural size. This worm was still alive when photographed.

THE "ATLANTIC PALOLO," EUNICE FUCATA.

VII. RHYTHMICAL PULSATION IN SCYPHOMEDUSÆ

By ALFRED GOLDSBOROUGH MAYER

Director of the Department of Marine Biology,
Carnegie Institution of Washington

13 text figures

RHYTHMICAL PULSATION IN SCYPHOMEDUSÆ. — II.

By Alfred Goldsborough Mayer.

The following paper presents the results of a continuation of certain studies, the first report of which appeared in publication No. 47 of the Carnegie Institution of Washington, 1906. The present paper aims to correct certain errors in the previous report, and to announce some new results.

CONCLUSIONS.

(1) Sea-water is a balanced fluid neither inhibiting nor stimulating pulsation in *Cassiopea xamachana*. This is due to the fact that the sodium chloride of the sea-water is a powerful nervous and muscular stimulant; but the magnesium, calcium, and potassium are inhibitors, and exactly counterbalance the effect of the sodium chloride thus producing a neutral fluid.

The sea-water itself, being indifferent, permits any weak, constantly present, internal stimulus to produce the nervous responses which cause rhythmical pulsation of the muscles.

(2) The stimulus which causes pulsation is due to the constant formation of sodium oxalate in the terminal entodermal cells of the marginal sense-organs. This sodium oxalate precipitates *calcium*, as *calcium oxalate*, thus setting free sodium chloride and sulphate which act as nervous stimulants. Pulsation is thus caused by the constant maintenance at the nervous centers in the sense-organs of a slight excess of sodium over and above that found in the surrounding sea-water.

(3) If we cut a strip of heart tissue, or of subumbrella tissue of a medusa in such manner as to give it the shape of a ring or of any closed circuit, and then start a contraction-wave moving in any *one* definite direction through this circuit, the wave will continue to travel at a uniform rate around the circuit. This wave will maintain itself indefinitely, provided the circuit be long enough to permit each and every point in the path of the wave to remain at rest for a certain period of time before the return of the wave through the circuit. No one localized point on the circuit acts as a dominant center for maintaining the wave, but all points on the path of the wave take an equal share in passing the wave onward to points beyond them. In nature the structure of pulsating organs, and their manner of stimulation,

are designed especially to prevent such a circuit-wave from taking possession of the organ.

(4) In *Cassiopea* the pulsation-stimulus is conducted by the diffuse nervous network of the subumbrella, and is independent of the muscles which may or may not respond to its presence by contraction. In other words, conductivity of the pulsating tissue is independent of its contractibility.

(5) Strong primary nervous and muscular excitement followed by exhaustion and sustained muscular *tetanus* is produced in *Lepas* or in *Cassiopea* by a solution containing the amounts and proportions of NaCl + KCl + $CaCl_2$ found in sea-water. This tetanus may, however, be cured and normal pulsation restored by adding the amount and proportion of *magnesium* found in sea-water. Magnesium relaxes the muscles, and prevents tetanus. It has but little direct effect upon the nervous elements which alone transmit the pulsation-stimulus.

EXPERIMENTS.

Romanes and Eimer found that if we remove the marginal sense-organs of a scyphomedusa, the disk becomes paralyzed and does not pulsate spontaneously in sea-water. In 1906 the writer found, however, that any strip of subumbrella tissue of a scyphomedusa cut in the shape of a ring, or closed circuit, will pulsate rhythmically in sea-water, provided a contraction-wave be once started in the circuit.

Any stimulus, such as that given by contact with any soluble salt of potassium, sodium, lithium, barium, platinum, hydrogen (acid), or an electrical or mechanical shock, will produce a contraction-wave in the disk of the scyphomedusa *Cassiopea* and will serve to start rhythmical pulsation in a ring-shaped strip of paralyzed subumbrella tissue.

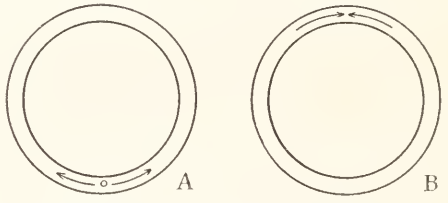


FIG. 1.—Annulling of pulsation which occurs when two waves of equal magnitude, coming in opposite directions, meet each other.

The contraction-wave arises from any point upon the ring of paralyzed subumbrella tissue which we may choose to stimulate. Two waves of *equal magnitude* may start from the stimulated point and travel in opposite directions from their common point of origin, as is shown in fig. 1, A. Under these conditions each wave travels around the ring until it meets the other wave coming in the opposite direction, as is seen in fig. 1, B. All movement ceases when these waves meet, for tissue which has been in contraction can not again contract until after an appreciable interval of rest; hence neither of the waves can stimulate the tissue which has only upon the instant previous been set into contraction by the other wave. Under the conditions described above, therefore, the whole ring gives a single contraction, and then ceases to pulsate.

More commonly, however, the two waves which arise from the stimulated point are *unequal,* one being strong and the other weak. This is doubtless caused by an inequality in the transmitting power of the nervous network on either side of the starting-point, and the stronger wave goes in the direction of the least resistance, as is shown in fig. 2, A. Under these conditions, when the strong contraction-wave meets the weak one (fig. 2, B), it is still capable of stimulating the tissue over which the weak wave has traveled, but the weak wave can not stimulate the tissue which has, only the instant before, been exhausted in responding to the stimulus of the strong

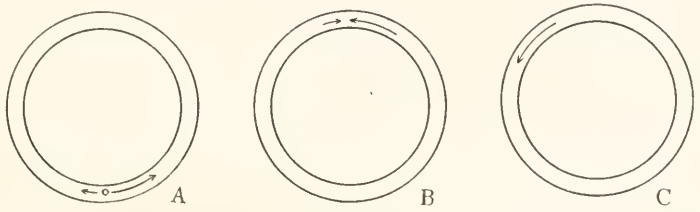


FIG. 2.—Showing that when a strong wave meets a weak one it suppresses the weak wave, and remains the only wave in the circuit.

wave. Thus the weak wave is annulled, and only one (the strong) wave remains to travel continuously around the ring in one direction, as is shown in fig. 2, C.

This single wave going constantly in one direction around the circuit may maintain itself for days traveling at a uniform rate. The circuit must, however, be long enough to allow each point to rest for an appreciable interval of time before the return of the wave. The wave is actually "trapped" in the circuit and must constantly drive onward through the tissue.

The ventricle of the extirpated heart of the loggerhead turtle (*Thalassochelys caretta*), if cut into ringed-shaped strips may also be caused to maintain itself in sustained pulsation in the manner described above for the scyphomedusa *Cassiopea.* The initial wave in the ring-shaped strip of turtle's heart may be started by an electrical or a mechanical stimulus, and will continue to travel at a uniform rate in a single direction around the ring until the dying of the tissue causes it to cease.

It is interesting also that this wave through the strip of turtle's heart passes mainly, if not wholly, through the dense, peripheral, muscular layer of the heart, the inner cavernated tissue being practically inert in so far as the pulsation-stimulus is concerned. The heart may be likened to a sponge inclosed within a periodically contracting bag. We meet with a parallel condition in the subumbrella tissue of Scyphomedusæ, where the peripheral nervous and muscular layers are all that are concerned in the pulsation; the thick gelatinous substance of the umbrella being a non-conductor and inert.

The point which was stimulated and from which the contraction-wave first arises is of no more importance in maintaining the rhythmical movement than is any other point on the ring. My conclusions of 1906 are erroneous in this respect, for the wave is *not* reinforced and sent forth anew every time it returns to its place of origin, but is maintained by each and

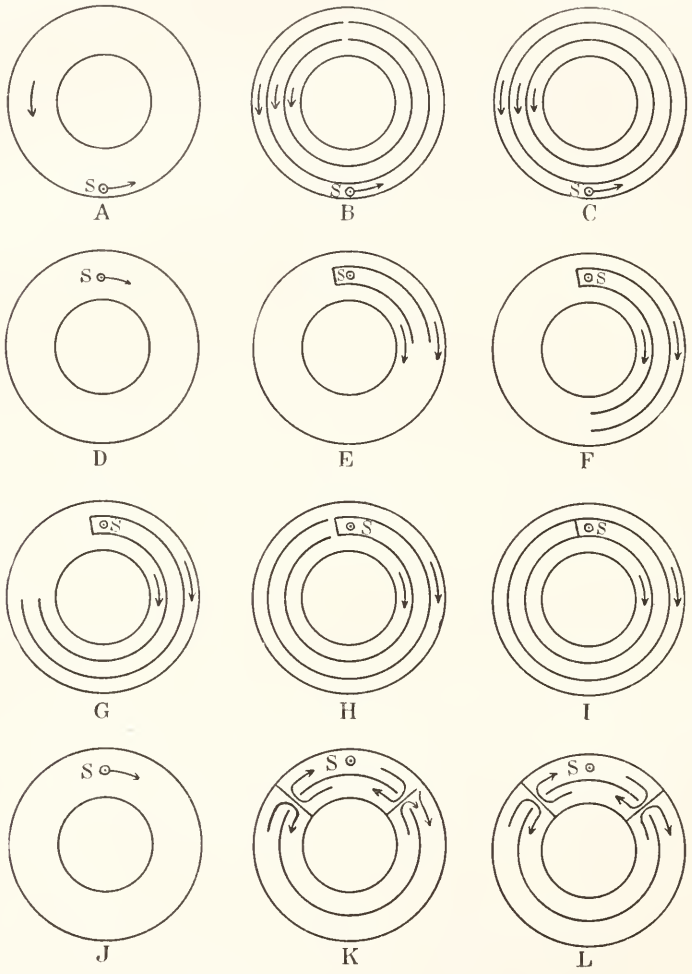


FIG. 3.—Showing non-importance of any definitely localized center in maintaining rhythmical pulsation.

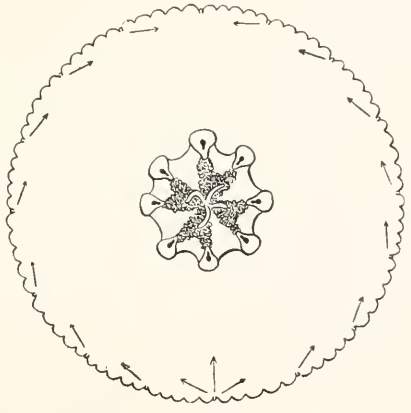
every part of the ring in succession as it passes. We may prove this by causing the wave to originate at some definite point and then cutting away the tissue around this point; yet the wave continues unhindered through the circuit. Fig. 3, A–L will serve to illustrate this non-importance of any definitely localized center in maintaining the circuit-waves. For example,

in fig. 3, A–C, we see how a single broad ring may be finally divided by two annular cuts into three separate rings, all of which remain in sustained pulsation. In this case *s* marks the stimulated point whence the contraction-wave started, yet the two inner circles which are finally isolated from the point *s* continue to transmit and maintain the wave.

Similarly in fig. 3, D–I, we may completely isolate the stimulated point and prevent its sending out any stimuli, yet the narrow inner and outer annuli, made from the original broad ring, still remain in pulsation.

Fig. 3, J–L illustrate the same point by showing that by a series of cuts we may obtain two independent pulsating circuits in the place of the original simple ring-circuit. In this case it is evident that the original center of stimulation can be in but one of these circuits, yet both can remain in pulsation.

It is remarkable that these isolated circuit-waves, moving constantly in one direction through a circuit, are *not met with* in nature. Each pulsation of the heart, or of the medusa, is a thing separate and distinct from the contraction which preceded or from that which is to follow it. Indeed, the



A

Fig. 4.—Showing that under normal conditions interference of contraction-waves coming in opposite directions prevents a rotary wave from being entrapped in the circuit.

heart, or pulsating medusa, contains within itself the means to *prevent* any single pulsation-wave from coursing constantly in one direction through the tissue. In the scyphomedusa, for example, the pulsation-stimuli originate in the marginal sense-organs, and the fastest-working sense-organ controls the rate of the pulsations. For example, the course of events in the case of each separate contraction is shown for *Cassiopea* in figure 4, where A is the sense-organ which has originated a contraction-wave. The wave of contraction spreads out on both sides of A and the wave of each side travels half the way around the subumbrella, where it meets with its fellow coming

in the opposite direction. When the two waves meet 180° away from their common point of origin they interfere with and annul each other, and a period of quiescence ensues until another contraction-stimulus is sent forth from a marginal sense-organ.

Under normal conditions the two side waves are of practically equal magnitude, and thus one can not overpower the other and travel constantly around the circuit in one direction. Such an accident is prevented by the interference and consequent suppression of the two waves, one by the other; but the protection is not perfect, for on several occasions I have started such a wave through a severe electrical or mechanical shock, and then the sense-organs, being exhausted by the wave which set them into play one after another, were powerless to control the pulsation, and the *single* wave rushed constantly around the subumbrella annulus, causing each and every part of the medusa to pulsate successively as it passed. The rate of pulsation under these unusual conditions was fully twice that of the isolated, *recurrent* contractions initiated by the sense-organs.

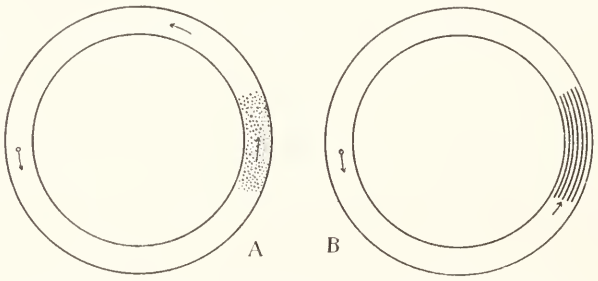


FIG. 5.—*A*, showing that a pulsation-wave may pass across newly regenerated tissue (dotted area) which contains no muscles. *B*, showing that a pulsation-wave can not pass through muscles (ruled area) from which the nervous network has been peeled away.

Such a circuit wave can not take possession of the vertebrate heart, for here each wave of contraction normally originates in the region of the sinus, then spreads over the auricles, and finally over the ventricle, whence it can not immediately return over its path. The pulsations of the heart are *recurrent*, and are rhythmical only in the sense that the separate pulsations follow one another, at sensibly equal intervals of time.

In the Scyphomedusæ the pulsation-stimulus is conducted by the diffuse nervous system of the subumbrella, and this stimulus causes the muscles to contract. The stimulus will pass through tissue which contains no muscles and can not contract, or through tissue wherein the muscles have been rendered incapable of contracting through the effects of distilled water, magnesium, curare, carbon dioxide, alcohol, etc.

On the other hand, the pulsation-stimulus can not pass through or be conducted by a muscle from which the nervous connections have been

peeled away. Thus in figure 5, *B*, the area ruled with annular lines represents a part of the ring from which the epithelial layer with its nervous network has been peeled away, leaving the muscles intact. Under these conditions the contraction is at once destroyed as soon as it reaches the border of the raw muscles and all movement ceases.

If, on the other hand, we cut away both muscles and epithelium, and allow the cut area to regenerate, the nervous network and epithelium will regenerate *before* the muscles reappear. Thus in figure 5, *A*, the dotted area represents a recently regenerated area, which contains no muscular elements, but over which the epithelium and nervous network has regenerated. The contraction-stimulus passes readily through this region, although it can produce no contractions in the dotted area where there are no muscles to contract; all other parts of the circuit wherein the muscles are found contract as soon as the wave reaches them. Indeed, the pulsation-stimulus is independent of the muscles, and passes through the nervous network whether the muscles respond to it by contraction or remain inert. This is illustrated by figure 6, where the dotted sector AD represents newly regenerated epithelium, which contains the diffuse nervous network, but has no muscular elements and therefore can not contract. The undotted part of the sector ABC is normal tissue containing muscular elements, but it is immersed beneath a 5/8m $MgSO_4$ solution, which renders the muscles incapable of contraction, although the pulsation-stimulus can still pass through the sector. The sector CD and the small undotted area around s is normal subumbrella tissue. If, now, a wave be started in this circuit, it will pass constantly around the ring, and wherever it passes through the sector CD or over the area s these regions contract, for they are normal tissue, but no contractions or other visible signs of the presence of the stimulus are exhibited by the sectors which lack muscular elements, or in which the muscles are rendered incapable of contraction through the effects of magnesium.

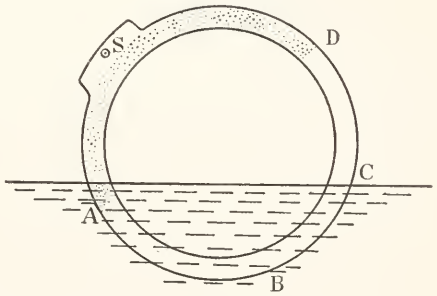


FIG. 6.—Showing how a pulsation-wave may pass from normal tissue (plain area), through tissue deprived of muscles (dotted area), and over muscles which have been rendered incapable of contracting through the effects of magnesium (immersed area).

But to return to our subject, if we cut a ring from the medusa's disk such as is shown in figure 7 and leave a long narrow strip (*AB*) attached to it, and then start a contraction-wave traveling around the ring, every time the wave passes the point *A* a side-tracked portion of the wave will pass along the strip from *A* to *B*. When each side-tracked wave comes to

the end *B* it dies out, for it can not return over the recently stimulated tissue along which it has just passed. Thus we see that the index strip *AB* simply serves to catch a portion of each wave which passes its base.

Now suppose we place the ring in a pure solution of magnesium chloride, and allow the index strip *AB* to remain in natural sea-water. Then the contraction-wave gradually dies out in the pulsating ring, for the magnesium paralyzes the muscles; and at the end of about a quarter of an hour all movement will have ceased in the ring, but for from 12 to 15 minutes after this we find that the strip *AB* still continues to transmit contractions at regular intervals of time. We see, then, that whenever the something which produced the contraction in the ring comes around to the point *A* it is still capable of setting up a contraction in the strip *AB*, although it can not now cause the muscles of the ring itself to pulsate.

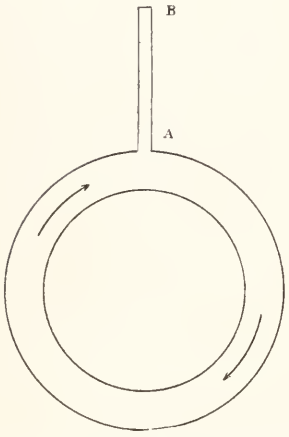


FIG. 7.—Showing that the pulsation-stimulus is nervous, not muscular, in nature.

The explanation is that the stimulus which produces pulsation is nervous in nature, and travels through the nervous tissue quite independent of the presence or absence of the muscles. When, therefore, the magnesium paralyzes the muscles the nervous stimulus still travels around the ring even though the muscles can not now respond to it by contraction.

The pulsation-stimulus is nervous, not epithelial, for in the exumbrella we find the epithelial but *no nervous* elements, yet the exumbrella tissue can neither pulsate nor conduct the pulsation-stimulus. The transmission of the stimulus which produces muscular contraction is therefore dependent upon the presence of nervous elements in the tissue.

Bethe, 1903,[1] in his important research upon the pulsation of *Rhizostoma* and *Cotylorhiza*, concludes that the pulsation-stimulus is nervous; for it readily passes over parts of the subumbrella where there are no muscles. Moreover he shows that the radial muscle-strands contract *before* the circular muscles, although the latter lie closer to the sense-organs. This is due to the longer latent-period of the circular muscles, and it is evident that this latent-period is a property of the *muscles, not* of the nerves. On the other hand, Bethe shows that Marey's refractory period during systole is a property of the *nerves*, not of the muscles.

T. Brailsford Robertson, 1905,[2] demonstrated that *contraction* may be abolished, and yet *conduction* of a peristaltic wave will take place at the

[1] Allgemeine Anatomie und Physiologie des Nervensystems.
[2] Trans. Roy. Soc. South Australia, vol. 29, p. 34.

same rate as before. Robertson carried out his experiments upon the intestine of a fly, and showed that if the intestine be placed in a decinormal solution of NaCl, peristaltic waves of contraction proceed down its entire length. If, now, any point near the middle of the length of the intestine be wetted with $CaCl_2$ or $BaCl_2$, the wave of contraction is observed to completely disappear on entering this region, but on reaching the other end of the affected area the wave emerges with its initial rate and vigor.

In *Cassiopea* the *conductivity* of the subumbrella tissue is *independent* of its *contractibility*. This is shown in figure 8, where a series of radial cuts extending part way in from the margin, or out from the center, oblige the pulsation-stimulus to travel inward and outward around the subumbrella. Then on stimulating the subumbrella by touching it with a crystal of KCl in each sector successively, the major wave is fully as likely to go inward toward the center of the subumbrella, where the tissue is relatively incapable of pulsating, as it is to go outward toward the margin, where the muscles are well-developed and the tissue contracts actively. Figure 8 represents the conditions actually observed in a disk with 16 sectors. It will be seen that the major initial wave went inward in 7 of the tests, outward in 7 other experiments, and in both directions in the case of 2 trials.

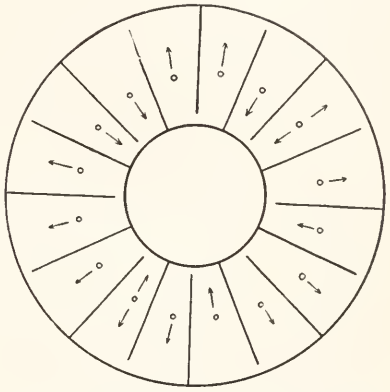


FIG. 8.—Showing the observed directions of pulsation-waves in a disk stimulated at various points in succession.

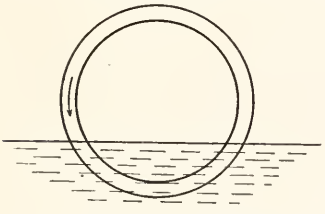


FIG. 9.—A pulsating ring of subumbrella tissue partially immersed in order to determine the effects of a dissolved salt upon the pulsation.

In so far as is known, all recurrently pulsating animal tissues contain or are surrounded by the elements Na, Ca, K, and Mg. Marine animals at Tortugas, Florida, live in a solution which is well represented by Van't Hoff's solution $\frac{5}{8}$m (100 $NaCl + 7.8$ $MgCl_2 + 3.8$ $MgSO_4 + 2.2$ $KCl + 3$ $CaCl_2$). On the other hand, the pulsating organs of terrestrial or fresh-water animals exist in the presence of the same salts, but in amounts and proportions other than those of the above formula.

We may readily test the influence of any solution upon pulsation in *Cassiopea* if we merely cut out a ring of subumbrella tissue, deprived of marginal sense-organs, set it into sustained rhythm, and then partially immerse the ring beneath the solution whose effects we wish to test (see figure 9). For example if the pulsating ring be partially immersed beneath

a pure 5/8mm $MgSO_4$ solution, the immersed portion of the ring gradually loses its *contractibility,* but it still *conducts* the pulsation-stimulus. After ten minutes' immersion the immersed portion of the ring can not be observed to contract, even if it be viewed under a microscope, but the *unimmersed* part still responds by normally vigorous contractions at each passage of the pulsation-stimulus; and it is evident that the pulsation-stimulus is transmitted through the non-contracting immersed part of the ring. Indeed, the pulsation-stimulus will usually continue to pass through the inert, immersed part of the ring for fully half an hour after all response to its presence has ceased. This experiment gives the same result if the ring be partially immersed beneath $MgSO_4$, $MgCl_2$, or $MgBr_2$. It is evident that magnesium chiefly affects the *muscles,* rendering them incapable of contracting and producing a state of inert relaxation. Magnesium has, however, less effect upon the pulsation-stimulus itself, which is *nervous* in nature. That it has *some* effect upon the nervous elements is, however, evident, for the immersed part of the ring, *after* losing its ability to contract, finally ceases even to *conduct* the pulsation-stimulus. Moreover, the rate at which the pulsation-wave travels around the ring always declines. For example, one ring partially immersed beneath 5/8m $MgSO_4$ slowly declined in rate from 67 to 57 per minute after 35 minutes immersion. In another case the rate declined from 93 to 78 per minute after 28 minutes' immersion, and in another from 88 to 40 in 31 minutes, etc.

Weaker solutions of magnesium, made by adding $MgSO_4$ or $MgCl_2$ to natural sea-water, may not cause any decline in rate, although they will destroy the contractibility of the muscles in the immersed part of the ring. The effect of these weaker solutions, such as 66.6 sea-water + 33.3 of 5/8m $MgSO_4$ is about wholly confined to rendering the *muscle* inert, and not to hindering the pulsation-stimulus, which is nervous in nature.

It is remarkable that the normal medusa, pulsating by means of stimuli set forth from its marginal sense-organs, can not pulsate for more than 20 seconds in a pure 5/8m $MgSO_4$ solution, whereas a ring-shaped strip of subumbrella tissue *without* marginal sense-organs can pulsate for at least ten minutes in the above solution. The marginal sense-organs can not send forth the pulsation-stimuli unless they be surrounded by calcium in solution, and one office of this calcium is to offset the anesthetic effects of the magnesium. Indeed, if magnesium be absent, calcium may *also* be absent, and the sense-organs will continue to send forth their pulsation-stimuli for a long time; but if magnesium be present, calcium must also be present if pulsation is to endure long. Calcium produces tetanus, as has been shown by Loeb; while magnesium produces muscular relaxation, as has been shown by Meltzer and Auer and by myself. In this sense calcium and magnesium are antagonistic in their effects and offset one the other. Both are necessary for maintaining that delicately balanced state which

permits of *recurrent* ("rhythmical") pulsation, for both magnesium and calcium are inhibitors of pulsation, and reduce the stimulating effect which the NaCl tends to exert.

We see that, in the absence of calcium, magnesium produces a profound relaxation of the muscles, rendering them incapable of pulsating. An exactly opposite effect is produced by the remaining elements Na + Ca + K. If a *Cassiopea* medusa be placed in a solution lacking magnesium, but containing sodium, potassium, and calcium chlorides, its pulsation is at first greatly increased both in amplitude and rate; but finally the rate and amplitude decline and become very slow and slight, while at the same time sustained tetanus sets in. This tetanus becomes so severe that after being 24 hours in the solution lacking magnesium the circular muscle fibers of the subumbrella are torn across, as is shown in figure 10, A; and soon thereafter the whole medusa-bell is drawn up into a crumpled mass, as is shown in figure 10, B. Under these conditions the medusa may give not more than 3 weak pulsations per minute, whereas its normal rate may have been been 80. The pulsations soon become so weak that they do not involve the entire margin, but spread only a little way on both sides of those organs which still initiate them. Even under these conditions, however, when death is imminent, the tetanus may be completely cured and normal pulsation restored by simply introducing any magnesium salt in the amount found in sea-water.

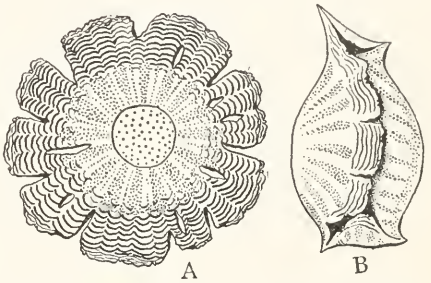


FIG. 10.—*A*, and *B*, successive stages of tetanus produced by a partial sea-water composed of NaCl + KCL + $CaCl_2$ but *lacking* magnesium. Upon adding magnesium, this tetanus was completely cured, and normal pulsation restored.

Tetanus and a final lowering of the rate of pulsation is also produced in the rhythmical movement of the branchial arms of *Lepas* by NaCl + KCl + $CaCl_2$; and in this case also the tetanus is cured and normal pulsation restored by magnesium. The tetanus is caused mainly by calcium, for it takes place in *Cassiopea* placed in sea-water + 3 per cent 5/8m $CaCl_2$, or in any solution *lacking* magnesium but containing calcium. Nevertheless, the tetanus is not due *solely* to calcium, for it is far more severe in medusæ subjected to NaCl + $CaCl_2$ + KCl than it is if we leave out the potassium, and place the medusa in NaCl + $CaCl_2$. However, calcium is the element *chiefly* responsible for the production of the tetanus, for no tetanus occurs in medusæ subjected to a solution of NaCl + KCl. The interesting fact remains true, however, that the most *severe* and constantly sustained tetanus is produced by a solution containing all three elements—sodium, calcium, and potassium.

A solution containing the amounts and proportions of NaCl + KCl but *lacking* magnesium and calcium is very toxic and medusæ can not live for more than two hours in it; yet if we merely add calcium this solution will sustain life for more than 24 hours.

Most important studies upon the beneficial effects of magnesium in overcoming the tetanus of lockjaw have been carried out by J. A. Blake, 1906, and by Meltzer and Auer, 1906.[1] These authors find that intraspinal injections of $MgSO_4$ in doses which do not affect the respiratory center, or other vital functions, are capable of abolishing, temporarily but for the time completely, all clonic convulsions and tonic contractions in cases of human tetanus, and experimental tetanus produced by tetanus toxin in monkeys. The palliative effects of the injections may last 24 hours or longer.

Dr. J. A. Blake, 1906,[2] gave five successive intraspinal injections of 4.5 to 8 c.c. of 25 to 12.5 per cent $MgSO_4$ to a boy suffering from tetanus. The injection was renewed whenever the relaxing effects of the previous dose disappeared, and a complete cure was effected in about 14 days.

Flexner and Noguchi, 1906,[3] find that the fatal constituent in tetanus toxin is the convulsive agent tetanospasmin, which has an especial affinity for nervous tissue; but that certain fluorescent aniline dyes, especially eosin, have the power to destroy the poisonous effects of this substance.

It would seem that the beneficial effects of magnesium in the case of human tetanus is due to its reducing the excitability of the nerves and muscles, and not to any direct effect in neutralizing the poison of the toxin. It would be important to know whether the tetanospasmin which produces the convulsive tetanus has the power to *precipitate* magnesium or to produce a relative increase of the soluble calcium, for my experiments indicate that it is the rôle of magnesium to offset and neutralize the effects of calcium. Loeb, 1906,[4] states that the margin of the medusa *Polyorchis* has a tendency to remain permanently contracted in a mixture of NaCl + KCl + $CaCl_2$, and this effect is due to the calcium. Loeb found, however, that upon the addition of $MgCl_2$ this tendency to a contracted condition lessened, and the medusa showed a more normal type of contraction.

I find that 5/8m (100 NaCl + $3CaCl_2$ + 2.2 KCl) is a powerful stimulant for *Cassiopea,* producing, at first, a very rapid, strong pulsation, and rendering the contractile tissue highly sensitive to all stimuli. The final effect of this solution is, however, to exhaust the tissue and produce sustained tetanus. This tetanus and exhaustion takes place even when the medusa is prevented from pulsating by removing its marginal sense-organs before it was placed in the NaCl + $CaCl_2$ + KCl. It appears that NaCl +

[1] See Meltzer, S. J., and Auer, John, 1906; Journal of Experimental Medicine, New York, vol. 8, p. 692–706. Also 1907; Reprints of Studies, Rockefeller Inst. Medical Research, New York, vol. 6, p. 692.

[2] Surgery, Gynecology and Obstetrics, vol. 5, p. 541.

[3] Journal of Experimental Medicine, vol. 8, p. 1.

[4] Dynamics of Living Matter, p. 91.

$CaCl_2 + KCl$ is a stimulant for nerves and muscles, although not so powerful as a *pure* NaCl solution, and that magnesium is a relaxing, or anesthetic, agent, which renders the muscles incapable of contraction. Calcium-tetanus is muscular not nervous in nature.

We see that magnesium is as essential to recurrent ("rhythmical") pulsation as is sodium, potassium, or calcium, for it holds the tissue in check, and guards it against the too powerful stimulus and tetanus produced by $NaCl + KCl + CaCl_2$. It is thus a counterbalancing reagent.

The importance of magnesium in vital phenomena is at present underestimated, despite the researches of Tullberg, Meltzer and Auer, and others. For example, Loeb, 1906,[1] lays special stress upon the importance of Na, K, and Ca in maintaining pulsation, but regards magnesium as of minor importance.

It is true that a Ringer's solution, consisting of chlorides of sodium, potassium, and calcium, will maintain pulsation longer than will any combination of *any two* of these elements with magnesium, but if pulsation is to endure indefinitely the pulsating organ must contain or be surrounded by sodium, potassium, calcium, *and magnesium*. In this connection it is interesting to see that Burnett, 1907,[2] finds that strips of the ventricle of the turtle's heart will live as long in isotonic, diluted sea-water as in Ringer's solution; and indeed my own experiments upon the heart of the embryonic loggerhead turtle confirm this observation.

A pure NaCl solution produces *the most* rapid initial pulsation possible for the tissues to sustain, but in less than one hour the medusa is thoroughly exhausted, and all movement ceases. In $NaCl + KCl$, or in $NaCl + CaCl_2$, pulsation is *slower* but *endures longer*, and in $NaCl + KCl + CaCl_2 + MgSO_4 + MgCl_2$ in the amounts and proportions found in sea-water, pulsation is still slower, and is normal in all respects. It is evident that the NaCl of the sea-water is a powerful stimulant; and that the Mg, Ca, and K are inhibitors which restrain its affects.

We can prove that the NaCl of the sea-water is a powerful nervous and muscular stimulant. If, as in figure 11, we cut a strip of subumbrella tissue leaving a sense-organ (*s*) at the one end only, then lay this strip across three shallow glass dishes, *A*, *B*, and *C*; and place natural sea-water in the two end dishes, *A* and *C*, and a solution of 5/8m NaCl in the middle dish, *B*, the sense-organ in the dish *A* gives forth pulsation stimuli in a normal manner, but each pulsation-wave is greatly increased as it passes through the NaCl in *B*, and it still maintains some of this increased amplitude in the dish *C*, although here it passes through normal sea-water.

If, on the other hand, we placed pure solutions of Mg, Ca, or K, or any appreciable excess of these salts in sea-water, in the middle dish *B* the

[1] Dynamics of Living Matter, p. 95.
[2] Biological Bulletin, vol. 13, No. 4, p. 203–210.

pulsation-wave is decreased both in rate and amplitude as it passes through *B*, but this is effected by each of these elements in its own peculiar manner. For example, magnesium soon renders the muscles incapable of contraction, but only later does it exert an inhibiting effect upon the nerves. Calcium, on the contrary, chiefly affects the nerves, and stops pulsation very suddenly. At first the wave extends throughout the length of the strip immersed in the sea-water containing an excess of calcium, but soon it can penetrate

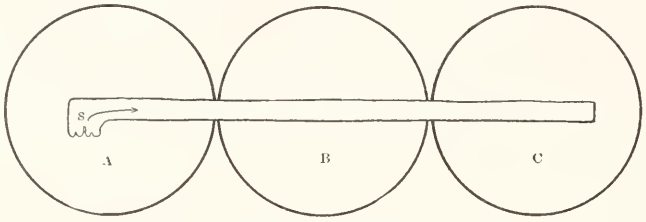


FIG. 11.—Test for nervous or muscular nature of effects of various salts of sea-water.

only part way through the calcium-affected portion of the strip, and the distance it can travel steadily decreases as time goes on until it is checked almost immediately after entering *B* from *A*. This gradual dying-out of the pulsation-stimulus is well seen in a strip immersed in 100 volumes of sea-water to which 40 volumes of a $\frac{5}{8}$m solution of $CaCl_2$ has been added. In this solution the tissue ceases to transmit the pulsation long before tetanus is produced, but a stronger solution of calcium quickly produces tetanus. This calcium-tetanus is purely muscular, and is not transmitted to portions of the strip other than those immersed in the calcium solution itself.

A similar experiment with an excess of *potassium* shows that the first effect of this salt is to stimulate pulsation, but its final effect is both inhibitory and toxic. Its toxic influence is prevented by calcium, and its effect in sea-water is simply to aid in the restraining of the stimulus due to sodium.

We can prove that of the depressants to pulsation in sea-water, the most powerful inhibitor is magnesium, while calcium is moderately and potassium only weakly depressant. This is shown (fig. 12) when we take a long strip of subumbrella tissue having a single sense-organ (*s*) in the middle of its length, and stretch the strip across five shallow glass dishes (*A–E*). If, then, we place natural sea-water in the middle dish *C*, and also in the two end-dishes *A* and *E*, we will be in a position to test the relative inhibiting powers of any two solutions placed in dishes *B* and *D*, respectively. In this manner we can show that a solution containing the amounts and proportions of NaCl + Mg of the sea-water is a more powerful inhibitor, and stops the pulsation-stimulus sooner than solutions of Na + K, or Na + Ca. Moreover, a solution of NaCl + $CaCl_2$ stops pulsation sooner than a solution of NaCl + KCl, the amounts and proportions being in all cases those found in sea-water.

It is, then, evident that the relative powers of the inhibitors in sea-water are from strongest to weakest—magnesium, calcium, and potassium. Indeed, the stimulating effect of the sodium chloride in the sea-water is exactly offset by the subduing tendency of the magnesium, calcium, and potassium; and thus it is that the sea-water as a whole neither stimulates nor inhibits the pulsation of the jelly-fish. The sea-water is, indeed, a delicately

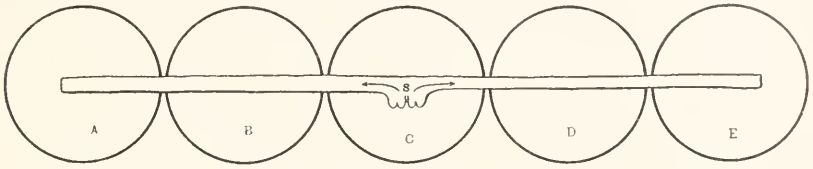


FIG. 12.—Test of relative inhibiting power of magnesium, calcium, and potassium of sea-water.

balanced fluid in all respects, for it contains poisons and antidotes which exactly counteract one the other.

The pulsation-stimulus is evidently not derived *directly* from the sea-water, but is engendered within the sense-organs of the bell-margin. Experiments show that the sense-organs can not maintain pulsation unless they be immersed in a fluid containing calcium in solution. Indeed they must constantly be *supplied* with calcium. On the other hand the pulsation-stimulus once it *leaves* the sense-organs and travels through the diffuse nervous network of the subumbrella is relatively *independent* of the amount of calcium in solution, for such a wave may endure for more than two hours if traveling through subumbrella tissue, whereas the sense-organs can not continue to send forth pulsation-stimuli for more than 6 to 10 minutes in a solution which lacks calcium, but contains all the other elements of sea-water.

We are now in a position to state that each pulsation is due to a nervous stimulus that originates somehow in the sense-organs. The question is how does it originate?

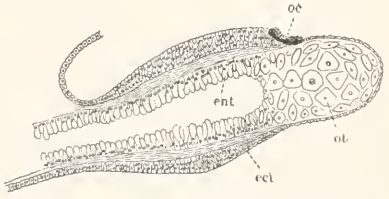


FIG. 13.—Median section of marginal sense-organ of *Cassiopea xamachana*. *ect.*, ectoderm; *ent.*, entoderm; *oc.*, ocellus; *ot.*, concretionary crystals.

In all of the Scyphomedusæ the marginal sense-organs are little clubs, the hollow entodermal cores of which contain a terminal mass of concretionary crystals. It has been commonly supposed that these crystals are composed of calcium carbonate, but I find that they are actually *calcium oxalate* with a certain small proportion of urea and uric acid. In nitric and hydrochloric acids they dissolve slowly without evolution of gas, but in sulphuric acid they slowly give off bubbles of carbon dioxide. In short, they respond to all of the chemical tests for oxalates.

Urea and uric acid are relatively passive in so far as pulsation is concerned, but the presence of crystals in the sense-organs containing calcium oxalate acquires a meaning when we recall the fact that the sense-organs can not maintain pulsation unless they be constantly *supplied* with soluble calcium from the sea-water.

We see at once that there must be some oxalate which is constantly forming in the sense-organs, and which is precipitating the soluble calcium chloride and sulphate derived from the sea-water to form the insoluble calcic oxalate crystals of the sense-club.

The question before us is, what oxalate is being formed in the sense-organs? We know that in certain tissues in the bodies of animals oxalic acid and other oxalates are formed apparently through the incomplete oxidation of carbo-hydrates. I find that 1 part by weight of oxalic acid in 1000 parts by weight of sea-water quickly paralyzes the sense-organs so completely that they do not recover the power of initiating pulsation even after they are returned to sea-water. So weak a solution of oxalic acid is, however, not a stimulant to the subumbrella tissue, nor is it appreciably poisonous to the medusa as a whole.

From 1 to 5 parts by weight of the oxalates of potassium and magnesium in 1000 parts of sea-water also inhibit pulsation after a short initial stimulation, and it can not be that these are the cause of pulsation in the sense-organs.

If, however, we immerse the sense-organs in a solution of from 1 to 5 parts by weight of sodium oxalate in 1000 parts by weight of sea-water, they are powerfully stimulated, and give forth pulsations at a rapid rate; but on the other hand this weak solution has no stimulating effect if applied to the subumbrella alone.

Now sodium oxalate precipitates the calcium which enters the sense-organ from the sea-water, forming calcium oxalate, and sets free sodium chloride, and sodium sulphate; both of which are powerful nervous and muscular stimulants. The formula for this reaction is as follows:

$$Na_2C_2O_4 + CaCl_2 = 2NaCl + CaC_2O_4,$$
$$Na_2C_2O_4 + CaSO_4 = Na_2SO_4 + CaC_2O_4.$$

It thus appears that each sense-organ normally maintains a certain slight excess of sodium over and above that found in the sea-water, and this acts as a stimulant which is prevented from becoming too concentrated by the fact that being in solution it is constantly passing out into the surrounding sea-water.

We can prove experimentally that this suffices to explain the phenomenon of pulsation, for if we simply add from 1 to 5 parts of sodium chloride to 1000 parts of sea-water, we find that this slight excess of salt acts as a powerful stimulant if applied to the sense-organs, but produces no pulsation if placed upon parts of the jelly-fish other than the sense-organs.

It is well known that Romanes, 1885,[1] demonstrated that the stimulus of a weak faradaic current of electricity applied to the subumbrella would cause Scyphomedusæ deprived of sense-organs to resume rhythmical pulsations.

The nervous stimulus which causes pulsation can not be produced at the extreme outer end of the sense-club where the calcic oxalate crystals are forming, for the calcium in solution must be relatively reduced at this place, and this would permit the magnesium to repress the stimulating effect of any slight excess of sodium. The free sodium salts must pass backward by osmosis to the nervous center near the base of the sense-club where the calcium is normal in concentration. We may prove this experimentally, for if we cut off the tip of the sense-club, removing the entire otolith mass, we may still stimulate the stump of the club into activity by a solution of one part by weight of sodium chloride in 1000 parts by weight of sea-water.

We are now in a position to state that the nervous stimulus which produces pulsation is caused by a slight excess of soluble sodium at the ganglionic center, but the chemistry of the change that takes place in the nerve itself while the pulsation-stimulus is passing through it remains undiscovered.

[1] International Scientific Series, vol. 49, New York. Also:—Philosophical Transactions Royal Soc., London, vols. 166, 167, 171.

VIII. NOTES ON MEDUSÆ OF THE WESTERN ATLANTIC

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Plates 1-4

NOTES ON MEDUSÆ OF THE WESTERN ATLANTIC.

By H. F. Perkins.

The Marine Biological Laboratory of the Carnegie Institution of Washington in the Dry Tortugas is admirably situated for the study of many of the lower marine animals, their behavior, and the conditions of life, and in none of the great groups are there better opportunities than in the Cœlenterates. In addition to the conditions ordinarily found in a region of coral reefs and coral islands, one quite unique feature occurs in the Tortugas in the presence of the old fortification and surrounding moat which occupy the entire surface of the islet known as Garden Key. This ruined structure, Fort Jefferson, dates back to the days of cast-iron cannon and vertical-walled forts of brick. The moat affords remarkably favorable conditions for the growth and multiplication of the lower forms of plants and animals, sheltered as it is from the violence of storms by the sea-wall, its shallow water warmed by the fierce rays of the sun and kept from stagnation by the agitation and partial change of the tides. Thus an unusual set of conditions obtains, and many of the minute forms which are daily swept in by the tide must find this an ideal place to grow and increase and become permanently established as part of the population of the moat.

Another fortunate circumstance is the ease with which cultures of eggs and larvæ may be maintained at the laboratory, the water in the culture-jars being cooler than that in the surrounding sea and considerably cooler than that in the moat. The distance of 4 miles separating the two islets occupied by the fort and by the laboratory is made less of a difficulty by the use of the laboratory launch, which makes it possible to transfer material from the moat to the aquaria at Loggerhead Key with little delay.

The writer has for several years been interested in the causes of migration and segregation of Medusæ. There are many instances of species such as *Gonionemus murbachii,* of Woods Hole, which have become established in some locality of very limited extent, and at a great distance from the nearest allied species. The circumstances which have caused first the distribution and then the segregation of the form offer fascinating fields for study and speculation. A particularly interesting phase of the problem is offered by the special adhesive organs which occur in several of the species which

have come under the observation of the writer, as in the case of the species mentioned above. In his attempt to gather data for this study the writer has been most generously aided by the Carnegie Institution of Washington, a debt which he acknowledges with gratitude. An appointment as research assistant in 1903 made it possible to collect and study the Medusæ of various points on the coast of New England, the somewhat voluminous notes upon which have not yet been published because so few of the many questions which arose could be satisfactorily answered by the work of a single year. During the summer of 1905 the hospitality of the same Institution, courteously extended through the director of the Tortugas Laboratory, made it possible to continue study upon some of the same questions in subtropical waters, and the following sections present the data accumulated at that time. It is a pleasure to acknowledge that the facilities liberally and wisely provided by the laboratory, and the stimulating interest and helpful suggestions of the director were of the utmost assistance in carrying out the research.

CLADONEMA MAYERI, New Species.

(Plates 1 and 2; Plate 4, figs. 21 and 22.)

I. THE MEDUSA STAGE.

CLADONEMA Dujardin, 1843. Ann. des Sci. Nat.
CORYNE Gosse, 1853. Naturalist's Rambles on the Devonshire Coast.

Generic characters.—Anthomedusæ of the family Cladonemidæ, having 8 to 10 stout inflexible marginal tentacles arising from an ocellated basal bulb. Tentacles bear terminally short prehensile processes, and usually also long, branching, filamentous, nettling processes. Bell deep hemispherical. Manubrium long as height of bell, ending in 4 to 6 oral tentacles or knobs beset with nematocysts. A circle of 4 to 6 gastric pouches located about half-way up the manubrium. Radial canals, 8 to 10, frequently arising in pairs from single canals at top of manubrium. All of small size (under 4 mm. in height of bell).

Cladonema mayeri, new species.[1]

Specific characters.—*Cladonema* with minute bell, 9 tentacles with both prehensile processes and branching terminal filament. Radial canals, 6 at origin, of which every other one bifurcates near the origin, making 9. Tentacles weighted with concretions of spherical or rounded shape produced and held within the endoderm cells of the larger part of the tentacle. Reddish-colored ocellus at the base of each tentacle. Manubrium with 6 gastric pouches and 6 oral knobs of nettling organs. Velum wide and strong.

Color lacking, except in the ocelli, which are reddish. Bell and ten-

[1] The species is named in honor of Doctor Alfred Goldsborough Mayer, Director of the Tortugas Laboratory of the Carnegie Institution of Washington.

tacles transparent, except for pattern of opaque white markings caused by parasitic protozoa.

Habitat.—Very limited; not found, so far as recorded, outside the moat of Fort Jefferson, Tortugas Islands, except, perhaps, in a single case. (A specimen of *Cladonema* was taken by Dr. C. O. Whitman in 1883 on the shoals near Fleming's Key, north of Key West, Florida. This was described by Fewkes,[1] and from the similarity between this specimen and our species in point of arrangement of canals it is not at all unlikely that it may be the same. The other points of anatomy are not so clear, and there are no figures.) Living in shallow water, close to the bottom, amongst tangled masses of filamentous algæ.

II. THE HYDROID STAGE.

CLADONEMA Hincks.[2] British Hydroid Zoophytes, 1868.
STAURIDIUM Dujardin. Ann. des Sci., 1843.

Generic characters.—Minute *Stauridium*-like hydroid arising from a creeping stolon attached to alga, stone, or other supporting substance. Invested by a perisarc. Hydranth club-shaped, tapering from above downward. Oral extremity rounded into a hypostome. Two series or verticils of tentacles, a capitate set at the oral end, four in number, forming a cross, thickly set with nematocysts; at a distance down the column a second verticil of four stiff, rod-like tentacles, set opposite the angles between the upper set.

Cladonema mayeri, new species.

Specific characters.—There does not appear to be any great difference between the various species of *Cladonema,* in the hydroid stage. Its constitution is so simple in comparison with that of the rather complicated medusa form that it is not surprising to find fewer points of contrast between representatives of different species. Like *Stauridium, Coryne,* and *Clavatella,* this genus offers a direct contrast to such hydrozoa as *Obelia,* in which it is hard to recognize any differences between gonosomes which develop upon trophosomes of very distinct character. The minute proportions of the hydroid under discussion, the absence of tactile hairs on the tips of

[1] Fewkes, J. W. 1883. On a few Medusæ from the Bermudas. Bull. Mus. Comp. Zool. Harv. Col., XI, p. 87.

[2] I am aware that the name *Stauridium* has the authority of older usage. In fact it was this name that was originally applied to the hydroid "nurse" of the free-swimming *Cladonema* found by Dujardin in his aquarium. It is unfortunate that it did not appeal to this astute naturalist as a convenient and permissible practice to call two stages in the development of the same animal by the same name. Had there not arisen confusion in the application of the name which Dujardin gave to his hydroid, to other similar but not identical forms, it might be best to continue to use two different names for the medusa stage and the hydroid stage of the animal in question. The old name has, however, been applied (Haeckel: System der Medusen) to hydroids whose progeny are not *Cladonema,* but *Sarsia.* It is certainly desirable to simplify our nomenclature to the utmost. I have thought that in this case it was by far the better plan to follow Hincks in his very logical decision in the matter.

the lower tentacles, and the location of the gonophores considerably above the latter, are points distinguishing the species from those described by other observers.

No specimens appeared in which there was any sign of a branching colonial stock, such as is described as an occasional form of the trophosome of *C. allmani*[1] and *C. dujardinii*.[2]

Habitat.—Not found thus far in any other locality than that given above for the medusa form, viz, the growing filamentous alga, abundant in the Fort Jefferson moat. Owing to its small bulk and unobtrusive appearance, it would be difficult to discover this hydroid upon any other than a very delicate foundation. It may be abundant upon stones and shells, etc., in the bottom of the moat, but it would be only by rearing the medusæ in aquaria containing nothing else that one would be likely to find it there.

III. GENERAL ACCOUNT OF THE MEDUSA STAGE OF CLADONEMA MAYERI.

The occurrence in the moat at Fort Jefferson of this species of *Cladonema*, or of any species of the genus for that matter, is certainly surprising. It would be difficult to find a part of the ocean more unlike the habitation one would select as that for which the structure of this creature seems to fit it. Here is a creature very unusually equipped for life in the open sea, capable of resisting ocean-currents, tides, and boisterous waves, provided with ballast and a whole battery of anchors against the assaults of tempests. It has established itself in cowardly fashion within the sheltering walls of a placid ditch, well out of harm's way.

The moat at Fort Jefferson, surrounding the hexagonal fortifications, is a relic of the ancient days of short-range artillery. It was constructed by throwing a substantial wall of masonry around the vertical face of the fort, founding it upon the natural bottom of coral rock. The moderate tides have access through the generous sluiceways, built large enough to permit the passage of small boats. Although shallow, the moat is never empty, nor is its bottom, which rises to within a few feet of the surface at low water, ever entirely uncovered, even in the shallowest parts. The water is warmed by the sun and by reflected heat from the shallow, sandy bottom and the brick walls of the fort. The temperature of the water is often high enough to make it feel decidedly warm to the hand when the air is well up in the eighties, Fahrenheit.

The ecology of the moat offers a most interesting problem. A number of species have become established here which never make their appearance in the waters outside the wall of the moat. The warmth and quietness of the water is partly the cause of this condition, but there is also to be taken into account the presence, because of that same warmth and protection from

[1] Allman, J. G., 1871. Monograph of Gymnoblastic Hydroids.
[2] Dujardin. Ann. des Sci. Nat., 1843, p. 370.

storms, of plant and animal life which encourages the growth and development of other species.

An interesting opportunity is offered by our species to see whether the concretions in the tentacles, increasing the weight of the organism, and the strong suctorial processes on the tentacles—as many as 25 to 30 in each individual—will disappear or become reduced as a result of their withdrawal from the rough weather they seem intended to combat. Observations extending over a series of years would be of value in determining this point. And yet, while apparently no longer a necessity as a protection against the elements, it is by no means certain that these peculiar modifications are not useful to the jelly-fish for other reasons. It seems not at all improbable, indeed, that the daily and normal activities of the medusa are to some extent dependent upon the heavy tentacles and bell-margin, and that the prehensile organs upon the tentacles are of much use in the feeding habits. The suctorial processes are very strong, much stronger than would seem at all necessary for the carrying out of the routine suggested, and evidently capable of far greater resisting power than that which is brought into requisition in the quiet life of the animal. How long is it likely that these organs will retain their strength or be kept in their present numbers, when there is no longer any tax upon their strength? It seems improbable that they will keep their present efficiency for long in the absence of such requirements.

Distribution of the genus.—The remarks in the last paragraph upon the possibility of degeneration of the suctorial powers of *Cladonema mayeri* apply with particular force to any representative of this genus. This is the case because of the evident tendency to variation amongst its members. Every writer upon the group calls attention to the large percentage of individuals having some other numerical arrangement of tentacles, radial canals, and parts of manubrium, than the typical one. In the species under consideration the irregularity did not seem to be so great as in the others which I have seen. Several counts showed a varying number of parts in about 20 per cent of the individuals. Instead of 9, there are present 8, 10, or 11 ultimate branches of the radial canals, and a like number of tentacles are present, or, in cases of normal numbers of tentacles and canals, the 6-parted manubrium may be varied into one possessing 5 or 7 parts. No single type of variation exceeded 8 per cent.

In view of this tendency to vary, it has been thought by some that it was a mistake to give specific or even lower rank to the different types. They should rather be regarded as nothing more distinct than varieties. I admit that it is possible that transitional types are in existence. It would be rather troublesome to apply breeding tests to the different types, and establish their identity or separateness by their sexual affinity or antagonism. The only course that is left open to us seems to be to decide whether the percentage of varying individuals out of any very large number is sufficient to

warrant us in holding such fortuitous variation responsible for the occurrence of large communities of the genus, in which so many individuals show a definite numerical arrangement. Perhaps that is a matter of opinion.

The first discovery of the genus, and the later study of it that has been carried on by several different observers, has had the peculiarity of depending upon aquarium material. Dujardin, in 1843, found the first medusa and later the hydroid in an aquarium stocked with material from the coast of France. The eggs of the genus must be capable of extended travels, judging by the great distances separating the localities where the hydroids have become established. It was doubtless in the egg stage, or possibly as a free planula larva, that the species was introduced into the aquarium where Dujardin discovered it. The other localities where representatives of the genus have been found are as follows: Brittany, Belgium, Messina in the Mediterranean, and the Bahama Islands. This latter habitat is so near the Tortugas that it might be expected that the two related species found in these neighboring localities would show closer similarity than two which occurred at a greater distance apart. Reference to the following table will show that this is not the case. The only point of agreement between the two, as regards numerical arrangement, is in the gastric pouches. Another point not indicated in the table is the difference between the tentacle processes of the two species. In the Bahama form,[1] the prehensile branches are developed at the expense of the floating nettling-threads, whereas in the species from the Tortugas these terminal filaments are the most conspicuous feature. The habitat of the species found in the Bahamas is in open, exposed shallows with sandy bottom. The long, floating filaments would increase the risk of the creature's being swept away by the waves. Their reduction must be an advantage.

Comparison of species of Cladonema.

Species.	Number of canals at margin.	Number of canals at top of bell.	Number of oral tentacles.	Number of gastric pouches.
C. gegenbauri Haeckel.	8	8 or 4 × 2	4	4
C. krohnii Haeckel.....	10	10 or 5 × 2	4	4
C. dujardinii Haeckel...	8	8 or 4 × 2	5	5
C. allmani Haeckel.....	10 or 5 × 2	10 or 5 × 2	5	5
C. perkinsii Mayer.....	8	8	5	6
C. mayeri sp. n.....	9	6 (3 & 3 × 2)	6	6

Discovery of C. mayeri.—The peculiar conditions of temperature, freedom from wave-action, and bottom-growth tempted me to investigate into the ecology of the Fort Jefferson moat. Inasmuch as I was particularly interested in the cœlenterate fauna, I worked at first with a fine-meshed tow-net. The first day this was tried some specimens of the medusa came into the net

[1] Perkins, 1902. Johns Hopkins University Circulars No. 21, Vol. XXI, No. 155.

and were found on examination of the washings. The towings were made just before sunset. The moat was visited next day, and careful search failed to bring to light any specimens. A mass of the filamentous alga was pulled up from the bottom and put into a separate jar. The water in the jar was soon observed to contain several of the minute medusæ, but great was my surprise to find, after returning to the laboratory and allowing the jar to stand for a short time, not a few individuals only, but nearly a hundred in various attitudes on the surface of the glass or amongst the weed. The exquisite appearance of the delicate creatures, their tentacles fully extended and interlacing at the tips, was most striking. Examined with a lens, the tiny bubble-like bell was seen to stand upright, sturdily braced upon the stocky pillars of the tentacles, which spread out at an angle with the perpendicular so as to give an absurdly stable foundation to this frail body. At the base of each tentacle a speck of color was displayed, the ocellus, red-brown in hue. Exquisitely slender threads extended out radially from the tips of the tentacles, each one branching into several similar threads, and all strung at intervals with glistening beads of nettling cells. These little organisms reminded one of nothing so much as the finest frost tracery.[1]

Swimming reactions.—And yet, this diaphanous delicacy of appearance is coupled with remarkable activity when the creature releases its hold upon its foundation and sets out to swim. It is only when disturbed, or when the light conditions effect a stimulus which is transmitted from the eye-spots to the nervous system of the medusa that the swimming reactions are to be observed. The most of the time the creature holds fast to its place in the weed, the long slender manubrium swaying about, apparently in search of food. The behavior of *Cladonema* suggests that of *Gonionemus* in many respects, and one of these is the habit of reacting to the light-stimulus, or to some impulse of a kindred nature, and going through a series of vigorous swimming reactions for a longer or shorter period in the morning and at dusk. It seems to require an extra effort on the part of *Cladonema* to break loose from its moorings and set out upon its periodic quest for food. The cause of this apparent inertia may possibly be the unusual heaviness of the apparently frail body.

When setting off, the jelly-fish makes one or two spasmodic attempts to pull itself away, then suddenly shoots off at a great rate, sometimes leaving behind a speck of tissue from one of the adhesive processes. The tentacles,

[1] The delicacy and vigorous activity of *Cladonema* are well portrayed by Van Beneden (1866, Rech. sur la Faune litt. de Belg.: Polypes): "Rien n'est gracieux comme un Cladonème nonchalamment étalé au milieu de son bassin, fuyant devant quelque danger imaginaire ou réel, ou solidement tapi par ses ventouses pour résister au courant, pendant qu'il étale soigneusement ses longs cirrhes dans toutes les directions. On peut rester des heures entières en contemplation devant ces organismes infimes, qui semblent moins solides qu'une bulle de savon, et qui se conservent cependent en dépit des vagues, des chocs et des tempêtes."

which while at rest were extended radially in a circle four or sometimes five times as wide as the bell, are shortened to half their extreme length. A succession of rapid, jerky contractions of the bell drives it forward for a short distance. It then settles slowly down until something solid is reached, when it either fastens itself for a short rest, or starts off at once on another voyage. The bell changes in shape by about one-fourth of its diameter at each contraction. The main part of the tentacles seem to be rather a hindrance than a help in locomotion. They are held stiffly out at less of an angle than when fixed to some solid object. With the slender terminal processes, they extend backward in the water and probably assist the swimming movements to the extent of steadying them somewhat.

In case the jelly-fish chanced to be hanging suspended by one or two tentacles from a bit of seaweed when the swimming commenced, it seemed to possess no means of knowing that it was not in the proper position. The course of the swimming was never seen to be changed from downward or sidewise to upward. From the horizontal position, as when resting upon the bottom, the dozen or so of impulses given to the bell before stopping usually sufficed to drive it upward to the surface, or near to it, in a fairly regular fashion, and with moderate directness. But the sidewise or downward course was much more erratic, the little creature bobbing first this way, then that, in tipsy style. The fact that the center of gravity is, as will be noted later, so low down on the bell is probably the occasion of this difficulty in maintaining a straight course when the bell is in any other position than the normal one, right side up. A number of counts were made to find the rate of the swimming contractions, and it was found that at the average temperature of the water in the moat during the summer the pulsations of the bell averaged a rate of 200 per minute, the activity being continued for only a few seconds. The jelly-fish appeared to become fatigued after from ten to twenty pulsations.

"Fishing" reactions.—As in the case of the famous fishing medusa of Woods Hole, *Gonionemus*, each period of active swimming is succeeded by a period of passive floating in the water, the outspread tentacles ready to seize any prey that might chance to come in the way. As soon as the pulsations cease, the tentacles stretch out into the water and are swept upward over the top of the bell by its downward course. At this time the position is similar to that shown by Allman[1] as the typical *resting* attitude of "*C. radiatum*" (now known as *C. allmani*). This "hands-up" posture is, I believe, never taken during periods of rest by the species under discussion.

Resting attitude.—*Cladonema* comes to rest margin down, instead of inverted as in the case of *Gonionemus*. The suctorial appendages apply themselves to the bottom the instant the medusa touches. There are from two to five of these on each of the nine tentacles, arising from the lower or axial surface of the tip end. These processes are smooth, devoid of nema-

[1] Allman. 1871. Monograph of the Gymnoblastic Hydroids, plate XVII, fig. 4.

tocysts, and terminated by a suctorial cushion of the type common to many of the Hydrozoa, having both muscular and glandular cells. At rest, the end is cupped slightly.

The arrangement of tentacle processes is not uniform in the several tentacles of any individual. Sometimes no two tentacles exhibit just the same plan in number and position of the two different kinds of appendages. In the case of the branches of the filamentous terminal process, they are seen to follow a generally alternate plan. This is only roughly followed, however. It is more noticeable in the first lateral processes that appear in the immature medusa than it is later in life. In some instances two or three processes grow out of the filament just at its junction with the main part of the tentacle, but ordinarily a little space intervenes. There are from three to eight of these branches on the average mature tentacle. In floating downward in the water, the slender branches reach out far enough to cover an area about three times the diameter of the bell, and as has been mentioned, a considerably wider field is covered when the jelly-fish is resting on the bottom. In this latter attitude, the filaments are held in such a position that they just clear the bottom. They are strung with minute clusters of nematocysts, with a slightly larger bead-like cluster at the end of each branch. The whole system forms a very beautiful and at the same time very efficient apparatus. Any luckless worm or copepod that happens to touch this spider-web is instantly treated to a vigorous nettling by the discharge of numbers of the nematocysts. Although these are small in size, they do their work in thoroughly efficacious fashion, the victim succumbing with hardly a struggle.

Feeding reactions.—After the discharge of the nettling cells there is no trouble in getting the prey to the mouth. The slender snares are instantly retracted, the entire tentacle shortens and curves towards the mouth, the bell-muscles contract spasmodically, the manubrium is set in eager motion, and the whole organism evinces the keenest interest in the prospect of a meal. Upon coming into contact with the spherical masses of stinging cells at the end of the manubrium, around the mouth, still further punishment is dealt out to the victim.

When one realizes that the warm waters of the moat are even more richly supplied with small creatures than the ordinarily teeming tropical seas in the neighborhood of coral shoals, it is easy to see that so well-equipped a fisherman as this should have no trouble in making a living. It is no wonder, then, that this species has become well established. Although the first individuals may have come into the locality within comparatively recent times, as we may conclude from the retention of the open-sea characters already mentioned, the genus being evidently a readily mutable one, such favorable conditions as have been described for the species might easily explain the presence of such very great numbers as were observed in the moat.

Specific gravity.—The reason for the apparent sluggishness of the medusa, mentioned above, is not far to seek. The tentacles are so laden with concretions that they must be a good deal of a burden to the small creature. The center of gravity is so far down on the bell and tentacles that a specimen, inverted in the water and released, will right itself at once. That this is not a muscular act is seen in experiments upon specimens which have been anesthetized. After treatment with menthol or chloretone the same power was exhibited. Examination of the tentacles shows the presence of large numbers of rounded concretions. These are packed tightly into the endodermal cells. Their composition has not been determined.

Experiments were made with a view to determining the specific gravity of the organism. Solutions of magnesium sulphate in sea-water were prepared, of various degrees of saturation. Inasmuch as no change in bulk, and consequently none in density, was effected by temporary immersion in this solution, it was concluded that the best way to determine the specific gravity of the medusa would be to find a solution in which it would be just suspended, without either sinking deeper or rising to the surface, and then determining the specific gravity of this solution. This was the method suggested by Dr. Mayer. It was found in this way that a solution, equal in density to the jelly-fish, weighed 106.4 grams per 100 c. cm. That is to say, the medusa, having the same density as the weighed solution, has a specific gravity of 1.064, or 3.9 per cent greater than that of sea-water. It seems likely that the extra weight of the creature makes up for the deficiency in the strength of the suctorial apparatus as compared with the corresponding parts in the species from the Bahamas.

IV. LIFE HISTORY.

After finding the medusa of *Cladonema* in the moat of Fort Jefferson, it was naturally a matter of interest to discover the other stages in the life-history, if possible. So far as I have been able to determine, the hydroid stage of the genus has never been reported from the open sea. The only cases in which it has been seen have been those in which the creature has made its appearance in captivity. The descriptions of these examples would seem to indicate that they were entirely normal in all respects. It is of interest, however, to find the hydroid growing in its natural environment.

Many trips were made from the laboratory on Loggerhead Key to the moat of the fort on Garden Key, 4 miles distant, and many hours were consumed in a fruitless search for the polyp. A microscopic examination of quantities of stones, sticks, and other débris from the bottom of the moat, and of the plants and animals that make it their abode, failed to show any sign of its existence. After some weeks had passed, however, the finding of another hydroid on the alga which grows in abundance on the bottom of the moat led to the discovery of the one I was more particularly anxious to

locate. I had not entertained any idea of finding a large and conspicuous hydroid, but the minute proportions of the creature when finally discovered surprised me. The polyp, growing singly, was so exceedingly delicate that it was almost invisible to the unaided eye. Only one specimen of the entire number that came to light, over a dozen in all, was large enough to be at all readily seen without a lens. This, though slender and transparent, measured 1.5 mm. in height. It is no wonder, then, that this form has not been a familiar sight to visitors in these waters!

During the time that the search was being carried on in the moat, careful watch was being kept over the medusæ which were brought into the laboratory every day. Only one individual was found in which the gonads showed any sign of activity. In this, a single spherical mass appeared upon the manubrium, above the gastric enlargements. This medusa was kept under frequent observation for some days, but the only perceptible change was an increase in the size of the egg, if such it was. The specimen disappeared, finally, without throwing any light upon the laying or development of the egg.

Filiform tentacles.—The absence of tactile hairs from the tips of the lower row of tentacles has been mentioned. The function of these processes, or " false tentacles," as Hincks[1] terms them, is problematical. It does not seem to be at all certain that they are intended to perform the function of tactile organs, inasmuch as their sensitiveness does not exceed that of the adjacent parts. Hincks says: " Their function seems to be to give notice of the presence of animalcules or other prey. If anything touches them, the head and upper arms are instantly bent towards it." I tried to find out whether this same reaction occurred in our species, and found that it did. But it did not make any difference whether the stimulus was applied to the tip end of the process, or to some other part of it, or to the column of the polyp nearby. It is likely that in the other species the tactile sense is more localized.

Feeding reactions.—The same eagerness in the presence of food which was noted in the medusa also characterizes the hydroid. The column of the polyp stands up stiffly and without any sign of life when there is no prey near. The capitate tentacles around the mouth droop a little at the tips, and the filiform tentacles below are straight and stiff. But let the smallest speck of an animalcule come along and touch the polyp, and it suddenly becomes flexibility itself. The column bends and twists, the oral tentacles reach after the prey, and even the slender tentacles below manifest signs of life. Plate 2, figure 7, is a drawing made to show the attitude of one of the polyps at the instant that a small worm, which had become partially fixed to the column by the nematocysts, made good its escape.

Reproduction.—I was unable to see that any definite gonophores were de-

[1] Hincks, *loc. cit.*, p. 64.

veloped. Several stages of budding were noted, but not more than one bud was found to occur at a time. The proliferation of the endoderm cells at a point above the lower set of tentacles was the first sign of budding, and this was soon followed by the protuberance of the ectoderm. The endoderm cells are small and rich in protoplasm, making a slightly opaque spot in the middle of the developing bud (plate 2, fig. 8). The nine tentacles make their appearance early, and their gradual lengthening, accompanied by internal changes, marks the subsequent growth. There are no points of especial interest in the history of the bud while attached to the parent stalk (see plate 2, figs. 9, 10, and 11). The youngest free medusæ that were seen bore no sign of their attachment to the hydroid nurse. They were about one-half the adult size, and had only one or two processes on each tentacle. The bell was more tall and slender than in the adult.

We have, then, the more important stages in the life-cycle of one of the two species of this remarkable genus which occur in the western hemisphere. For efficiency combined with delicacy it would be difficult to imagine a more successful work of nature.

CAMPANULARIA MACROTHECA,[1] New Species.

(Plate 3, figs. 12 and 13.)

Specific characters.—Minute, colorless, unbranched Campanularian hydroid, arising from a single creeping stolon. Stem short. Cup long and slender, vase-shaped, cylindrical, tapering at the point of attachment in a graceful curve. Hydrocaulus with seven rings just above stolon, and just below hydranth a second series of equal number. Margin of cup crenelated in six U-shaped indentations of moderate depth (plate 3, fig. 13).

Hydranth exceedingly slender, with 16 slender flexible tentacles, length, fully extended, somewhat greater than that of cup. Base of hydranth forms a slender flaring column within the hydrotheca. The manubrium is prominent, oval or pear-shaped. The stolon is filamentous, creeping on the stems of algæ. The gonotheca is elongate clavate, largest diameter at the free end, which is rounded. The attached end tapers gradually to the colony stem. The gonotheca is about twice as long as the hydrotheca, which it resembles in general shapeliness of outline. The annulations which are a characteristic marking of the stem in the hydranth are lacking in the case of the gonotheca, which is connected with the colony stem by a smoothly tapering branch. In some cases this slender connection was curved around the colony stem very much as some vine-leaves curve about the main stem at the base (see plate 3, fig. 12).

The blastostyle extends through the gonotheca as a slender column, flaring at the base to the wall of the cup, where it rests upon the circular shelf

[1] Derivation: From μακρος, long, and θηκη, case.

which is so characteristic of the genus, and flaring also at the distal end into a trumpet-shaped closure for the capsule.

Only two of the hydroids that were seen showed anything of value as to the reproductive process. In these, two medusa buds were developing upon the blastostyle within the gonotheca. Both were more than half-way out on the blastostyle, and behind the smaller, more proximally situated bud there was no sign of more progeny ready to begin growth.

The bud farther from the base of the capsule was about twice as far along in the matter of size and development as was the younger individual. It was my misfortune to be unable to find specimens in later stages of growth than that of the older bud represented in the figure. No free medusæ were taken in the tow-net, which was plied patiently in the waters of the moat, so that the specific characters of the mature jelly-fish can not be described at this time.

There were, in the specimens observed, four radial canals fully developed, each one ending in a large cushion of ectodermal tissue, evidently the basal enlargement, possibly sensory in function, which the tentacles of Campanularian medusæ always carry at the point of emergence from the bell-margin.

The nearly spherical shape of the medusa buds should be mentioned as a point in contrast with the very long buds which are found in the capsules of some of the Campanularidæ.

Comparison of characters of Campanularia macrotheca with those of other species nearly related to it.

Species.	Height.	Hydrotheca margin.	Annulations on hydranth stem.	Annulations on gonotheca stem.
	Inch.			
Campanularia raridentata[1]....	0.05	Serrated.....	4 distally ..... / 5 or 6 proximally	None
Platypyxis cylindrica[2].....	0.125	Crenelated.....	2 or 3 distally....	2 or 3
Campanularia macrotheca.....	0.062	Crenelated.....	7 proximally..... / 7 distally	None

Habitat.—The specimens here described were all found in the moat of Fort Jefferson, Tortugas Islands, Florida. The stolons were found creeping upon the same filamentous alga upon which the hydroids of *Cladonema* were growing.

The above species differ in the matter of habitat as well as in morphological characters. The habitat of *C. raridentata* is given by Hincks as "other zoophytes and on corallines, between tide marks." The two other species given in the table have the same habitat.

[1] Alder, J. A catalogue of the Zoophytes of Northumberland and Durham. Trans. Tynes. Nat. F. Club, 1857.

[2] Agassiz, A. 1862. Contrib. Nat. Hist. U. S.

AGLAURA CILIATA,[1] New Species.

(Plate 3, figs. 14–16.)

Specific characters.—*Aglaura* with bell, provided with a prominence at the top; gastric pouches at upper extremity of manubrium; four pendulous oral lappets bearing nettling organs and lined with strong cilia. Velum not strongly developed; tentacles, 24 in number, short and not vigorous; lithocysts, 8 in number, placed midway between the marginal endings of the radial canals. Four small masses of glandular tissue hang down from the walls of the manubrium, above the middle, into the lumen of that organ. Eight similar masses hang from the radial canals, one from each canal, into the space within the bell. Color, steely blue, uniform throughout.

Habitat.—Open sea around the Tortugas Islands, Florida. Taken in tow-net near ship-channel by Dr. W. K. Brooks, July, 1905. The specimens were amongst a quantity of material taken in the tow, and very kindly given to the writer for examination. This opportunity is taken to acknowledge my gratitude to Dr. Brooks for this and very many other favors. His helpful suggestions were keenly appreciated.

The occurrence of the peculiar knotted masses of tissue in the two different parts of this medusa, on the radial canals and within the manubrium, with every indication of being glandular rather than gonadial tissue, is of considerable interest. It would not be strange if either or both of these two groups of protuberances had sometimes been mistaken for gonads. Again, the slender pendulous pouches upon the manubrium have undoubtedly been called by that name. On the placing of these organs a distinction has been made between medusæ, which were therefore concluded to constitute separate genera. Thus, Haeckel[2] has separated *Aglaura* and *Agalma* partly on account of the presence of eight gonads in the latter, located on the radial canals. *Aglantha* is another genus which is distinguished principally on the basis of this character. The species under discussion partakes of the characters of both *Agalma* and *Aglantha*.

A comparison between the old species *Aglaura hemistoma* Péron[3] and *A. ciliata* shows that the differences group themselves as follows:

Aglaura hemostoma.—Height not greater than breadth. Lips not provided with nematocysts (?). Gastric pouches ("Geschlechtsorgane," Leukart;[4] "Tentakeln," Eydoux u. Souleyet[5]) not higher than the middle of manubrium. No glandular protuberances on radial canals or interior of manubrium.

Aglaura ciliata new species.—Bell not parallel-sided, higher than broad. More decided apical protuberance than in *A. hemistoma*. Lips strongly ciliated and set with clusters of nematocysts. Gastric pouches near the upper end of the manubrium. Glandular protuberances projecting from inner walls of manubrium, and pendant from radial canals, near their origin.

[1] Derivation: *ciliatus,* having cilia. From the character of the inside of the manubrium.

[2] Haeckel, E. 1880. System.

[3] Péron. Annales du Museum, t. XIV.

[4] Leukart, Rud. 1856. Archiv für Naturgeschichte.

[5] Eydoux u. Souleyet. Voyage de la Bonite. Zool. Zoophyt., Pt. I.

In the above reference to the absence of nematocysts from the lips of the older species, it is purely negative evidence that governs. Nettling organs so large and conspicuous as those which stud the lips of the Tortugas species would hardly have been overlooked by the careful observers who have described the genus.

The species *peronii* of *Aglaura*,[1] established by Leukart, appears to be the same as that for which we already had the name *hemistoma* Péron.

It should not be inferred from the application of the name *ciliata* to this new species of *Aglaura* that the presence of cilia is peculiar to this one species of the genus. In the figures of *A. hemistoma* which accompany the descriptions by Leukart and Metschnikoff[2] the lips are represented as being lined with large and numerous cilia.

[1] Es ist dieselbe für die ich hier mit unterdruckung des ziemlich nichtssagenden Speciesnamens die obige Bezeichnung gewalt habe." Leukart, *loc. cit.*

[2] Metschnikoff, E. 1886. Arbeiten Zool. Inst., Wien, Bd. VI, Hf. II.

NOTE ON THE OCCURRENCE OF CASSIOPEA XAMACHANA AND POLYCLONIA FRONDOSA AT THE TORTUGAS.

(Plate 4, figs. 17–20.)

Amongst the various forms of plants and animals which find a convenient and salubrious abode in the warm storm-proof waters of the Fort Jefferson moat, none is more characteristic than the rhizostomous scyphozoan medusa *Cassiopea xamachana* Bigelow. The favorable conditions which Bigelow[1] found to prevail in the Salt Ponds of Jamaica must have been very much the same as those which are so marked in the sheltered moat in the Tortugas.

Besides the large bronzy-black ascidians that grow upon the rock walls of the moat at tide-mark, no creature is so conspicuous to the eye of the zoologist as the feathery brown disks that fairly carpet the floor of this place. When the surface of the water is unruffled, these jelly-fishes can be counted by the hundred as they lie on the warm sand or amongst the masses of algæ, the fluffy branches of the oral arms uppermost, the edge of the disk lazily fanning at the rate of a few strokes to the minute. "Moss cakes" the marines at the fort called the great creatures. Judging by both size and numbers, this species has here found an ideal breeding-ground.[2]

The medusæ vary in size through a wide range, and the extremes are as apt as not to be found resting side by side on the sand.

The largest examples measured 145 to 155 mm. in diameter, and there were very many of this size. The smallest specimens were less than 25 mm. in diameter, and they were characterized by less distinct markings, oral arms of smaller proportionate size, and greater activity of habit. The parts of the moat where the bottom was composed of clean sand, with only a fathom of water, seemed most favorable to the small individuals. In these younger cassiopeas the number of marginal sense-organs was from 13 to 15, while the largest and oldest ones possessed from 18 to 22.

Sexual multiplication.—Very little is known about the reproductive processes of the rhizostome medusæ. Bigelow, in his admirable monograph on this species, has given us a most entertaining as well as thorough account

[1] Bigelow, R. P. 1900. Memoirs Boston Soc. Nat. Hist., vol. 5, No. 6. Anatomy and development of *Cassiopea xamachana.*

[2] The first record of the occurrence of this species in this locality is given by Fewkes, J. Walter, 1882: Notes on Acalephs from the Tortugas (Bull. Mus. Comp. Zool. IX, 7). While his determination of the specimens found at Fort Jefferson was as *Cassiopea frondosa,* his description and figures, and the occurrence of *C. xamachana* in the same locality, make it clear that Bigelow was justified in assuming that the species was in fact *C. xamachana.*

of the larval forms, their multiplication and metamorphosis, but he lacked material for a study of the development of the sexual organs in the adult, and the early larval phases. Indeed, strangely enough, there seems to be no certainty as to the sexual character of the creatures—whether they are hermaphrodite or have separate sexes. The latter condition is assumed to obtain by some writers on the genus.

In the hope of determining some of the main points in the sexual multiplication of *Cassiopea,* large numbers of medusæ were taken from the moat and transferred to aquaria and live-cars at the Carnegie Institution Laboratory on Loggerhead Key. As Dr. Mayer has beautifully demonstrated,[1] no more favorable material can be imagined for all sorts of laboratory observations and experimentation than this same *Cassiopea.* It lives remarkably well in small aquaria.

Parasitic(?) larvæ.—When the medusæ are left for only a short time in a jar, and then removed, the water is found to contain floating masses or clouds of mucus. Microscopic examination of this mucus shows multitudes of nematocysts, discharged or intact, singly or in small clusters, floating in it. Also included in this substance, or suspended in the water outside of it, there appeared great numbers of very small organisms which I, and others, took for embryos of the medusa. These small objects appeared in several shapes, suggesting successive stages in growth and metamorphosis, and it looked as if it should be an easy task to get the full series of phases in the development of the *Cassiopea* egg. After some days of careful watching it was necessary to conclude, in disappointment, that these creatures were the parasitic young of some other animal; that they were probably not even of cœlenterate origin.

These organisms were bilaterally symmetrical, not radial, having three lobes separated by clear-cut incisions at one end, the larger, and two at the other. They were clear, almost entirely transparent, and colorless in the earliest stages. With increasing size the number of lobes accessory to the first set increases, much as in the echinoderm larva, and there appear in the interior of the creature unicellular zoanthellæ, which give a yellow and later a brown cast to the organism.

The surface was granular in appearance, a condition which was due to the presence of innumerable minute spherical bodies, arranged in regular pattern, suggesting in a general way the follicle cells of ascidians. Around the margins of the rounded lobular projections, cilia in bands served to drive the larva through the water in a rotating motion. Comparison of these objects with the ovarian eggs of the medusa, together with a consideration of the surface appearance and the peculiar contour of the body, made it impossible to regard this organism as of cœlenterate affinities. Further

[1] Mayer, A. G. 1906. Carnegie Institution Publication, No. 47, Rhythmical Pulsation in Scyphomedusæ.

than that I was unable to go, and am still quite in the dark as to the nature of these curious bits of animal life.

Mucus masses.—The presence of these larval creatures in the mucus clouds excreted by *Cassiopea* gave rise to the notion that the reproductive organs must secrete this mucus, which was useful as a vehicle for the sperm-cells. It was afterwards seen, however, that the clouds of mucus originated not in the genital pouches but from the oral arms. They probably serve to entrap and hold minute animalcules and other prey for food, having the same origin and function as the similar product in corals.[1] These sluggish rhizostomes come nearer to the actinians in point of habit than do most of the cœlenterates, and it would not be surprising to find that both their food and their mode of capturing it were somewhat similar.

Failing in my attempt to get the larval stages by natural means, I tried artificial fertilization of the eggs, or rather I took the preliminary steps thereto. But I was unable to discover any sperm! All the medusæ that I examined proved to be females, and no organs but the ovaries appeared within the genital pouches. Over one hundred of the medusæ were opened in the laboratory, with the result that every individual was found to have ovarian eggs in the vermiform gonads, in all stages of maturation.

It was disappointing to fail of getting the larvæ of this interesting medusa, and that, too, without having any explanation to fall back upon. True, it did not seem to be the normal breeding-season, prolonged search having brought to light only a few larvæ in the scyphistoma stage, and only a single free ephyrula. But these few young stages indicated that there was some activity in the reproductive functions of the members of the species there in the moat. At the height of the breeding-season there would undoubtedly be no difficulty in finding many immature stages. Bigelow reports that at the time of his observations in Jamaica the stones and sticks in Salt Pond were thick with the scyphistomas. It certainly looks as if the creature were hermaphrodite, and the indications are that it is also protogynous.

Polyclonia frondosa Agassiz.

While collecting in the moat one morning early in July, 1905, my eye caught the sparkle of clear white spots upon the oral arms of a medusa on the sandy bottom. When it was brought to the surface, these white spots were found to be small scales about the size and shape of an apple seed, except that they were flatter, attached to the surface of the arm by the small pointed end. The fleshy yellow tentacle-like appendages, which are so characteristic in *Cassiopea* were entirely absent, and the scales seemed to take their place. The general color-tone of the medusa was noticeably different from that which prevails in *Cassiopea*. There was a more transparent appearance, with the brownish yellow turned to olive-brown, and the

[1] Duerden, J. E., 1904. The Coral Siderastrea radians and its Postlarval Development. Carnegie Institution of Washington. Publication 20, page 6, footnote.

white markings upon the surface were much fewer. These points of difference were so important that it was concluded that this specimen must represent a different genus of Scyphomedusæ. It was determined to be *Polyclonia frondosa.* Much time was spent in trying to find other specimens of the same form without avail.[1] The single individual found must have grown up in the moat from a larva brought in accidentally by the tide. The habit of these creatures is so excessively sedentary that it is inconceivable that the adult creature could have been carried thither by ocean currents. There may, of course, have been others in the same location, but the most painstaking search failed to reveal them, and this in spite of the fact that so large a part of the bottom is visible from a boat in clear weather.

Oral scales.—These noticeable flakes of white serve to distinguish the species at a glance from the evenly yellow hue of *Cassiopea.* Their presence, together with the absence of the digitate yellow appendages of *Cassiopea,* is sufficient, to my mind, to separate the genera from one another. The shape of these scales has been described. They are scattered over the arms, between forty and forty-five on each arm. They serve as little lids, guarding the openings into the oscula or oral funnels. They are sensitive to touch, contracting and bending away from anything that touches them. This reaction serves to bring the scale over the opening of the oral pores, preventing the ingress of the disturbing object. There is nothing in *Cassiopea* which shows so great a degree of sensitiveness as these oral scales in *Polyclonia.*

Oral arms.—Another point of difference was to be noted in the appearance of the oral surface of the two forms of Rhizostome, viz, the relative shortness of these processes in *Polyclonia.* In the individual examined the arms were not visible projecting beyond the disk, as in *Cassiopea.* Their length was a little less than the diameter of the disk. In *Cassiopea,* on the other hand, the oral arms project beyond the margin.

Surface of disk.—There is a conspicuous band or ring of darker color in *Cassiopea,* three-fourths of the distance from the center to the margin of the smooth surface of the disk. It is just outside of a circle of large oval white spots, which are more or less sharply separated from one another, and it is bounded outwardly by a clean-cut band of whitish hue, which extends to the margin. This ring is slightly raised above the rest of the surface, and when a jelly-fish is put into a glass jar it usually applies this part to the surface of the glass, the disk inside the ring and the margin being left free. The center of the disk is slightly concave, and acts, in this attitude, as a cupping organ. If one tries to remove the creature from its position, it will be found to require a vigorous pull to dislodge the disk from its hold upon the glass. The cells of the dark ring secrete mucus, which aids in giving a firm hold to the disk.

[1] Dr. Mayer reports the finding of six individuals of this species in the moat during July, 1907.

In *Polyclonia,* on the other hand, it is impossible for the disk to assume any such shape as that just described for the other genus. There is no raised ring, no concave center, no mucous tissue. The top of the disk is quite flat and smooth.

Color pattern of Polyclonia.—Instead of the circle of 12 to 20 rounded spots which mark the inner portion of the disk in *Cassiopea,* we find that in *Polyclonia* the dark coloration of the center extends in eight broad rays nearly to the margin, and the spaces between the rays are evenly yellowish in hue. Close to the edge of the disk are many oval or round white spots, of small size and regular arrangement, one large one opposite each of the sense-organs, smaller ones distributed between them. This part of the surface in *Cassiopea* is, as has been indicated, entirely free from color markings.

Marginal sculpturings.—Whereas the margin of the disk is smooth in the case of *Polyclonia,* a characteristic arrangement of radial grooves, extending various short distances in from the edge, is noticeable in the other form under consideration.

Marginal sense-organs.—The marginal sense-organs in the two genera show a difference in number. The specimen of *Polyclonia* which was examined, measuring 76 mm. in diameter, bore 12 sense-organs, while in an average specimen of *Cassiopea* of this same size the number is 18. Even in the smallest specimens, less than one-third as large as the example of *Polyclonia,* 13 was the smallest number noted, and very many counts were made.

CASSIOPEAS FROM DIFFERENT LOCALITIES.

The writer has had the privilege of examining specimens of this genus from Jamaica, and has studied the characteristics of the specimens found in the Bahama Islands and at the Tortugas. In the first and last-mentioned localities the conditions were much the same, but in the Bahamas there was much less protection afforded the waters in which the medusæ were found. There was less of peculiarity in all the surroundings, the temperature of the water, storm influence, and food supply being normal for the shores of coral islands. The only points of difference to be noted in the medusæ are with reference to size and color-pattern. The average size of the Bahama specimens taken at the same time in the summer was considerably smaller than in the case of the others, and, while the markings, described by Bigelow as caused by the presence of zoanthellæ in the cells, were of the same general character, the spots and bands were less sharply marked.

The appended table gives the main features of the two species, *Cassiopea xamachana* and *Polyclonia frondosa.*

Comparison of morphological characters of Cassiopea xamachana and Polyclonia frondosa.

Cassiopea xamachana.

Color:
Yellowish-brown.
Pattern:
Concentric bands, light and dark. White spots near center. Margin white without spots.
Shape of disk:
Concave above, with raised circular band.
Appendages of oral arms:
Yellow digitate appendages. Not sensitive.
Oral arms:
Projecting beyond margin of disk.
Sense-organs:
Not less than 13 in adult.

Polyclonia frondosa.

Color:
Olive-brown.
Pattern:
Dark center without spots. No bands. Margin with numerous distinct spots.
Shape of disk:
Flat. No band.

Appendages of oral arms:
White scales guarding oral funnels. Irritable and contractile.
Oral arms:
Shorter than diameter of disk.
Sense organs:
12 in recently matured individual.

Zoological Laboratory,
University of Vermont.

EXPLANATION OF PLATES.

Plate 1.

Cladonema mayeri sp. n.

1. Medusa of *Cladonema mayeri.* × 25.
2. *Cladonema mayeri.* Aboral view.

Plate 2.

Cladonema mayeri sp. n.

3. Manubrium, much enlarged, showing gastric pouches, oral tentacles and bands of markings.
4. Top of bell. Dilations and branching of canals and pattern of white markings.
5. Top of abnormal bell, showing eleven canals arising from seven primaries.
6. Mouth and oral tentacles of medusa.
7. Hydroid stage of *C. mayeri,* showing two verticils of tentacles, lower set contracted.
8. Hydroid with tentacles fully extended. Bud in early stage of development upon column, showing granular endoderm.
9. Medusa-bud, further developed, with tentacles forming.
10. Medusa-bud with tentacles elongating. Oral view.
11. Medusa bud with tentacles developing. Oral view.

Plate 3.

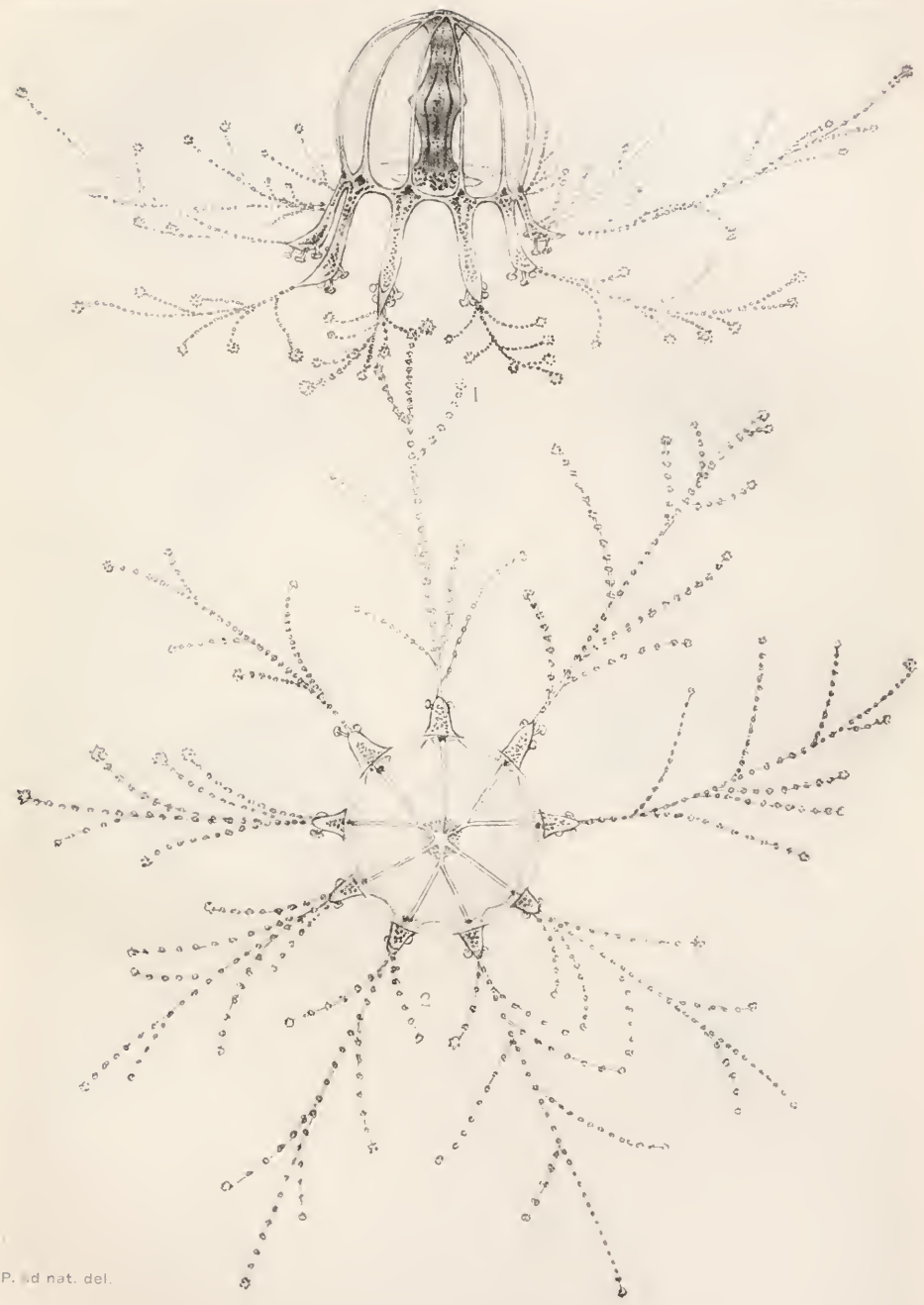
Campanularia and *Aglaura.*

12. *Campanularia macrotheca,* new species. Stalk with nutritive and reproductive zooids. × 100.
13. Empty hydrotheca of *C. macrotheca.*
14. *Aglaura ciliata,* new species. Enlarged.
15. Oral lappet of *A. ciliata.*
16. Manubrium of *A. ciliata,* sectional view.

Plate 4.

Cassiopea, Polyclonia, Cladonema.

17. *Polyclonia frondosa.*
18. *P. frondosa,* oral view.
19. *Cassiopea xamachana,* Bigelow.
20. *C. xamachana.* Oral view.
21. *Cladonema mayeri* sp. n. Side view. × 62. Photo. From life, by author.
22. *C. Mayeri.* Oral view. × 62. Photo. From life.



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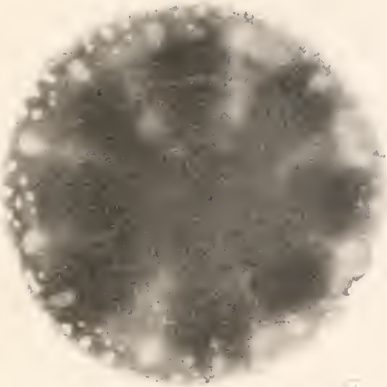

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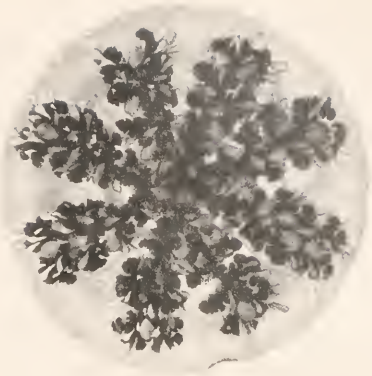

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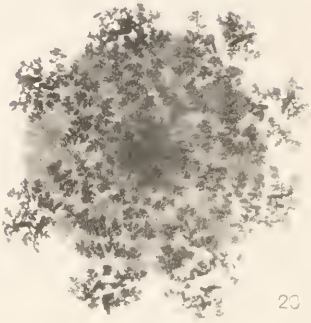

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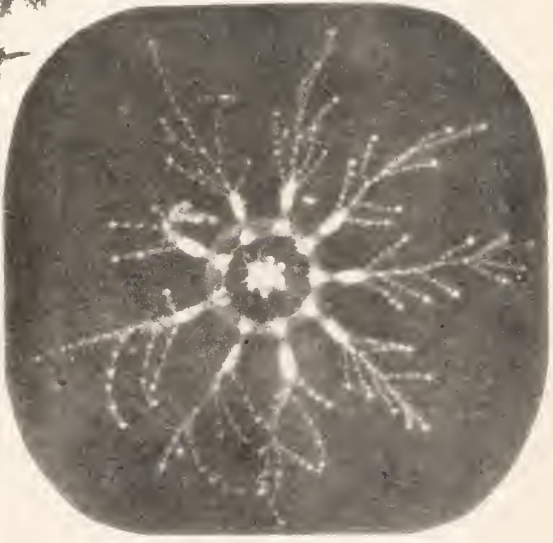

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IX. HELMINTH FAUNA OF THE DRY TORTUGAS

By EDWIN LINTON
Professor of Biology, Washington and Jefferson College

I. CESTODES

Plates 1–11

HELMINTH FAUNA OF THE DRY TORTUGAS.

By Edwin Linton.

INTRODUCTION.

The material upon which the following report is based was collected at the Marine Biological Laboratory of the Carnegie Institution of Washington, Tortugas, Florida, June 30 to July 18, 1906. A preliminary report was published in Year Book No. 5, of the Carnegie Institution of Washington, pp. 112–117.

This report, with a few emendations, follows:

REPORT ON ANIMAL PARASITES COLLECTED AT TORTUGAS, FLORIDA, JUNE 30 TO JULY 18, 1906.

In the table on pages 162, 163 will be found a list of the hosts which were examined for parasites, and a summary of the results of that examination, together with a few food notes. Where no food is recorded it is to be understood that either the alimentary canal was empty or the nature of its contents could not readily be identified.

While a more comprehensive search, extending over not only a greater range of species than is included in the accompanying list of hosts but also over a larger number of individuals under each species, is desirable, and would doubtless add very many species of parasites, enough, I think, may be learned from the table to warrant the following general remarks on the helminth fauna of the Tortugas.

I shall record also in this connection a few extracts from notes made at the time the material was collected.

Acanthocephala.—Representatives of this order appear to be rare at the Tortugas. The species found in the frigate mackerel was *Echinorhynchus pristis,* which seems to be eminently a southern form, since it was found to be the most frequently recurring species at Beaufort, while a closely related species has a similar distribution in the fishes of Bermuda.

Neither in the fishes of Beaufort, Bermuda, nor Tortugas have I found Echinorhynchi as abundant as in the fishes of northern waters. There thus appears to be the same contrast between tropical and northern forms shown in the distribution of the Echinorhynchi as in many other groups of organic forms. In this case, however, there does not appear to be a multiplication of species along with relative paucity of individuals, a condition which is characteristic of many tropical forms.

Nematodes.—But few nematodes were found. Those found in the nurse-

List of hosts examined for parasites in 1906, and summary of results.

Host.	No. of hosts examined.	Acantho-cephala.	Nematodes.	Cestoda	Trematoda.	Ectozoa.	Food notes, etc
Ginglymo-toma cirratum (nurse-shark).	3 large, 3 small, on five different dates.	.....	23, one species attached to wall of stomach.	8 species, represented by numerous to few individuals, in spiral valve.	....	One leech on tongue.	Small specimens with fish, crustacea, and annelids in the stomach; large specimen empty, except spine of sea catfish and fragment of nemertean.
Galeocerdo tigrinus (tiger-shark).	1 (9 feet)...	.....	.....	3 sp., very numerous in spiral valve.	.....	.....	Stomach contained two tin cans, one bottle, one large shark hook with swivel, and numerous fragments of Palinurus.
Carcharhinus platyodon (cub-shark).	1 (10 feet)	.....	.....	5 sp., few in spiral valve.	.....	.....	Lenses of fish eyes in intestine.
Dasyatis say (southern sting-ray).	1 (18 in. broad)...	.....	.....	9 sp., few in spiral valve.	....	2 isopods.....	Crabs in stomach.
Lycodontis moringa (spotted moray).	1 (2 feet).....	.....	.....	.....	1 sp , few in stomach.	.....	
Lycodontis funebris (black moray).	1 (4 feet).....	.....	1, in rectum	1 encysted on rectum.	3 sp., numerous in stomach and intestine.	.....	A spotted moray was ejected from the stomach. It had probably been swallowed after entering the trap.
Sphyræna barracuda (great barracuda).	3 large, 4 small...	.....	1 sp., immature, few.	.....	1 sp., few.....	.....	Fish in stomach of large specimens.
Chanodon pseudo-hispanicus (Spanish sardine).	33 on two dates ..	.....	Immature, very small, encapsuled, few.	1 encysted on viscera.	2 sp., few.....	2 copepods	Small annelids.
Tylosurus marinus (garfish).....	1 (18 in.)	.....	.....	.....	2.....	.....	Stomach empty, fish caught with fish bait.
Atherina laticeps (cabezote).....	33 on two dates ...	.....	.....	Larval forms encysted on viscera, few.	... .	Isopods, from mouth, few.	
Auxis thazard (frigate mackerel).	1.....	8.....	.....	Larvæ, few.....	2 sp , few.....	.....	
Epinephelus striatus (grouper)...	9 on five dates	.....	Few, immature ...	3 sp., larval and encysted	2 sp., few..... ...	.....	Fish and crabs.
Mycteroperca venenosa (yellow-finned grouper).	1.....	....	2, encapsuled.....	Many cysts on viscera.	.....	2 isopods on gills.	Fish.
Mycteroperca bonaci (black grouper).	1.....	.....	Few, immature ...	4 sp., encysted and larval	1 sp., numerous...	.....	

List of hosts examined for parasites in 1906, and summary of results.—Continued.

Host.	No. of hosts examined.	Acantho-cephala.	Nematodes.	Cestoda.	Trematoda.	Ectozoa.	Food notes, etc.
Lutianus griseus (gray snapper).	41 on six dates	1	3 sp., few	2 sp., larval and encysted.	4 sp., numerous		Fish (Atherina), crabs, isopods, spines of sea-urchins.
Ocyurus chrysurus (yellow-tail).	3		Few, immature	Few larval	1		
Hæmulon macrostomum (striped grunt, "porgy").	2 on different dates.		3, immature		2 sp., few		Annelids.
Hæmulon plumieri (white grunt)	35 on six dates		Few, immature		8 sp., mostly represented by few individuals		Crustacea and annelids.
Hæmulon sciurus (yellow grunt)	1		1, immature				
Leiostomus xanthurus (spot)	6 on three dates		2 sp., one adult, few of each.		1		Fish and algæ.
Abudefduf saxatilis (cow-pilot)	1				1		
Lachnolaimus maximus (hog-fish).	1		1		1		Broken mollusk shells and fragments of crustacea.
Chlorichthys bifasciatus	1		2 fragments				
Pomacanthus arcuatus (black angel-fish).	1				4 sp., one very numerous.		The very long intestine was filled with material browsed from the reef, mainly sponges.
Angelichthys isabelita (angel-fish).	1				3 sp., few		Alimentary canal filled with sponges, etc.
Thalassochelys caretta (logger-head turtle).	1				2 sp., one of them represented by 1 specimen, the other by over 3,000.		
Shrimp—common, on gulf-weed	12 on three dates					Isopod, near Phryxus, found on most of the shrimp.	

shark, a species of *Ascaris,* were firmly attached to the stomach-wall, their heads penetrating at least as far as the muscular layer.

Representatives of the genus *Heterakis* were found sparingly in the green moray, gray snapper, spot, and hog-fish. Some of those from the gray snapper and one from the spot agree closely with *H. foveolata.*

A species of *Ichthyonema* was found on three different dates in the ovaries of the gray snapper; one was also found in the gar.

Immature nematodes were found, usually encysted on the viscera, in the following fishes: Barracuda, yellow-grunt, yellow-tail, grouper, cabezote, white grunt, striped grunt, black grouper, yellow-finned grouper. In all cases the number of these immature nematodes was few. The most common type was characterized by having an elongated basal bulb on the œsophagus and a diverticulum from the anterior end of the intestine.

One very singular form was found in *Chlorichthys bifasciatus,* which had a subglobular, chitinous pharynx which was marked with spiral ribs running from left to right anteriorly, thus crossing in optical section.

Cestodes.—The larval forms usually referred to by the name *Scolex polymorphus* are not so abundant as they would be in an equal list of northern fishes. Only a few were seen and only in the gray snapper, yellow-tail, grouper, and frigate mackerel.

Encysted stages, belonging for the most part to the genus *Rhynchobothrium* were found in eight of the species of fishes examined. *R. speciosum* was recognized in a number of instances. Encysted cestodes were found only on the viscera. No cases of flesh parasites comparable with that of the butter-fish (*Poronotus triacanthus*) of the northern coast, or of the hound-fish (*Tylosurus acus*) of Bermuda, were met. The selachians here as elsewhere are bearers of many species of adult cestodes, whose favorite place of lodgment is in the spiral valve.

I had the opportunity of examining but one sting-ray and that a small specimen. It yielded, however, a list of nine species of cestodes belonging to seven genera. This list is as follows: *Acanthobothrium brevissime* sp. nov., *Anthocephalum gracile, Phyllobothrium foliatum, Spongiobothrium variabile, Symbothrium filicolle,* two species of *Rhinebothrium* and two species of *Rhynchobothrium.* It may be inferred therefore that the sting-ray, if a sufficient number were to be examined, would yield as long a list of entozoa as it does at Beaufort or Woods Hole.

Some interest may attach to the fact that one lot of parasites is credited to the tiger-shark in the table, although the shark from which they were obtained was not identified.

On June 2, before my arrival at the laboratory, a 9-foot shark was captured. Its spiral valve was opened and placed in 5 per cent formaldehyde. Upon examining this material I decided that it had come from a tiger-shark. As this is an unusual method of identifying a fish it may be worth while to record my reasons for having confidence in this identification. In the first place, the valve itself is of the same type as that of the tiger-shark. This fact, however, does not exclude the cub-shark, which is common in these waters. In the second place, the varied contents of the stomach (see table) agree with what has been recorded for this species (U. S. Fish Commission Bulletin for 1899, pp. 270, 271, 425).

Again, there were a large number of both adult and young and free ripe joints of the singular cestode *Thysanocephalum crispum.* In all the tiger-sharks which I have examined in the Woods Hole region I have found

this parasite abundant and varying from young specimens a few millimeters in length to adults with ripe segments and measuring as much as a meter in length. There were also large numbers of ripe proglottides free in the chyle of the intestines. Furthermore, I have never seen this cestode in its adult stage, in any other host than the tiger-shark.

Since tiger-sharks are rather common in the waters about the Tortugas this vicarious identification is probably correct.

In like manner the finding of the cestode *Discocephalum pileatum* in the cub-shark, while not justifying a change in any record of habitat, at least calls in question the validity of a former identification.

This species was based on four specimens obtained from material brought to the laboratory of the United States Fish Commission at Woods Hole, Massachusetts, July 19, 1886, and taken from what was reported to me to be a dusky shark (*Carcharhinus obscurus*). The viscera only were brought to the laboratory.

No other entozoa were found associated with them. Twelve specimens were found on another occasion in a shark which was identified as a dusky. They were associated with a few examples of *Anthobothrium laciniatum* and *Orygmatobothrium angustum*. In all other specimens of dusky shark which I have examined at Woods Hole I have found numerous cestode parasites. As a rule there were several different species, usually represented by numerous examples, in each shark. The same conditions were found to prevail in the dusky sharks which I examined in 1901 and 1902 at Beaufort, North Carolina.

The third find of *D. pileatum* was made in 1903, when I collected seven specimens from a cub-shark (*C. platyodon*) in Bermuda. In that case also the worms were not associated with any other cestodes, and the heads, as in the first instance, were firmly attached to the walls of the intestine. These conditions were repeated very closely in the cub-shark which was examined at Tortugas. The single specimen of *D. pileatum* was firmly attached to the intestinal wall, the disk-like head being embedded in the submucosa. There were, however, associated with this specimen, five other minute cestodes, representing four species and as many genera. They were *Anthobothrium laciniatum, Phoreiobothrium lasium, Otobothrium crenacolle,* and another which was not identified at the time of collecting and concerning whose systematic position I am not yet certain.

Leaving the species *D. pileatum* out of the account, it will be observed that two of the above species, viz, *A. laciniatum* and *P. lasium,* have been found in the dusky shark, both at Woods Hole and at Beaufort, and one other (*O. crenacolle*) at the latter place. While there is thus established a close resemblance between the cestode parasites of the dusky and the cub-shark, the species *D. pileatum* must, at present, be regarded as a parasite of southern range and of rare occurrence in the dusky shark.

Trematodes.—Beyond the preliminary examination made at the time of collecting, and often of necessity hastily given, the collection has not been studied.

From notes made during the preliminary examination it would appear that there are about 33 species, many of which are new. Of these, all but nine could be referred to the old genus *Distomum*. Three species of *Gasterostomum* were noted. Appendiculate distomes were seen in but two instances, one in the green moray and the other in the Spanish sardine. Those from the moray were numerous and resembled the form which I have been

recording under the name *D. monticelli;* those from the sardine were few and agree with *D. appendiculatum.*

Many of the species are represented in the collection by but one or at most few specimens, and it may be advisable to refrain from giving them names until more material is secured.

A distome, probably represented by more than one species, found in most of the lots of gray snappers, grunts, and groupers, is unique in that the ova, as they lie in the folds of the uterus, present a wreath-like appearance, and each ovum has a long, slender filament, such as is common on the ova of monogenetic trematodes.

Trematodes were found in large numbers in only two instances, a black angel-fish, examined July 18, and a loggerhead turtle, examined July 1.

In general it may be said that the trematode fauna of Tortugas is rich in species.

Ectozoa.—Parasitic Isopods were found on the sting-ray, cabezote, yellow-finned grouper, and a small shrimp common in the gulf-weed.

Parasitic Copepods were found on only one fish, the Spanish sardine.

One leech,[1] colored vivid green and red-brown with blotches of white, was found on the tongue of a nurse-shark.

General Observations.—The groupers of the Tortugas, like those of Bermuda, especially the older specimens, are characterized by having more or less abundant cysts on the viscera and often in the walls of the stomach and intestine. These cysts are, as a rule, dark brown, often nearly black. The color is due to the abundant pigment which is deposited in the cyst. While these cysts are more often than otherwise due to cestodes, accumulations of pigment and degenerate connective tissue were also found associated with other entozoa, viz, nematodes and acanthocephala in Bermuda, and nematodes in Tortugas.

It is perhaps worthy of remark that the great barracuda, which is a very voracious and predatory fish, appears to harbor but few parasites, either as a final or intermediate host. This conclusion is warranted also from the results of the examination of 5 barracuda in Bermuda in 1903. The largest Tortugas specimen measured about 1.5 meters in length; the Bermuda specimens were about one-half that length.

It would be of interest to know whether the apparent immunity from parasites of the barracuda and other fish is correlated in any way with the digestive ferments.

I take advantage of the opportunity offered by the passage of the proof of this paper through my hands to add the following extract from the preliminary report on the results of my work at the Tortugas laboratory in the summer of 1907 (Year Book No. 6, p. 114).

No selachians were taken while I was at the laboratory this year. One small nurse-shark (*Ginglymostoma cirratum*) had been taken before my arrival, from which were obtained a few specimens of a species of *Ascaris*, not found last year. After I left the laboratory, Mr. Davenport Hooker collected from a cub-shark (*Carcharhinus lamia*) some cestodes, which I have since examined. As I have already made a report on the cestodes

[1] This has been identified for me by Dr. J. Percy Moore as *Pontobdella muricata*, a widely distributed parasite of selachians.

collected in 1906, I take this opportunity of recording the cestodes from this species of shark, which is not included in last year's list:

Crossobothrium angustum, or near it; 3 scoleces and a few fragments from the spiral valve.
Phoreiobothrium lasium, 15, from the spiral valve.
Otobothrium penetrans, 1 adult from stomach. This name was given to an immature form found in the flesh of the gar (*Tylosurus acus*) in Bermuda. This is therefore the first record of the adult of this species.

In the Year Book of the Carnegie Institution for 1906, page 116, I stated that the spiral valve of a shark which had been kept in formalin until my arrival at the laboratory belonged to a tiger-shark (*Galeocerdo tigrinus*). As the identification was a somewhat unusual one, being based in large part on the character of the stomach contents and the entozoa, I embrace this opportunity of confirming the identification. Having learned from Dr. Mayer that the jaws of the shark in question had been sent to the museum of Harvard University, I wrote to Professor Samuel Garman, who replies that the jaws are the jaws of *Galeocerdo tigrinus.*

In the present paper only the cestodes of the collection are described.

A more critical study of the material than was possible at the time of collecting reveals that the cestodes from the nurse-shark are, for the most part, new, as is the case also with a nematode from the same host, a species of the genus *Acanthocheilus,* which will be described in a subsequent paper.

It has been found necessary to establish a new genus, *Pedibothrium,* to accommodate certain cestodes found in the nurse-shark. This genus is represented by three distinct species.

The species of *Acanthobothrium,* which was recorded in my notes at the time of collecting as *A. paulum,* proves to be a new species. Several species of the genus *Rhynchobothrium* were found, very few of which could be referred to any known species.

It has long been recognized that the hooks on the proboscides of the Tetrarhynchidæ are indispensable in determining the species. This makes it extremely difficult to identify species in this family, since it is frequently impossible to get the specimens to unroll their proboscides. Even when the scoleces have been made transparent, so that the hooks in the inverted proboscides can be seen, it is usually not possible to make out their arrangement, and unless there is something characteristic in the outlines of the hooks, one must often remain uncertain about the species where the everted proboscides have not been seen.

Again, the appearance of a given proboscis at different levels may be very different. It follows that species which have been described and figured when only the basal portions of the proboscides were seen, may not be recognized when examples are seen for the first time with proboscides completely unrolled.

So far as my observation extends, there is little variation in the arrangement of the hooks in the individuals of a given species, although, at present,

I am inclined to allow considerable variation in the sizes of corresponding hooks in different individuals.

It is, perhaps, worthy of note that, while I have found the encysted stage of *R. speciosum* in several hosts, especially the groupers, both at Tortugas and Bermuda, I did not find the adult of this cestode at either place. The number of sharks examined was so small, however, that this apparent absence of adults is not significant.

The large number of cestodes in contrast with the small number of individuals is significant and agrees with the characteristics, in this particular, of tropic fauna in general.

The multiplication of species of the genus *Rhynchobothrium* is distasteful to the writer, but seems to be unavoidable, as will appear when the figures of characteristic hooks are consulted.

It is to be hoped that another season's collecting will result in the addition of sufficient material to permit of a study of the comparative anatomy of the proglottides. Reports on the trematodes, nematodes, and acanthocephala will be made as soon as the material can be studied.

It is a genuine pleasure to acknowledge in this place the unfailing courtesy and interest shown by the director of the laboratory, Dr. Alfred G. Mayer, as well as the assistance which was given so freely by my fellow-workers in the laboratory, in the way of supplying me with material for study. I am especially indebted to my friend, Dr. Ulric Dahlgren, for many valuable favors.

DESCRIPTION OF SPECIES.

1. Dibothrium sp.

(Plate 1, figs. 1 and 2.)

The only representative of this genus seen was a larval form, a few examples of which were found encysted on the viscera of *Atherina laticeps*, June 30.

The cysts were elongated and filled with yellowish granular plastic material, which surrounded the elongated larva. The parenchyma of the larva contained numerous calcareous bodies, which were relatively large, especially at the posterior end.

The following dimensions, in millimeters, are of the living specimen: Cyst, length 2.8; diameter, anterior 0.47, middle 0.70, posterior 0.35. Larva, length 2.60; diameter, anterior 0.32, middle 0.42, posterior 0.28; length of anterior part, head 0.30.

A specimen (fig. 2), which had been fixed over the flame, under pressure, and afterwards stained with hematoxylin and mounted in balsam, exhibits a peculiar glandular structure much like that noted in a *Rynchobothrium* from the sand-shark (Proceedings of the National Museum, vol. XIX, p. 797, plate 63, figs. 14, 15. See also Bull. U. S. Fish Com. for 1899,

p. 300, plate 42, fig. 100; Pintner, Sitzungsbr. der kaiserl. Akad. der Wissensch., Bd. CXII, p. 563, Taf. 1, fig. 1). In this specimen, which is about 3 mm. in length, these structures are not found in either the anterior fifth or the posterior sixth. Anteriorly they are very closely crowded together, posteriorly they are less crowded, and the pyriform shape and racemose clustering can be seen.

These larvæ probably represent the encysted stage of a cestode which is adult in the tern or some other fish-eating bird.

2. Anthobothrium laciniatum Linton.

Report Commissioner of Fish and Fisheries, 1887, pp. 754–759, plate III, figs. 10–13, and plate IV, figs. 1–3. Proc. U. S. Nat. Mus., vol. XX, p. 439. Bull. U. S. F. C. for 1899, p. 411. Bull. Bureau of Fisheries, vol. XXIV, pp. 339. 343.

One example of this species was found in the spiral valve of a cub-shark (*Carcharhinus platyodon*), July 12.

Dimensions, in millimeters, of living specimen: Breadth of head 0.23; bothrium, variable, length, at rest, 0.35, breadth 0.23; neck, length 0.28, breadth 0.07; first segment, length 0.14, breadth 0.07. This specimen, mounted in balsam, has the following measurements: Length 3.5; distance to first laciniæ 0.25; length of first segment 0.11.

There are about eight laciniate segments. Behind these the segments become more and more crowded, the laciniæ become indistinguishable, and the segments are represented by transverse striæ about 0.014 mm. or less apart, and making the margins bluntly serrate. There is continuity, however, from the laciniate anterior segments through the compressed segments to the larger segments at the posterior end. Of these there are six, averaging about 0.16 mm. in length and 0.08 mm. in breadth. The greatest breadth of the strobile is in the region of compressed segments, where it is 0.26 mm. in breadth.

3. Rhinebothrium flexile Linton.

Report U. S. F. C., 1887, pp. 768–771, plate V, figs. 3–5. Bull. U. S. F. C., 1899, pp. 275, 433. Bull. Bureau of Fisheries, vol. XXIV, pp. 342, 347.

One specimen was found in the southern sting-ray (*Dasyatis say*), July 10, with numerous loculi, which appears to belong to this species. Unfortunately the specimen was lost before further notes were made on it.

4. Rhinebothrium sp.

(Plate 1, figs. 3 and 4.)

A few specimens found in the southern sting-ray (*Dasyatis say*), July 10, resemble a species found in this ray at Beaufort, North Carolina. A brief description was published in the Bulletin of the Bureau of Fisheries, vol. XXIV, p. 347, No. 5. The condition of this material is such that the bestowal of a specific name seems to be hardly justified.

The bothria are mounted on distinct cylindrical pedicels, and appear as if hinged in the middle. There are twelve loculi visible in a side view. There are therefore probably ten pairs of loculi with an odd loculus at each end, making 22 loculi on each bothrium. The neck is distinct from the body and in life had two small red pigment spots at the base. Pedicels and neck minutely spinose. Fine transverse lines occur very close behind the neck, preceding the first segments, which are very short. The succeeding segments increase in length until they are about as long as broad, then increase in length but decrease slightly in breadth. The last segment seen measured 0.35 mm. in length and 0.07 mm. in breadth. Another strobile was about the same breadth throughout.

Dimensions, in millimeters, of specimen mounted in balsam: Length 1.28; length of bothria 0.20; length of pedicel 0.11, diameter 0.05; length of neck 0.14; diameter of neck, anterior 0.05, base 0.07; breadth of body just behind neck 0.08; last segment, length 0.35, breadth 0.08.

5. Spongiobothrium variabile Linton.

Report U. S. F. C., 1886, pp. 462–464, plate II, figs. 13–16. Report U. S. F. C., 1887, pp. 778–780. Proc. U. S. Nat. Mus., vol. XX, p. 442. Bull. U. S. F. C., 1899, pp. 275, 432. Bull. Bureau of Fisheries, vol. XXIV, p. 347.

Four specimens were found in the spiral valve of a sting-ray (*Dasyatis say*), July 10. The bothria of these specimens were in unusually fine condition, making possible the following note on their structure: The bothria of the living worm are very flexible and bear some resemblance to those of *Rhinebothrium*. They are without costæ, but are provided with numerous loculi along the margins. When they are placed in alcohol they assume the characteristic crumpled appearance of the type.

6. Phyllobothrium foliatum Linton.

Report U. S. F. C., 1887, pp. 787–794, plate VI, figs. 5–10. Proc. U. S. Nat. Mus., vol. XX, p. 443. Bull. U. S. F. C., 1889, pp. 275, 433. Bull. Bureau of Fisheries, vol. XXIV, pp. 340, 347.

One strobile, with the scolex missing, was found in the spiral valve of the sting-ray (*Dasyatis say*), July 10.

7. Discocephalum pileatum Linton.

(Plate 1, fig. 8.)

Report U. S. F. C., 1887, pp. 781–787, plate X, figs. 1–7. Year Book of Carnegie Institution of Washington for 1906, p. 116.

One specimen was found in the spiral valve of the cub-shark (*Carcharhinus platyodon*), July 12.

The worm was very flat and thin and the muscular head was firmly embedded in the intestinal wall; color white, except the corrugated neck, which was olivaceous. The posterior segments contained ripe ova, which were of a faint greenish tint. On the morning of the 13th they were seen to

be segmenting. A few of the early segmentation stages are shown in fig. 8.

A few measurements, in millimeters, were made on the living worm with the following results: Length 178; diameter of head 4.5; greatest breadth of body 5, about the middle; last segment, length 2.5, breadth 2.25, with ripe ova, which were 0.035 and 0.032 in the two principal diameters.

8. Anthocephalum gracile Linton.

Report U. S. F. C., 1887, pp. 794–796, plate VII, figs. 1–2. Bull. U. S. F. C., 1899, pp. 275–411. Bull. Bureau of Fisheries, vol. XXIV, p. 347.

Twelve specimens were found in the spiral valve of the sting-ray (*Dasyatis say*), July 10.

The following measurements, in millimeters, were taken from a specimen mounted in balsam: Length 6; diameter of head 0.040; breadth of body just behind head 0.11; last segment, length 1.49, breadth 0.11. Strobile linear.

9. Undetermined Cestode.

(Plate I, figs. 5, 6, 7.)

One immature strobile from the spiral valve of the cub-shark (*Carcharhinus platydon*), July 12, probably belongs to a new genus. It was nearly dark when this specimen was collected, and it was not in good enough condition to risk keeping it in sea-water over night. The appearance of the living worm was that of having a lappet at the posterior end of each bothrium. In the preserved and mounted specimen this proves to be a loculus produced by a transverse costa near the posterior end of the bothrium. Each bothrium is also provided with a single auxiliary acetabulum at the anterior end. There are no hooks on the bothria. So far as I know, there is no genus of cestodes in which the bothria are without hooks and at the same time possess auxiliary acetabula and costa.

The strobile is filiform, with ten segments, all of which are singularly long and slender. The neck is also long and slender, and both neck and segments are finely serrate. The reproductive apertures are marginal and are situated at about the posterior third. The last segment is attenuate at the posterior end.

Dimensions, in millimeters, of the living worm: Length 15; breadth of head (variable) 0.4; bothrium (variable), length 0.29, breadth 0.19; distance to first segment 5.8; first segment, length 0.35, breadth 0.12; last segment, length 1.40, breadth 0.15. In balsam the diameter of the head is 0.40, while that of the neck near the head is 0.08.

PEDIBOTHRIUM[1] gen. nov.

Body tenuiform, articulate, head separated from the body by a distinct neck, and provided with four distinct, cruciform, armed bothria, without auxiliary suckers, costæ or loculi.

[1] πέδιος = even, *i. e.*, without costa.

Each bothrium is strengthened by a strong muscular ring, with a thin, more or less leaf-like border, and is armed at the anterior end with a pair of compound hooks. Each hook consists of two unequal prongs, which rise from a flattened base. This basal part of the hook has a characteristic shape in each species. The neck is traversed by conspicuous bundles of longitudinal muscle fibers.

This genus is separated from the genus *Acanthobothrium* by the absence of costæ, and from *Phoreiobothrium* by the character of the hooks, which have two instead of three prongs, and further by the absence of loculi on the bothria.

The species *P. globicephalum* suggests in its general habit of body the genus *Onchobothrium*, but there are no costæ on the bothria, as in that genus.

It is worthy of note that the hooks of *P. globicephalum* closely resemble those figured by some authors, *e. g.*, Zschokke, for *Onchobothrium uncinatum*.

Diesing's genus *Cylindrophorus*, based on Wagener's *Tetrabothrium* sp., is suggested as being possibly near this, but the character of a tubular bothrium, as that must be understood from Wagener's figures, indicates an essentially different structure from that shown by this genus.

In like manner Diesing's genus *Prosthecobothrium* (*Bothrium cornutus* Duj., *Onch. coronatus* Duj., *Acanthobothrium*, Dujardin, van Beneden, etc.), while resembling it in the absence of costæ and in the presence of forked hooks on each bothrium, differs in having a foliaceous appendage on the posterior end of each bothrium.

10. Pedibothrium globicephalum gen. et sp. nov.

(Plate 2, figs. 9-16.)

Head, especially in preserved specimens, globular. Bothria ovate, projecting in front of hooks, and supplied with prominent marginal border; each armed with a pair of small two-pronged hooks. The prongs are only moderately curved and are of unequal size, the inner one being the shorter. The common base is somewhat elongated. The neck is distinct, but the first segments begin as faint transverse lines at a distance from the head equal to three or more times its length. Strong muscular bundles lie in the neck near the head.

The first segments are broader than long, then squarish, then longer than broad, with rounded angles. Ripe segments much longer than broad, in some cases slightly narrowed at the extremities, especially the anterior. Genital cloaca on lateral margin, a little behind the middle, vagina in front of the cirrus, at first at right angles to the axis of the segment, then parallel with it to the paired ovaries near the posterior end of the segment.

The vitelline glands form a marginal border throughout, except at the extremities. As a rule they extend but a short way back of the ovaries.

One free segment was noticed, which appeared to belong to this species, in which there was a slight elongation of the postovarian region, as in *P. brevispine*.

The uterus is spacious and lies between the ovary and the angle of the vagina. The ova are amber color, thin-shelled, mostly collapsed, and consequently difficult to measure. The cirrus is long, slender, enlarged at the base, with exceedingly minute spines, if any. Testes numerous, occupying the middle space in front of the vagina. Cirrus pouch behind vagina and in its angle, but most of the coils of the vas deferens are in front of the vagina. Length in life as much as 60 mm.

Dimensions of a mounted specimen in millimeters: Length 30; head (compressed), length 0.96, breadth 0.96; bothrium, length 0.80, breadth 0.40; breadth of neck 0.56; distance to first segment, about 1.6; first distinct segment, length 0.04, breadth 0.6; mature segments, or maturing segments, length 0.80; breadth 0.40; free segments with ripe ova, length 1.8, breadth 0.6; length of hooks 0.035; ova about 0.025 and 0.018 in the two principal diameters.

This species was found on three occasions in the spiral valve of the nurse-shark (*Ginglimostoma cirratum*).

July 6, sixteen, in middle of spiral valve and a little below the middle, longest about 60 millimeters.

July 15, two, small. The hooks agree with those collected on the 6th, but the worms are much smaller. Length 16 millimeters.

July 18, three, smallest 30 millimeters in length, longest 35. Hooks very small, almost obsolete.

11. Pedibothrium longispine gen. et sp. nov.

(Plate 3, figs. 17, 18, 19.)

Bothria in life elongate, with crenulate borders in fresh specimens, flexible, often reflected; at rest and in alcoholic specimens usually longer than broad, projecting but little in front of hooks, but in life probably capable of being protruded so as to make a small cup. Free margin outside of muscular ring narrower than in the other species. Hooks relatively long, in some cases equal to half the length of a bothrium. The two hooks on each bothrium have their bases apposed and projecting forward to the anterior end of the bothrium. The two prongs on each hook are long as compared with the oblong base and are strongly recurved; the outer prong is about twice the size of the inner, and both are curved in the same manner, so that the two would lie in the same curved surface and be nearly parallel. The character of the hooks may be best understood from the figures. The neck exhibits various contraction stages in life, but at rest appears to be slightly larger than the succeeding part of the strobile. In the mounted specimens it was seen to be minutely spinose and distinct from the body,

with strong longitudinal muscle bundles of relatively coarse strands. Strobile, so far as certainly seen, filiform. First distinct segments about as long as broad, nearly circular, so that the first five, in one specimen, made a moniliform portion of the strobile; succeeding segments rod-shape, very much longer than broad, and rather loosely attached, margins finely crenate. Details of the anatomy were not certainly made out for ripe segments, but are probably much like those of *P. brevispine*. The two species may be distinguished from each other by means of the hooks, which present quite marked differences besides that of size.

From the nurse-shark (*Gingliostoma cirratum*), July 2, two; July 5, six. All small, with no mature segments.

Dimensions of living specimen in millimeters: Head, length 0.35, breadth 0.35; bothria, length 0.35, breadth 0.21; length of hooks, base not included, larger 0.06, smaller 0.03; diameter of neck 0.09; distance to first distinct segment 0.42; first segment, length 0.07, breadth 0.07; number of segments 9; last segment, length 0.63, breadth 0.06. In two mounted specimens the length of the bothrium in each was 0.35, and the hooks, including the base, 0.15.

12. Pedibothrium brevispine gen. et sp. nov.

(Plate 3, figs. 20–22; plate 4, figs. 23–25.)

Bothria much as in *P. longispine*, except that, in the alcoholic specimens at least, they project farther in front of the hooks, and the free margins of the bothria are perhaps wider. The hooks are much smaller than those of *P. longispine*, and the prongs are unequal and unequally curved. The outer prong of each pair is curved much as in that species, but the inner prong is nearly straight and abruptly enlarged at the base. The common base of the two prongs of a hook is irregularly triangular. Neck distinct, with very conspicuous muscle bands, and is minutely spinose.

There appears to be a considerable variety in the strobiles, some being short, with relatively few segments, others longer, with many immature segments. In the longer strobiles the last segments are no farther advanced in the development of the reproductive organs than those on the shorter strobiles.

The first segments broader than long, and, especially in the shorter type of strobile, have a tendency to become moniliform. Later they are much elongated, becoming six or more times as long as broad. The free segments are long-fusiform, eight or more times as long as broad, the posterior end being the more slender. The vagina and cirrus have their common aperture on the margin a little behind the middle. The vagina opens in front of the cirrus, proceeds almost at right angles to the long axis of the segment to the median line, then turns almost at right angles and passes near the median line to the ovaries. The ovaries are paired and lobed organs and lie nearly half-way between the reproductive cloaca and the posterior end. The vitel-

laria form a narrow band near the margin, and extend from near the anterior end quite to the posterior end.

Behind the ovaries the segment usually narrows and the only reproductive organs there represented are the vitellaria.

The uterus lies in the median region between the ovaries and the angle of the vagina. The ova are elliptical, amber-colored, with rather thin shells, and are about 0.023 by 0.013 mm. in the two principal diameters.

The cirrus is long and slender and is armed with exceedingly minute spines. The cirrus pouch lies in the angle of the vagina, but the vas deferens, for the most part, lies in front of the vagina.

The testes occupy the median portion of the segment in front of the reproductive aperture nearly to the anterior end, and are bordered by the marginally placed vitellaria.

The extreme anterior end of the proglottis is sometimes rounded and slightly constricted. There is always a short anterior portion which contains no genitalia.

Dimensions of living worm in millimeters: Length 5 to 10; head, length 0.28, breadth 0.28; diameter of neck 0.11; length of hook 0.064. In a specimen mounted in balsam the length of a bothrium was 0.26, its hooks 0.10; another, bothrium 0.26, hook 0.07. The base of the hook is here included.

From the nurse-shark (*Ginglimostoma cirratum*), July 15, numerous; July 18, several.

13. Acanthobothrium brevissime sp. nov.

(Plates 4, figs. 26–29.)

This specific name is used to accommodate a few very minute cestodes collected from the spiral valve of the sting-ray (*Dasyatis say*), July 10.

Bothria with characters of the genus, that is, with two transverse costæ, a pair of two-pronged hooks, and a triangular cushion in front of the hooks. The first segment begins at once, without being preceded by any noticeable transverse lines or divisions of any kind, and the following segment is adult, with a well-developed cirrus pouch and relatively large testes.

In the specimen which was sketched (fig. 26), the enlargement which is shown at the base of the neck is evidently due to contraction. Since this part contains the rudiments of reproductive organs, it is to be regarded as the first segment.

Dimensions of specimens mounted in balsam, in millimeters: Length 1.40; head, length 0.20, breadth 0.11; diameter of neck 0.04; length of neck, from the head to the point where it merges into the body, 0.42; length of last, and, in this specimen, the only segment, 0.60; breadth, anterior 0.11, middle 0.12, posterior 0.07; hooks, all more or less broken, about 0.05. The cirrus pouch is at about the middle of the length.

As these worms were thought at the time of collecting to be small examples of *A. paulem,* few notes were made of the living worms, and the

mounted material does not show as many details of structure as could be desired.

14. Phoreiobothrium lasium Linton.

Report U. S. F. C., 1886, pp. 474–476, plate IV, figs. 24–29. Report U. S. F. C., 1887, pp. 819–820. Proc. U. S. Nat. Mus., vol. XX, p. 447. Bull. U. S. F. C., 1899, pp. 272–273, 426, 427, 428. Bull. Bureau of Fisheries, vol. XXIV, pp. 340, 343.

One specimen was found in the spiral valve of the cub-shark (*Carcharhinus platyodon*), July 12.

Dimensions, in life, in millimeters: Length 9.8; bothrium, length 0.50, breadth 0.20; breadth of head 0.42, of neck 0.16, narrowing to 0.12 at a distance of 0.35 from the head, then enlarging again; first distinct segment 4.9 back of head, but indications of segments in front of this; first segment, length 0.35, breadth 0.22; last segment, length 0.84, breadth 0.36; length of longer prong of hook 0.10, of shorter prong 0.03.

15. Thysanocephalum crispum Linton.

Report U. S. F. C., 1886 (*Phyllobothrium thysanocephalum*), pp. 464–468, plate II, figs. 1–12. Report U. S. F. C., 1887, pp. 823–824. Report U. S. F. C., 1888, pp. 543–556, plates LXI–LXVII, figs. 1–43. Bull. U. S. F. C., 1899, pp. 271, 426. Year Book of Carnegie Institution of Washington for 1906, p. 116.

Found in what was presumably a tiger-shark (*Galeocerdo tigrinus*).

This shark was captured on June 2, before my arrival at the laboratory, and the spiral valve was preserved in formalin. While the shark had not been identified, the type of the spiral valve, the nature of the stomach contents, and, particularly, the presence of this entozoan in great numbers, both of large and small examples, all point to the tiger-shark as the host (see pp. 164, 167).

These worms were found to be very numerous, large and small together, and still attached to the mucous membrane of the spiral valve. The folds of the pseudoscolex are preserved expanded and are in an unusually fine state of preservation. This condition is the result of the intestines having been placed in formalin while the worms were still adhering to the intestinal walls. Not only do the specimens exhibit the structure of the pseudoscolex better than would have been the case if the worms had been detached before they were placed in the preserving fluid, but they also illustrate the mode of attachment of this singular worm to its host. When this parasite attaches itself to the intestinal wall the minute head penetrates the mucous membrane while the fimbriated folds of the pseudoscolex are spread widely, thus making an adhering, and probably, at the same time, an absorbing organ.

One of these scoleces was mounted in balsam. The diameter is 9 mm. There are eight pairs of primary divisions of the pseudoscolex. These are simply outgrowths of the anterior end of the strobile, being preceded

by the head and neck, which are so small as to be easily overlooked by the collector. In its expanded condition this peculiar organ is a disk with fimbriated edges. Each of the primary divisions has a tendency to divide, some of them nearly to the base. The diameter of the central, undivided part is 4 mm. The radiating divisions and the undivided central portion are all profusely frilled and folded. The minute scolex is often lost in detaching the worm from the mucous membrane of its host.

16. Scolex polymorphus Rudolphi.

Report U. S. F. C., 1886, pp. 3, 4, plate VI, figs. 8, 9. Proc. U. S. Nat. Mus., vol. XIX, pp. 789–792, plate I, figs. 4–15. Bull. U. S. F. C., 1899, pp. 270–284, and 413, etc., noted under 28 hosts. Bull. Bureau of Fisheries, vol. XXIV, p. 332, etc., noted under 34 hosts.

The literature of this title is very extensive. Without doubt it has been used as a specific name to designate the larvæ of a great variety of cestodes belonging to many different genera. It is a convenient term, however, and in my papers is to be understood to refer to small larval cestodes found free in the alimentary canal and bile-duct of many fishes.

Since these forms are evidently possessed of great powers or resistance to the digestive juices of fish in general, they doubtless often pass a longer or shorter time of sojourn in each of many hosts, related to each other as eater and eaten. Ultimately they attain the adult state in some selachian.

These larvæ were not found in many of the Tortugas fishes which were examined in the season of 1906, nor were they at all abundant in those situations in which they were found.

Following is a list of the finds of this larva:

July 7.—A few small larvæ were found in the intestine of a grouper (*Epinephelus striatus*). The bothria were without costæ, and there was no red pigment in the neck.

July 10.—Several larvæ were observed in washings from the alimentary tract of a frigate mackerel (*Auxis thazard*). These were small, active, with no red pigment, but with a distinct costa on each bothrium.

July 11.—Several small larvæ were seen in washings from the alimentary canal of a black grouper (*Mycteroperca bonaci*).

July 5 and 9.—A few were found on the first date and several on the second in the gray snapper (*Lutianus griseus*). They were small, active, with prominent and distinct anterior sucker, and simple bothria. The water vascular system was distinct, especially at the posterior end.

July 6.—A few were found in the yellow-tail (*Ocyurus chrysurus*). These were small, with the rudiments of a costa on each bothrium, and two small red pigment spots in the neck. They were very active.

17. Rhynchobothrium speciosum Linton.

(Plate 11, figs. 78, 79.)

Proc. U. S. Nat. Mus., vol. XIX, pp. 801–805, plate LXIV, figs. 13, 14, and plate LXV, figs, 1–7. Bull. U. S. F. C., 1899, p. 413, etc., noted in 11 hosts. Bull. Bureau of Fisheries, vol. XXIV, pp. 369, 373, 384.

This species is comparatively easy to recognize on account of the highly characteristic arrangement of the hooks. The encysted stage only was found. The following notes were made at the time of collecting:

1. *Epinephelus striatus.*

July 7.—Elongated cysts, colored with brown pigment, were found on the liver and mesentery. They were left over night in sea-water, and on the following morning five larvæ had crept out of the cysts.

July 11, several; July 12, two. Long-pyriform cysts with dark pigment were found on the viscera. The blastocysts (*plerocerca*) were very active after they had been freed from their cysts.

Dimensions of a living larva, in millimeters: Length 40; breadth, varying with the length, about 1 when the length was 40; length of head and neck 7; length of bothria 0.84; breadth of head, flattened, 1.12; diameter of neck, flattened, anterior 0.77, at bulbs 0.84; contractile bulbs, length 1.40, breadth 0.14; proboscis, length 3.5, diameter, near base, exclusive of hooks, 0.068.

There is much variety of size and shape of bothria and neck in the alcoholic specimens.

Many cysts, dark-brown and filled with waxy degenerate tissue, were found in the stomach wall of the grouper on July 7 and 8, some of which may be due to this parasite.

2. *Mycteroperca venenosa.*

July 18.—Many elongated cysts were found on the viscera. These cysts were all very dark-brown, some of them even almost blue-black. One larva was released and proved to belong to this species.

3. *Mycteroperca bonaci.*

July 11.—Several large, long-pyriform cysts were found on the viscera. Most of these cysts were dark-brown, slightly iridescent, and associated with mats or tangles of filiform cysts which had been occupied by immature nematodes.

A small larva from this lot was thought at the time of collecting to be specifically different from the larger specimens, but after mounting the worm in balsam and studying the hooks, I have concluded to record it under this species. While it is much smaller than the others, the arrangement of the hooks is in close agreement.

Diameter of proboscis, exclusive of hooks, in millimeters, 0.04; length of largest hooks 0.021, as against 0.05, the usual size.

4. *Lutianus griseus.*

July 5.—One small larva from this host, while much smaller than usual for this species, agrees so closely with it in the arrangement of the hooks that it also is recorded here.

Another like it was collected on July 10.

Dimensions of living specimen, slightly compressed, in millimeters: Length 3.64; head, length 0.49, breadth 0.63; neck, length 1.96, breadth, anterior, 0.35; bulbs, length 0.51, breadth 0.14; proboscis, length, estimated, 1.40, diameter, exclusive of hooks, 0.04; length of largest hooks, about 0.021.

18. Rhynchobothrium simile sp. nov.

(Plate 5, figs. 30–37, and plate 6, fig. 38.)

This tetrarhynch belongs to the group of comparatively large forms, represented by *R. imparispine* and *R. speciosum,* to which it bears a close resemblance. It resembles the latter in the general habit of the scolex and neck, but is more like the former in the character of the hooks. Indeed, at the time of collecting it was thought to belong to that species, and it was not until the hooks were examined critically that the great difference between them and those of *R. imparispine* was revealed. The general character and arrangement of the hooks may be seen in the figures.

Bothria, in alcoholic specimens, about as broad as long, emarginate on the posterior border, with margins raised so as to make the face a deep cup; in marginal view distinct from each other, the posterior ends flaring slightly. Head in both marginal and lateral views wider than neck. The bothria are attached on those sides of the head which correspond to the flat surfaces of the strobile. Contractile bulbs rather long and slender, the retractor muscle of the proboscides being attached at the posterior ends of the bulbs.

The first segments begin near the base of the neck, at first as faint transverse lines, then as distinct segments much broader than long, lengthening posteriorly, soon becoming squarish, and ultimately longer than broad. The entire strobile is linear and the segments squarish. In a typical specimen, measuring 50 mm. in length, the last segment was 2 mm. in length and 1.5 mm. in breadth. The average length of the last four segments was a little less than 2 mm.

The genital cloaca is at about the posterior third, and is a shallow notch with abrupt sides. In glycerin the segments show longitudinal striæ like those noted in *R. imparispine* (Report U. S. Fish Com. for 1887, pp. 840–843, plate XII, figs. 6–9).

The proboscides are long and beset with hooks of many different patterns, the longest of which measures as much as 0.2 mm.

Dimensions of a specimen mounted in balsam, in millimeters: Length of head and neck 6; bothria, length 1.20, breadth 1.00; bulbs, length 2.5,

breadth 0.4; proboscis, length, estimated, 3, diameter, exclusive of hooks, 0.19; length of longest hook 0.20. In an alcoholic specimen, somewhat more slender than usual, the average length of the last four segments was 3.5, the breadth, 0.09.

From the nurse-shark (*Ginglimostoma cirratum*):

July 2, one scolex and strobile, and a fragment, spiral valve.

July 6, 59, in upper part of spiral valve.

19. Rhynchobothrium tenuispine Linton.

Report U. S. F. C., 1887, pp. 837–838, plate XII, figs. 1, 2. Proc. U. S. Nat. Mus., vol. XX, pp. 448–449, plate XXXIV, fig. 8. Bull. U. S. F. C., 1899, pp. 426, 433. Bull. Bureau of Fisheries, vol. XXIV, p. 348.

Numerous small tetrarhynchs from the nurse-shark (*Ginglimostoma cirratum*) agree very closely with this species, and are for the present referred to it.

The neck is rather long and slender, often long-pyriform, tapering forward, with red pigment at the base in front of and beside the contractile bulbs. The neck in many cases was strongly spinose, the spines on the neck being considerably larger than those on the proboscis. These neck-spines are much less dense on some than on others, and are therefore evidently an evanescent character. The proboscides are relatively very long, with bulbous base, and are armed with minute hooks. The hooks on the base of the proboscides agree closely with this species, while those towards the distal end are, perhaps, a little smaller and more slender. The first segments are in some cases moniliform.

Measurements were made of specimens mounted in balsam and showed a close correspondence with the dimensions given for this species. A few of these measurements, in millimeters, are here given: Length of head and neck 1.10; breadth of head 0.28, of neck, behind head, 0.16, at contractile bulbs 0.22; length of contractile bulbs 0.40; length of first segment 0.09, breadth 0.12; diameter of proboscis at tumid base 0.025, in front of tumid base 0.018. The length in life is about 5 mm.

Seventy-five specimens were collected from the spiral valve of a nurse-shark on July 5, and numerous specimens were obtained on July 6. In both cases the shark was large.

20. Rhynchobothrium lineatum sp. nov.

(Plate 6, figs. 39–43.)

Bothria elliptical, entire, widely flaring at base; neck cylindrical, with evanescent spines; contractile bulbs long and slender; sheaths in close spirals; proboscides very long, slender, and slightly enlarged near the base, hooks very minute and closely set. Body continuous without any constriction behind the bulbs, linear, increasing in breadth very little posteriorly and

that very gradually. First distinct segments begin a short distance back of neck, at first much broader than long, soon becoming squarish, then longer than broad, all with crenulate margins. Genital aperture marginal in a deep rounded notch at about the posterior third; ripe segments with ova not seen; longest segment two and one-half times as long as broad.

There are many points of resemblance between this species and *R. longicorne*. Length of living specimens as much as 30 mm.; of alcoholic specimens 18 to 20 mm.

Dimensions, in balsam, given in millimeters: Length 20; length of head and neck 3.28; bothrium, length 0.27, breadth 0.27; diameter of neck, anterior 0.23, posterior 0.40; bulbs, length 1.36, breadth 0.14; first distinct segment, length about 0.08, breadth 0.40; last segment, length 1.12, breadth 0.56; proboscis, length (estimated) 2.5; diameter at base 0.05, near distal end 0.03; length of longest hooks 0.017.

The segments, in this specimen, are twenty in number, and are somewhat irregular in length, the last one not being the longest. The longest segment measures 1.6 in length, and 0.67 in breadth.

From the nurse-shark (*Ginglimostoma cirratum*).

July 6, four. July 15, seven and fragments.

21. Rhynchobothrium curtum sp. nov.

(Plate 6, figs. 44–47.)

Bothria broad-elliptical or oval-elliptical and somewhat thick, placed on the sides of the head which correspond to the lateral margins of the strobile. Head, in side view, heart-shaped; neck very short, shorter than bothria; bulbs oval-elliptical, sheaths nearly straight; proboscides short, hooks small. Body at first a little narrower than neck; first segments begin near neck, at first much broader than long, but soon becoming as long as broad, then very soon becoming longer than broad, with rounded corners and more or less crenulated or indented margins; marginal vessels conspicuous; reproductive aperture at about the posterior fourth.

Only preserved specimens were seen; the largest specimen noted measured 10 mm. in length.

Measurements, in millimeters, of a specimen mounted in balsam: Length 6; head, length 0.21, breadth 0.22; neck, length 0.06, breadth 0.07; breadth of body behind neck 0.10; distance to first distinct segment 0.08; first segment, length 0.02, breadth 0.11.

The hooks were not seen very distinctly, as no proboscis was everted. A number of measurements showed that the longer hooks were about 0.024 in length. Only one was found which measured as much as 0.04. It was near the base of the proboscis.

As the increase in length and breadth of segments is much the same in different specimens, the following measurements of all the segments in one example are given:

Segment.	Length.	Breadth.	Segment.	Length.	Breadth.
1	0.02	0.11	7	0.16	0.16
2	.03	.11	8	.21	.17
3	.05	.12	9	.43	.19
4	.06	.14	10	.62	.24
5	.11	.16	11	.72	.30
6	.12	.16	12	1.23	.32

The general outline of the head and neck of this species bears a strong resemblance to that of *Otobothrium crenacolle*. There are no accessory organs on the bothria, however, and the contractile bulbs do not diverge at their posterior ends; furthermore, the bothria correspond in position to the lateral margins of the strobile instead of to the flat surface.

From the tiger-shark (*Galeocerdo tigrinus*), June 2, five, spiral valve. See remarks under *Thysanocephalus* (pp. 164, 167).

LARVAL STAGE.

On July 11 a small amber-colored cyst was found on the viscera of a black grouper (*Myctroperca bonaci*). The cyst contained a blastocyst in which was a small larva which appears to belong to this species, although the general appearance, not only of the larva, but of the cyst as well, was almost identical with that of the larva and cyst of *O. crenacolle*. There is, however, no indication of accessory organs on the bothria, while the hooks, thick-margined bothria, short neck, and undivergent bulbs, all agree with the species from the tiger-shark.

Measurements of living specimen, in millimeters: Cyst, length 0.84, breadth 0.50; blastocyst, length 0.32, breadth 0.18; larva, length 0.16, breadth 0.08.

On July 11 one larva of this species was obtained from a cyst on the viscera of a grouper (*Epinephelus striatus*).

22. Rhynchobothrium exile sp. nov.

(Plate 7, figs. 48–54.)

Bothria with thin, flexible margins, thus giving to the preserved specimens a variety of shapes, nearly parallel in marginal view, with posterior ends sometimes slightly divergent. Neck two or more times the length of the head, cylindrical, and as wide as or wider than the body, and thicker; bulbs long-oval or elliptical, sheaths in close spirals; proboscides long, only the basal portions seen everted; bulbous enlargement at base of proboscis armed with many small and a few large hooks. On the everted part of the proboscis the hooks are of very diverse shape and size, on the inverted part they appear to be more regular in shape than they are at the base. Body linear, filiform; first segments begin near base of neck, at first very short, increasing in length rapidly, ultimately becoming many times as long as

broad; breadth of body varies but little; free segments with ova very much elongated. The reproductive cloaca is near the posterior end of the proglottis; the vagina opens behind the cirrus pouch. The ovary is situated about half-way between the cirrus pouch and the posterior end of the segment. The testes occupy the median axial region of the entire segment, except a short space at the anterior end. The vitellaria lie along the margins, and also spread peripherally over the median axial region, thus obscuring the other organs, especially in the mature segments. The uterus lies along the median line, and, in one of the free segments, extended as a slender tube containing ova, at least as far forward as the anterior third. In others the ova lay in an elongated mass from just in front of the reproductive cloaca to about the anterior fifth.

The lateral vessels are very conspicuous, except in the free segments, also the last segment in the strobiles examined did not have as conspicuous lateral vessels as the preceding segments.

Living specimens not seen.

Dimensions, in millimeters: Length of longest about 30; length of head and neck 1.12; head, length 0.45, breadth 0.40; bothrium, length 0.45, breadth (estimated) 0.40; breadth of neck, anterior 0.24, base 0.27; bulbs, length 0.32, breadth 0.11; breadth of body near neck 0.16; distance to first distinct segment 0.3; first segment, length 0.03, breadth 0.16; a middle segment, length 1.20, breadth 0.19; last segment, length 3.68, breadth 0.32; proboscis, length (estimated) 1, diameter, behind and in front of bulbous enlargement, 0.04, at bulbous enlargement 0.06. In a strobile 18 mm. long, the last segment was 2.24 long and 0.4 broad; the last segment in another of 26 mm. in length was 4 mm. long and 0.3 mm. broad. A free segment measured 5.5 in length and 0.6 in breadth.

Mature segments resembling these have been noticed before in the chyle of the spiral valve of the tiger-shark at Woods Hole, but this is the first time I have seen the scoleces.

From spiral valve of tiger-shark (*Galeocerdo tigrinus*), captured June 2. Eighty-five specimens were collected, all filiform, with a conspicuous enlargement at the base of the proboscides and edges of the bothria folded as if rather thin and flexible.

For remarks on the identification of the host see under *Thysanocephalum* (pp. 164, 167).

23. Rhynchobothrium binuncum sp. nov.

(Plate 8, figs. 55–64.)

Strobile small, slender, with few segments. Bothria short, rather widely separated in front, at least when compressed; neck relatively long, with very long, slender, contractile bulbs, equaling in length half the total length of the head and neck; sheaths in loose spirals; proboscides long and for the most part with small, slender spines, but with a few larger spines near the

base; among the latter are two which are larger than the others and stand side by side. These two spines are quite conspicuous. They are of nearly uniform size from the base to near the tip, where they terminate in a short recurved, almost acuminate hook. These characters are best seen in the figures. A third spine of the same general shape, but much smaller, stands near the large pair.

The segments begin immediately behind the neck, are few, four or five in the example studied, increase in length rapidly, but remain rather narrow. The last segment may be as long as the rest of the strobile; reproductive aperture marginal, a little back of the middle, making a shallow notch with gently sloping sides.

Dimensions, in millimeters, of a living specimen, a strobile with four segments: Length 7.31; length of head and neck 1.68; length of bothrium 0.28; breadth of head 0.56, of neck 0.39; length of bulbs 0.84; distance from base of neck to first distinct segment 0.07; length of first segment 0.14, of second 0.42, of third 1.40, of fourth 3.60; breadth of third 0.19, of fourth 0.42; diameter of proboscis, exclusive of hooks, 0.04; length of longest hook noted 0.023. In a specimen, mounted in balsam, there were five segments, which had the following lengths: 0.08, 0.13, 0.45, 1.23, 3.48; length of head and neck 1.25.

Dimensions of a specimen in balsam: Length 5.97; length of bothrium 0.16; of head and neck 1.44; of bulbs 0.84; length of last segment 2.41. In a specimen, which measured 6.62 in length, the fifth and last segment was 3.48 in length. The largest hook measured was 0.035 in length.

From a sting-ray (*Dasyatis say*), July 10, ten, in spiral valve.

24. Rhynchobothrium sp.

(Plate 9, figs. 65–69.)

This is probably a new species, but since only one specimen was found, and it a scolex with the rudiment only of a strobile, it does not seem advisable to bestow a specific name upon it at present. The specimen was flattened at the time of collecting and is now mounted in balsam. The head can be seen only in side view. The bothria approach each other anteriorly and are widely flaring posteriorly. Their shape can not be made out exactly, but they evidently have flexible borders and are probably about as broad as they are long. The neck is also flattened and expands a short distance behind the head until it is as wide or wider than the head. The bulbs are long-fusiform, and the retractor muscles take their origin from the posterior ends. The sheaths are coiled in loose spirals. The proboscides are relatively very long and are armed with hooks which are short and of nearly uniform size and shape. The strobile is rudimentary, shorter than the head and neck and tapers to a blunt point at the posterior end. The lateral vessels there meet and open by a terminal pore. Segments have begun to form, but they are all very short.

The species has some suggestion of *R. lomentaceum.*

Dimensions, in millimeters, of specimen mounted in balsam: Length 3.5; head, flattened, breadth 0.56; length of bothria 0.40; bulbs, length 0.72, breadth 0.12; diameter of neck, flattened, anterior 0.38, posterior 0.56; proboscis, length (estimated) 3, diameter, exclusive of hooks, base 0.05, near apex 0.04; hooks (at base shorter and more crowded than at apex), length, at base 0.014, near apex 0.028.

From spiral valve of nurse-shark (*Ginglimostoma cirratum*). July 5, one.

25. Rhynchobothrium sp.

(Plate 10, figs. 70–74.)

Bothria foliaceous, but with margins somewhat thickened; head much broader than neck; neck slender, cylindrical, enlarging at bulbs; sheaths in close spirals; bulbs long-oval, with retractor muscle attached at about the middle of the length on the median wall; proboscides long, hooks of different sizes and shapes. The most marked differences are to be seen in those hooks which are near the base of the proboscides. On one side there are some small, straightish spines; on the other they are much larger; long and nearly straight, but with an abrupt curve at the apex. A single row of these large hooks extends around to the opposite side a short distance from the base. The proboscides were not seen fully extended. So far as seen, the hooks on one side remain small, slender and very sharp-pointed, but grow larger toward the apex, so that in the completely everted proboscis the difference between the hooks of the opposite sides is probably slight. The large hooks with abruptly recurved ends are confined to the basal region. Beyond the base the larger hooks become rather broad, in lateral view, and are strongly and uniformly curved. On the other hand, among the small hooks some distance from the base are hooks which are straightish with abruptly curved tips. Towards the tip of the proboscis, as may be seen in the retracted part, a prevailing form is a slender hook curved in two directions, like a letter **S** nearly straightened out.

Transverse striæ begin immediately below the neck. The first distinct segments are shorter than broad, but soon become as long as broad. They then rapidly and uniformly lengthen, but remain about the same breadth. The posterior segments are nearly ten times as long as broad, and their anterior ends are abruptly larger than the posterior end of the preceding segment. None of the segments were mature, although rudiments of reproductive organs could be made out. In the next to the last segment the rudiment of the cirrus bulb was a little behind the posterior third, and the ovary was at the posterior fifth. The anatomy of the posterior segments, so far as it could be made out, is much like that of *R. exile.*

Dimensions, in millimeters, of specimen mounted in balsam: Length 15; length of head and neck 2.4; breadth of head 0.73; bothrium, length 0.48,

breadth 0.48; diameter of neck, anterior 0.32, posterior 0.64; bulbs, length 0.64, breadth 0.16; first distinct segment, length, about 0.06, breadth 0.24; fifth segment, length 0.14, breadth 0.20; tenth segment, length 0.35, breadth 0.24; twentieth and last segment, length 2.08, breadth 0.22; proboscis, length, estimated, 3, breadth, exclusive of hooks, base 0.05, near apex 0.04; length of longest hooks, base 0.035, at apex of everted part, about 0.6 from base, 0.028.

From spiral valve of nurse-shark (*Ginglimostoma cirratum*), July 6, one.

ENCYSTED STAGE.

(Plate 10, fig. 75, and plate 11, figs. 76, 77.)

A larva taken from a cyst in the walls of the rectum of the green moray (*Lycodontis funebris*) is in such close agreement with this species that I do not hesitate to place them together.

The blastocyst resembles that of the genus *Synbothrium*. Its posterior end was orange-yellow, which perhaps has no special significance.

Dimensions, in millimeters, of living larva, flattened: Length 4.34; bothrium, length 0.75, breadth 0.75; neck, length 3.16, breadth 0.47; bulbs, length 0.63, breadth 0.16; proboscis, length, estimated, 1.96, diameter, exclusive of hooks, 0.056; length of longest hooks 0.035.

26. Rhynchobothrium sp.

(Plate 11, figs. 80–82.)

A single minute tetrarhynch, found July 12, in the spiral valve of a large cub-shark (*Dasyatis say*), has many points of resemblance to *R. hispidum*, but the hooks, while showing close relationship, are not in sufficiently close agreement to permit the specimen to be referred with certainty to that species. Only the head and neck and a very short piece of the strobile were secured.

The bothria are separated by a space at the anterior ends, and are widely divergent at their posterior ends. The neck is nearly linear, only slightly larger at base than in front, and is spinose. The bulbs are parallel and equal to about half the length of the neck; sheaths loosely spiral; proboscides relatively long; hooks very small, for the most part slender and spinose, a few larger hooks, broad, in lateral view, near the base. Only the basal portion of the proboscides was seen. The hooks on the inverted portion do not show much variety. None of the broad variety, seen near the base, and characteristic of *R. hispidum*, could be made out through the transparent walls of the sheaths.

Dimensions, in millimeters, of specimen mounted in balsam: Length of head and neck 0.72; length of bothrium 0.19; bulbs, length 0.32, breadth 0.04; diameter of proboscis, exclusive of hooks, 0.017; length of largest hooks 0.007.

27. Rhynchobothrium sp.

(Plate 11, figs. 83–87.)

A larval tetrarhynch was obtained from a cyst on the viscera of a Spanish sardine (*Clupanodon pseudohispanicus*), on July 9, which I have not been able to refer to any described species.

Bothria about as long as broad, emarginate on posterior border, approaching each other in front, divergent behind. The neck is cylindrical, increasing slightly in diameter to base, with a slight hint of a fold at the base, minutely spinose. Sheaths very densely coiled spirals; bulbs moderately short, arcuate; proboscides relatively long; hooks small, but of several different kinds. The most of the hooks are slender, those at the base sharp-pointed and slightly arcuate. Elsewhere they are slender, straightish, with a tendency to have abruptly recurved tips. There are a few, two or three, longitudinal rows of shorter hooks.

Dimensions, in millimeters, of specimen mounted in balsam: Length of head and neck 0.88; length of bothrium 0.17; diameter of head 0.22; of neck, anterior 0.14, base 0.20; bulbs, length 0.19, breadth 0.05; proboscides, length (estimated) 1, diameter at base, exclusive of hooks, 0.017; length of longest hook 0.01.

28. Otobothrium crenacolle Linton.

Report U. S. F. C., 1887, pp. 850–853, plate XIII, figs. 9–15, plate XIV, fig. 14. Bull. U. S. F. C., 1889, pp. 273, 428. Bull. Bureau of Fisheries, vol. XXIV, p. 331, etc., encysted in 13 hosts, adult in 1.

Two specimens of this species were found in the spiral valve of a cub-shark (*Carcharhinus platyodon*), July 12.

Dimensions of living specimen, in millimeters, flattened: Length 2.24; head, length 0.28, breadth 0.30; neck, length 0.16, breadth, anterior, 0.16, at base 0.19; bulbs, length 0.084, breadth 0.046; breadth of body behind neck 0.12; last segment, length 0.86, breadth 0.21.

This species is of very wide distribution. At Beaufort it was found encysted in thirteen species of teleosts and adult in one selachian. At Woods Hole it is of frequent occurrence encysted in a variety of teleosts and adult in the hammerhead shark. It infests the muscles of the common butter-fish (*Poronotus triacanthus*) to an unusual degree.

29. Synbothrium filicolle Linton.

Report U. S. F. C., 1887, pp. 861–862, plate XV, figs. 2–4. Proc. U. S. Nat. Mus., vol. XIX, p. 819, plate LXVIII, fig. 10. Bull. U. S. F. C., 1899, pp. 275, 413, 414, etc., noted in 10 hosts. Bull. Bureau of Fisheries, vol. XXIV, p. 333, etc., noted in 9 hosts.

One adult of this species was found in the spiral valve of the sting-ray (*Dasyatis say*), July 10.

Dimensions of living specimen, in millimeters: Length 6; length of head and neck 0.84; diameter of head 0.56, of neck 0.21; length of bulbs 0.42; distance from base of neck to first distinct segment 0.14; first segment, length 0.21, breadth 0.08; second segment, length 0.42, breadth 0.08; third segment, length 0.84, breadth 0.11; fourth segment, length 1.54, breadth 0.35, variable; ova 0.034 by 0.022, and 0.039 by 0.020 in the two principal diameters.

The proboscides were but slightly everted, but the hooks, so far as seen, agree with this species. Length of hooks about 0.04.

EXPLANATION OF PLATES.

Letters which have the same meaning in the different figures.

b. Muscular bulbs, whose use is to evert the proboscides by pressure on fluid with which they are filled.
c. Cirrus.
c. p. Cirrus pouch.
g. Rudiment of genitalia.
l. m. Longitudinal muscle.
l. v. Lateral vessel.
o. Ovary.
r. a. Reproductive aperture.
r. m. Retractor muscle of proboscides.
sh. Proboscis sheath.
t. Testes.
u. Uterus.
v. Vagina.
v. d. Vas deferens.
v. g. Vitelline glands.
y. d. Vitelline duct.

In all cases the actual size of the object sketched in the several figures is given.

Plate 1.

Dibothrium sp., from *Atherina laticeps.*

1. Larva in cyst, sketched from life, actual length of larva 2.8 mm.
2. Same, flattened, mounted in balsam, length of larva 3 mm.

Rhinebothrium sp., from *Dasyatis say.*

3. Head, neck, and anterior segments sketched from mounted specimen. Actual length of neck 0.14 mm.
4. Posterior segments, balsam. Actual length of last segment 0.35 mm.

Undetermined cestode from *Carcharhinus platyodon.*

5. Memorandum sketch of living worm; length of bothrium 0.29 mm.
6. Sketch of same, specimen mounted in balsam; diameter of head 0.40 mm.
7. Fourth segment from last, in balsam; actual length 0.60 mm.

Discocephalum pileatum from *Carcharhinus platyodon.*

8. Segmenting ova; actual size 0.035 by 0.032 mm., in the two principal diameters. Sketches made from living ova. *a,* July 12; *b,* July 13; *c,* July 14.

Plate 2.

Pedibothrium globicephalum gen. et sp. nov., from *Ginglimostoma cirratum.*

9. Head and neck, flattened, life; actual diameter of head 1.8 mm.
10. Head and neck, balsam; actual diameter of head 1 mm.
11, 12. Single bothria, life; actual lengths 0.86 and 0.92 mm.
13. Typical hook, side view; actual length 0.086 mm.
14. Pair of hooks, from small specimen; actual length 0.085 mm.
15. Segments with rudiments of reproductive organs, balsam; actual breadth 0.35 mm.
16. Free, mature segment, balsam; actual length 1.8 mm.

Plate 3.

Pedibothrium longispine gen. et sp. nov., from *Ginglimostoma cirratum.*

17. Sketch of a strobile, life; actual length of bothrium 0.35 mm.
18. Head, balsam; maximum diameter of head 0.32 mm.
19. Hook; actual length 0.16 mm.

Pedibothrium brevispine gen. et sp. nov., from *Ginglimostoma cirratum.*

20, 21. Single hooks, different views; actual length 0.068 mm.
22. Free, ripe segment, balsam; actual length 2.8 mm.

PLATE 4.

Pedibothrium brevispine, continued, from *Ginglimostoma cirratum.*

23. Pair of hooks; actual length 0.064 mm.
24. Head, balsam; maximum diameter 0.21 mm.
25. Free, ripe segment with cirrus exserted; actual length 2.8 mm.

Acanthobothrium brevissime sp. nov., from *Dasyatis say.*

26. Strobile, balsam; actual length 1.43 mm. The enlargement at the base of the neck is probably a contraction character.
27. Head, balsam; length of head 0.25 mm.
28. Single hook.
29. Pair of hooks, prongs broken.
Actual length in figs. 28 and 29, 0.05 mm.

PLATE 5.

Rhynchobothrium simile sp. nov., from *Ginglimostoma cirratum.*

30. Head, neck and anterior part of strobile; lateral view of the head; actual diameter of neck behind head 0.6 mm.
31. Head, showing margins of bothria; diameter of neck behind head 0.65 mm.
32. Proboscis, near base; diameter, exclusive of hooks, 0.19 mm.
33. Proboscis, near base, showing side opposite to that shown in figure 32; diameter, exclusive of hooks, 0.17 mm.
34. Proboscis 0.8 mm. from base; diameter, exclusive of hooks, 0.17 mm.
35, 36. Two types of hooks shown in fig. 34, more enlarged; lengths 0.16 and 0.17 mm.
37. Last segment, in glycerin; length 2 mm.

PLATE 6.

Rhynchobothrium simile sp. nov., continued.

38. Base of proboscis; diameter, exclusive of hooks, 0.17 mm.

Rhynchobothrium lineatum sp. nov., from *Ginglimostoma cirratum.*

39. Head and neck, balsam; length of head and neck 3.5 mm.
40. Proboscis at base; diameter, exclusive of hooks, 0.05 mm. Partly diagrammatic.
41. Proboscis, middle; diameter, exclusive of hooks, 0.04 mm.
42. Proboscis, near apex; diameter, exclusive of hooks, 0.04 mm.
43. Segment toward posterior end of strobile; length 1 mm.

Rhynchobothrium curtum sp. nov., from *Galeocerdo tigrinus.*

44. Head, neck, and anterior part of strobile, alcoholic, showing marginal view of bothrium; length of head 0.20 mm.
45. Lateral view of bothrium, and anterior end of strobile, alcoholic; length of bothrium 0.19 mm.
46. Same view of another specimen mounted in balsam; length of bothrium 0.19 mm.
47. Last segments of strobile mounted in balsam; breadth of last segment 0.33 mm.

PLATE 7.

Rhynchobothrium exile sp. nov., from *Galeocerdo tigrinus.*

48. Head and neck sketched from alcoholic specimens; diameter of neck, anterior, 0.28 mm.

49. Base of proboscis; diameter of enlargement, exclusive of hooks, 0.07 mm.
50. Another view of base of proboscis; diameter of enlargement, exclusive of hooks, 0.07 mm.
51. Another view of proboscis; diameter, exclusive of hooks, 0.031 mm.
52. Types of hooks; length 0.020 to 0.028 mm.
53. Segments near posterior end of strobile; maximum diameter 0.35 mm.
54. Free segment, partly diagrammatic. Testes represented a little larger than natural; vitelline glands partly cover testes in nature; length 5.5 mm.

PLATE 8.

Rhynchobothrium binuncum sp. nov., from *Dasyatis say.*

55. Strobile, balsam; actual length 5 mm.
56. Head, neck, and anterior end of the body, balsam; length of head and neck 1.25 mm.
57, 58. Different views of base of proboscis, showing characteristic paired hooks; length of largest hooks 0.03 mm.
59. Another view of proboscis; diameter, exclusive of hooks, 0.03 mm.
60. View of proboscis towards distal end; diameter, exclusive of hooks, 0.035 mm.
61, 62, 63. Different views of paired hooks which are situated near the base of each proboscis. The pairs shown in these sketches are from different proboscides; length of longest 0.030, 0.031, and 0.035 mm. in the several pairs.
64. Posterior segment, specimen mounted in balsam; actual length 2.41 mm. Length of strobile from which this sketch was made 5.97 mm.

PLATE 9.

Rhynchobothrium sp., from *Ginglimostoma cirratum.*

65. Entire specimen, showing head, neck, and rudimentary strobile, balsam; actual length 3.5 mm.
66. View of proboscis near base; diameter, exclusive of hooks, 0.056 mm. The hooks become yet denser a little anterior to the point shown in the sketch.
67. View of proboscis near distal end, opposite to view shown in fig. 68; diameter of proboscis, exclusive of hooks, 0.045 mm.
68. View opposite to that shown in fig. 67.
69. Posterior end of strobile, much enlarged, showing terminal pore of the excretory system; length of segment 0.26 mm.

PLATE 10.

Rhynchobothrium sp., from *Ginglimostoma cirratum.*

70. Head and neck, balsam; actual length 2.10 mm.
71. View of proboscis near base; diameter, exclusive of hooks, 0.06 mm.
72. View of proboscis 0.5 mm. from base (as much as was exserted); diameter, exclusive of hooks, 0.05 mm.
73. View of proboscis opposite to that shown in fig. 72.
74. Next to last segment, balsam; actual length 2.24 mm.

Rhynchobothrium sp., from *Lycodontis funebris.* (Encysted stage of preceding species.)

75. View of proboscis on opposite side from that shown in fig. 76, and a little in front of it; diameter, exclusive of hooks, 0.038 mm.

PLATE 11.

Rhynchobothrium sp., from *Lycodontis funebris,* continued.

76. View of proboscis opposite to that shown in fig. 75, near base; diameter, exclusive of hooks, 0.040 mm.
77. Blastocyst with larva, sketch from life; actual length 4.34 mm. *l,* larva.

Rhynchobothrium speciosum, from cyst in *Mycteroperca bonaci.*

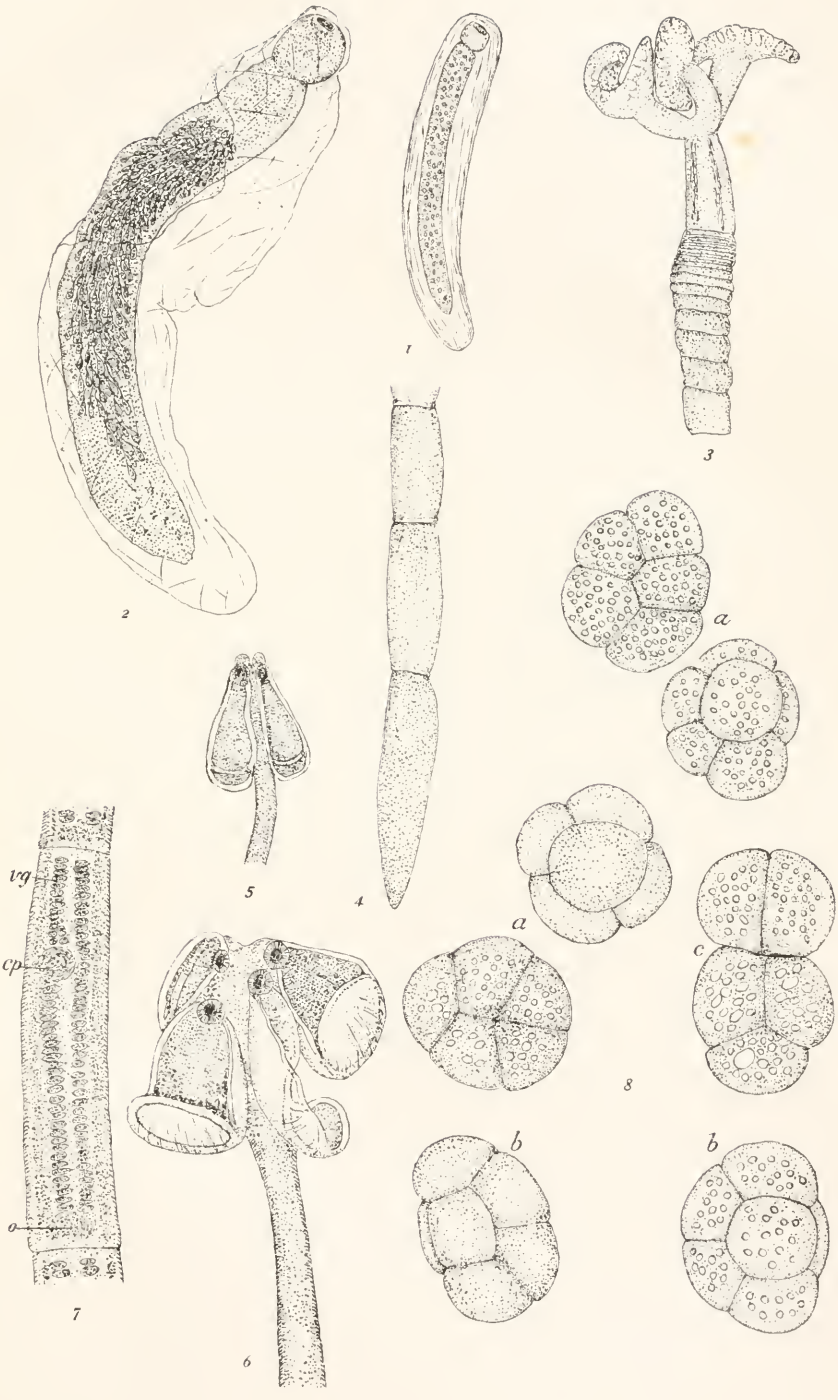
78. Cyst, sketch from life; actual length 22 mm.
79. Same, flattened so as to show blastocyst. *cy,* cyst; *l.* larva; *pg,* brown pigment surrounding *bl,* the blastocyst.

Rhynchobothrium sp., from *Dasyatis say.*

80. Entire specimen, balsam; actual length 0.90 mm.
81. View of proboscis at base, partly diagrammatic; diameter, exclusive of hooks, 0.017 mm.
82. Another view of proboscis near base; diameter, exclusive of hooks, 0.014 mm., length of longest hooks 0.007 mm.

Rhynchobothrium sp., from *Clupanodon pseudohispanicus.*

83. Head and neck, balsam; actual diameter of head 0.22 mm.
84. View of proboscis near base; diameter, exclusive of hooks, 0.017 mm.
85. 86. Other views of proboscis.
87. Characteristic appearance of margins of proboscis; length of longest hooks 0.010 mm.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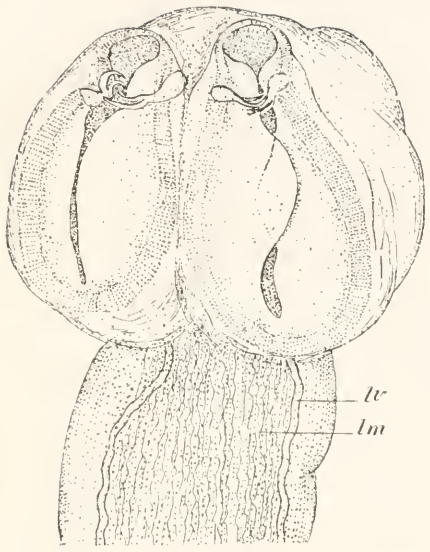

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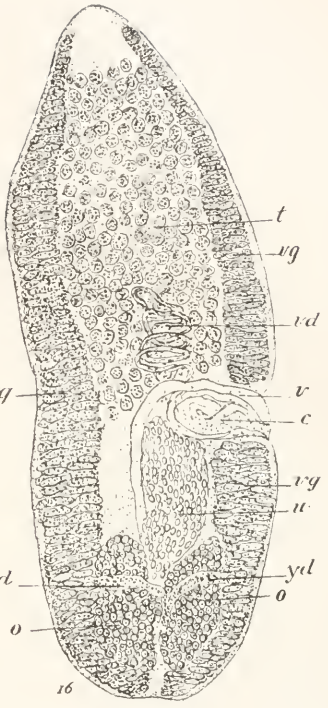

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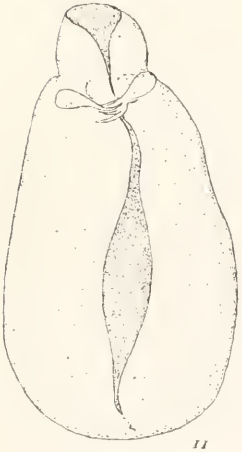

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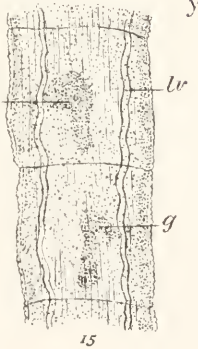

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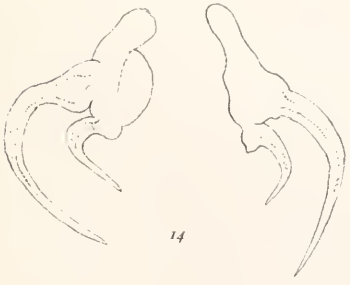

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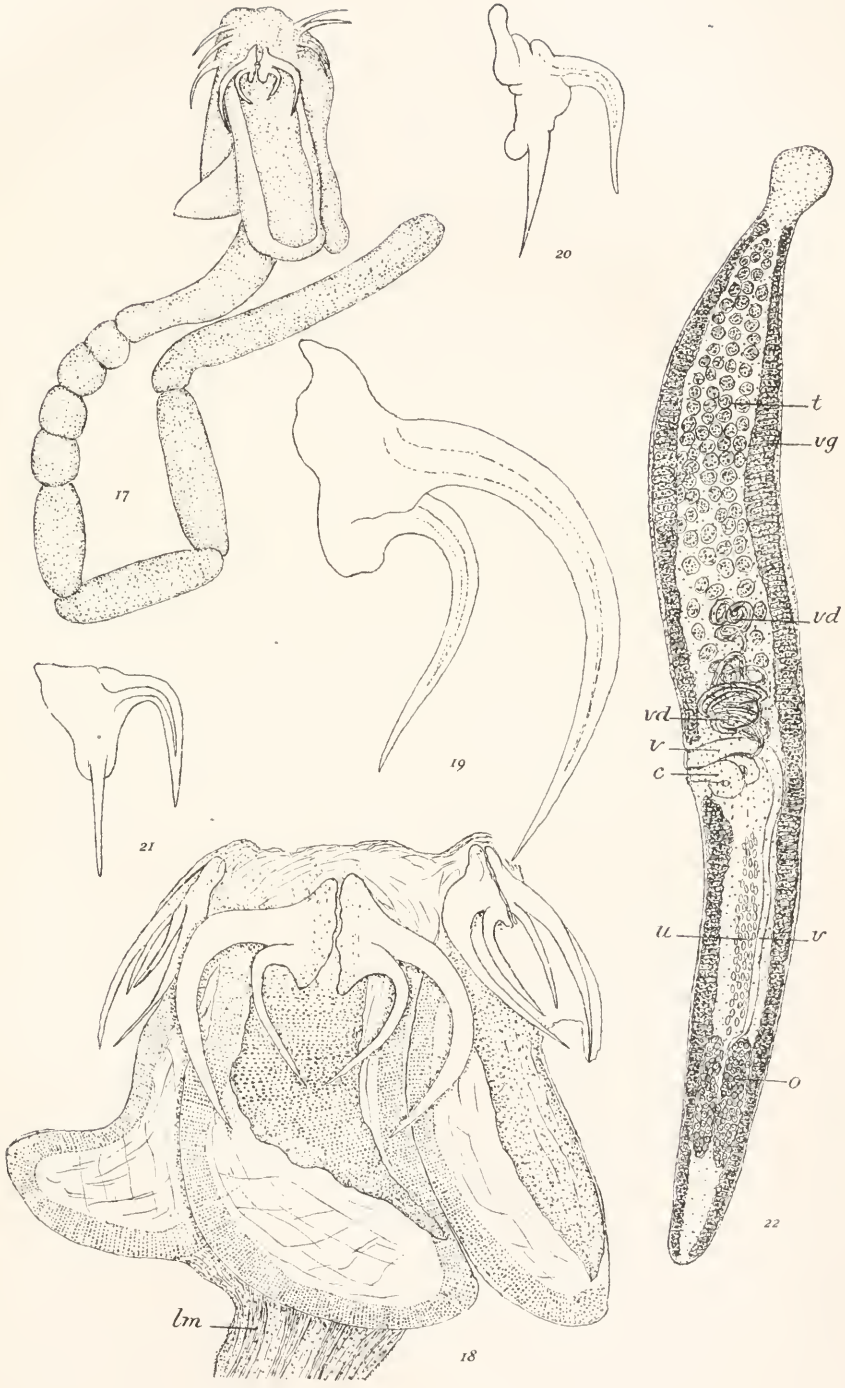
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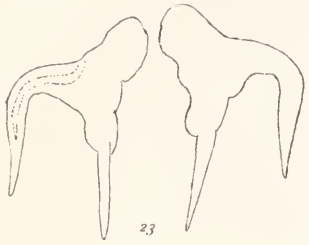

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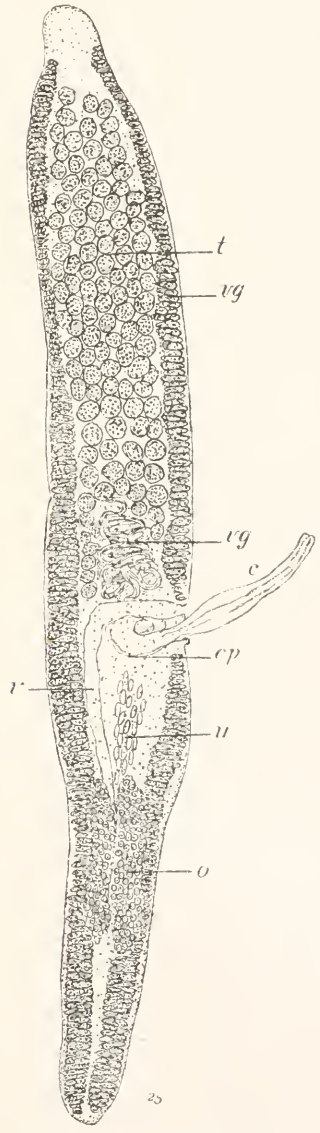

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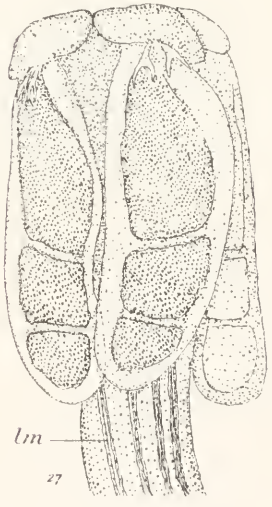

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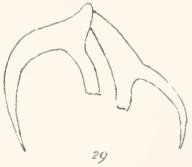

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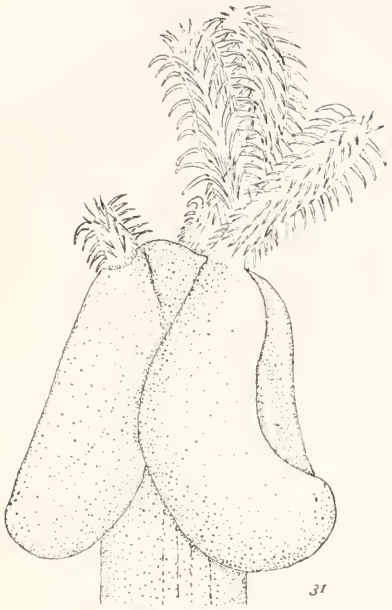

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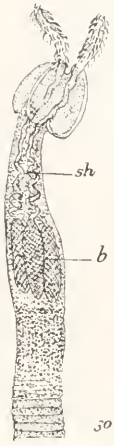

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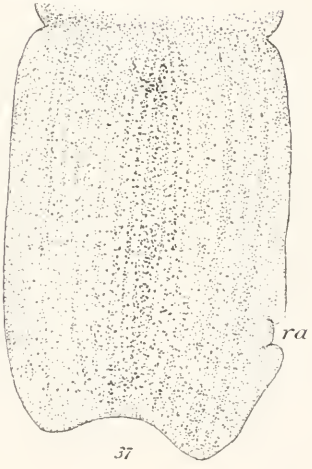

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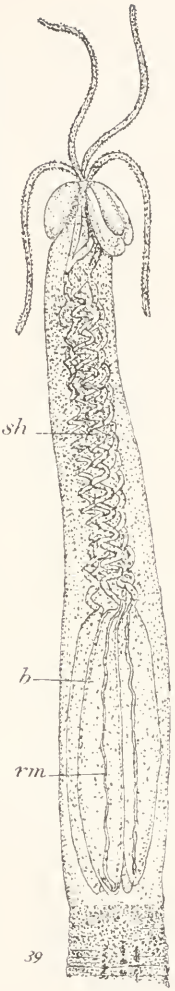

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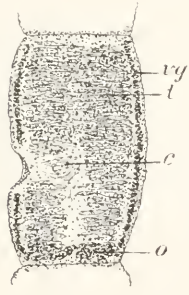

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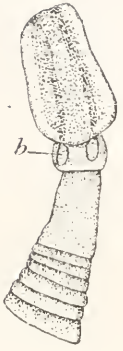

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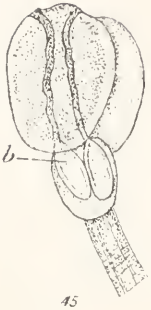

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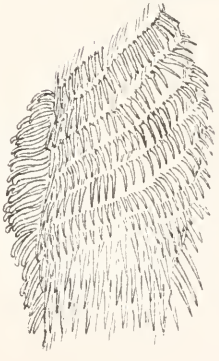

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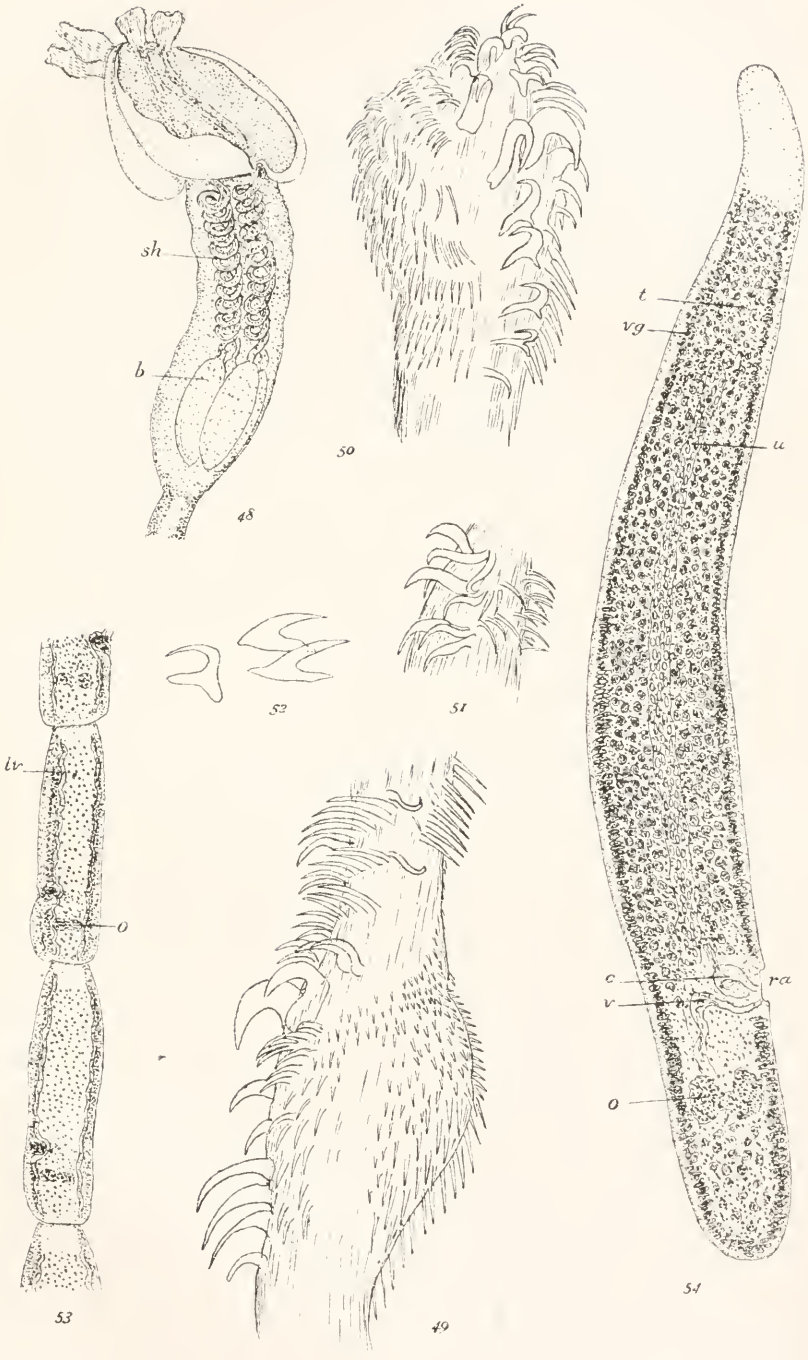
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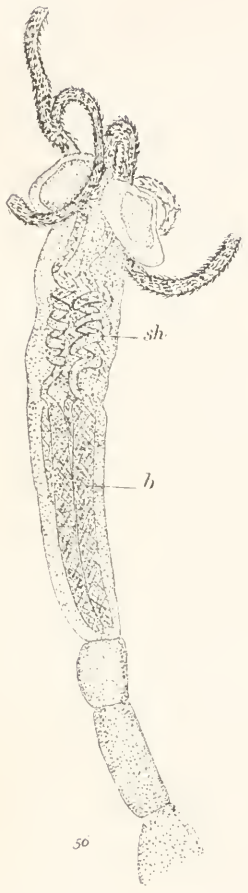

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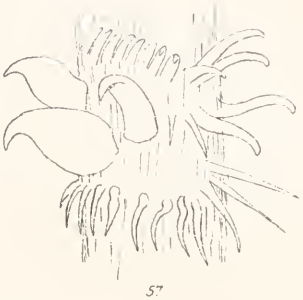

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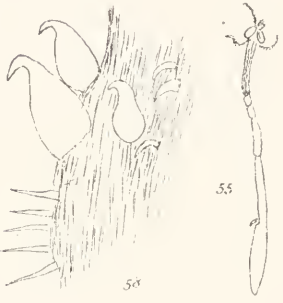

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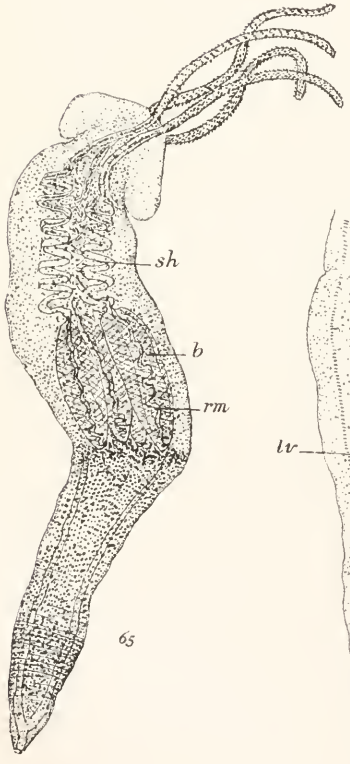

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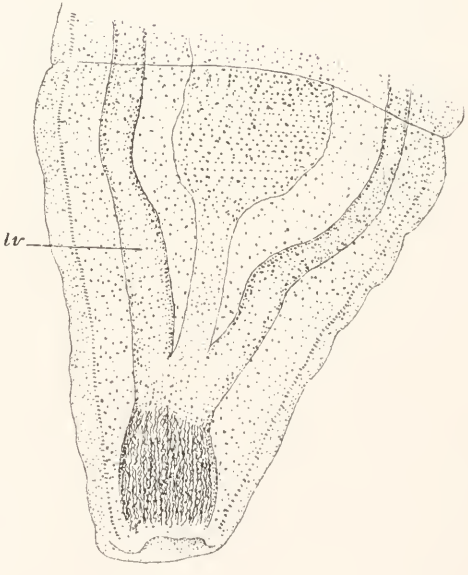

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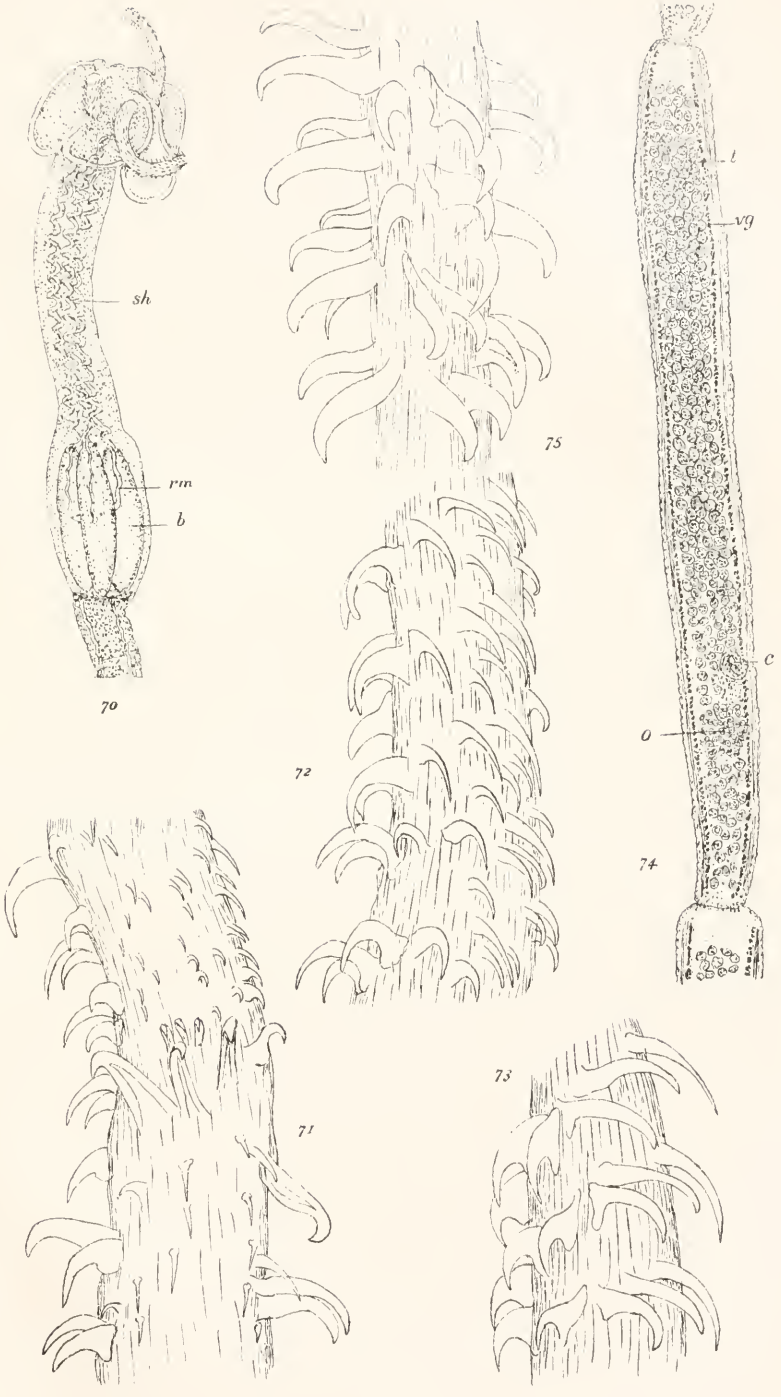
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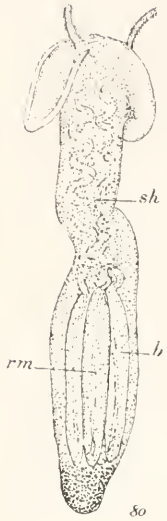

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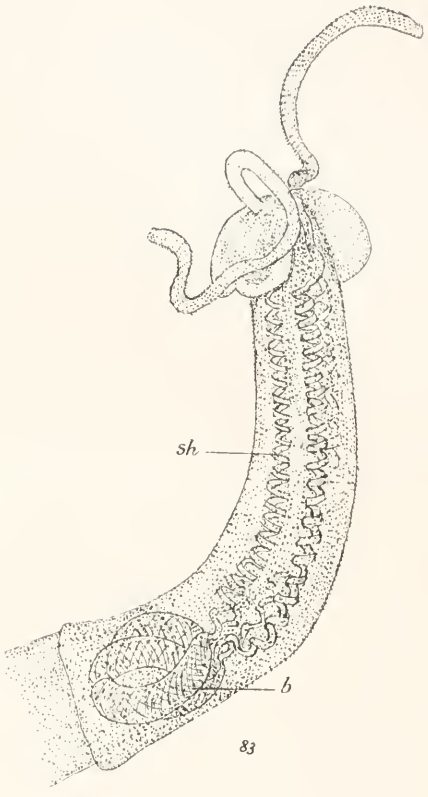

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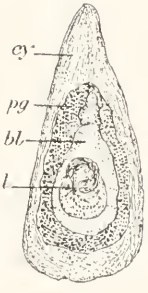

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A VARIETY OF ANISONEMA VITREA.

By C. H. Edmondson.

Anisonema vitrea (Dujardin) is a flagellated protozoan, elongate-oval in form, the anterior end broadly rounded, the posterior more acutely rounded. An oral groove is present on the ventral surface, and near the anterior end of this groove are inserted two flagella of unequal length. The longer and stouter of the two flagella is curved backwards, being non-vibratile, trailing along behind as the animal advances. The shorter flagellum is directed forward, its vibrations causing the oscillating movement of the organism. A contractile vacuole is present, as is also a spherical nucleus, the latter being central in position.

1 2

Anisonema vitrea is distinguished from other species of the genus by eight furrowed surfaces extending in a slightly spiral manner from one end of the body to the other. This species is a salt-water form and has been observed at Woods Hole by Calkins.

During the summer of 1906, while working on marine Protozoa at the Tortugas, Fla., I made a careful study of a form which, no doubt, should be considered as a variety of the above species and which I would entitle *Anisonema vitrea* (Duj.) var. *pentagona.*

The body of this variety is somewhat shorter and thicker than the species reported from Woods Hole, the Tortugas form measuring 40 μ in length by 30 μ in width. The chief distinction, however, between the species and the variety is that the latter possesses but five longitudinal furrows which are well marked and very deep. In other respects the variety resembles the species.

The eight-furrowed form was not observed at the Tortugas, but the pentagonal variety was one of the most common species, being found abundantly within the moat surrounding Fort Jefferson and also in infusions of gulf-weed.

Reproduction of the organism takes place by longitudinal division.

Figs. 1 and 2 represent lateral and transverse views, respectively.