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Notes on Histology of *Lingula anatina* Brugière.

By

Naohidé Yatsu, *Rigakushi.*

With Plates I—II.

CONTENTS.

I. INTRODUCTORY.

Regarding the histology of *Lingula* there have hitherto appeared only two articles. One of them by BEYER ('86) is very far from being satisfactory, considered from the present state of our science. The other is the beautiful work of BLOCHMANN ('00), thanks to which the main organization and some of the finer structures of *Lingula* have been made thoroughly clear. It must however be confessed that a greater part of the histology of this form still remains in the dark. The present piece of work, which has been undertaken in connection with my embryological studies, is thought, fragmentary though it be, to be worth publishing, as it may help to throw some new light upon the subject in question. As for other points I shall reserve them for a future occasion.

II. FORMED ELEMENTS OF THE CŒLOMIC FLUID.

In the cœlomic fluid of *Lingula*, which fills up the body cavity, the pallial sinus and the peduncular cavity, and bathes all the viscera and muscles, we find three kinds of formed elements: viz. 1) blood corpuscles, 2) leucocytes, and 3) spindle bodies. Besides these there occur encysted sporozoa, and very rarely a *Distomum*, closely allied to those found in *Balanoglossus*, *Amphioxus*, and *Gephyrea*.

1. Blood Corpuscles.

The blood corpuscle (Pl. I., Fig. 1) exceeds in number all the other elements. It is colorless, transparent and somewhat spherical in form, measuring 10–20 μ in diameter. It is usually depressed in one or two places. In section, therefore, it gives a crescent or biconvex shape. Just within the wall of the corpuscle there is a small refractive spherule, which is probably a nutritive oil drop (Pl. I., Fig. 2, *r. g.*). Treated with acidulated methyl green it swells up and appears like a hollow sphere (Pl. I., Fig, 2), for the cytoplasm is very transparent and is consequently invisible. When a section of blood corpuscles is stained with erythrosin the cytoplasm stains homogeneously red in well fixed specimens and looks rather compact. But under certain conditions of precipitation by the action of some fixing reagents the entire cytoplasm breaks up into coarse granules (Schollen) which also take a deep erythrosin stain. The nucleus is very compact, and the stain intense and uniform. The blood corpuscle has a well defined and rather thick membrane.

When the mantle of a young specimen (4.5–10 mm. in shell length) is examined under a low power, the circulation of corpuscles can be very clearly seen through the shell, as SEMPER ('61) has described. In every branch of the pallial sinus the observer can make out two opposed currents of blood corpuscles, although there is no septum which divides the sinus into two canals. The non-existence of a septum can be demonstrated in the living specimen, for it is seen that, when occasionally a corpuscle goes astray, it is quickly caught up by the opposed stream, as FRANÇOIS ('91) observed at Numea. The presence of the two currents is made possible by a low ridge (Epithelleisten of BLOCHMANN) which arises from the floor (next the shell) of the pallial sinus. The mantle of a young living *Lingula* is a good object for class demonstration of blood circulation. When sections are studied the corpuscles are sometimes found crowded together in a pallial sinus and they here have the appearance of a fat-tissue, whose contents have been dissolved out.

2. Leucocytes.

Of the second element, the leucocyte, a small number are found in the cœlomic fluid, while a considerable number occur in the marginal lacuna (Randlacune). It is very probable that SEMPER ('61) observed the leucocytes in the lacuna, which he called the "Lymphraum." BROOKS ('78) on the other hand confounded this element with the ordinary blood corpuscles (p. 49). The leucocyte measures 13–20 μ in diameter, and is sometimes spherical in form with a wrinkled surface. It clearly shows amœboid movements giving off a few blunt pseudopodia and sometimes presenting fine sharply pointed, stiff pseudopodia which cluster together in twos or threes (Pl. I., Figs. 3 *a*, *b*, and 4). The leucocyte is always found filled with erythrophilous granules, therefore, and treated with erythrosin, gives a very beautiful appearance, as in Figs. 4 and 5 (Pl. I.). In some cases a swarm of leucocytes is attached along the epithelial ridge (Epithelleisten) of the pallial sinus (Pl. I., Fig. 5): not only do they attach themselves to it, but some of them also force their way into the ridge, probably performing the collection of waste products. This is proved by the following fact. The leucocyte is phagocytous in nature: the yellow pigment, of which more or less is found in the epithelial ridge, is carried out by the element, as shown in Fig. 5 (Pl. I.): in the epithelial ridge we often meet with a leucocyte taking up within itself a compact body, which will be described further on: in another case I was able to observe in the pallial sinus a leucocyte surrounding a spindle body and a compact body (*vide infra.*) at the same time (Pl. I., Fig. 6).

3. Spindle Bodies.

The third element to be described is the enigmatical corpuscles, known as the spindle bodies. These bodies are so characteristic of *Lingula*, that they have been referred to in every work concerning this Brachiopod. I shall go somewhat in detail into the history and description of these bodies. But before doing so I may state in advance the main conclusion, that they are formed out of blood-corpuscles in two separate and independent places: one of these being the special areas on the dorsal and ventral body walls, and the other along the ridge which passes along the middle line of every branch of the pallial sinus.

A. Historical.

C. Vogt ('45) observed the proliferating ridge of the pallial sinus and figures it in his anatomy of *Lingula*. But he mistook the ridge for a blood vessel, an error, natural perhaps, since he did not study the ridge by means of sections.

A. Hancock ('59), in his beautiful monograph on the Brachiopods, considers the proliferating zones in the body walls as a part of the reproductive organs and indeed as the testes. He referred to these zones under the name of the "dendritic organ" and studied it in detail: "......but further experience," he says, "has led to the conclusion that they are really a portion of the genital mass and that from the pressure of the valves, on their being closed, they become occasionally adherent to the membrane......the cells, however, in *Lingula* appeared to present different stages of development, varying much in size and form. Some were oval, others perfectly elliptical, the larger ones were pointed at both ends, and exhibited a double line in the centre, placed longitudinally; while the largest measuring $\frac{1}{180}$th of an inch [=141 μ] in length, were fusiform, with the extremities more or less sharply pointed. These corpuscles were filled with numerous delicate hair-like bodies, resembling spermatozoa. From these facts it can scarcely be doubted that the dendritic organ is the testis, and that the fusiform cells are fully developed spermatophora, containing spermatozoa" (p. 819). As far as the microscopical structure of the dendritic organ is concerned, his observations are correct in all essentials, but from the vague premise that the corpuscles contain "*hair-like bodies resembling spermatozoa,*" it was certainly a dangerous conclusion that the "*dendritic organ is the testis.*" It is strange that HANCOCK's erroneous conclusion passed over to BEYER's view, as will soon be seen. HANCOCK studied, on the other hand, the epithelial ridge and rightly saw the difference between it and the sexual organ found there in other Brachiopods, recognizing the difference in position dependent on the fact that the sexual organ hangs from the inner side of the pallial sinus while the epithelial ridge rises from the outer side (nearer the shell). But unfortunately as he did not study the ridge microscopically he was not able to ascertain that the ridge was the equivalent of the dendritic organ.

In the year following the publication of HANCOCK's memoir there appeared the work of GRATIOLET ('60) on the anatomy of *Lingula*. In this he expressed the curious view that the spindle bodies were young *Lingula*. These, he thought, were produced in the body cavity as the result of self-fertilization: he even goes so far as to discover two valves in the spindle bodies! For these he has evidently mistaken surface depressions which occur not infrequently in the spindle bodies.

In BRONN's Klassen und Ordunung des Thierreichs the question is raised: "ob nicht diese zellen, bei deren bildlicher Darstellung die Angabe von Vergrösserungsgrades vermisst werden, etwa für Prosospermien oder parasitische Pseudonavicellen zu nehmen worden" (p. 281). This interpretation seems *à priori* probable. But if the spindle body were a Sporozoon it should contain a nucleus or nuclei in some form or other. Such a structure is not found at any stage under any treatment.

MORSE ('73 *b*) noticed the presence of the spindle bodies and added that "these are amoeboid in their appearance, and may be seen bending and turning as they course through the more delicate ramifications in the pallial membrane" (p. 25). He advances no view as to the nature and orgin of this body.

BROOKS ('78) also observed the spindle bodies and states that when the bodies are crowded together they give an exceedingly faint violet tint. He noticed that "running out from one or both of the pointed ends of many of the corpuscles are long delicate filaments of variable length" (p. 49), and found the bodies, which he supposed to be on point of division.

BEYER's investigation ('86) made known to us that the dendritic organ of HANCOCK and the epithelial ridge are equivalent structures. Like HANCOCK he came to the conclusion that the spindle bodies are the spermatophores. He does not seem to have himself studied the structure of the spindle bodies. In no part of his paper does he adduce the slightest evidence that these bodies contain spermatozoa. He refers, however, to the "Spermatogenesis" in a final paragraph. He also mistook the

the compact bodies (*vide infra*) for young ova. It seems hardly necessary to enter further into the criticism of his paper.

FRANÇOIS ('91) observed the spindle bodies in the blood of *Lingula* at Numea. "Le sang qui circule dans l'axe du peduncule, outre les globules sanguins ordinaires, contient des corpuscules fusiformes plus ou moins régulier, dont la taille varie de 20 à 100 μ de longueur. Ils present parfois quelques striés longitudinals. Faul-il voir là des globules transformés ou des fibres musculaires en formation qui se seraient détachés de la paroi ?" (p. 239).

In the same year CORI ('91) described the spindle bodies in his anatomy and histology of *Phoronis*. In this genus the body [=Spindelförmiger Körper (CORI)] was first described by KOWALEVSKY. It is formed in the "Gefässperitonealzellen," set free into the body cavity and then carried to the exterior through the nephridium. It measures 11–43μ, as I calculate form CORI's figures. In some cases near the middle part refractive granules are found, and sometimes it is enclosed by a membrane which presents a double contour. He concludes: "Die kleinen doppelt kontourirten Formen dieser Körper lassen mancher Merkmale erkennen, welche die Umbildung der rothen Blutkörperchen in diese wahrscheinlich machen könnten" (p. 557). My friend, Mr. I. IKEDA, who devoted himself during the past spring chiefly to the studies of the "Gefässperitonealgewebe" of the Japanese *Phoronis*, tells me that he has never met with the spindle bodies in his slides.

BLOCHMANN ('00) observed the spindle bodies in the coagulated cœlomic fluid of *Lingula* and positively proves the absence of a nucleus in it (p. 118). But he gives no further account of the structure and origin of the bodies. It seems he was not able to observe the spindle bodies proliferating from the epithelial ridge. The dendritic organ, HANCOCK's supposed testis, BLOCHMANN considers as merely the coagulum of the cœlomic fluid (p. 122).

B. OBSERVATIONS.

a. **Form and Structure.**

The spindle bodies assume all imaginable forms, ranging from the spherical to the greatly elongated. Sometimes they are triangular (Pl. I., Fig. 7 *b*), sometimes delicately spindle shaped (Pl. I., Fig. 19), often with one or two constrictions (Pl. I., Fig. 7 *c*); but in the majority of cases they are shaped like a spindle, or like a grain of rice; hence the name. The flagellum-like prolongation of the spindle body at one or both ends is often met with, as observed by BROOKS. This appearance is, I believe, probably due to an artefact, since the spindle body is very soft and plastic as MORSE has stated, and either end of it is, therefore, very easily drawn out. The doubly elongated spindle, which was supposed to be in a stage of division by BROOKS, was not infrequently observed. This I regard merely as a more elongated form.

The spindle bodies vary greatly in size, measuring 55 μ in length and 16 μ in breadth, on an average. The longest with a constriction often attains twice the usual length. On the other hand there are found specially small and slender ones (Pl. I., Fig. 11). Such considerable variations in size make it difficult for us to believe that they can have a definite structure in connection with a definite function.

The spindle body consists of a thin cell membrane with distinctly fibrous contents. The fibres themselves are in a majority of cases arranged in a single bundle parallel to one another, and converging toward either extremity (Pl. I., Figs. 10, 11), but in some instances there have been observed two bundles, so arranged as to form an angle with each other (Pl. I., Fig. 7 *a*): or in rare instances three bundles are present, arranged like the sides of a triangle (Pl. I., Fig. 7 *b*); finally there are those in which several bundles appear running in various directions (Pl. I., Figs. 7 *f*, *g*). Sometimes a bundle of fibres is bent within the membrane (Pl. I., Fig. 7 *d*). Rarely only a fragment of a bundle is found (Pl. I., Fig. 7 *e*). The shape of the spindle bodies, as just described, depends upon the length and arrangement of the fibres. There are also modifications in the mode of combination of fibres with one another: when they are compact they assume the normal spindle shape, while when loosely arranged at one or both ends the bundle does not taper but the ends of the fibres stand widely apart (Pl. I., Fig. 7 *g*). The fibres, it should be stated, are best seen in fresh material just taken out of a living specimen, or in formalin preparations stained with acidulated methyl green. Under this treatment the fibers become somewhat loose and hence more distinct, staining light green with a darker tone in the middle. In favorable preparations treated in the same way, a series of fine easily stained refractive granules can be seen arranged on the equatorial surface (Pl. I., Figs. 8 *a*, *b*, *c*) as observed by CORI in *Phoronis*. These granules cannot be taken as nuclei, since they are not demonstrated by any other method. The fibres in the living state give evidence of a great plasticity: in fixed material, on the other hand, they appear stiff and brittle. Their behavior toward the stains suggests that of a muscle. They are stained red with erythrosin, as the blood corpuscles usually are: in very favorable cases in hæmatoxylin-erythrosin-orange G preparations, they are differentiated from the blood corpuscles and compact bodies (*vide infra*) by their being stained red, while the latter two become yellow (Pl. I., Fig. 10). If the fibres were actually modified nuclei as BEYER maintains, they should be stained green with BIONDI-EHRLICH's mixture, while as a matter of fact they become a homogeneous red.

b. Occurrence.

In the body cavity and the pallial sinus the spindle bodies are found sparingly intermingled with other corpuscles. Among the lobes of the testis they occur in numbers. In the peduncular cavity almost all the floating elements of the fluid are the spindle bodies, which seem to increase in number with age. The reason why they accumulate in this region may, I suppose, be that the spindle bodies are larger than the blood corpuscles and are heavier than the lattter. As to their fate, whether they are retained in the body or at times ejected is quite unknown.

c. Development.

During the larval life the spindle bodies seem to be produced from any part of both the dorsal and ventral body walls. In the adult, on the other hand, the proliferating regions are restricted to two independent places, as I have already stated. The one is the epithelial ridge and the other is the dendritic organ of HANCOCK.[1]

1. BEYER ('86) states that the spindle bodies are proliferated from the lateral body wall, but as this region is occupied by the parietal muscle layer, no proliferating zone can exist there. He perhaps means the dorsal and ventral body-walls instead of the lateral.

In young *Lingula*, or near the tip of the branches of the pallial sinus in the adult, the epithelial ridge is composed of tall columnar cells with enlarged tips and narrowed bases, showing no difference from the structure described by BLOCHMANN ('00). In older individuals, however, this region gives quite a different aspect. In sections of the mantle cut perpendicular to the epithelial ridge (Pl. I., Figs. 5, 9, 10), the following relations will best be seen. The boundary between the tall columnar cells forming the epithelial ridge vanishes as if the cells had been disorganized to a certain extent. The nuclei placed in the lateral portion of the ridge point away from the middle line of the latter. Near the base of the ridge there are found yellowish brown pigment granules. A number of the spindle bodies occur imbedded in the epithelial ridge, and, in a majority of cases lie in a cavity which has no membrane delemiting it from the neighboring part. What attracts special attention is the fact that the spindle bodies are found lying in any position in the ridge: sometimes they stand almost perpendicular to the surface, while in other cases they are placed nearly parallel. In an extreme case the spindle body is so elongated that its length exceeds the thickness of the ridge and appears to find hardly room enough for itself there (Pl. I., Fig. 11). From these facts alone one may consider that the spindle bodies do not belong to the epithelial ridge, but that they are introduced from without. That the spindle bodies, or at least their antecedents, are thus introduced will be shown directly. When they attain full growth they are set free into the pallial sinus. And very often there are seen holes which are formed after the escape of the spindle bodies. The sections through the middle line of the epithelial ridge also give very instructive figures, spindle bodies of different sizes being met with throughout almost the entire length of the section.

In young *Lingula* (4.5–10 mm. in shell length) at the posterior region of the dorsal and ventral body walls, where the dendritic organs of HANCOCK are to be developed, the peritoneal layer, a very thin epithelium (Pl. I., Fig. 14), is separated from the outer epithelium of the body wall, next the shell, and in the lumen thus formed many spindle bodies are found together with the leucocytes and the compact bodies. In preparations of the adult *Lingula*, especially in those fixed with sublimate, when all the viscera are taken out before or after the fixation, the dendritic organs come distinctly into view as three irregularly pinnated ridges (Pl. I., Fig. 12); the central being the longest, runs along the median plane; the lateral ones along the lateral body wall. Differing much from the structure seen in the young individuals, the organ in sections shows that it is composed of a many layered epithelium with vesicular nuclei and granular cytoplasm without cell-boundaries (Pl. I., Fig. 13). In this epithelium an enormous number of spindle bodies are found: a few of them are directly imbedded in the epithelium, while the rest, in groups of twenty or thirty lie in hollows. In the neighborhood of the cavity cytoplasm becomes disorganized as in the case of the epithelial ridge.

To the curious phenomenon that such enigmatical bodies of various sizes are produced in such a remarkable number from definite regions, I paid not a little attention, and at last I came to discover nearly *all stages of transformation from the ordinary blood corpuscles up to the spindle bodies.* Unfortunately the blood corpuscles were not found in the epithelial ridge, but in the dendritic organ of young *Lingula* they were distinctly seen enclosed in the cavity (Pl. I., Fig. 14). As the first step of transformation the blood corpuscles become spherical: the cytoplasm shows a granular appearance, and the nucleus is pushed aside until it lies just inside the cell-membrane (Pl. I., Fig. 15). Then the nucleus seems to undergo degeneration though I was not able to obtain sections favorable for studying the karyolysis, and the granular cytoplasm changes into a very compact homogeneous and strongly refractive sphere. This I shall call the compact body (*c. p.*). Such bodies occur, in a tolerably large number, constantly imbedded directly in the epithelial ridge and the dendritic organ (Pl. I., Figs. 10, 13). They take a darker erythrosin stain than the spindle bodies and in all their behavior toward stains the compact bodies show no difference from the blood corpuscles. The bodies vary greatly in size (Pl. I., Fig. 13) although the blood corpuscles are of an uniform size. In what way such differences in size arise I do not understand: they may perhaps be caused by the fact that different portions of cytoplasm are used for the formation of the compact bodies. Sometimes, but not always, the compact bodies then undergo eccentric splittings, as the result of which a sickle-like space is left between the two portions in the section (Pl. I., Figs. 17 *a*, *b*). Sometimes a small spherical portion is formed near the periphery and this acts as the centre of splitting (Pl. I., Fig. 16). Next the fibres are formed at the expense of the compact metamorphosed cytoplasm; at this time an enormous growth in length certainly takes place, as the fibres are not found coiled up from the outset of their formation. From each portion of the compact body thus split, a bundle of fibres is formed. Consequently all imaginable forms of spindle bodies are produced according to the degree and mode of splitting. In some cases I found spindle bodies, in which one portion had already turned into fibres, while the other remained in its orginal state (Pl. I., Fig. 18); and in other cases lying beside the perfect spindle bodies, the remnants of compact bodies were found enclosed in the cell-membrane. Although from the fact just described it is obvious that the fibres are formed from the compact bodies, yet as to the manner in which the growth in length of the fibres takes place I cannot at present give any positive datum. Judging, however, from the fact that while the compact bodies are directly imbedded in the epithelium, and the spindle bodies are found in many cases lying in a cavity, it is probable that the spindle bodies in their growing stage emit a certain enzyme in order to dissolve the neighboring cytoplasm and to take it up in themselves. Owing to this nutrition, if it may be so called, the fibres of the spindle bodies increase in length, at the same time becoming more or less loose.

d. **What is the Spindle Body?**

As above stated the spindle body is a metomorphosed blood corpuscle, as Cori has observed in *Phoronis;* that is, it is distinctly *a cell, whose nucleus has degenerated and whose cytoplasm has been turned into a fibrous structure.* Since in every individual the spindle bodies are produced in such great numbers in definite regions, they cannot be looked upon as pathological products. As they are enucleated cells they are destined sooner or later to die out. They must, however, as such or at the time of their formation, play an important part in the economy of *Lingula.* It is certain that the blood corpuscles, leucocytes and spindle bodies have their respective offices. Among several hypotheses which suggest themselves in regard to the function the of spindle bodies the following seems to me the most probable. As the cœlomic epithelium of other animals often perform the excretory function, so the cells forming the dendritic organs and the epithelial ridges accumulate waste products found dissolved in the cœlomic fluid. The blood corpuscles now force their way into these regions in order to take up the waste substance accumulated there. At the same time they are turned into the spindle bodies and set free into the body cavity again, being in the end collected in the peduncular cavity; in short, *the spindle bodies function only at their formation as the eliminators of waste products.*

III. OTOCYSTS.

MORSE ('78) is the only investigator who describes the occurrence of the otocysts in the adult *Lingula.* Unfortunately his paper has not been published in full, so that no details are forth-coming. The only thing I can learn is that "their [otocysts'] position and general appearance recall the auditory capsule figured by *Claparède* in certain tuticolous Annelids" (p. 157).

On the other hand, in his recent work BLOCHMANN ('00) pronounced thus positively upon the absence of the otocysts: "Nun kann ich zunächst mit voller Sicherheit behaupten, dass bei erwachsenen *Lingula* Otocysten nicht vorhanden sind" (p. 124). He ('98) regarded the vesicles found in the larvæ of *Discinisca* and referred to as the otocysts by FRITZ MÜLLER ('60, '61) as the funnels of the nephridium. Moreover he claimed to have found a duct leading from this organ to the exterior.

In the free-swimming larvæ of *Lingula* BROOKS ('78) rightly observed the otocysts, but he was not able to study the young *Lingula,* and was very cautious in his statements about the fate of these vesicles.

For the purpose of determining how long the otocysts which are present in the larvæ of the 5–15 p. c. stage persist, I searched after the structures in many individuals at the identical place where they are situated in the larvæ, and in all cases I could discover there a pair of sharply outlined vesicles which can be identified as the otocysts. The structure I was able to find in our *Lingula* is probably one and the same organ that Morse discovered twenty three years ago, since the vesicles are very conspicuous and there are no other organs which can be mistaken for the otocysts.

The otocysts are found even in the oldest individuals I have examined (45 mm. in shell length). They are a pair of vesicles of so conspicuous a size that an experienced observer can easily see them with the naked eye. In young *Lingula* (4.5–10 mm. in shell length) with translucent shells, we can readily detect with strong reflected light a pair of otocysts under the dorsal valve, the otoliths in constant dancing motion and the blood corpuscles running about the otocysts. In older individuals the shell increases in thickness and prevents the transmission of light: in such cases we are able to detect the vesicles only in fixed specimens. To have the best surface aspect of the organ the material should be fixed with chromo-acetic or some other mixture with chromic acid, since in a specimen thus treated the connection between the valve and animal becomes loose, so that the shell can easily be peeled off from the soft part without danger of injuring the latter.

With this general remark I shall enter upon a detailed description of the otocysts. In a dorsal view of the fixed specimen whose dorsal valve is taken off we can at once make out the otocysts imbedded in the supporting substance, upon the gastoparietal band, a little posterior to the median half of the *occlusor anterior,* and near the bottom of the lateral indentation of the body cavity. Fig. 20 (Pl. II.) shows the position of the organ. Just distal to this vesicle the pallial sinus comes into communication with the body cavity.

Generally speaking the organ is lenticular, but there are not wanting variations: in a dorsal view it is sometimes nearly triangular with rounded angles, while in other cases it is almost circular. As a rule it is broader than it is long. Its walls are not of equal thickness, but are thickest on the median and lateral walls, and thinnest on the dorsal and ventral sides. There is also a great variation in size: the following is an average of the measurements of the organ in various sections.

Length	... ...	145 μ
Breadth	... ...	160 μ
Height	... ...	40 μ
Thickness of the walls	thickest portion ...	35 μ
	thinnest ,, ...	8 μ

In tracing a series of longitudinal sections (Pl. II., Figs. 21, 22) as soon as the valve at the entrance of the pallial sinus comes to an end, and in the same section in which the nephridium is about to open to the exterior on the ventral side the otocyst becomes visible. Following the sections toward the median plane we soon find that the otocyst is a closed sac, as is seen on a surface view, and that it bears not a little resemblance in structure to the same named organ occurring in *Arenicola grubii* studied by E. EHLERS (Taf. XIII., Fig. 37). It is situated just anterior to the attachment of the gastroparietal band to the dorsal body wall, so that it has a lamella of the supporting substance at the posterior end in common with the above band. The greater part of the ventral face, the entire anterior face and a small part of the dorsal face of the otocyst is free and coated with a layer of longitudinal muscle which attains a considerable thickness at the anterior end. The otocyst itself is composed of an external layer of the supporting substance and an inner sac formed by a thick epithelium. The supporting layer is of different thickness at different places: it is thickest at the posterior edge and on the median and lateral sides, while in other parts it remains thin. Interior to the supporting layer there is a very thick epithelial sac, composed of tall columnar cells with nuclei near their bases and placed at several different levels. The nuclei are of a spindle shape, and have a great affinity for stains. The epithelial cells are highest along the edge formed by the meeting of the two surfaces of the lens-shape. The cells are throughout of the same structure and no other elements occur among them. At the tip of these cells facing toward the cavity there is no indication of the cuticula formation, but there fine rod-like granules are seen, which with a high degree of certainty are to be regarded as the basal pieces of cilia, indicating that the inner cavity of the otocyst was covered with cilia, although in fixed specimens no cilia could be detected. In the cavity there are found some thirty spherical otoliths, varying greatly in diameter, the largest measuring 3–4μ. They are compact, highly refractive and take a somewhat deep hæmatoxylin stain which proves that they are organic in composition and hence not introduced from without, but secreted from the walls. Along the margins of the lenticular vesicle there is left a ring-shaped space filled with granular substance and in one region a few muscle fibres. The former is no doubt nervous in nature, but I must confess that but little is known about the origin and termination of the nerve fibres among the epithelium.

From the above description no one would doubt the presence of the otocysts in the adult *Lingula,* at least in the Japanese form, and my account is confirmatory of MORSE's discovery.

Physiologically the organ just described would probably subserve a static function, as in similar organs found in other animals. The terms "otocyst" "otoliths" are employed here simply in the morphological sense.

IV. HEART.

Into the macroscopical feature of the heart I shall not enter, as it has already been fully described by HANCOCK ('59) and BLOCHMANN ('00). In sagittal sections of the young *Lingula* (Pl. II., Fig. 23, *h.*) the finer structure of the organ is best seen. In the region which we designate as heart, the blood vessel increases slightly in calibre. The constrictions of the vessel are not constant, varying according to the state of contraction at the time of killing. The vessel is here composed of a tube of columnar or cubical cells and the inner muscular layer. The epithelial cells vary considerably in thickness in different places. As a rule the cell has an enlarged tip, and an attenuated base. The nuclei placed in the middle of the cells are rather compact and stain intensely. Interior to the epithelial layer a thin coating of muscle fibres is found. The fibres do not appear to run longitudinally, but to take a somewhat screw-like course. The movement of the fluid therein contained is due to the contraction of the muscle. At the transverse dorsal ridge of the stomach, upon which the heart is situated, the layer of the supporting substance attains a considerable thickness projecting for a short distance posteriorly (Pl. II., Fig. 24). To this protuberance small bundle of muscle secures its attachment. The muscle has a sheath of cell layer as in other muscles. It runs ventral to the heart vessel, and posteriorly gradually attenuating into a fine string, is finally inserted on the dorsal wall of the stomach. The muscle serves probably to draw the posterior part of the stomach nearer the region of the gastroparietal band, facilitating the movement of food particles in the alimentary canal, and indirectly of the lymph fluid in in the heart tube.

V. OVARY AND TESTIS.

On the sexual organs and elements of *Lingula* BLOCHMANN did not touch at any place in his recent paper ('00). It may, therefore, not be superfluous to devote a few pages to this point. As for finer studies on ovogenesis and spermatogenesis I shall put them off for some future time.

Lingula is an animal of distinct sexes as is the case with other Brachiopods. MORSE was the first to emphasize this fact, for he was able to study *Lingula* in life (73 *b*, p. 38). Thirteen years later BEYER ('86), advanced another view, maintaining that *Lingula* is hermaphrodite. This conception originates from HANCOCK's statements. As both the sexual elements are very much alike in appearance, the last named author did not distinguish the testis from the ovary; or, if not, the specimens he studied were all female. At any rate he did not notice the true testis in his monograph ('59) and regarded the dendritic proliferating zone of the spindle bodies on the walls of the body cavity as the testis. BEYER found the same structure as HANCOCK's supposed testis in the epithelial ridge of the pallial sinus and mistook the spindle bodies for spermatophores. Moreover he extended his view, regarding the round compact bodies also found in the epithelial ridge as young ova. As I have explained at great length in Section II, these two enigmatical bodies are not sexual elements at all.

In *Lingula* the formation of sexual elements is restricted entirely to the ileo-parietal band, and the sexual organs in the pallial sinus found in other Brachiopods are entirely wanting. Both the ova and spermatozoa are nothing else than modified epithelial cells on either side of the ileo-parietal band, as has already been described by many writers on the Testicardines.

The sexes of *Lingula* are after some experience easily distinguished from without. Seen through the translucent shell the females appear somewhat dark brown, while the males lighter brown or even whitish. The distinction can best be seen in individuals of a medium size, for in older animals the shell increases in thickness and obscures these features.

a. Ovary.

As to the structure and development of the ovary: in free-swimming larvæ with eight pairs of cirri there are distinctly seen, though few in number, primary germ-cells,[1] some which are distinguished from other epithelial cells by their larger size, granular cytoplasm, and vesicular nuclei.

In young *Lingula*, 4.5 mm. in shell length the sex is already differentiated; the undifferentiated condition, therefore, must be sought for in individuals younger than this. In Pl. II., Fig. 26

1. *Vide* N. YATSU. On the Development of *Lingula anatina*. This volume, Art. 4, p. 60.

the ileo-parietal band and a portion of the nephridium from a transverse section through a young *Lingula* (5.5 mm. in shell length) is shown. Here we see at once that the ileo-parietal band is nothing but a folding of the visceral layer of the mesoblast, which covers the nephridia as well as the alimentary canal. Moreover in the figure we can distinguish two kinds of cells which constitute the ileo-parietal band: the one with vesicular nuclei, and the other with compact nuclei. The former are those which have already acquired the character of ova, while the latter kind of cells are those which still remain as ordinary epithelial cells. The supporting substance between the two layers of the ileo-parietal band is not as yet formed.

In young *Lingula* of 9–14 mm. shell length the layers of the ileo-parietal band bearing the young ova are subjected to a high degree of folding, so that in sections they give a dendritic appearance.

In the female which has almost attained maturity, the ovary appears as a conspicuous mass of cells of a darker brown than the liver, filling up the main part of the body cavity, and protruding even into the "Erker." The ova (Pl. II., Fig. 27) are prism-shaped, pentagonal or hexagonal in section, and slightly rounded out on their free surfaces. They measure 60 μ in diameter and 90 μ in height. Their cytoplasm (Pl. II., Fig. 28) contains minute yolk granules uniformly distributed. In larger ovarian ova vacuoles are found scattered throughout the peripheral portion. The vacuoles apparently result from the dissolving away of the yolk granules.

The nucleus, enclosed by a definite membrane, lies always near the free surface: a portion which must bear some relation to the metabolic function of the ovum (Pl. II., Fig. 27). The nucleus is vesicular and stains less intensely than cytoplasm, as if it were quite devoid of chromatic substance.

In young ova the nucleolus is single but in older ovarian ova there appear accessory nucleoli (*a. nl.*) which are usually found closely apposed to the principal nucleolus (*p. nl.*) (Pl. II., Figs. 28, 29 *a*, *c*). But sometimes they detach themselves from the principal one (Pl. II., Fig. 29 *b*). The accessory nucleolus is not always spherical, in some cases assuming an irregular outline (Pl. II., Fig. 29 *b*). The two kinds of nucleoli can easily be differentiated by gentian violet, the principal one staining darker than the accessory, as is shown in the figures (Pl. II., Figs. 28, 29). Consequently it seems that the accessory nucleoli are less dense than the principal. In the Testicardines the presence of two kinds of nucleoli is clearly figured by VAN BEMMELEN ('83), but he says nothing about it. VOGT and JUNG ('88) mention that often two nucleoli are present. The supporting substance (Stützsubstanz) of the ileo-parietal band is found as a thin membrane between two layers of ova (Pl. II., Figs. 27, 28). This membrane BEYER ('86) neither mentions nor figures. At some places the membrane increases in thickness and gives a vacuolated appearance (Pl. II., Fig. 30).

Even in the region where ova are developed cells are of course not all converted into them. The follicle of the ovum is formed at the expense of interstitial cells, which become extremely flattened, so that their presence is perceived only by their compact and spindle-shaped nuclei (Pl. II., Fig. 28). Of the cells which have started to become ova, some lying pressed between two adjacent ova are arrested in their early development and remain small. That these degenerating ova take a hæmatoxylin stain more readily than others, shows that they are at the beginning of yolk-formation.

Yellowish brown pigment granules are found scattered here and there along the supporting substance of the ileo-parietal band and among the ova. They are compact and polygonal granules of various sizes (Pl. II., Figs. 27, 30). These granules must have been formed by a process of pigment degeneration of the young ova (early arrested in their development) and even of the ripe ova. In large specimens of *Lingula* collected at the end of the summer or in the fall, the ovary is found very much reduced in size as dirty brown masses with black spots. On cutting such an ovary into sections we meet with diverse stages of karyolysis and plasmolysis of the ova; the latter at first provided with vesicular nuclei come to increase in consistency and break up into granules. But I could not determine whether it is only the ova left behind after the ripe ova had been deposited that are subjected to degeneration or whether losing the opportunity of discharging, the entire ovary, with both ripe and unripe ova, turns into pigment granules. Must probably both these things take place. LANKESTER ('73) found in *Terebratula vitrea* the yellow matter among the ovarian ova and this he considered as the envelope of escaped ova (p. 93). JOUBIN ('86) states that on keeping *Crania* the eggs atrophied and turned into brown bands (p. 255).

b. **Testis.**

In male individuals the spermatozoa are formed only in the region corresponding to that in which the ova are produced in the female. About the supporting substance of the ileo-parietal band is a tolerably thick layer of cells with vesicular nuclei; this layer consists of the spermatogonia and spermatocytes. Exterior to this there is another thicker layer of compact nuclei, being the clusters of the heads of spermatozoa. The outer surface of the latter layer has a ciliated appearance owing to the presence of the tails. Spindle bodies force their way into the narrow spaces of the testes, probably bearing some important physiological relations to sperm cells. The above two layers of testis are noted by VOGT and JUNG ('88) in their Lehrbuch (p. 724).

The spermatozoon has a head, slightly pointed at the anterior end and rounded posteriorly (2 μ), a very short middle piece (1 μ) and a comparatively long tail (40 μ) (Pl. II., Fig. 25).

At what age individuals discharge their sexual elements I could not determine. It is certain that *Lingula* of about 30 mm. in shell length discharge eggs, as I have observed the act. As to the male, the spermatozoa are met with even in so young an individual as one 7 mm. in length. Whether spermatozoa which are so precociously developed are retained in the body for a long time or are soon discharged, is quite unknown.

VI. LITERATURE CITED.

'86. BEYER.—A Study of the Structure of *Lingula* (*Glottidia*) *pyramidata* STIM (DALL).—Studies from the Biol. Lab. Johns Hopkins University. Vol. III. No. 5. pp. 227–265.

'98. BLOCHMANN.—Die Larve von *Discinisca* (Die Müller'she Brachiopodenlarve).—Zool. Jahrbücher. Bd. 11. pp. 417–246.

'00. ——— Untersuchungen über den Bau der Brachiopoden. Zweiter Theil. pp. 67–124.

'62. BRONN.—Die Klassen und Ordnungen des Thierreichs. Bd. III. Abth. I. pp. 255–316.

'78. BROOKS.—The Development of *Lingula* and the Systematic Position of the Brachiopoda.—Scient. Results of the Session of 1878, Chesapeake Zöol. Laboratory. pp. 35–112.

'91. CORI.—Untersuchungen über die Anatomie und Histologie der Gattung *Phoronis*.—Zeit. wiss. Zool. Bd. LI.

'92. EHLERS.—Die Gehörorgan der Arenicolen.—Zeit. wiss. Zool. Bd. LXII. pp. 169–249.

'96. FRANÇOIS.—Choses de Nouméa.—Arch. d. Zool. expérim. et générale. (2) Tom. IX. pp. 229–245.

'60. GRATIOLET.—Recherches pour servir à l'Histoire des Brachiopodes. 2me Monographie. Etude anatomique sur la *Lingula anatine* (*L. anatina* LAM.).—Journ. de Conchologie. VIII. 2me Série. IV. pp. 49–107, 120–172.

'59. HANCOCK.—On the Organization of the Brachiopoda.—Philosophical Transactions. London. Vol. CXLVII. Part II. pp. 791–869.

'73. MORSE.—The Systematic Position of the Brachiopoda.—Proc. Boston Soc. Nat. Hist. Vol. XV. pp. 315–371.

'78. ——— On Japanese *Lingula* and Shell Mounds.—Am. Jour. Sc. and Art. Vol. XV. pp. 150–157.

'60. MÜLLER F.—Beschreibung einer Brachiopodenlarva.—Müller's Arch. f. Anat. n. Physiol. pp. 72–80.

'61. ——— Die Brachiopodenlarve von St. Catarina. Zweiter Beitrag.—Arch. f. Naturgesch. Vol. XXVII. pp. 53–56.

'61. SEMPER.—Reiseberichte.—Zeit. wiss. Zool. Bd. II. pp. 100–104.

'83. VAN BEMMELEN.—Untersuchungen über den anatomischen und histologischen Bau der Brachiopoda Testicardinia.—Jen. Zeit. Bd. XVI pp. 88–161.

'45. Vogt.—Anatomie der *Lingula anatina*.—Neue Denkschrift der schweizer Gesellschaft. Naturwiss. VII. pp. 1–18.

'88. Vogt und Jung.—Lehrbuch der praktischen vergleichenden Anatomie. pp. 699–733.

Abbreviations used in Plates.

a. nl..................accessory nucleolus.
ant. dr. lv. ......anterior dorsal lobe of liver.
bl. cp. ....blood corpuscle.
cl. m. ....circular muscle of the stomach.
cp..................compact body.
d. o. ....dendritic organ.
dr. sh. ....dorsal shell.
ep. rd. ....epithelial ridge.
foll. ....follicle cell.
gl. cll. ....gland cell of mantle.
gm. cll............germ cell.
gst. pr. bd. ......gastroparietal band.
h. ....heart.
il. pr. bd.........ileo-parietal band.
lcy..................leucocyte.
lat..................M. lateralis.
lv. ....liver lobule.
mg. l. ....marginal lacuna.
m. pr. ....parietal muscle.
n. ....nucleus.
nph. ....nephridium.
obl. int............M. obliquus internus.
occ. ant. ....M. occlusor anterior.
ot. ....otocyst.
otl...................otoliths.
p. nl...............principal nucleolus.
pll. sn. ....pallial sinus.
pig...................pigment.
r. g. ....refractive granule.
spd. ....spindle body.
spl. ....supporting layer.
stm. ....stomach.
vt. sh. ....ventral shell.

PLATE I.

Plate I.

Fig. 1.—Blood corpuscles taken from a living specimen of the adult *Lingula*. × 310.

Fig. 2.—Blood corpuscle treated with acidulated methyl green. × 800.

Fig. 3 *a, b*.—Two leucocytes taken from a living specimen. × 380.

Fig. 4.—Star-shaped leucocyte, from a hæmatoxylin-erythrosin preparation. Blood corpuscle is also drawn for comparison of size. × 729 (immers).

Fig. 5.—Transverse section through the epithelial ridge. × 729 (immers).

Fig. 6.—Leucocyte embracing a spindle body; a compact body by it. × 729 (immers).

Fig. 7 *a*.—Spindle body with two bundles. × 380.

b.—Spindle body with three bundles. × 800.

c.—Spindle body with a constriction. × 380.

d.—Spindle body with a bundle bent within the membrane. × 380.

e.—Fragment of spindle body. × 380.

f.—Spindle body with fibres running in various directions. × 380.

g.—Spindle body in the dendritic organ with very loose fibres in the cell membrane. × 729.

Fig. 8 *a*.—, *b*.—, *c*.—Three spindle bodies treated with acidulated methyl green. × 490.

Fig. 9.—Transverse section through the mantle perpendicular to the pallial sinus. Here two epithelial ridges are seen as the section passes the point of bifurcation of the pallial sinus. × 80.

Fig. 10.—Transverse section through the mantle perpendicular to the epithelial ridge. Hæmatoxylin-orange G-erythrosin preparation. × 490.

Fig. 11.—Portion of a section of the mantle. × 420.

Fig. 12.—Dendritic organs (*d. o.*) on the ventral body wall exposed. Sublimate preparation. × 2.

Fig. 13.—Transverse section through a dendritic organ of the adult. × 500.

Fig. 14.—Transverse section through the young *Lingula* of 5.5 mm. shell length, showing only the dendritic organ. × 729 (immers).

Fig. 15.—Blood corpuscle in a stage of transformation into the compact body. The nucleus is pushed aside and the cytoplasm becomes granular. × 729 (immers).

Fig. 16.—Transverse section through the epithelial ridge of the adult. A compact body is imbedded in the epithelium. Three blood corpuscles are shown for comparison. × 729 (immers).

Fig. 17.—Two compact bodies of different sizes; both are much smaller than usual compact bodies. The splitting can here be seen. × 729 (immers).

Fig. 18.—Compact body from the dendritic organ, half of which has been metamorphosed into fibres. × 729 (immers).

Fig. 19.—Spindle body with the remnant of a compact body from a section through the dendritic organ. × 729 (immers).

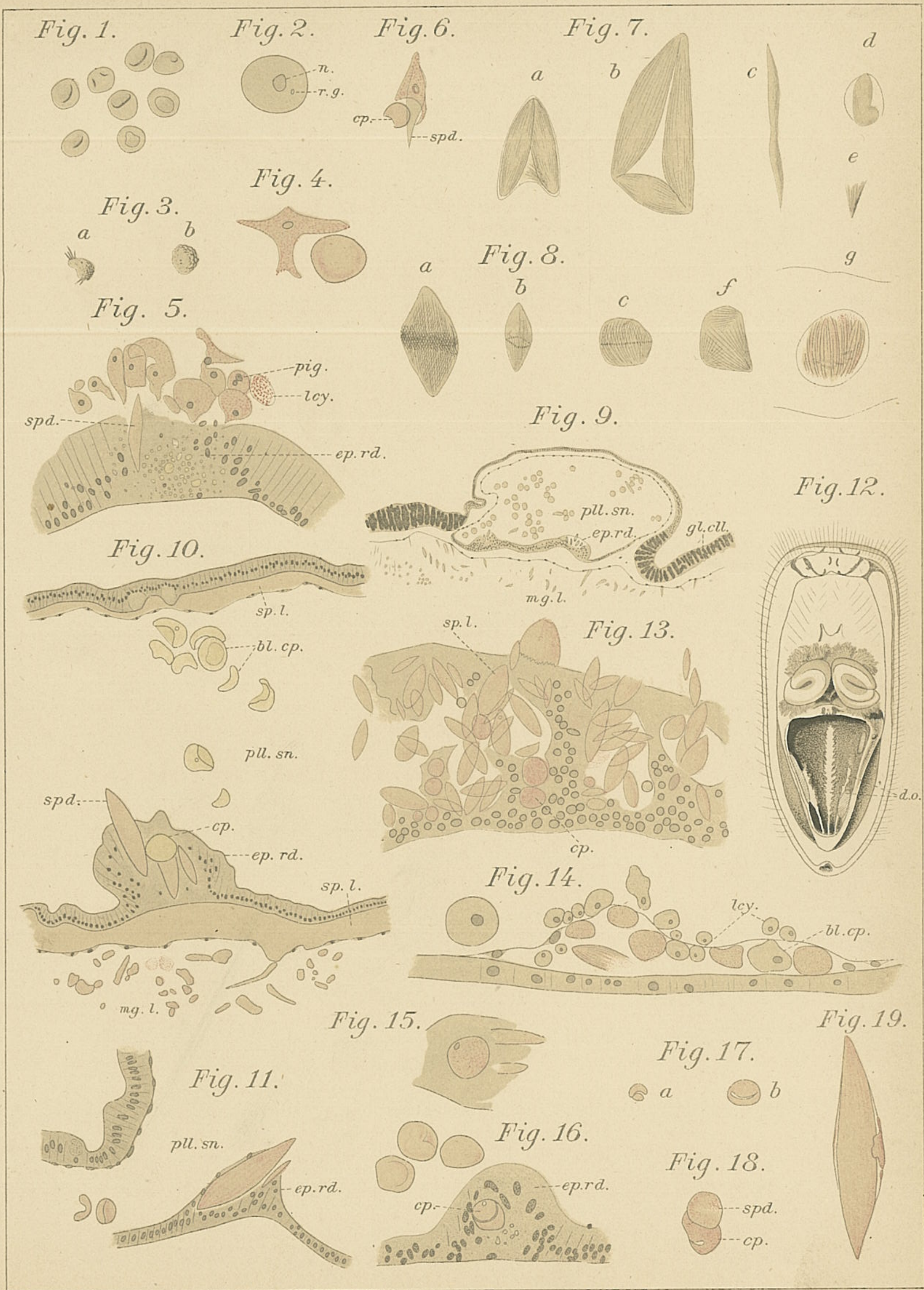

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PLATE II.

Plate II.

Fig. 20.—Left anterior portion of a young *Lingula* of 5 mm. shell length, showing the otocyst. Unstained clarified preparation. × 80.

Fig. 21.—Sagittal section through a young *Lingula* of 6.5 mm. shell length. Acetic-sublimate preparation. × 80. *lv.* liver lobule.

Fig. 22.—Otocyst of the same section as that drawn in Fig. 21. × 500. *otl.* otoliths.

Fig. 23.—Portion of sagittal section through a young *Lingula* of 6.5 mm. shell length, showing the heart. × 380.

Fig. 24.—Portion of sagittal section through a young *Lingula* of 7.5 mm. shell length, showing the heart and the muscle running below it. × 729 (immers).

Fig. 25.—Spermatozoa, from living material. × 500.

Fig. 26.—Portion of transverse section through young *Lingula* of 5.5 mm. shell length, showing the young ova in the ileo-parietal band. A portion of the nephridium is also represented. × 729 (immers).

Fig. 27.—Section through the ovary of the adult. × 729 (immers).

Fig. 28.—Full grown ovarian ovum from the above section. × 490.

Fig. 29.—Three nuclei of ovarian ova, each with a principal nucleolus and accessory nucleoli.

Fig. 30.—Section through the ovary of an old individual killed late in the autumn. × 490.

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