On the Development of Limulus Longispina.

By

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With Plates V—XI.

After working out a general outline of the development of spiders,* I undertook the study of the development of the *Limulus* of this country with the object of ascertaining, if possible, how it is related to the Arachnida. In order to collect materials for this investigation, I was enabled, by the liberality of the University authorities, to visit Ajino in the province of Bizen, in the summer of 1889, and again in the same season of 1890. In that place, I had exceptional opportunities of collecting and observing the eggs of *Limulus*, by the kind assistance of Mr. B. Nozaki and Mr. T. Nozaki to whom my best thanks are due.

Limulus longispina is generally known by the name of *kabutogani*, but there are many local names such as *umidongame*, *mangoyei*, *hachigani*, *unkyū*, etc. In Bronn's "Die Classen und Ordnungen des Thierreichs" we find it stated that the Chinese call their *Limulus* as *Umi-dogame* or *Unkiie;* but this is evidently a mistake, these terms being a corruption of the Japanese names, *umidongame* and *unkyū*.

* Kishinouye—*On the Development of Araneina.* This Journal, vol. IV. Part I.

Of the spawning of the American *Limulus* there is a minute description by Lockwood in the *American Naturalist* vol. IV ; but as the spawning process of the Japanese *Limulus* differs a little from that of the American species, I shall give a brief account of it.

The *kabutogani* deposits its eggs chiefly in August. On calm nights during that month, males and females, the former on the abdominal shield of the latter, come in with the rising tide towards sandy shores. Judging from appearances after deposition, the process of spawning is as follows : Egg-laying takes place between the tide-marks, but only as this space becomes covered with water. The female excavates a hole, about 15 c.m. deep, and deposits eggs in it, while the male fertilizes them. The female afterwards buries them and begins to excavate the next hole. This process is repeated many times.

As *Limulus* can not dig deeper than about 15 cm. when the diameter of the hole is less than the breadth of the cephalothorax, the eggs deposited in such holes are liable to be washed out of the sand, and carried away by waves, when the wind is rather strong. This is especially the case with older eggs the vicarious chorion of which being inflated acts as a sort of float.

As a general thing, fishermen do not pay much attention to the *kabutogani* on account of its small economic value, and even an experienced fisherman does not know the place where it spawns. Such being the case, Mr. T. Nozaki kindly detailed a fisherman to make special search for the *kabutogani's* eggs along the shores of the Inland Sea. The fisherman succeeded in finding spawn of the animal on the shore at Obatake, a village not far from Ajino. I was taken by the fisherman and Mr. Nozaki to this spot. When we reached the place, the fisherman began to dig at random those parts of the shore, from which the tide had retired.

While I was looking about, my attention was caught by peculiar markings on the shore, such as are represented in fig. 1. On a close examination these markings proved to be the tracks of the spawning *kabutogani*. They consisted of a number of small roundish elevations (about 35 cm. in diameter) stretching in a line, which is always perpendicular to the coast-line and never parallel with it. The length of the line is $1\frac{1}{2}$–4 m.

The elevations are highest at one extremity and become gradually lower towards the other. The higher end is the spot where the *kabutogani* began egg-laying. Near the end of spawning, the legs of the female animal are no doubt tired, so that she cannot cover eggs with as much sand as at the beginning of the operation. The lower end of the spawn is often lost in scratches from legs, which show that the direction of movement has been from the higher to the lower end. The higher end may be either near to or away from the shore. From this fact we may conclude that the *kabutogani* in depositing eggs proceeds either towards or away from the shore. The latter will probably be the case when the spawning begins at the time of the receding tide, and the former when it begins at the time of the rising tide.

Under each elevation of the spawn there are about one thousand eggs aggregated and compressed together into a mass. The mass of eggs is not placed just under the centre of each elevation, but is deposited either to the left or to the right side of the median line of the whole spawn. On digging out one whole series of a spawn, I observed that in the higher half of it the egg-mass deviated towards the left side, and in the lower half towards the right side. From this I concluded that in this case the *kabutogani* deposited the eggs of the left ovary first, and then those of the right ovary.

As eggs are easily washed out by waves, it is very difficult to obtain them in their later stages. Even when not washed out, the

peculiar and very convenient marks of the spawn are lost after three or four days. Therefore I found it necessary to dig out eggs as soon as possible after the time of deposition and to transfer them into a large wooden box, especially made for this purpose. The box was filled with sand and was placed at the mouth of a canal. The place was happily chosen as the box was covered by water during high tide and exposed to the air during low tide, and was always exposed to the sunshine in the day-time.

In the summer of 1889 I buried eggs of different stages in the box; but in the year following I buried only two series of spawns which were deposited on the same day, and thus I could easily find out how many days old my eggs were.

Eggs one or two days old are not spherical but polyhedral by mutual pressure. At this time they are connected together by the coagulation of a fluid with which they were covered, when deposited. The average diameter of an egg is $2\frac{1}{2}$ mm. Its yolk is yellowish and glutinous; but when exposed to the air it soon loses water and changes its color to dirty green, and becomes hard, and brittle. It is covered with a thick, tough chorion which with the abundant yolk makes observations either from the surface or by section very troublesome.

Eggs were fixed by heating in water to 60°–70° C., or by plunging into hot water of the same degree. After cooling they were transferred into 70 % alcohol, in which they were left one or two days. Then in the case of eggs of early stages some of them were pierced through into the yolk with the point of a fine needle at two or three spots, care being taken not to hurt the germinal disc. These perforated eggs were left in 70 % alcohol for one or two days more, and afterwards were dehydrated in ascending grades of alcohol. The chorion is split naturally or by heating and is easily removed; but at these stages the vicarious chorion is formed. This membrane was perforated

before heating by applying the points of two fine needles from opposite points at the same time.

For the surface-view of the embryo, the ventral plate was peeled off from the underlying yolk of the preserved egg, and was stained with borax carmine, then washed in acidulated alcohol, and after dehydration and clarification was imbedded in Canada-balsam.

For the sections the eggs were stained with borax carmine or haematoxylin *in toto*. As the yolk is very abundant, the sectioning was very troublesome. In the case of the early stages in which the dorsal part consists only of the one-cell layered epiblast, I cut away this part as much as possible. By using the celloidin-paraffin method of imbedding I obtained very good sections.

I am sorry that I could not observe thoroughly the whole developmental history of the *kabutogani*, for want of sections of eggs of very early and of very late stages. My observations were made chiefly on the stages from the formation of the germinal layers to about two weeks before hatching.

External Changes.

On July 29th, 1890, I dug out two series of the spawn, which had been laid on the morning of the same day, transferred them into the box before mentioned, and examined and preserved eggs from them every day.

On August 2nd (5 days after spawning), I saw many irregular furrows on one pole of the egg (fig. 2). This was the first surface change I observed. These furrows were observed by Osborn[1] in the American *Limulus*.

1) Osborn—*Metamorphosis of Limulus polyphemus*. Johns Hopkin's Univ. Circ. vol. V. 1885.

On August 7th (10 days after spawning), I found a small round whitish disc (fig. 3). This gradually enlarged and on August 10th (13 days after spawning) it had assumed an oval shape. The longer axis of the disc corresponds to the future median line of the embryo, and the disc itself is the rudiment of the ventral plate. The disc had divided into two unequal parts by a transverse line (fig. 4). The smaller portion of the disc corresponds to the cephalic lobe and the first appendage-bearing segment. New segments are successively cut off from the anterior end of the larger, posterior portion.

On the morning of August 11th (14 days after spawning), three transverse lines and a median longitudinal line were visible (fig. 5). The median line did not extend posteriorly beyond the third transverse line. Thus at this stage the ventral plate is divided into four segments, three anterior ones of which are divided into two lateral halves by the median longitudinal line. About noon of the same day one more segment had been added (fig. 6), and the median line had been produced to the posterior end of the new segment.

On August 12th (15 days after spawning), five transverse lines were seen, and the median line was produced beyond the penult segment (fig. 7). Although the change is very gradual, yet when we compare the two ventral plates represented in fig. 5 and fig. 7, we find that the ventral plate is much larger in fig. 7. Moreover while the angle made by the lateral halves of a segment with the median line is almost a right angle in fig. 5 and in the anterior region of fig. 6, the angle has become acute in fig. 7—the acuteness increasing more and more towards the posterior end.

On August 13th (16 days after spawning), many important changes had occurred. The external ends of the segments had united with each other forming a white marginal band on each side of the

ventral plate (figs. 8, 9, *mar. bd.*). Packard[2] describes the band accurately. He says: "Around the edge (of the 'primitive disk') is a pale areola, destined to be the lower edge of the carapace; it is most distinct along the middle, merging imperceptibly into the anterior and posterior end of the disk. It is a thin ridge, due to a local heaping up of cells." Almost simultaneously with the appearance of the marginal bands, two paired longitudinal grooves are formed just internal to the band and parallel with the median line of the ventral plate. I shall call the outer of these longitudinal grooves the first, and the inner of them, the second, lateral longitudinal groove (figs. 8 & 9, *1 lat. gr.*, *2 lat. gr.*). The first appendage-bearing segment had become cut off from the cephalic lobe, *i.e.*, the first segment of the earlier stages (figs. 4 & 7) had thus been divided into the first appendage-bearing segment and the cephalic lobe. The cephalic lobe which was earlier clearly divided into two lateral halves had now begun gradually to unite (fig. 8), and in fig. 9 is seen completely united, no dark line being found in the median part of it. The sixth appendage-bearing segment had become cut off. The median line had been much produced and almost reached the posterior end of the ventral plate. The posterior part of the line is grooved.

On August 14th (17 days after spawning), the stomodaeum (*st.*) was distinctly seen in the cephalic lobe, at the anterior end of the longitudinal median line (fig. 10). It is a very shallow, roundish depression. Rudiments of the distal end of the appendages had now become distinct as round protuberances within the marginal band and were very conspicuous features of the ventral plate. In the marginal band and external to the fourth appendage, paired, round protuberances (*lat. h.*) appear. They are a little larger than the rudiments of appendages. As the function as well as homology of these peculiar protuberances

2) Packard—*The Development of Limulus polyphemus.* Mem. Bost. Soc. Nat. Hist. vol. II.

are uncertain I shall call them the *lateral humps*. The second lateral longitudinal grooves had now reached the posterior end of the fifth appendage-bearing segment. The seventh appendage-bearing segment had been newly cut off. At this time all the appendages are post-oral.

The external changes that occur before the appearance of the rudiments of all the ambulatory appendages are very hard to observe, as the ventral plate is small and pale at this time. I could not observe them in the summer of 1889, except the irregular fissures found in a very early stage.

As the result of the gradual enlarging of the ventral plate, the egg gradually flattens on the ventral side, thus producing a space filled with a clear fluid between the ventral plate and the vicarious chorion which is by this time already present. The vicarious chorion dilates greatly while the chorion outside it cannot expand equally; thus the chorion is necessarily ruptured. Two specimens of the broken chorion are represented in fig. 11. The lines of rupture are seen to go round the chorion irregularly and do not form closed circles.

On August 15th (18 days after fertilization), the eighth appendage-bearing segment had appeared. The second lateral longitudinal groove had reached the posterior end of the sixth appendage-bearing segment, beyond which the groove never elongates. The seventh appendage had appeared on this day.

On August 16th (19 days after fertilization), the ninth appendage-bearing segment had appeared (fig. 12). The eighth appendage had been produced. The median longitudinal groove, which had appeared four days before in the posterior part of the ventral plate, had become very distinct in the last undifferentiated segment. The lateral humps were not as elevated as before.

On August 17th (20 days after fertilization), the tenth appendage-

bearing segment had appeared (cf. fig. 13). The ninth appendage had been produced. The second, third, fourth, and fifth appendages had become chelate. The rudiment of the flabellum was found as a small round prominence on the outside of the sixth appendage. A similar prominence was also found in the segment of the fifth appendage. The nervous system was seen as indistinct, white, longitudinal bands near the median line. In the brain, paired invaginations were found. The marginal band had moved much farther outward, so that the outer lateral longitudinal groove had become very wide and shallow. The first appendage was found at the level of the stomodæum.

On August 18th (21 days after fertilization), mesoblastic dissepiments were found, and the homodynamous organ to the flabellum was seen in the segments of the fourth, third, and second appendages (fig. 13).

From August 19th onwards, the abdomen became gradually prominent by the development and addition of new segments. The hatched embryo has ten abdominal segments. This number corresponds to the number found in the adult animal. The sixth and the first appendages became chelate. The seventh appendage gradually degenerated from the distal end, and at last a small proximal portion only remained as the metastomum. As the ventral plate increased its surface very rapidly, the at first spherical egg became flat dorso-ventrally, and the egg assumed the trilobite-like shape (fig. 17). The lateral hump separated gradually from the marginal band and was found in the trilobite stage on the dorsal side of the embryo.

Internal Changes.

A section cut through the white disc found in the embryo (fig. 3) of August 7th is represented in fig. 21. It shows that in the disc cells

are much smaller and more crowded than in other parts of the egg. Thus the white disc is nothing more than a blastodermic thickening. This blastodermic thickening is not produced by the addition of cells from within, but by the rapid cell-division in a certain space of the surface. The cells forming other parts of the egg, both superficial and internal, are large, spherical, and all of about the same size. At this stage all the cells contain yolk granules which are smaller in the vicinity of the nucleus.

On August 8th (11 days after fertilization), the blastodermic thickening had become larger and also deeper (fig. 22), but on the next day it had increased only in the horizontal plane. At about this stage a thin transparent membrane, the vicarious chorion, is secreted from the blastodermic cells. As the blastoderm-cells become pressed together, and in consequence, polygonal grooves are formed around each cell, the vicarious chorion is tucked in to fit these grooves and presents polygonal markings with a double contour.

On August 10th (13 days after fertilization), an important change had taken place. The cells forming the blastodermic thickening had separated into two layers (fig. 24). The upper layer is composed of a layer of columnar cells and represents the epiblast, the lower layer is many cells thick and represents the mesoblast. Thus the blastodermic thickening gives rise to the epiblast and mesoblast of the ventral plate. The remaining germinal layer, the hypoblast is represented by the large yolk-cells in the interior of the egg. Outside the ventral plate, the epiblast rests directly on the hypoblast, the mesoblast being not yet found in these parts. The epiblast cells throughout are smaller than the hypoblast cells. Every cell of the egg had at this stage a distinct nucleolus. Late, on this day, the epiblast of the ventrum had become composed of cells in many irregular rows, as a consequence of rapid cell-proliferation (fig. 23).

On August 11th (14 days after fertilization, cf. fig. 5), the mesoblast layer had divided into two lateral bands (fig. 25). Since the preceding day the mesoblast had already divided into as many transverse bands as there are segments externally seen.

Late on August 12th (15 days after fertilization, cf. fig. 7), the mesoblast of the fifth appendage-bearing segment had split into two layers, somatopleur and splanchnopleur, producing a cavity—the cœlomic cavity—between them (fig. 26). As the mesoblast is divided into two lateral halves the cavity is of course paired. The fifth appendage-bearing segment was well developed, and was separated from the fourth and sixth appendage-bearing segments by deep grooves.

On August 13th (16 days after fertilization, cf. figs. 8 & 9), a short median groove had formed in the posterior part of the ventral plate. Cells underlying the groove had become loose, wandered in, and expanded into a sheet between the epiblast and the yolk-hypoblast (fig. 27). I shall call the groove the *primitive streak*. The epiblast cells of the ventral plate and most of the mesoblast cells have by this time lost their nucleolus, while the epiblast cells of the dorsum and hypoblast cells have still distinct nucleoli (fig. 41). The mesoblast of the sixth appendage-bearing segment had split into two layers, producing the cœlomic cavity.

On August 14th (17 days after fertilization, cf. fig. 10), a pair of the cœlomic cavities, common to the cephalic lobe and the first appendage-bearing segment, had appeared (figs. 29, 30), and another pair in the seventh appendage-bearing segment (fig. 31). The primitive streak had receded partly towards the posterior end of the ventral plate. The mesoblast produced from the primitive streak had separated into two lateral halves and then divided into many transverse segments. I am inclined to believe that the mesoblast belonging to the

region of the body posterior to the sixth segment (exclusive) is formed from the primitive streak. The white marginal band (figs. 8 & 9) externally observed on the preceding day was a thickening of the epiblast (*lat. bd.*) as represented in fig. 30.

The lateral hump consisted of very high columnar cells forming a little protuberance (fig. 42). The cells stain faintly. The lateral hump is a problematic organ which was formerly erroneously described by Kingsley[3] as the rudiment of the lateral eye. It was homologised to the so-called "dorsal organ" of other Arthropods by Watase.[4] Recently Patten[5] and Kingsley[6] have described it as serially homologous with the lateral eyes. I can not corroborate the last two authors, as I could not find such a peculiar organ in other segments, and because it appears at first as a *protuberance* and not as an *invagination* which it should be, if it were homologous to the eye.

The yolk granules in the cells of the ventral plate had mostly disappeared. Late on this day the nervous system had become marked off from the general epiblast by a peculiar grouping of cells (fig. 46). The arrangement of cells in the nervous system is just like that found in the retina of the compound eye for the ommatidium. Conical shaped groups of cells with spherical nuclei are surrounded by compressed and slender cells with long and deeply staining nuclei. The top of the cones is directed exteriorly. The brain and the ventral nerve-cords are formed at the same time, and are connected with each other, though their two lateral halves are formed independently (figs. 28–30).

On August 15th (18 days after fertilization), the cœlomic cavities

3) Kingsley—*Notes on the Embryology of Limulus.* Quart. Journal of Micro. Sc. 1885.

4) Watase— *On the Morphology of the Compound Eye of Arthropods.* Stud. from the Biolog. Lab. Johns Hopkin's Univ. 1890.

5) Patten—*On the Origin of Vertebrates from Arachnids.* Quart. Journ. of Micro. Sc. 1890.

6) Kingsley—*On the Phylogeny of Limulus.* Zool. Anz. 1890.

of the eighth appendage-bearing segment had appeared. Since the preceding day an incomplete septum had been produced in the first common cœlomic cavity, separating the cephalic portion from that of the first appendage-bearing segment (fig. 30).

On August 16th (19 days after fertilization), three pairs of invaginations were visible in the cephalic region. One pair was in the external part of the cephalic lobe (figs. 12, 34, 59, *ex. gr.*). I shall call it the external cephalic groove. It is the groove for the ganglion of the lateral eye. Another pair was a small roundish depression, external and posterior to the first pair (figs. 12, 35, 59, *lat. e.*). It is the invagination for the lateral eye. The third pair was also a small roundish depression in front of the cephalic lobe near the median line (figs. 12, 34, *med. e.*). It is the invagination for the median eye. The mesoblast which had been divided into lateral halves had now reunited in the cephalic region (fig. 59), and in the second (fig. 33), third, and fourth (fig. 39) appendage-bearing segments. The ninth pair of the cœlomic cavities had appeared (fig. 32).

On August 17th (20 days after fertilization), one more pair of the grooves had appeared in the internal, anterior portion of the cephalic lobe (figs. 36, 38, *int. gr.*). The new groove is long and crescent-shaped. I shall call it the internal cephalic groove. It is the groove for the brain. The tenth pair of the cœlomic cavities had appeared. The rudiment of the flabellum had appeared as a small, round protuberance formed of high, columnar cells (fig. 47). A similar, and undoubtedly homodynamous organ may be seen in the segment anterior to the segment of the flabellum, though much smaller (figs. 13 and 48).

On August 18th (21 days after fertilization), the ventral nerve-cord had divided into ganglia (fig. 13). The paired invaginations for the median eye had united (figs. 13, 36, 37). A pair of round protuberances, homodynamous with the flabellum, was found in the seg-

ments of the fourth, third, and second appendages (fig. 13). The mesoblast belonging to the fifth and sixth appendage-bearing segments had united in the median line.

Formation of the Germinal Layers.

No gastrula mouth is found in the formation of the germinal layers, but the blastodermic thickening, shown in figs. 3 and 21, similar to the primary thickening[7] of the spider's blastoderm, may be considered as a modified gastrula mouth, though in addition to the epiblast it gives rise only to the mesoblast, while the similar thickening of the spider gives rise to both the mesoblast and hypoblast besides the epiblast. Though I was unable to study the segmentation of the egg of *Limulus*, I am inclined to believe that after segmentation the egg is a solid sphere consisting of many spherical balls of similar size, each of which contains a nucleus and abundant yolk granules in it. In one spot of the egg's surface these balls are divided again and again and form the blastodermic thickening. Therefore we find many cells in the interior of the egg.

In the spider all the segmentation nuclei come to the surface of the egg, after segmentation is over, and form the one-cell layered blastoderm enclosing the yolk which is free from nuclei. At one spot of the blastoderm, cells are very rapidly multiplied and form the primary thickening. Some cells forming the lower part of the thickening wander in again into the yolk and form the hypoblast.

These two types in the formation of the germinal layers, exemplified in *Limulus* and in the spider, are often found in closely related groups of Arthropods, and neither of them is of rare occurrence. I think that the type of *Limulus* is an abbreviation of that of the spider. In the former the hypoblast cells, instead of coming out of the

7) Kishinouye—*On the Development of Araneina.* This Journal, vol. IV.

yolk and afterwards returning to it as in the spider, remain in it from the time of their formation.

The epiblast in *Limulus* is derived in two different ways. The epiblast of the ventrum arises from the superficial layer of cells of the blastodermic thickening, separating from the lower cells, while the epiblast of the dorsum is differentiated from the immediately underlying hypoblast cells by rapid multiplication, thus forming a single layer of columnar cells (fig. 41).

The mesoblast of *Limulus* has three sources. One portion arises from the cells forming the lower part of the blastoderm thickening. This portion forms the mesoblast of the cephalothorax. The second portion arises from the primitive streak. After the separation and differentiation of the cells of the blastodermic thickening into the epiblast and mesoblast, and after the metamerism of the ventral plate has already begun, a median longitudinal groove, the primitive streak, is found near the posterior end of the ventral plate. From the bottom of the groove many cells become loose and proliferate vigorously between the epiblast and hypoblast. These cells form the mesoblast of the abdomen (probably the mesoblast of the last thoracic segment also). The primitive streak is found even in an embryo about three weeks old (fig. 32). It recedes gradually posteriorly giving rise to paired sheets of the mesoblast. It may be compared to the secondary thickening, the caudal thickening, of the spider's blastoderm, though the latter is produced before the differentiation of the germinal layers, thus of course before the appearance of metamerism. The primitive streak of *Limulus* is probably homologous with the structure of the same name in the *Chordata*. The section shown in fig. 27 has a striking resemblance to a section across the primitive streak of a chick. The third portion of the mesoblast arises from some yolk cells. In later stages some yolk-cells losing the yolk granules come out of the

yolk and mix themselves with the mesoblast cells (figs. 40, 41, *mes'*). This takes place chiefly in the dorsum. These secondary mesoblast cells may be compared to the fat cells of the spider. I was unable to trace the fate of these cells, but think that some of them probably become blood corpuscles.

In early stages, just after the separation of the epiblast and mesoblast in the blastoderm thickening, we find often some large cells in the deep part of the mesoblast (fig. 24), and these gigantic cells seem to play an active part in the multiplication of the mesoblast cells.

Mesoblast and Segments.

The mesoblast after its formation extends very rapidly, chiefly by division, which takes place mostly near the ventral median line. The dorsal (distal) end of the mesoblastic somites is gradually pushed towards the dorsal surface. In these parts the karyokinetic figures are rarely found, the cell multiplication taking place, as just stated, chiefly by the division of cells in the ventral plate. The mesoblast cells in the dorsal part of the second, third, and fourth appendage-bearing segments become loose and spherical (fig. 39, 40).

I can not say whether the metamerism arises from the change in the epiblast or from that in the mesoblast. When the metameric division takes place, the mesoblast of the ventral plate is divided into transverse segments between which the epiblast is grooved.

The order of the separation of the segments is as follows ;—First a common segment for the cephalic lobe and the first appendage-bearing segment is cut off as the smaller anterior part of the ventral plate (fig. 4). Then from the larger posterior portion the second, third, fourth, fifth, &c. appendage-bearing segments are successively cut off. When the sixth appendage-bearing segment is formed, the first

appendage-bearing segment is separated from the cephalic lobe. After two or three segments are cut off, the mesoblast of the ventral plate is divided in the median line into two lateral halves, beginning from the anterior end. In early stages the dark lines in the ventral plate, where few or no mesoblast cells are found, show from the exterior the division of the mesoblast into transverse segments as well as into two lateral halves (figs. 4–7).

The mesoblast which was separated into lateral moieties unites again from the anterior end of the ventral plate. Afterwards there is found a special compact accumulation of mesoblast cells in the median line of the cephalothorax above the nervous system (figs. 51, 52, 56, 68, *entost.*). This accumulation is laterally very thick. It is the rudiment of the entosternite.

About three weeks after fertilization, the mesoblastic dissepiments are formed in the cephalothorax by the sinking in of mesoblast cells into the yolk (figs. 52, 57). They are intersomitic, dividing the yolk into as many compartments as there are segments. But there is no dissepiments between the cephalic lobe and the first appendage-bearing segment, and the dissepiment between the second and third appendage-bearing segments and that between the sixth and seventh appendage-bearing segments are short. In the abdomen the dissepiments are not well developed.

Cephalothorax and Abdomen.

The cephalothorax consists of the cephalic lobe and the succeeding seven segments. It forms the greater part of the egg, and becomes gradually flat by the horizontal increase of the ventral plate.

The development of the abdomen takes place very late. At about the time when the two mesoblastic plates of the seventh appendage-

bearing segment unite in the dorsal median line, the abdominal part becomes prominent, as the yolk is then forced into it and as the epiblast and mesoblast increasing by rapid cell-division produce a projection (figs. 15, 16). Thus the abdomen comes to be distinguished from the cephalothorax. The yolk always plays a passive part.

The peculiarity of the abdomen is that the yolk is found in the later stages in the middle portion (rachis) only (figs. 54, 55), while the cephalothorax is entirely filled up with the yolk. In the abdomen the epiblast with the enclosed mesoblast extends as two large wings *(pl.)* on either side. In these wings there is no yolk. The coelom is also not found in these wings.

The appendages borne on the abdomen differ greatly from those on the cephalothorax, remaining to the last as flat, band-like prominences, compressed antero-posteriorly, and the two lateral members afterwards uniting more or less at the ventral median line (fig. 55).

A little before hatching the abdomen consists of ten segments, (the number equal to that found in the adult animal) the four anterior ones of which bear the appendages (fig. 56).

Appendages.

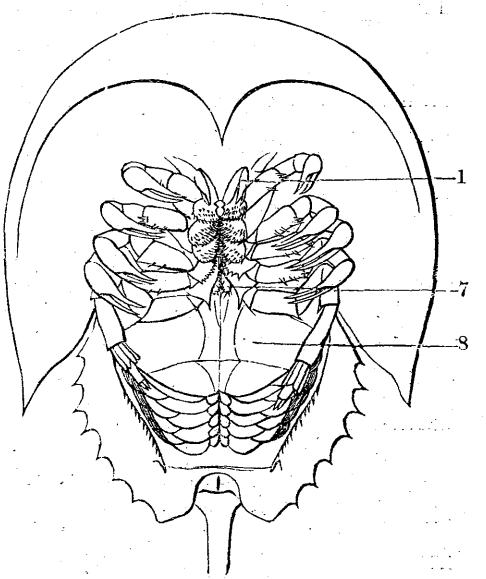
The first rudiments of the appendages appear in the stage of fig. 7. It is not right to consider the round protuberances found in figs. 8, 9, 10, as the first rudiments of appendages, and the bases of the protuberances as the external extremities of the segments. A groove is produced between every two segments. It is deeper towards the lateral ends. Thus a pair of band-like prominences may be found in every segment. Such a prominence is the rudiment of an appendage. When the appendage is thus far formed, a groove, the first lateral groove, appears at the lateral edge of the segment on each side (figs. 8, 9, 10,

1 lat. gr.). Shortly after this, a second lateral groove is formed, parallel and internal to the first lateral groove (figs. 8, 9, *2 lat. gr.*). Thus in some segments arise round protuberances bounded by the lateral grooves and transverse grooves. These protuberances are the rudiments of the *distal* portion of the appendages. The epiblast is now tucked in from the external side of the protuberances, thus separating the band-like portion internal to the protuberances from the general surface of the subjacent body (fig. 33). The band-like portions thus cut off are the rudiments of the *proximal* portions of the appendages. Three distal joints (four in the last ambulatory leg) are developed from the protuberances, and three proximal joints from the flat band-like portions.

Though the appendages (especially the anterior six pairs) appear almost simultaneously, yet by a close examination I found that the fifth appendage appears first, and gradually forwards the fourth, third, second, and first appendages are formed; then gradually backwards the sixth, seventh, eighth, &c. appendages. The rudiments of all the appendages are almost alike. The order of the differentiation of the distal portion (round protuberances) of the appendages does not coincide with the order of the appearance of the appendages themselves. The differentiation begins at first in the fourth appendage, and proceeds in others successively to the first appendage, then gradually in the opposite direction in the fifth and sixth appendages. From the seventh appendage (inclusive) posteriorly, the second lateral groove is not formed. Thus all the abdominal appendages develop from the band-like prominences and finally assume a leaf-like form.

The six anterior appendages become afterwards chelate. The first appendage does not develop as vigorously as the others. It remains small and slender and is afterwards placed before the mouth and becomes the chilaria or antenna. The following five appendages become

the ambulatory legs, the four anterior ones of which are later six jointed, while the last one is seven jointed. The last ambulatory leg differs from the other ambulatory legs also by having four rod-like appendices at the base of the sixth joint. The presence of these appendices enables the last ambulatory leg to serve for digging. The seventh appendage gradually degenerates from the external side (fig. 14), leaving a small proximal portion only, which becomes the metastomum of the adult.



Limulus longispina. 1/5.
(Ventral side).
1, Chilaria; 7, Metastoma; 8, Operculum.

The appendages from the eighth backwards belong to the abdomen. The eighth pair of appendages becomes the operculum (figs, 10, 12–14, 53–57, *op.*). The right and left members of it unite secondarily with each other to a great extent and form a single plate (figs. 54, 55). The proximal portion of the operculum contains the mesoblast cells, but the distal portion does not. Hence the proximal portion is very much thicker than the distal portion (figs. 53, 54). The mesoblast is differentiated into muscles. The 9th–13th appendages bear the gill-lamellae. Among them the ninth pair is the earliest developed. In embryonic stages, the two lateral members of the pair are united at the median line only for a slight length (figs. 54, 55). Like the operculum the appendage is thick at the proximal portion (figs. 49, 53, 57). The epiblast of the posterior side of the thick portion is invaginated by many transverse furrows. The least

number of the furrows which I observed was three. The number increased to five, then six (in the stage of figs. 17, 54, 57), new furrows being added posterior to the older ones. The embryo, soon after hatching, has only four pairs of the abdominal appendages, and the second pair alone bears the gill lamellae.

The peculiar appendix, the flabellum, at the exterior base of the sixth appendage develops from a small round protuberance (fig. 13). The homodynamous protuberances are also found in the segments of the 2nd–5th appendages; but they do not develop any further and disappear.

Nervous System.

The lateral halves of the nervous system develop independently of each other. Each half of the brain with its corresponding ventral nerve-cord is produced as a continuous long cord of epiblastic thickening, just inside the base of the appendages (figs. 12–14). It is easy, however, to distinguish the brain from the ventral nerve-cord. When they are first formed, the former is very much broader than the latter, occupying almost the whole of the segment of the cephalic lobe, while the ventral nerve-cord occupies only about one third of the breadth of each appendage-bearing segment (fig. 12).

In the stage of fig. 12 we see most distinctly that the nervous system consists of peculiar cell-groups like the ommatidia of the eye (figs. 34, 36, 38, 46). These peculiar cell-groups of the nervous system were first noticed by Patten. They disappear when the ventral nerve-cord is divided into ganglia and begins to be separated from the epiblast.

A paired small invagination appears in the lateral part of the

brain (fig. 34 *ex. gr.*). The invagination is in the margin of the brain where none of the peculiar cell-groups above referred to is found. After the appearance of this invagination there is found another paired epiblastic invagination, along the anterior internal corner of the brain (figs. 13, 36, 38, *int. gr.*). This new invagination is also on the margin of the brain, where the peculiar cell-groups are not found. I believe that the external invagination is homologous with the invagination of the lateral vesicle of the spider and the internal one to the semicircular groove. These cephalic invaginations of Limulus, however, are very shallow and disappear before the separation of the nervous system from the epiblast takes place. They are already not found in the embryo in the stage of fig. 14. But the two cell-masses which were thus invaginated are found separate in the deep part (figs. 37, 51). The part of the brain formed by the external groove becomes the optic ganglion of the lateral eyes as in the case of the spider. The optic ganglion of the median eyes is produced from the epiblastic thickening forming the united mouth of the invagination for the median eyes (fig. 69, *g. med. e.* see infra). The thickening is always between, and touches on each side, the two cephalic ganglia (figs. 14, 37, 69).

At first the anterior and lateral borders of the brain were directly on the anterior and lateral borders of the ventral plate; but as the growth of the brain lags behind that of the ventral plate, it becomes gradually separated from the margin of the ventral plate.

The brain which was almost circular in its outline, as its height was almost equal to its breadth (figs. 12, 34), becomes afterwards semicircular (fig. 13), later still, sickle-shaped (fig. 14), and shortly before hatching almost straight (fig. 69). This is caused probably by the peculiar development of segments. All the segments seem to develop most vigorously at the line midway between the ventral and

dorsal median lines, *i.e.*, all the segments have their greatest antero-posterior extension on this line, and become shorter and converge, like the frame-pieces of a folding fan, towards both the ventral and dorsal median lines. Therefore the external ends of the brain are pushed anteriorly by the growth of the succeeding segments. About two weeks before the hatching of the embryo the brain proper becomes divided into two transverse portions by a constriction (fig. 69). Thus the brain of *Limulus* may be divided into four parts—the ganglion of the median eyes, the ganglion of the lateral eyes, and the anterior and posterior portions of the brain proper. These four parts are arranged in three transverse rows. The anterior row is occupied by the two pairs of the optic ganglia, the middle row by the anterior portion of the brain proper, and the posterior row by the posterior portion of the brain proper (fig. 69).

Though the posterior portion of the brain proper is very early (earlier than any other part of the nervous system) cut off from the epiblast, and the fibrous commissure is developed between the right and left sides (figs. 45, 51, 69), its anterior portion as well as the two pairs of the optic ganglia are for a long time not separated from the epiblast (fig. 56).

The ventral nerve-cord consists at first of one continuous longitudinal thickening of the epiblast (figs. 12, 30, 33). The thickening is at this time distinguished by the peculiar cell-groups already mentioned. At about the stage of fig. 13, the thickening is divided into ganglia, as the cells composing it accumulate into a mass in every segment. A pair of such ganglia may be found in each of the segments of the first and seventh appendages as in the other segments. The ganglia of the first and the second appendage-bearing segments form the circumœsophageal ring (figs. 51 and 69).

Lying between the two lateral members of the ganglia there are

found some epiblast cells (fig. 68, *m.c.*). These epiblast cells seem to take no part in the formation of the lateral commissure. They form probably the membrane covering the nervous system.

Cells of the nervous system, facing the centre of the egg and lying near the median line of each nervous thickening, become changed into fibres. The fibres of the ganglia as well as those of the brain and of the optic ganglia of the lateral eyes communicate with one another and form the longitudinal commissure (fig. 51). The fibres develop internally and form the lateral commissures (figs. 14, 45, 52). There is only one lateral commissure in the brain :—at the posterior portion of the brain proper (fig. 69). The lateral commissure does not develop in the segments of the first and second appendages (fig. 51). The fibrous portion of the ventral cord develops externally and innervates the appendages (figs. 14, 52, 69).

Eyes.

The lateral eyes make their appearance as small epiblastic thickenings with shallow invaginations, just at the margin of the ventral plate, and at about the level of the posterior end of the external cephalic groove (figs. 12, 35, 59, 60). At this stage they are not distinctly seen in the preserved eggs nor in the surface-view preparations. They are distinctly seen in sections only. This is due to their very small size and also to their being placed near the cephalic thickening of the nervous system and near the anterior end of the thick marginal band. But in one or two days after their formation when they are a little removed exteriorly and posteriorly from the cephalic thickening (fig. 13), and then from the marginal band (fig. 14), they become easily recognisable in the surface-view preparations. As they are thus removed, the opening of their invaginations is directed posteriorly and dorsally. The thickening is more than one-cell thick.

It is interesting that the lateral compound eye of Limulus is at first an epiblastic thickening with an invagination like a simple eye, and not a group of thickenings or invaginations, and that it appears at the outer margin of the brain, as in the case of other Arthropods. The lateral eyes of Limulus are not thoracic eyes, but cephalic eyes. After their formation they shift their position gradually dorsally and posteriorly and at last they are behind the lateral hump, at the level of the fourth appendage (fig. 17).

Cells forming the bottom of the invagination of the lateral eye become large and are faintly stained by a colouring solution (fig. 62). The invagination is faintly divided towards the posterior end into two branches or depressions, ventral and dorsal. The large faintly stained cells are found in the ventral branch only. The dorsal branch is very short and is soon aborted. The invagination of the lateral eye is not deep, and its bottom prolonged anteriorly as a short solid tube (fig. 63).

After the disappearance of the dorsal branch of the invagination, cells forming the thickening, dorsal to the invagination, are pigmented and grouped into rudimentary ommatidia (figs. 64–66), smaller and more pigmented cells surrounding larger and less pigmented cells. Each of these groups of larger cells is spindle-shaped. Thus at this time there is not to be found a separate invagination for each ommatidium. The first differentiation in the lateral eyes is the grouping of their constituent cells into rudimentary ommatidia. I do not consider the invagination for an ommatidium as of much value, as it seems to me to be produced secondarily by the thickening of the cuticula into the lens. The changes which take place in the lateral eyes after the formation of the rudimentary ommatidia are not minutely known to me.

The optic nerve of the lateral eyes is formed from the lengthening of cells forming the solid prolongation of the invagination (fig. 66),

and in the embryonic stages does not communicate with the brain. From the adult anatomy and from the analogy of the spider, I consider the portion of the brain formed by the external groove as the optic ganglion of the lateral eyes.

The lateral compound eye of *Limulus* is homologous with the group of the lateral simple eyes of the Arachnida. For the lateral simple eyes of the Arachnida arise as a group from a common epiblastic thickening—as I have recently shown in the Dōbutsugaku Zasshi (the Zoological Magazine, Japanese) and as is set forth in the article immediately following in this Journal. The lateral eyes of the scorpion are most nearly related to the lateral compound eye of *Limulus*, as they are monostichous and are placed close to one another.

The median eyes appear as paired invaginations in paired epiblastic thickenings near the median line and in front of the cephalic thickening (fig. 12). They are formed just outside the brain (figs. 34, 38), so that they are not neural in origin. The paired invaginations afterwards approach each other (fig. 13), and finally form a median unpaired invagination (figs. 14, 36, 37). This unpaired invagination becomes deep and slender and develops anteriorly and dorsally, its blind end touching the epiblast (figs. 50, 56). The lumen of the invagination is lost as it becomes exceedingly slender, and at this stage the median eyes are represented by a slender solid rod (fig. 51). Later the extremity of the rod becomes enlarged and at the same time epiblastic cells lying on it becomes columnar (figs. 56, 67). The enlarged extremity becomes the retina and the columnar epithelium forms the vitreous body.

The mouth of the invagination is closed, when the tube of the invagination becomes solid. The epiblast where there was originally the mouth of the invagination is thick and slightly depressed (figs. 68, 69). This thickened area of the epiblast probably gives rise

to the ganglia of the median eyes, and the slender stalk which connects this area with the retinal portion to the optic nerve.

I was unable to study the later changes of the median eyes. Their development greatly lags behind that of the lateral eyes. I do not know whether the anterior end of the retinal cells is morphologically the anterior end or not. The unpaired retinal portion is afterwards divided into two and gives rise to two median eyes in the adult. That the median eyes of *Limulus* are homologous with those of the *Arachnida* is most probable; but it is a little doubtful whether they are exactly homologous in every respect. Thus the median eye of *Limulus* appears as a thickening anterior to the brain and outside the semi-circular cephalic groove, while that of the Arachnida appears as a thickening at the posterior end of the semi-circular cephalic groove.

I find no more eyes than the two pairs mentioned above, nor any other structure which I can consider as homologous with them.

Alimentary Canal.

The stomodaeum is first of all produced as a shallow round invagination in the cephalic lobe (figs. 10, 29, *stom.*). The invagination becomes gradually deep and takes an anterior and upward course (figs. 50, 68). When it almost reaches the dorsum it bends a little backwards. At first it develops with the surrounding mesoblast between the epiblast and the yolk-hypoblast (fig. 50); but later, the yolk finds its way into the region in front of the stomodaeum, so that in sections, we find masses of yolk before the stomodaeum (fig. 52, C+I; and fig. 56). The stomodæal wall consists of high columnar cells (figs. 45, 68). The stomodaeum gives rise to the œsophagus and proventriculus. As the upper lip grows posteriorly, the mouth-

opening which was at first pre-appendicular gradually shifts its position backward and becomes surrounded by the cephalothoracic appendages.

The proctodaeum is not well marked out. In the sagittal section represented in fig. 50, we see an invagination at the posterior end of the body. The invagination is crescent-shaped, as represented by the horizontal section, fig. 53, and is continued to the grooves produced by the ventral reflexion of the pleurae *(pl.)*. Therefore the invagination can not be considered as the proctodaeum.

I could not find any trace of the mesenteron at all, even in the embryo of the trilobite stage (fig. 17). According to Brooks and Bruce, the proctodaeum and the mesenteron do not appear in the embryonic stages.

Cœlom.

The early development of the cœlom may easily be understood by examining figs. 12–17. The cœlomic cavity is produced by the splitting up of the mesoblast into the somatopleur and the splanchnopleur. The somatopleur is thicker than the splanchnopleur, the former being many cells thick, while the latter consists of a single layer of cells (figs. 29–32). When the cœlomic cavity is produced, the mesoblast is already divided into many transverse segments, in every one of which the mesoblast is separated into lateral halves, so that the cœlomic cavities are always paired in a segment. Though the lateral halves of the mesoblast afterwards fuse together secondarily at the ventral median line, the cœlomic cavities of both sides remain always separate. The walls of the first cœlomic cavity fuse together along the whole line in which the two lateral members meet—from the ventrum up to the dorsum. From

the second cœlomic cavity backwards the walls of the two lateral members do not fuse together at the dorsal, though they do at the ventral median line.

A pair of cœlomic cavities appear in every segment, except the segments of the second, third, and fourth appendages, in which the cœlomic cavity does not appear at all. At least eleven pairs of these cavities are produced (fig. 53). The eleventh pair belongs to the seventh abdominal segment.

All the cœlomic cavities develop towards the dorsum between the epiblast and the hypoblast, and meet with one another at the dorsal median line (figs. 17, 43, 44). They develop just above the hypoblast and do not enter nor give branches into the epiblastic outgrowths, such as the appendages and the abdominal pleurae (figs. 53–55).

The first pair of cœlomic cavities is common to the cephalic lobe and the segment of the first appendage (fig. 12). A little later this common cœlomic cavity is partially divided into two by an incomplete septum between the cephalic lobe and the segment of the first appendage (figs. 12, 30); but as the cœlomic cavity gradually degenerates from the posterior end, the smaller portion belonging to the segment of the first appendage disappears soon after its formation (fig. 13). The first cœlomic cavity always accompanies the stomodæal invagination (figs. 45, 50, 52). As the stomodaeum does not develop always along the external epiblast and as its distal end takes gradually an upward and then backward direction, and grows into the yolk mass, the first coelomic cavity is necessarily embedded among the yolk (fig. 52). Taking the upward and then the backward direction the first cœlomic cavity reaches at last the dorsum and there meets with the wall of the second cœlomic cavity, which has grown forward to this point (fig. 17).

The second cœlomic cavity belongs to the segment of the fifth

appendage. It is well developed. From the segment of the fifth appendage backwards, a pair of cœlomic cavities develop in every segment. These cœlomic cavities develop at first in a dorsal and posterior direction. The second and third cœlomic cavities are divided into two portions each—ventral and dorsal—as the cells forming the middle part of their wall become loose and fill up the cavity (figs. 14, 58). The ventral portion of the second cœlomic cavity remains as the coxal gland, while that of the third cœlomic cavity disappears soon after its separation (fig. 14). From the fourth backwards cœlomic cavities gradually disappear from the ventral median portion (fig. 55). The dorsal portion of the cœlomic cavity remains long at the sides of the circulatory system (figs. 54–56); but it disappears before the embryo hatches. The dorsal portion of the second cœlomic cavity elongates anteriorly and meets with the posterior end of the first cœlomic cavity (fig. 17).

Coxal Gland.

The brick-red gland of Packard is mesoblastic in origin, and the lumen of the gland is the remnant of a portion of the second cœlomic cavity. The gland develops from the ventral portion of the second cœlomic cavity, which belongs to the segment of the fifth appendage. Almost simultaneously with the separation of the cœlomic cavity into the dorsal and ventral portions, one layer of cells enclosing the ventral portion is separated from the other mesoblastic cells, (which are used to form connective tissue, muscles, entosternite, &c.,) and causes that portion to have the shape of a sac or tube. The sac is the rudiment of the coxal gland. The cells forming the wall of the gland are

pressed close together. They have a high columnar shape near the ventral median line; but as we recede from that line they become almost suddenly low, flat, and loose, and are lost in loose, scattered, connective tissue cells (figs. 52, 58). This thin walled portion becomes the funnel of the gland.

The coxal gland is at first straight and obliquely directed; but soon afterwards a small axial portion of it near the median ventral line is bent backwards parallel to the median line making almost a right angle with the rest of the gland (fig. 14). The apex of the angle is now gradually shifted forwards; the result being that the angle becomes more and more acute, as the limbs of the angle increase in length, while the distance between their bases remains almost constant. The posterior end of the internal limb of the gland develops a little downwards and then backwards till it touches the posterior surface of the coxal joint of the fifth appendage, where it is connected with the epiblast and makes the lumen of the gland communicate freely with the exterior. The coxal gland at this stage is represented in fig. 19. Its anterior apex reaches the mesoblastic dissepiment between the third and fourth appendage-bearing segments. Its external limb is arched externally, the key-stone of the arch being at the mesoblastic dissepiments between the fourth and fifth appendage-bearing segments. The funnel is directed outwardly and posteriorly and is in the mesoblastic dissepiment between the fifth and sixth appendage-bearing segments.

In the next stage (fig. 20) the anterior apex of the gland has reached in its growth the mesoblastic dissepiments between the second and third appendage-bearing segments, and another curvature—this time towards the mesoblastic dissepiment between the third and fourth appendage-bearing segments—has been added to the external limb. When the gland of this stage is seen from one side, its external

limb presents many wave-like curves. The number of these curves corresponds with that of the metameres in which the gland extends. The limb presents a dorsally directed curvature at each mesoblastic dissepiment. There is thus a dorsal as well as outward curvature over each of these dissepiments. The coxal gland in such a stage of development is found in an embryo of about the stage of fig. 17. The further development of the gland I did not follow.

The coxal gland is probably a degenerated excretory organ, as in development, position, and structure it is similar to the excretory organs of other animals. It is on both sides of the entosternite (fig. 52).

The coxal gland of *Limulus* is not exactly homologous with that of the spider. In the latter, the gland opens at the base of the third appendage and reaches about to the base of the sixth appendage, while in *Limulus* the gland opens at the base of the fifth appendage and develops forwards to the base of the third appendage.

Vascular System.

The chief dorsal circulatory vessel is formed from the mesoblastic wall of the cœlomic cavity. As the walls of the lateral halves of the succeeding cœlomic cavities unite gradually, beginning with the posterior segments (figs. 15, 16), the dorsal circulatory vessel is formed, *pari passu*, from the posterior end of the body. Though the walls of the cœlomic cavities on both sides meet in the dorsal median line, they do not fuse together (figs. 17, 43, 44). A longitudinal and many transverse slits separate every cœlomic cavity from its fellow on the opposite side of the same segment, and from the preceding and succeeding cœlomic cavities of the same side (figs. 15–17). The

lateral halves of the first cœlomic cavity only are fused together and not separated by a longitudinal slit (figs. 16, 52). In later stages, however, the walls of all the cœlomic cavities fuse together at two lines, dorsal and ventral. Thus a tube is formed (figs. 54–56). The tube, as in the case of spiders, has many lateral slits (ostia), as the wall does not fuse together at the intersomitic lateral places. In the tube some wandering mesoblast cells are enclosed. They are destined to become blood corpuscles. Probably all the blood corpuscles are formed from these cells.

As no cœlomic cavity is developed in the segments of the second, third, and fourth appendages, the mesoblast of these segments plays no part in the formation of the wall of the dorsal circulatory vessel. The first cœlomic cavity, which belongs both to the cephalic segment and to the segment of the first appendage, also does not take part in the formation of the dorsal circulatory vessel, because the walls of its two halves are fused together as stated above (fig. 17).

When the dorsal vessel is not yet completely formed, the aortic arches (*ao. a.*) are produced at the sides of the œsophagus (figs. 17, 45). A tubular lumen appears in the mesoblastic wall of the first cœlomic cavity, where the somatopleur and splanchnopleur unite, between the cœlomic cavity and the œsophagus. The lumen is paired. The lumina of the two sides unite into one and communicate with the lumen of the dorsal circulatory vessel, at the posterior end of the blind stomodæum (fig. 17).

While the aortic arch is thus developing, a large unpaired blood space (*st. a.*) appears below the entosternite (*entost.*) and around the nerve cords on the ventral median line (figs. 50–52, 56, 58, 68). This is the sternal artery, which communicates anteriorly with the aortic arches in later stages.

I can not but think that Kingsley is mistaken in regard to the

vascular system of the American *Limulus*, and that it will be found to be formed in the same way as the Japanese species. The number and position of the ostia of the American *Limulus* (fig. 18) agree exactly with those of the embryo of the *kabutogani* (fig. 17).

Dorsum and Ventrum.

The compound eyes of the adult *Limulus* are very peculiar in this that they lie in the thoracic region and on the dorsal surface of the cephalothorax. Previous writers differ in their opinions, as to what thoracic segment the lateral eyes belong to, though all of them allow that the lateral eyes are thoracic. If now the lateral eyes really belong to a thoracic segment, it is an exceedingly interesting fact, for there is no parallel in other animals.

When we look into the matter more carefully, we are struck, first of all, with the fact that the lateral eyes are innervated from the brain —a fact well known for a very long time. Now if they really belong to a thoracic segment, they ought to receive their nerves from the ganglia of that segment, and it is, to say the least, very peculiar that they should be connected with the brain and not with the ganglia of a thoracic segment.

Examining the adult animal (fig. II) we find that the dorsal surface of the cephalothoracic shield is divided into five, while that of the abdominal shield is divided into three longitudinal lobes, as in the Trilobites (fig. V). These lobes I name after the corresponding lobes of the Trilobites—the median unpaired lobe as the *glabella* in the cephalothorax, and as the *rachis* in the abdomen, the first (internal) paired lateral lobes as the *fixed cheeks* in the cephalothorax, and as the

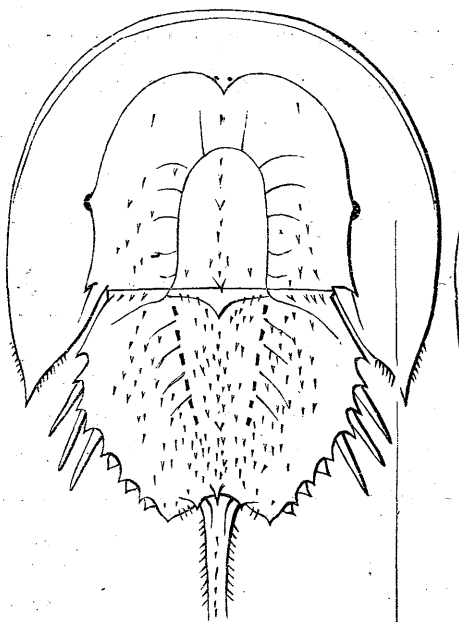


Fig. II.

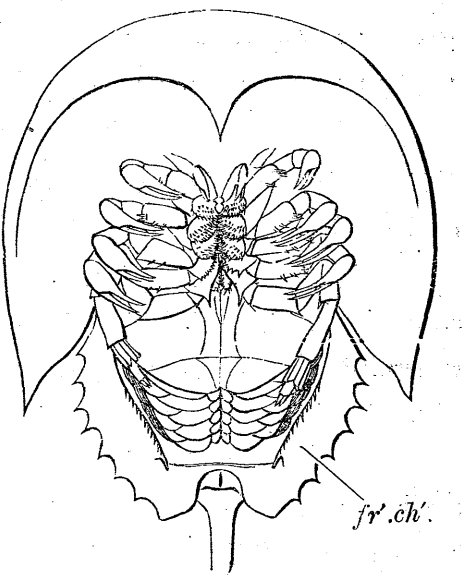


Fig. III.

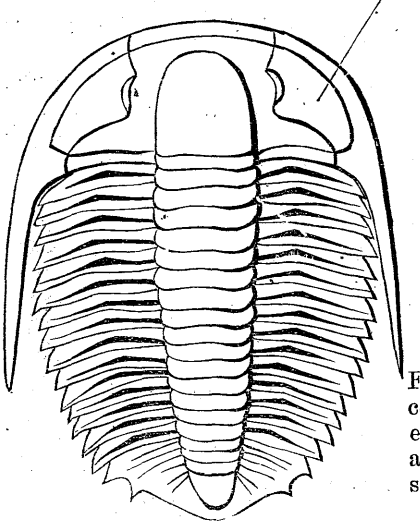


Fig. V.

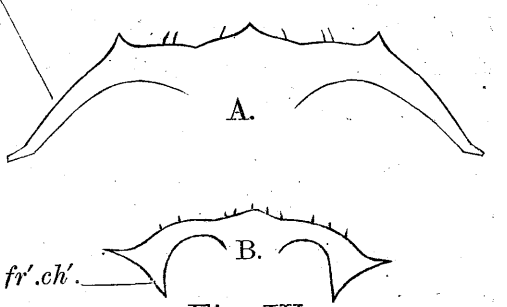


Fig. IV.

Fig. II.—IV. Limulus longispina 1/5. Fig. IV. A. Transverse section through the cephalothorax at the level of the lateral eyes. B. Transverse section through the abdomen at the level of the second lateral spine.

Fig. V. A Trilobite (Angelina Sedgwickii). *After Nicholson.*

pleurae in the abdomen, and the second (external) paired lateral lobes, which appear only in the cephalothorax, as the *free cheeks (br. ch.)*.

Also after the corresponding structure of the Trilobites, I name the furrow which separates the median lobe from the first lateral lobe as the *axial furrow*, and the suture between the first and the second lateral lobes as the *facial suture*.

It is remarkable that in *Limulus* the spines, movable and immovable, are found on the facial suture of the cephalothorax and the margin of the abdomen, and within these boundary lines only (figs. II–IV).

It is also very remarkable that the eyes are found below the facial suture only. The Trilobites, in which the facial sutures of both sides generally do not meet in the dorsal median line, have no median eyes (fig. V). *Harpes* among the Trilobites, and *Limulus* in which the facial sutures of both sides meet in the dorsal median line, have the median eyes.

In some Trilobites such as *Æglina*, the free cheeks are not well developed ; they are not seen from above, hence the lateral eyes are at the lateral margin of the cephalothorax. In these forms the fixed cheeks are also very feebly developed. I regard this type, which is found in most Arthropods as the prototype of the cephalothorax. In *Phacops* etc., the free cheeks are a little seen from the dorsal side. In the majority of the Trilobites the free cheeks are well developed at the lateral sides of the fixed cheeks ; and in *Harpes* and *Limulus* they are enormously developed, at the lateral sides as well as in front of the fixed cheeks. In these animals the cephalothorax is much broader than the abdomen.

In the ventral surface of the abdominal shield of *Limulus* we find a region (figs. III, IV, *fr.' ch.'*) corresponding to the free cheek of the cephalothorax. It is an elevated portion of the lateral edge.

The development of the eyes, which I have described above, shows that they (both lateral and median eyes) arise from paired epiblastic thickenings, just at the margin of the epiblast of the ventral

plate. The lateral eyes, however, gradually travel from the thickened margin of the ventral plate exteriorly and posteriorly, until they are found in the dorsal surface of the embryo, behind the lateral hump in the level of the fourth thoracic appendage. The invagination of the median eyes becomes gradually deep, and while the mouth of the invagination does not shift its place, with respect to the brain, the invaginated tube grows anteriorly and dorsally, keeping its blind end always in contact with the epiblast. Its development soon surpasses that of the thickened margin of the ventral plate, and at last its tip is found at the dorsal surface of the embryo. In the trilobite-stage (fig. 17) the epiblast is thickened in a line above the eyes (fig. 64).

I propose to interpret the above stated facts as follows: The epiblast, covering the body of *Limulus* may be distinguished into the dorsum and ventrum, and the line in which they meet is clearly marked out as the external margin of the pleurae in the abdomen, and as the facial suture (the outer margin of the fixed cheeks) in the cephalothorax. The ventrum is the epiblast which develops from the upper layer of cells of the blastodermic thickening, and the dorsum is the descendant of the epiblastic cells covering all the surface of the egg except the blastodermic thickening. Thus the free-cheeks which are usually looked on as a part of the dorsum are in my opinion, a part of the ventrum. If we accept the view that the original blastopore is in the ventral median line of the Arthropoda and the stomodæum and the proctodæum are its two ends, and that the segments of the Arthropoda are homologous with the compartments of the Cœlenterata, then the ventrum as defined above, corresponds to the subumbrella of the medusae, all the important organs being produced from it and the dorsum to the exumbrella. The margin of the umbrella, *i.e.*, the boundary line between the two, has all the sense-organs on it.

At the time when the eyes are first formed, the ventrum and the

ventral plate are one and the same; but later the ventral plate does not keep pace in its growth with the ventrum. The ventrum extends very quickly, as is known from many karyokinetic figures in it, while the dorsum seems to increase very little after its formation (after the differentiation from the underlying hypoblast), if it increases at all. As the result of this increase of the ventrum, the egg is flattened dorso-ventrally, and the ventrum is in the cephalothorax reflexed upwards and forms apparently a part of the dorsum, and in the abdomen is elevated as in fig. IV. The retinal portion of the eye is always at the margin of the ventrum, just as the eyes of the medusae are always on the umbrellar margin, between the exumbrella and the subumbrella.

The margin of the ventrum of the cephalic lobe develops not only dorsally but also posteriorly. Thus, though the lateral eyes are found at the level of a thoracic appendage in later stages, they are not produced there, but are formed in the cephalic lobe like the eyes of other bilateral animals.

Table showing the chief early changes of the Kabutogani's eggs, which were fertilized on July 29, 1890.

	SEGMENT.										CŒLOM.										APPENDAGE.								
	br.	1	2	3	4	5	6	7	8	9	10	*br.*	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9
Aug. 10																															
11			×	×	×																										
12						×											×														
13	×	×					×											×					×	×	×	×	×				
14								×											×									×			
15									×											×									×		
16										×											×									×	
17											×											×									×

Relation of Limulus with Other Arthropods, with Some Remarks on the Arthropod Ancestry of the Chordata.

The closest relative of *Limulus* among the Arthropoda is undoubtedly the Trilobites. The relations between them have already been given in the previous section. Though the appendages and the internal structure of the Trilobites are but little known, their external features match those of no other animal so well as those of *Limulus*. The five-lobed cephalothorax, the three-lobed abdomen, and the eyes under the facial suture, are the characteristic features of both *Limulus* and the Trilobites. These characteristics are not distinctly found in other animals, even if not entirely wanting. Moreover, *Limulus* and the Trilobites resemble each other in their habitat—swarming in the muddy bottom of a shallow sea.

The chief differences between *Limulus* and the Trilobites are as follows:— In the Trilobites, some anterior abdominal segments are separate, while in *Limulus* all the abdominal segments except the last one are united into a single shield. In the Trilobites some posterior abdominal segments are united and form the pygidium, while in *Limulus* the last segment is separate from all the other abdominal segments and forms the sword-like telson. In the Trilobites the two lateral lobes of the free cheeks are generally not united in the dorsal median line, and the median eyes are generally absent*, while in *Limulus* the two lateral lobes of the free cheeks are united in front and a pair of the median eyes is present.

Those differences are not of much importance and do not speak against a close relationship existing between the Trilobites and *Limulus*.

The next near allies of *Limulus* are the Merostomata (*Eury-*

* Present in *Harpes*.

pterida, Pterygotus, &c.). Two pairs of eyes (one, lateral and compound, the other, median and simple), the ambulatory appendages around the mouth, and the sword-like telson, are the points of resemblance between them. But there are many differences ; the chief ones among which are the want of the facial suture in the dorsal surface of the cephalothorax, and the fact that the appendages are divided into more than six segments in the Merostomata. The Merostomata resemble closely the Scorpionida, an order of the Arachnida. Hence we find a relation between *Limulus* and the Arachnida.

From early times the genetic relation between *Limulus* and the Arachnida has been maintained, and at present we do not doubt the reality of such a relationship; but whether *Limulus* ought to be classified among the Arachnida or among the Crustacea, or be made into an independent class is still an open question. In order to show at a glance in what points *Limulus* resembles, and in what points it differs from, the Crustacea on the one hand and the Arachnida on the other, I have introduced the table on the next page. I venture to think that there are some new points which have not been noticed by previous writers but which are nevertheless quite significant.

From the table it will be seen that *Limulus* differs in many points from both the Crustacea and the Arachnida. With the sole exception of the facial suture, there is no important point in which *Limulus* differs from both the Crustacea and the Arachnida. In those points in which *Limulus* differs from the Arachnida, it resembles the Crustacea, and where it differs from the Crustacea it resembles the Arachnida. Therefore it appears to me not advisable to place *Limulus* in either class, or to make it an appendix to either. On the whole it seems safe, or rather necessary, to make an independent class for *Limulus* and the Trilobita.

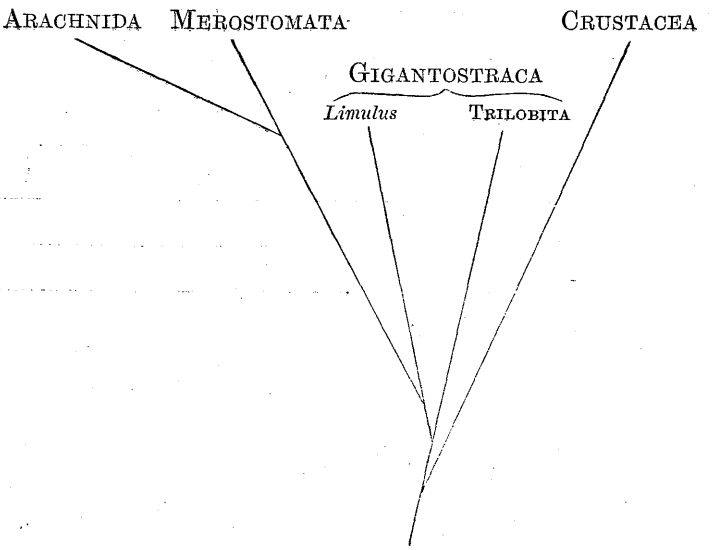
Hitherto the Merostomata have generally been considered to be

more nearly related to *Limulus* than the Trilobita are; but I venture to doubt the correctness of this view: my conclusions as set forth above point in just the opposite direction.

	ARACHNIDA	LIMULUS	CRUSTACEA
Lateral eyes	many, simple	two, compound	two, compound
Median eyes	two (paired)	two (paired)	one (unpaired)
The distal portion of the ambulatory appendage from the carpopodite consists of	four segments	three segments (in the last pair, four)	three segments
Yolk in the embryo remains long and abundant in	the posterior region of the body	the anterior region of the body	the anterior region of the body
Cœlomic cavity	one pair in every segment and enters into the appendage	does not enter into the appendage and is not developed in some segments.	does not enter into the appendage and is not developed in some segments
Malpighian tube	present	wanting	wanting
Brain and optic ganglia arise from	paired grooves in the paired epiblastic thickenings	paired grooves in the paired epiblastic thickenings	paired epiblastic thickenings
Nauplius stage	wanting	wanting	present
Breathe	in air	in water	most of them in water
Origins of mesoderm of the ventrum	two (in spiders)	two	one
Blood vessel ensheathes the nervous system	,,	,,	in most of them not
Entosternite	present	present	wanting
Facial suture	indistinct	distinct at the dorsal side	distinct at the margin

The name *Gigantostraca* may conveniently be retained for the new and somewhat restricted class containing *Limulus* and the Trilobita. The Merostomata excluded from the group ought, in my opinion, to be classified as an appendix to, or as an order of, the Arachnida.

The genealogical tree which I would construct is as follows:



What order of the Crustacea is most nearly related to the Gigantostraca I do not know, as the developmental history of different orders of the class is not yet well known.

I have already compared the eyes of *Limulus* with those of the Arachnida, and found that they are probably homologous with each other. But I do not know whether the eyes of *Limulus* are homologous with those of the Crustacea or not.

The homology between the pulmonary sac of the Arachnida and the lamelligerous gills of *Limulus* is most probable and is generally acknowledged. But as to the manner in which the former has been derived from the latter, whether by invagination or by subsidence, opinions of investigators do not agree. If a pulmonary sac of the Arachnida is formed by the invagination of a lamelligerous appendage of a *Limulus*-like ancestor, (1) the mouth of the invagination ought to look towards the head, (2) the gill lamellae ought to be formed on the posterior wall of the invagination, (3) new lamellae ought to be added ventral to those already formed, *i.e.*, the most dorsal gill lamellae must be formed first,

But none of these points so happen in the development of the pulmonary sac of the Arachnida. Hence I think the pulmonary sac of the Arachnida has been produced by the subsidence of the lamelligerous appendage of a *Limulus*-like ancestor.

The number and position of the eyes are exactly the same in *Limulus* and in the Chordata. The median eyes of *Limulus* are represented by the pineal eye of the Chordata, which is probably formed by the union of a pair of median eyes such as those in *Limulus*. The lateral eyes of *Limulus* are represented by the paired eyes in the Chordata. The paired eyes of the Chordata differ from the lateral eyes of *Limulus* by having their retinal portions inverted; but this is the effect of the closure of the medullary plate with the optic area into a tube. The retina of the paired eyes of the Chordata is also composed of ommatidia—a cone surrounded by many rods.

The lateral eyes of *Limulus* receive their nerves from the pre-oral cephalic lobe. Then the ancestral mouth of the Chordata must be searched for behind the optic chiasma, and within the ring of the nervous system. We have an invagination in the nervous system, which cannot be explained otherwise than by considering it as the degenerated stomodæum of the ancestor. It is the infundibulum. The view that the infundibulum is the remnant of the ancestral mouth is maintained by Cunningham, Patten, Gaskell, &c. The facts that the infundibulum is closely in contact with the anterior end of the mesenteron in the embryonic stage, and that it is behind the optic chiasma, favours this conclusion.

The origin of the mesoblast, separation of the nerve-cord from the epiblast, formation of the heart, the number and position of the eyes, the grooves in the brain, &c., make *Limulus* approach more closely to the Chordata than it was thought to do formerly, and accordingly make the Arthropoda more nearly related to the Chordata than to the Vermes.

TOKYO, JUNE, 19, 1891.

Explanation of Figures.

List of Abbreviations.

I, II, III, &c.	Segments of the 1st, 2nd, 3rd, &c., appendages.
ao. a.	Aortic artery.
app.	Appendage.
br.	Brain.
C.	Cephalic lobe segment.
cox. gl.	Coxal gland.
d.	Dorsal side of the embryo.
d. g.	Dorsal groove of the lateral eye.
entost.	Entosternite.
ex. gr.	External groove of the brain.
flab.	Flabellum.
flab'.	Structure homodynamous with the flabellum.
g.	Ganglia.
g. lat. e.	Ganglia of the lateral eye.
g. med. e.	Ganglia of the median eye.
ht.	Heart.
int. gr.	Internal groove of the brain.
lat. e.	Lateral eye.
lat. gr. 1.	First lateral groove of the ventral plate.
lat. gr. 2.	Second lateral groove of the ventral plate.
lat. h.	Lateral hump.
mar. bd.	Marginal band.
m. c.	Epiblastic cells between lateral ganglia.
med. e.	Median eye.
med. l.	Median line of the embryo.
mes'.	Mesoblastic cells derived from the yolk-hypoblast.
n.	Nerve.
oeso.	Œsophagus.
op.	Operculum.
ost.	Ostia of the dorsal circulatory vessel.
pl.	Pleura.
prim. gr.	Primitive groove.
prim. str.	Primitive streak.
prov.	Proventriculus.
st. a.	Sternal artery.
stom.	Stomodæum.
v.	Ventral side of the embryo.
v. g.	Ventral groove of the lateral eye.

Plate V.

Fig. 1. Peculiar markings on the shore, showing the place where eggs were laid.

Fig. 2. Surface view of an egg which has many irregular fissures (5 days after fertilization).

Fig. 3. Surface view of an egg with the blastodermic thickening (10 days after fertilization).

Fig. 4. Surface view of an egg, at the beginning of the metamerism (13 days after fertilization).

Fig. 5. Ventral surface view of an egg, 14 days after fertilization.

Fig. 6. The same as above, a little more advanced.

Fig. 7. Ventral surface view of an egg, 15 days after fertilization.

Fig. 8. Surface view of the ventral plate of an egg, at the commencement of the appearance of appendages (16 days after fertilization).

Fig. 9. The same as above, a little more advanced.

Fig. 10. Surface view of the ventral plate of an egg, 17 days after fertilization.

Fig. 11. Two specimens of the chorion, broken up by growth.

Plate VI.

Figs. 12–14. Three successive stages in the development of the ventral plate. The nervous system is marked dark red, while the mesoblastic somites are marked blue and the cavities in them light blue. Fig. 12, 19 days old; fig. 13, 21 days; fig. 14, 25 days, about × 30.

Fig. 15. Side view of an embryo, about 24 days old.

Fig. 16. Dorsal view of an embryo, about 25 days old.

Fig. 17. Dorsal view of an embryo of the trilobite stage, showing the formation of the dorsal circulatory system.

Fig. 18. Heart of *Limulus Polyphemus*, after A. Milne Edwards (much reduced).

Fig. 19. Coxal gland of an embryo, about 27 days old, constructed from sections. a. Ventral view. b. Side view.

Fig. 20. Coxal gland of an embryo, about 30 days old, constructed from sections. a. Ventral view. b. Side view.

Plate VII.

Fig. 21. Transverse section through the blastodermic thickening of an egg, 10 days old, shown in fig. 3. 2C.

Fig. 22. Transverse section through the blastodermic thickening of an egg, 11 days old. 2B.

Fig. 23. Transverse section through the rudimentary ventral plate of an egg, 13 days old, showing the separation of the blastodermic thickening into epiblast and mesoblast. 2B.

Fig. 24. Portion of a section, similar as above, showing the columnar epiblast cells and a mesoblast cell with 2 very large nuclei. 2D.

Fig. 25. Transverse section through the cephalic lobe of an embryo, 14 days old (fig. 5), showing the separation of the mesoblast into 2 lateral bands. 2B.

Fig. 26. Longitudinal section through the ventral plate of an embryo, 16 days old (fig. 8), showing the appearance of the cœlomic cavity in the 5th segment. 2B.

Fig. 27. Transverse section through the primitive streak of an embryo, 16 days old (figs. 8,9), showing the formation of the mesoblast cells in the posterior part of the ventral plate. 2D.

Figs. 28–31. Transverse sections through the ventral plate of an embryo, 17 days old (fig. 10). 2B.

Fig. 32. Transverse section through the posterior part of the ventral plate of an embryo, about 20 days old (fig. 14). 2B.

Fig. 33. Transverse section of the anterior part of an embryo, shown in fig. 12. 2B.

Plate VIII.

Figs. 34, 35. Horizontal sections of the cephalic region of an embryo, shown in fig. 12. 2C.

Figs. 36, 37. Horizontal sections of the cephalic region of an embryo, shown in fig. 13. 2C.

Fig. 38. Horizontal section of the cephalic region of an embryo, shown in fig. 14. 2C.

Plate IX.

Fig. 39. Portion of a transverse section of an embryo at the stage of fig. 12. 2C.

Fig. 40. Portion of a transverse section of an embryo, shown in fig. 13. 2C.

Fig. 41. Dorsal portion of a transverse section of an embryo, showing the columnar cells of the dorsum. 2D.

Fig. 42. Portion of a transverse section of an embryo, shown in fig. 10, showing the prominent lateral hump. 2D.

Figs. 43, 44. Dorsal, median portions of transverse sections of an embryo, at about the stage of fig. 16, showing the formation of the heart. 2D.

Fig. 45. Portion of a transverse section of an embryo, represented in fig. 17. 2C.

Fig. 46. Anterior portion of a longitudinal section, showing the peculiar cell group of the brain. 2D.

Fig. 47. Portion of a transverse section of an embryo, shown in fig. 13, through the fifth ganglia. 1C.

Fig. 48. The same as above, cut through the sixth ganglia. 1C.

Fig. 49. Longitudinal section of the second abdominal appendage, before the formation of the gill-lamellae. 2D.

Plate X.

Fig. 50. Median longitudinal section of an embryo at the beginning of the trilobite stage. 2aa.

Figs. 51, 52. Horizontal sections of an embryo in the same stage as above. 2aa.

Fig. 53. Horizontal section of the abdomen of an embryo of the trilobite stage. 2aa.

Figs. 54, 55. Transverse sections through the abdomen of an embryo, a little more advanced than the one above mentioned. 2aa.

Figs. 56, 57. Longitudinal sections of an embryo in the stage of fig. 17. 2aa.

Plate XI.

Fig. 58. Portion of a transverse section of an embryo, shown in fig. 14, through the coxopodite of the 5th appendage. 2C.

Fig. 59. Transverse section (a little oblique) of the cephalic region of an embryo, shown in fig. 12. 2B.

Fig. 60. Portion of a transverse section of an embryo, shown in fig. 13, through

the lateral eye. 2D.

Fig. 61. Lateral portion of a transverse section of an embryo, a little before the stage of fig. 15, through the lateral eye. 2D.

Fig. 62. Transverse section of the lateral eye of an embryo, shown in fig. 17. 2D.

Fig. 63. Two consecutive transverse sections of the anterior end of the lateral eye. 4B.

Fig. 64. Transverse section of the lateral eye of an embryo of the trilobite stage. 2D.

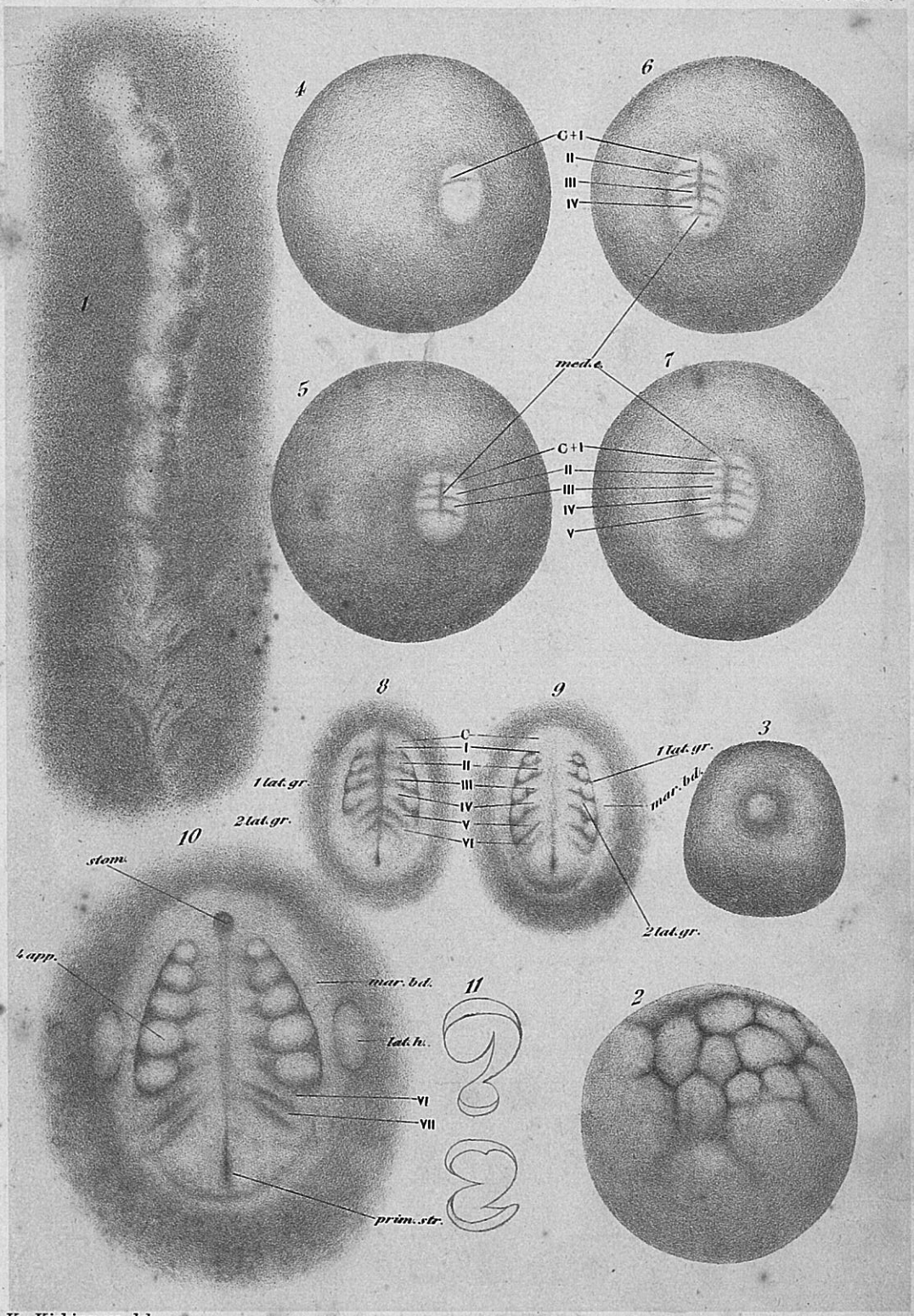
Fig. 65. Cross section of three ommatidia of the lateral eye of an embryo of the trilobite stage. 2F.

Fig. 66. Longitudinal section of the same. 2F.

Fig. 67. Longitudinal section of the median eye of an embryo, at about the stage of fig. 56. 2D.

Fig. 68. Portion of a longitudinal section of an embryo, shown in fig. 15. 2C.

Fig. 69. Diagrammatic representations of the anterior portion of the nervous system of an embryo of the trilobite stage. a. Frontal view. b. Side view of the right half.



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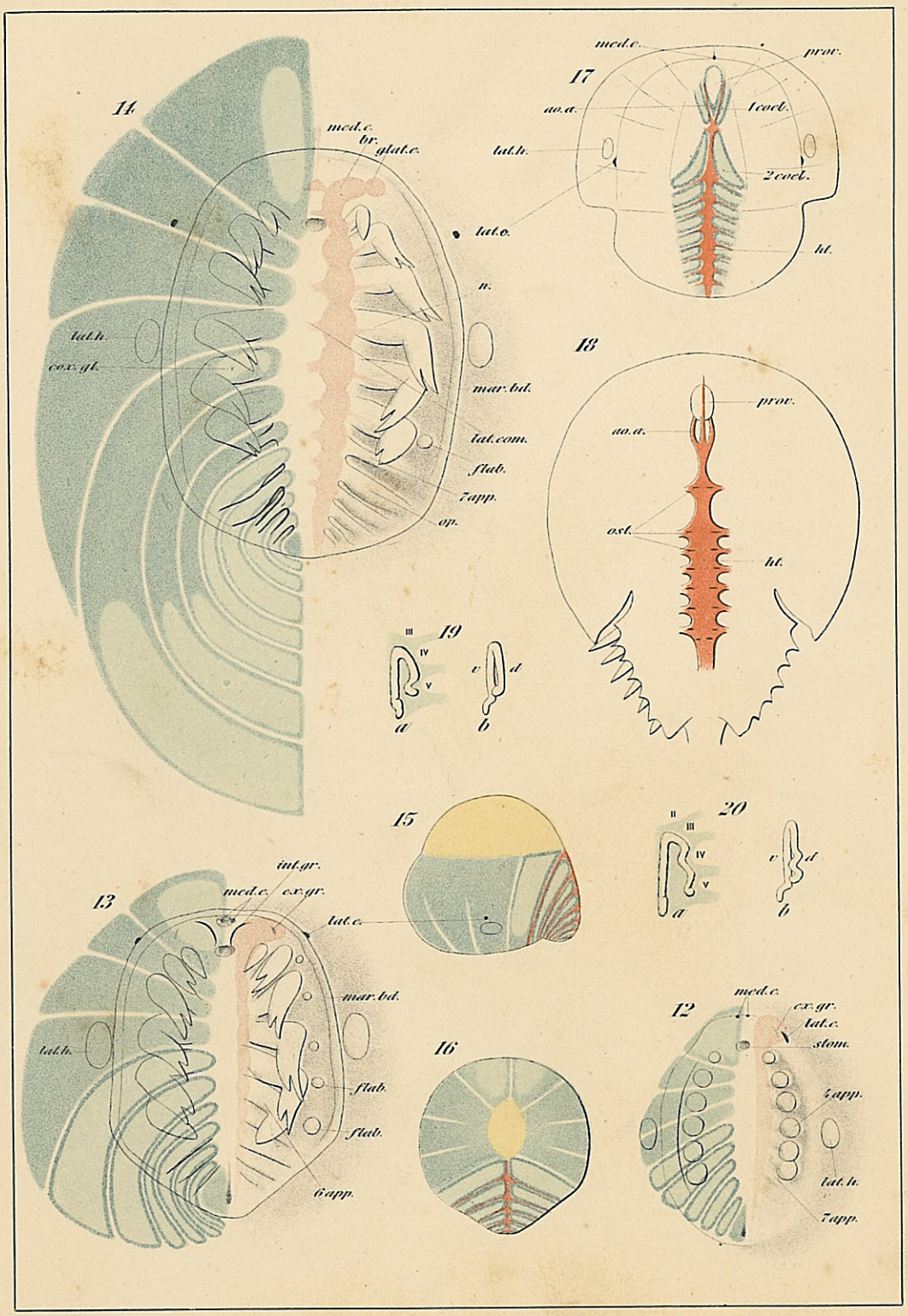

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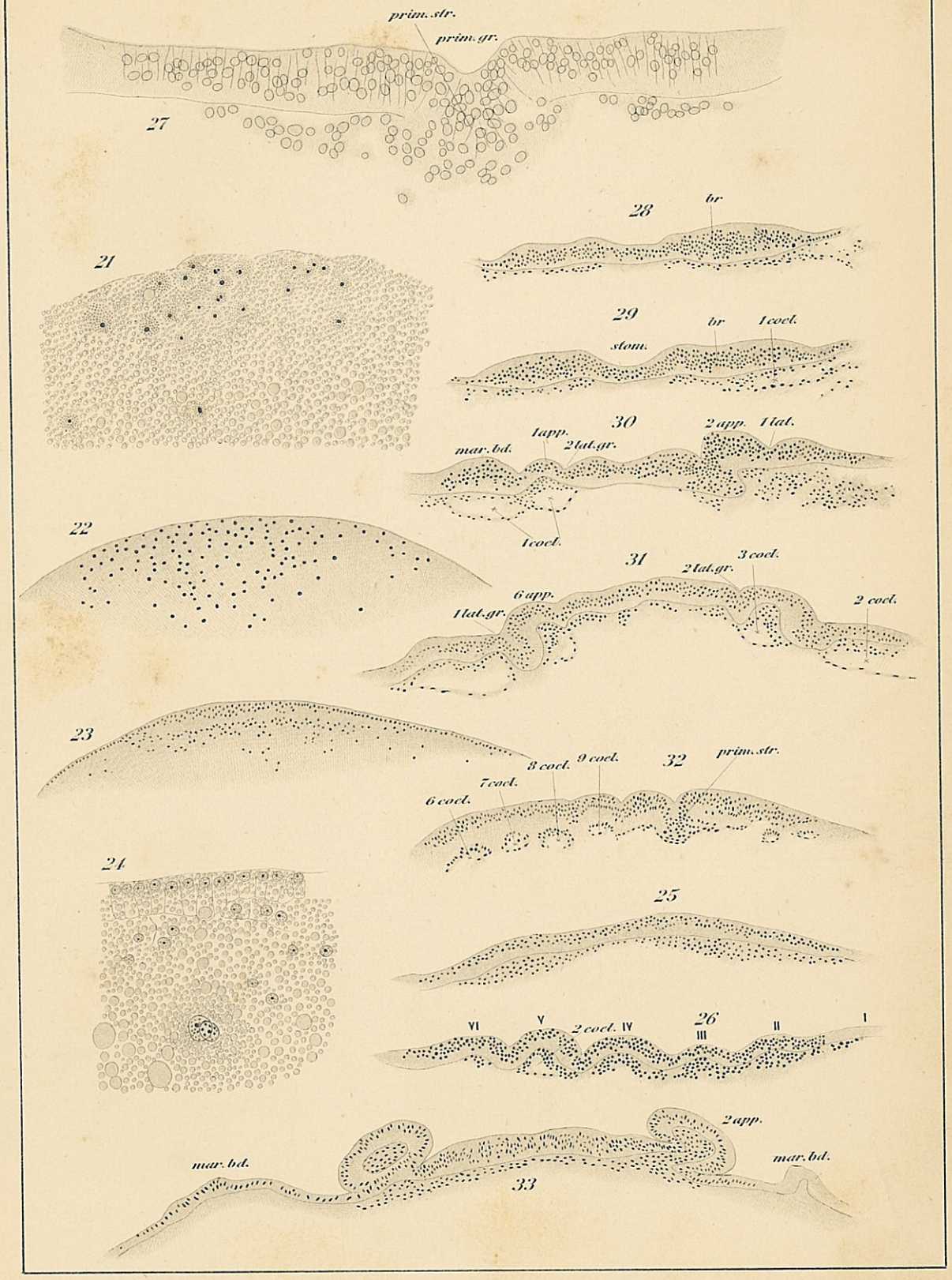

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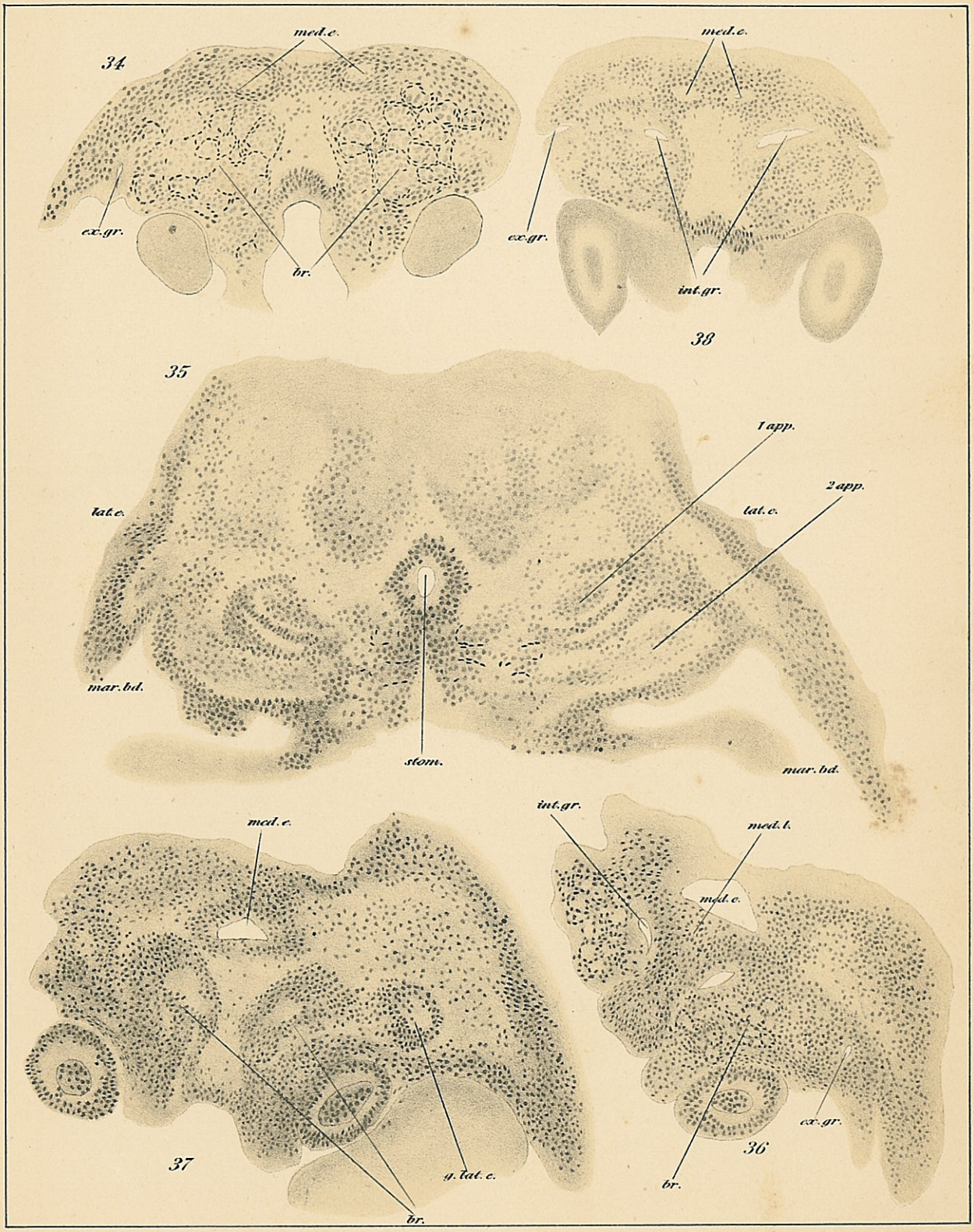

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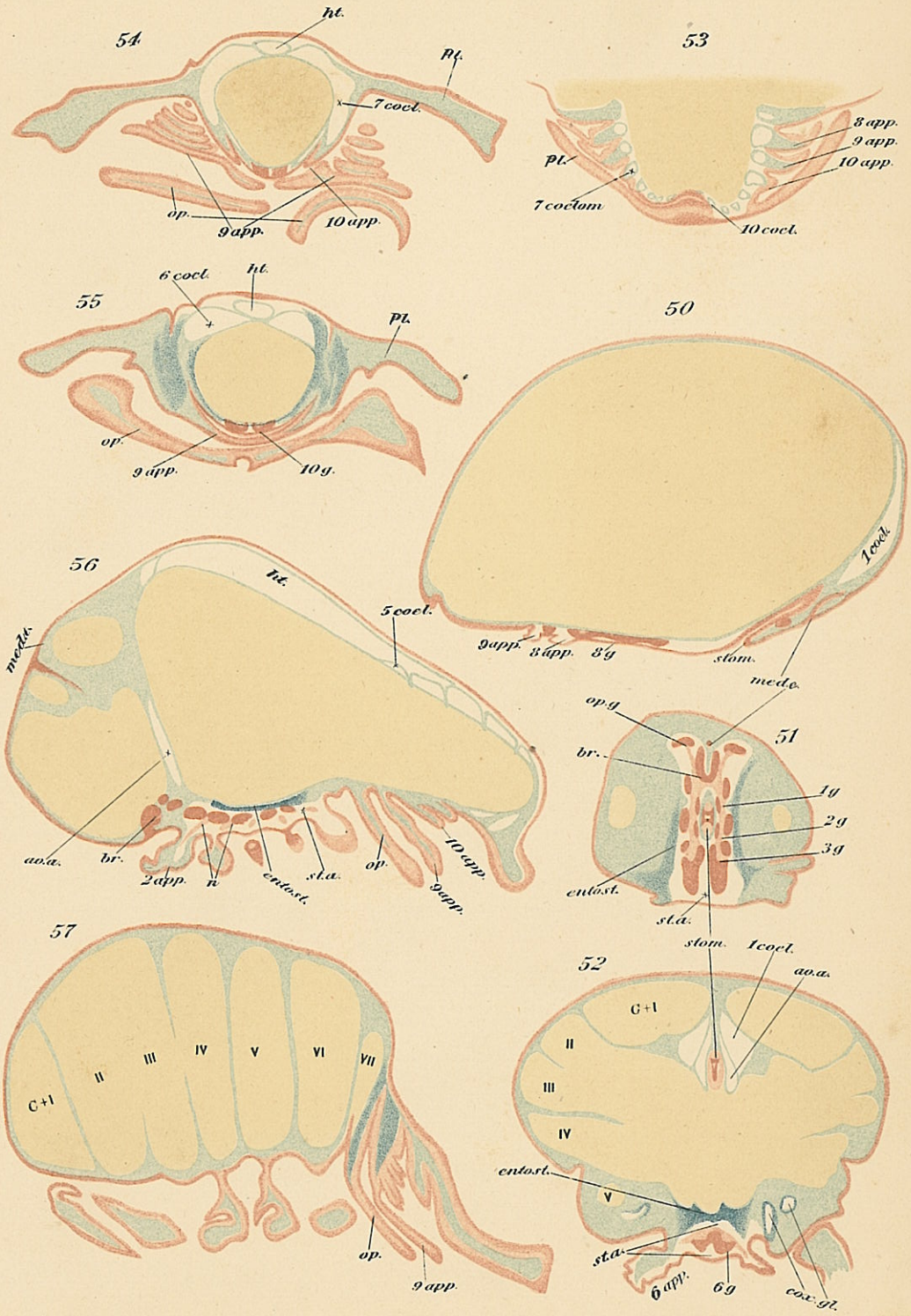

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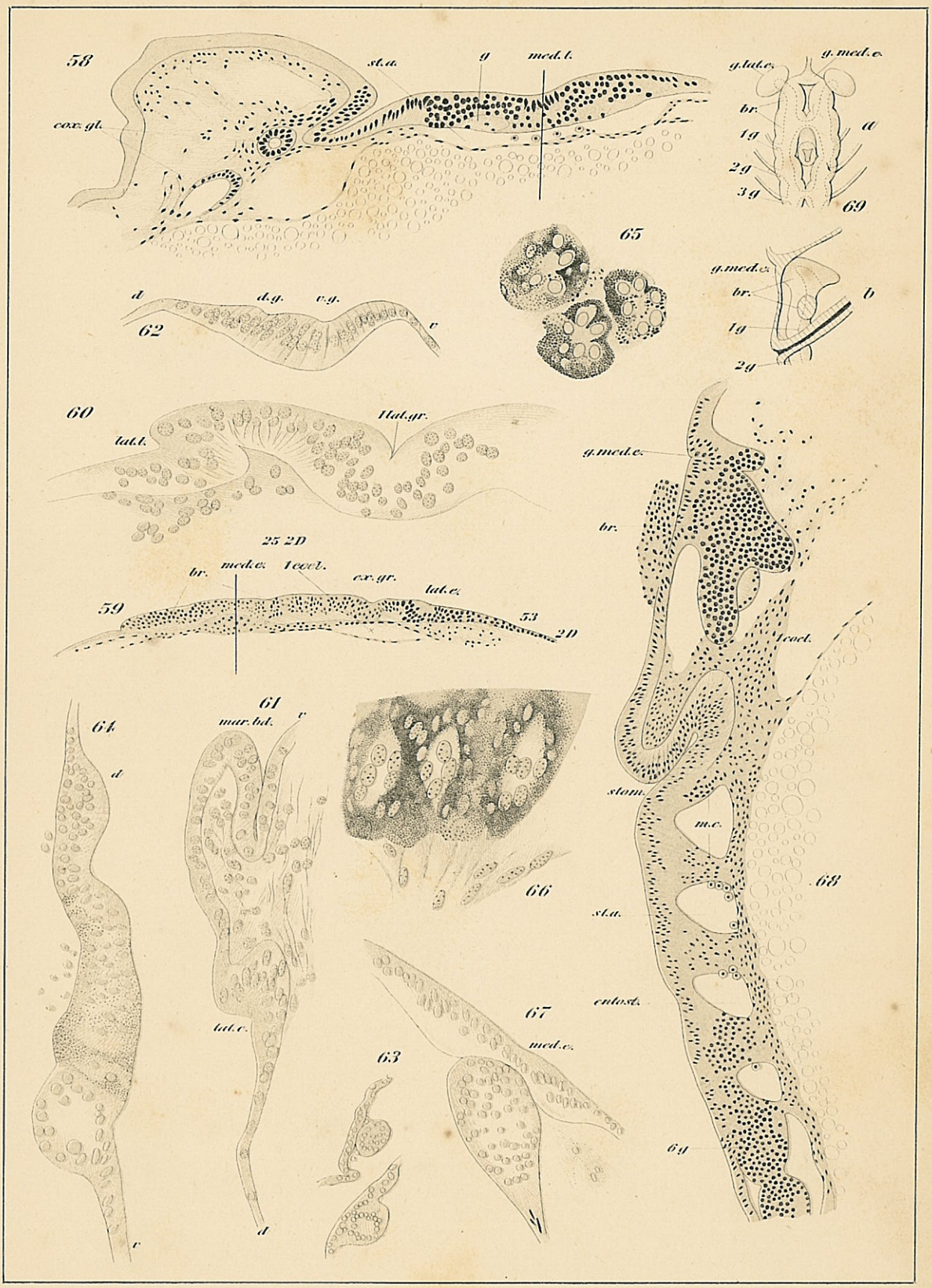

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