

THE EMBRYOLOGY OF LIMULUS.

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HISTORICAL.

THE embryology of *Limulus* has already given rise to considerable literature, but there still remain many problems to be solved. The first to describe any embryonic stage of this genus was Henri Milne-Edwards, who figured and briefly described ('38, '39, '40) a single larval stage. The next was Samuel Lockwood ('70), who gave an account of the oviposition, and the hatching of the egg, and described several of the larval stages. A. S. Packard followed with a long series of articles ('70^a, '70^b, '70^c, '71, '72, '73, '75, '80, '85), each of which added something to our knowledge. Dohrn ('71), who studied material supplied by Packard, was able to see some points which had escaped the latter. Alexander Agassiz ('78) described the habits of the young after the beginning of a free life, and contributed a figure of the larva to Faxon's ('82) compilation of drawings illustrative of the embryonic stages of Crustacea. The present writer made his first contribution to our knowledge of the development of *Limulus* in 1884, following it in October of the next year with a more extended paper ('85). In the same month H. L. Osborn ('85) and Brooks and Bruce ('85) published preliminary accounts of their investigations; the complete papers have not yet

appeared. S. Watase has made the structure and development of the visual organs the subject of three papers ('89, '90^a, '90^b). William Patten ('89) gave a brief note on the origin of the nervous system and sense organs, while in a later paper ('90), in which an attempt is made to derive the Vertebrates from the Arachnids, numerous facts relating to the early history of *Limulus* are given. Lastly, I have presented ('90) a brief abstract of some of the results to be described more at length in the present series.

HABITS, ETC.

The American horse-shoe crab (*Limulus polyphemus*) is distributed along our eastern shores, from Maine to the West Indies and the Gulf of Mexico (Vera Cruz, teste J. E. Ives), occurring at certain times and places in large numbers. Its habits have been described with some detail by Dr. Lockwood ('70). During most of the year it frequents deeper water, but during the breeding season—May until the middle of July—large numbers come to the shore for the purposes of oviposition. I have never been able to notice any connection between the hours when they frequent the shore and the state of the tide. Several times on moonlight evenings, in the height of their spawning season, I have sailed over their favorite spawning grounds, but did not see any of the "crabs."

I do not know where the couples meet. When first seen they come from the deeper water, the male, which is almost always the smaller, grasping the hinder half of the carapax of the female with the modified pincer of the second pair of feet. Thus fastened together, the male rides to shallow water. The couples will stop at intervals and then move on. Usually a nest of eggs can be found at each of these stopping-places, and as each nest is usually buried from one to two inches beneath the surface of the sand, it appears probable that the female thrusts the genital plate into the sand, while at the same time the male discharges the milt into the water. I have not been able to witness the process more closely because the animals lie so close to the sand and all the appendages are concealed beneath the carapax. If touched during oviposition, they cease the operation and wander to another spot or separate and return to deep water. I have never seen the couples come entirely out of the water, although

they frequently come so close to the shore that portions of the carapax are uncovered.

I have already commented upon the great vitality of the eggs and the young ('85, p. 522), but a few words more may prove of interest. When studying the development in 1884 the eggs I studied were transported 200 miles from the place they were laid. They were six days on the journey, packed in moist sand, but without any addition of salt water. On August 1 I left the shore, taking with me some 200 embryos and about a pint of salt water. By merely supplying the loss by evaporation with fresh water from the city supply I kept some of these alive until November 20, when the last were killed to supply material for study. In 1890 I fertilized some eggs on June 22. Some 200 of these were taken in half a litre of water to Lincoln, Nebraska, 1600 miles from the shore, where they lived from September 7 to the week of November 14-20, when they were killed by an accidental drying up of the water during a temporary absence. As it was, they lived over twenty weeks in confinement. It would not have been possible to keep them much longer, as the stock of food yolk was about exhausted. Adult specimens have been shipped alive to San Francisco, and now one meets occasionally with notices in the Pacific coast papers of the capture of horse-shoe crabs, probably those planted there several years ago by the U. S. Fish Commission. They have also been shipped alive to England and Germany. Professor E. Ray Lankester had three barrels of these animals sent him in London from Woods Holl, a large proportion of them surviving the voyage.¹

An observation made by Dr. Lockwood upon the retardation and vitality of the eggs should be repeated. He says ('70, pp. 271-272): "At the close of the warm season last year [1869] my jars must have contained not less than 200 young *Limuli*. . . . Hoping to continue observations upon the growth of my interesting family, the jars were carefully put away. Little regard, however, was paid to temperature, which, on several occasions, went down to the freezing-point. On the 3d of May, 1870, I emptied the jars to see how my charge was getting on, when lo! not one of the last year's hatching was alive! but, won-

¹ Mr. Vinal Edwards, who made the shipment, informs me that those packed without seaweed or other moist packing survived the journey the best.

derful to say, at least a dozen little fellows, all hatched this spring, and all alive, had taken their place. With these were also at least thirty eggs, in different, but all in advanced, stages of incubation. In some of them the young could be plainly seen revolving." Here was a retardation of development for almost a year!

METHODS.

The observations here recorded were made at the Marine Biological Laboratory at Woods Holl, Massachusetts, during the months of June, July, and August, 1889 and 1890, and in the zoological laboratory of the University of Nebraska. In writing up the results obtained I have been hampered not a little by my distance from the larger libraries, and hence the comparative portion of the paper is sadly deficient — a fact which no one can realize more than myself.

For my material I have relied partly upon the natural nests and partly upon artificial impregnation. With the former method one cannot be certain of the age of his material, for not infrequently two ovipositions become mixed. I have never succeeded in getting the crabs to oviposit naturally in confinement. In artificial impregnation the eggs and milt were sometimes obtained by squeezing individuals taken *in copulo*, or by sucking these products from the genital ducts with a pipette. Very severe squeezing will force out but a small number of eggs, — far fewer than are naturally laid in a nest, — while any attempt to remove them from the body by cutting covers the eggs with a layer of very rapidly coagulating blood (*vide* Howell, '85), which affords an excellent nidus for bacterial and fungoid growths.

The study of the early stages has proved very difficult from the fact that the eggs are the most refractory objects I have ever seen. Until the outlining of the germ there is no means of orientation, so that sections must be taken hap-hazard. The greatest care must be taken in hardening them in order to prevent the yolk becoming too hard for the section knife; and after numberless experiments with every reagent I could think of, I came to rely almost entirely upon killing the eggs by heating them in sea-water to 70°–75° C. and then passing them through successive grades of alcohol, from 30 per cent to 70 per cent, in which they were finally kept. Eggs thus treated afforded at

the moment of killing excellent, but evanescent, surface views, as a short immersion in alcohol renders the whole surface one uniform color. Hence, in order to orient these eggs for subsequent section, I marked each one, at the moment of killing, with India ink — not affected by alcohol — and subsequently arranged the egg with reference to the line thus afforded. For staining I used chiefly alum cochineal and Grenacher's borax carmine, while a short stay in osmic acid brought out certain details.

I found it impossible to cut the early eggs in paraffin. Absolute alcohol and the clearing reagents rendered the yolk extremely hard and brittle, while the paraffin refused to penetrate the centre of the egg. So for the early stages I had recourse to celloidin. For the main outlines of the process employed I am indebted to the suggestions of Dr. H. C. Bum-pus. The celloidin was hardened with chloroform and cleared with origanum oil or with a mixture of turpentine and carbolic acid *before* cutting. The sections were cut with the knife flooded with the clearing fluid, and then placed in order on the slide. Being already cleared, all that is now necessary is to apply balsam and the cover glass. In many respects this process is identical with that described by Eyclesheimer ('90).

To study the stages after the outlining of the germ, the chorion was removed by needles,¹ and then by careful manipulation the blastoderm was stripped from the yolk, stained, and either mounted *in toto* for surface views or sectioned as usual in paraffin. In the later stages the processes of development so modify the yolk that the whole embryo is capable of being sectioned in the usual manner.

As a result of the difficulties of manipulation the following account of the early stages is exceedingly fragmentary, yet it is hoped that the little here detailed will prove of value, especially as almost nothing is known of the processes involved in the formation of the germ layers. (See Postscript.)

¹ Owing to the great thickness of the chorion I found it difficult to control the action of eau de Javelle or Labbaracque's solution. Before the chorion was dissolved the solution would frequently affect the egg, interfering with staining and making it very crumbly.

OVIGENESIS.

I have made no extended observations upon the origin and development of either eggs or spermatozoa. The gross structure of the ovaries has been described by van der Hoeven ('38), Gegenbaur ('58), and Owen ('72), while Gegenbaur adds a short account of the origin of the egg, presented in abstract by Ludwig ('75). Owen gives a line or two to the testis, while Benham ('83) describes it more in detail. Packard ('72) figured the spermatozoa, Lankester ('78) noted the fact that they are motile, and Packard ('80) refers to the histology of the testis and speaks briefly of the development of the ovary. Aside from these and one or two older papers, at present inaccessible to me, I know of no published results upon the reproductive organs of *Limulus*.

In a female *Limulus* four inches long (not including the caudal spine) I find the ovarian cæca lined with columnar epithelium which secretes a delicate cuticle, and outside of this epithelium a connective tissue tunica propria. As in other higher Metazoa, this epithelium is the ovogenetic layer, certain of its cells becoming modified into primordial ova. These at first lie within and form a part of the parent epithelium, but with growth the eggs pass to the outside of the epithelium and, separating the tunica from the other layer, come to lie between the two. (Figs. 1 and 2.)

The primordial ova are distinguishable not only by their size, but by their more deeply staining cytoplasm, in which the yolk spherules, so characteristic of the mature eggs, are lacking, unless the minute granules are to be regarded as such. Around the cytoplasm of the older eggs, after leaving the epithelium, there is a delicate membrane, the origin of which I have not been able to decide, but I think it a true vitelline membrane. The nuclei of the ovarian eggs vary considerably with age. In the younger ones they are strongly staining bodies of about the size of the nucleoli of older eggs. In these no reticulum is visible. A little later this nucleus is surrounded by a clear space which separates it from the darker and more granular cytoplasm. This clear space shows processes radiating into the surrounding substance. In still older eggs a well-marked nuclear membrane is distinguishable, inside of which is a faintly staining chromatin (?) reticulum

and from one or two to five spherical and deeply staining nucleoli. There is no 'yolk nucleus' like that described in certain Arachnids.

As will be seen, the foregoing description differs *in toto* from Packard's brief account and figures ('80, p. 39, Pl. IV, Figs. 8, 8^a). In fact, I cannot determine what he had under the microscope. If I understand Gegenbaur ('58) aright, the eggs in his specimen¹ project into the lumen of the ovarian tube, a difference possibly explicable on account of the mature condition of his material. He was farther unable to recognize any membrane around the egg aside from the epithelial cuticle of the ovarian tube. In other respects there is no discrepancy between our accounts.

Making comparisons now with the Arachnida, we see no little similarity in the structure of the ovary and the relations of the ova. Metschnikoff ('71, pp. 207-208, Pl. XIV, Figs. 1 and 2) and Laurie ('90, pp. 108-111, Pl. XIII) describe and figure almost the same condition in the scorpion. The ovary consists of the same epithelium and tunica, and the eggs, as they increase in size, come to lie between these two layers. The differences are that in *Limulus* each egg is not enveloped in a separate follicle; but in the scorpion, where the eggs are few, such is the case. In *Limulus* the epithelium does not form such a well-marked "stalk" connected with the egg as in the scorpion; and the cells of this stalk are columnar, not stratified. Closely similar resemblances can be traced with the Araneida, as epitomized by Ludwig ('75) and the Acarina (Pagenstecher, '60-'61). In the Crustacea, on the other hand, a similar condition is not found, there being nowhere an ovary with a similar constitution. In short, so far as my observations on ovigenesis go, *Limulus* agrees well with the Arachnida and contrasts strongly with the Crustacea.

EARLY DEVELOPMENT.

The eggs of *Limulus*, as they come from the oviduct, vary considerably in size and shape. They are usually more or less oval,

¹ Twenty-five German inches long. Gegenbaur is in doubt about his specimen. In appearance it was clearly *L. molluccanus*, but so far as he was able to find out it came from the West Indies. As *L. polyphemus* and *L. molluccanus* are easily distinguishable, it is possible that a mistake was made in locality.

being somewhat flattened at first by mutual pressure in the oviduct. The average diameter is perhaps two millimetres. Each egg is enveloped in a tough chorion in which a laminated structure is readily recognizable. I have never been able to discover any opening or pores in this chorion through which impregnation can be effected, although it is certain that fertilization must take place outside the body of the female, and hence after the chorion is formed. The egg proper consists of a large mass of strongly refractive yolk globules of various sizes, and in the egg as it comes from the oviduct I have been unable to find a trace of a nucleus, nor of nuclear material. No matter what stain was employed, I could not recognize any chromatin granules scattered through or upon the yolk, while anything that might be considered as protoplasm was very scanty.

In this my experience is paralleled by that of certain other students of Arthropod eggs. The nucleus can be traced to a certain stage of ovarian development where, as Stuhlmann says ('86), "*Später verschwindet das Keimbläschen vor unseren Blicken, bis wir endlich am oberen Eipol der Furchungskern wieder finden.*" Of course this absence is apparent rather than real, as has been shown by numerous other observations.

I have been equally unsuccessful in my attempts to witness the phenomena of fertilization, nor have I seen any features undoubtedly characteristic of maturation, although I have sectioned many eggs. In one egg, an hour after fertilization, I found on one side a faintly staining structure which I have possibly thought may have been a polar globule (Fig. 3), but the fact that a nuclear stain brought out no chromatin inside the yolk renders this doubtful.

The various steps of development vary in time, not only with the temperature, but with eggs of the same lot exposed to exactly the same conditions. Hence the ages quoted in the following pages must be understood as averages. Thus, in one lot of eggs I have found phenomena occurring at four hours, which in others occurred at twenty-four hours, while in later stages there may be variations of a month or more.

At the time of impregnation, the surface of the egg is covered with dark yolk granules, each granule having a lighter boundary. The granules vary in size, and the egg completely fills the chorion. In fifteen minutes the chorion distends so as to

leave a space between it and the egg, and at the same time its outline becomes regular and ellipsoidal. In half an hour the granules begin to break up and become smaller, while the yolk begins to swell, and at the end of an hour completely fills the chorion.

In four hours begin those strange modifications of the surface already noticed by H. L. Osborn ('85) and by Brooks and Bruce ('85). Viewed from the surface the eggs exhibit a number of fissures, usually at one pole of the egg, which strongly simulate cleavage furrows (Figs. 4, 5, 6). I have not been able to kill such eggs quickly enough to preserve these furrows for section. Even when dropped into hot water the surface would become smooth before death ensued.

Sections of such eggs present some features difficult of interpretation. In the earlier phases near one pole there appears a clear line inside the yolk, concentric with the surface, which marks off a central from a superficial portion; while in older eggs (Fig. 7) the line has extended nearly around the egg. Inside of this line were no features worthy of mention, and in the several eggs sectioned no nucleus was to be found. Outside of the line the yolk becomes broken up into numbers of columnar bodies—like the cells of columnar epithelium—with rounded external ends. These yolk columns are separated from each other by a slightly staining protoplasm (Fig. 8), and the outer ends of these columns are more free from yolk spherules than are the deeper portions. I think, notwithstanding the apparent disparity of dates, that it was an early stage of this process which Brooks and Bruce describe when they say of an egg of twenty-four hours "protoplasmic processes or pseudopodia extend from the [protoplasmic] cap into the yolk, and surrounding and including the substance of the yolk divide this up into a number of yolk balls." After a short time these motions of the external surface cease, and the egg becomes as smooth as before, while in section no change is recognizable except that there is a thin layer of protoplasm—a true blastema¹—over the whole yolk.

¹ As I have already indicated ('86, p. 116, foot-note), I use the term blastema in the original sense. Patten defines ('84, p. 564) the blastema as "a thin nucleated layer of protoplasm covering the whole outer surface of the yolk, and not divided into distinct cells." He, however, suggests that it is not impossible that a

I am in doubt as to the interpretation of these phenomena. They are not connected with segmentation. Two possibilities have suggested themselves. One is that they may possibly be compared with those still unexplained polar rings described by Whitman ('78, p. 234), on *both* poles of the maturing egg of Clepsine (a suggestion of doubtful value). The other would view them as connected with the formation of the blastema. It is certain that a blastema surrounds the egg of *Limulus* after this process while none was visible before.

In one egg of about twelve hours I found what I regarded in my preliminary paper ('90) as the segmentation nucleus, occupying a subcentral position in the yolk, but I have not succeeded in connecting it with the later stages. In other eggs of the same age I find a thickening of the blastema on one side of the egg, but no stain serves to distinguish a nucleus in it, but still it may be present. The position of the segmentation nucleus has no great taxonomic importance, as in both Crustacea and Arachnida it may be either subcentral or superficial.¹

Stage A.—Between twelve and twenty hours I have not been able to get any sections showing anything. At twenty hours I found an egg containing eight nuclei. By drawing these in their relative positions and projecting them on a plane (Fig. 9), a marked polarity in their distribution is apparent. As will be seen, the nuclei are much nearer to one pole of the egg than to the other, and had the plane of projection been slightly different this polarity would have been more marked. This condition is intelligible on the view that the segmentation nucleus is subcentral as well as if it be regarded as superficial.

In the next twenty hours there are no phenomena to detail at length. From the surface no changes are visible, while sections reveal a gradual increase in the number of nuclei, the polarity just mentioned persisting in their distribution.

blastema may exist without nuclei. The term blastema was first used by Weismann ('63) for a non-nucleated layer in *Musca* and *Chironomus*, and such a layer has been shown to exist in many eggs by various authors, among them Metschnikoff ('66) in *Aphis*, *Aspidotus*, *Cæcidomyia*; Witlaczil ('84) in *Aphis*; Locy ('86) in *Agalena*; Heider ('89) in *Hydrophilus*; Voeltzkow ('89) in *Musca*, etc. A blastema, then, is a layer of anucleate protoplasm around the yolk.

¹ *E.g.* subcentral in *Cetochilus* (Grobbe, '81), *Crangon* (Kingsley), *Eupagurus* (Mayer, '77), *Porcellio* (Reinhard, '87), *Araneina*; peripheral in *Nebalia* (Metschnikoff), *Mysis* (Van Beneden, '69), *Scorpio* (Laurie), *Acarina* (Claparède, '68).

In from forty-two to forty-eight hours the eggs, as they lie in the dish, show on their upper surfaces the first traces of segmentation of the yolk. In this there is no regularity as to the direction of the furrows nor uniformity in their extent. At first the furrows are clean cut, with well-defined margins and some depth, but soon they become shallower, and the margins and bottoms become irregular by the formation of numerous yolk spheres of varying size (Figs. 10, 11). Gradually the furrows flatten out, and the yolk spheres become merged in the general yolk of the surface, and the egg is as smooth as before. In from four to six hours this process is repeated, the spaces between the furrows becoming smaller and the furrows embracing more of the egg than before (Fig. 12). This is repeated several times, until at last the whole surface is included in the segmentation (Figs. 13, 14). At each of these divisions there are at first the same clean-cut furrows followed by the same irregularity, and eventually by the apparent obliteration of the planes of segmentation.

Sections plainly show (Fig. 15) that this is a true segmentation of the yolk, the result being to divide the whole egg into a series of cells, each consisting of a mass of yolk (Fig. 18) with a central nucleus. It is also apparent that therewith is connected the appearance of the nuclei at the surface of the egg and the formation of a blastoderm (Fig. 15). In the projection of an egg of forty-eight hours (Fig. 16) twenty-six nuclei were seen. A little later ($2\frac{1}{4}$ days) a higher power shows some interesting phenomena. The nucleus is surrounded by an amœboid mass of protoplasm, sending processes into the surrounding yolk, while the planes of segmentation, as well as the external surface of the egg, are covered with a thin layer of faintly staining protoplasm (Fig. 17), apparently the blastema of the earlier stages. At the time when these furrows seem to disappear (*supra*), this protoplasm regains the surface, but the furrows themselves remain, and eventually the whole egg is divided into nucleated yolk cells (Fig. 18).

At first the central portion divides as rapidly as the peripheral, and in each portion of the egg the cells are about equal in size; at last, however, the central cells enter upon what may be called a resting stage, which condition persists until after the beginning of a free-swimming life. Their divisions occur at infrequent intervals, and the differences in size, from the appearance of

the germ until the caudal spine appears, are scarcely noticeable.

Stage B. — The peripheral cells, on the other hand, divide more rapidly, so that in five days from impregnation (Fig. 19) there is a marked difference between the cells on the surface and those deeper in the egg. A more careful study shows that this division of the surface cells has a peculiar character. In each instance (see Fig. 20, which represents a portion of an egg of $5\frac{3}{4}$ days) the first division of the peripheral cells occurs in a plane parallel to the surface of the egg. This is plainly shown in the cases of the cells marked *x*, where the direction of the mitotic spindle shows the direction of the future division — a view which is confirmed by a study of the other cells. Another feature is noticeable. The products of this division are unequal. There is a deeper and larger cell containing a large amount of food yolk and closely resembling the neighboring yolk cells; and a superficial smaller and flattened cell, richer in protoplasm and containing far less yolk. In this way a blastoderm is differentiated, but the process has in my opinion a deeper significance, for by it the entoderm is separated from the rest of the egg. In other words, in *Limulus* the two primary germ layers are differentiated by multipolar delamination.

This process is clearly allied to that multipolar delamination which Morgan ('90, '91) has described as occurring in the eggs of certain pycnogonids and Faussek ('91) in phalangids. While I shall discuss it later, I may say here, that it probably has at most a very distant relationship to the Cœlenterate delamination, but has arisen within the Arthropod phylum.

After the formation of the blastoderm, *i.e.* the separation of ecto-mesoderm from entoderm, I have not been able to add much to our knowledge until about eight days after impregnation. The absence of all features which would aid in the orientation of the egg makes it necessary to cut all sections at random, while the opacity renders surface views impossible. In general this time is occupied by a multiplication of the blastoderm cells and a consequent diminution in their size. This multiplication by division proceeds at a more rapid rate at one pole of the egg than at the opposite, the result being that soon a germinal pole may be recognized by the smaller and more columnar cells, those at other portions retaining, until later stages, more the appearance of pavement epithelium.

BLASTODERM CUTICLE.

With the formation of the blastoderm, the blastodermic cuticle is first laid down. Its history need not be given here, as I have already ('85, p. 524) detailed it, and have suggested for it and similar envelopes, Claparède's term "deutovum." The occurrence of these *Blastodermhauten* is frequent in the Arthropod phylum. In *Limulus* the envelope persists as a protective structure until a late stage in development, but it is omitted from my figures.

EARLY EXTERNAL DEVELOPMENT.

Stage C.—At from six to eight days after impregnation a lighter patch is visible on one side of the egg. Its outline is not distinct, but in general it may be said to be circular. The change which this undergoes in two or three days (eight to eleven days from impregnation) is slight; at the latter date a pit is apparently seen in the centre of the white spot (Fig. 21).

For this lighter patch I have taken the same name which was given by Claparède ('62) to a similar structure in the developing Arachnid egg.¹ The spot is the first appearance of what is to form the primitive streak. At first this spot is circular, but it soon becomes elongate. The next day a second cloud appears immediately adjoining the first and connected with it (Fig. 22). I am not positive in my identification, but believe that the primitive cumulus marks the anterior end of the embryo. At first the posterior, or secondary, cloud is smaller than the primitive cumulus, but it rapidly increases in size, while its outlines become more indistinct than shown in Fig. 23. At the same time the primitive streak extends backward from the spot mentioned above, into the posterior cloud; the anterior spot remaining the widest of the whole. For reasons which will appear farther on I regard the widest end of the primitive streak as marking the position of the future mouth. The posterior cloud continues to grow until the result is as shown in Fig. 24.

Next there appears a transverse line behind the primitive cumulus, cutting the embryo into an anterior, or cephalic

¹ I retain this term, 'primitive cumulus,' notwithstanding Kishinouye ('90) has shown that it is possible that Claparède has mistaken the order of appearance of his "cumulus primitif" and the 'calotte.'

region, and a posterior or thoracico-abdominal portion, the cephalic being the smaller and more sharply differentiated from the rest of the blastoderm. This occurs on the average about fifteen days after impregnation. Twelve hours later a second line occurs behind the first, cutting off from the thoracico-abdominal region the first somite of the body; and about twelve hours later a third transverse line appears (Fig. 25), and now there is a head region, two body somites, and an undifferentiated caudal region.

This figure (Fig. 25), taken from a blastoderm peeled from the egg and mounted in balsam, shows clearly that this appearance of somites and lines of separation results from the fact that the cells are abundant in certain regions and more scattered in others; in other words, from the outlining of the mesodermic somites. This process continues until six segments behind the head are formed, the sixth consisting of the united sixth 'thoracic' segment and the caudal plate.¹ At first these segments are quite short and correspondingly broad (Fig. 26), but later they increase rapidly in length. I may say in passing that owing to the difficulties of observation it was not possible to be certain of the limits and proportions in certain figures. Each egg had to be studied in strong sunlight, and the use of a camera was impossible. Such was the case with Figs. 22, 23, 26.

MESODERM.

The primitive cumulus is shown in section in Fig. 36. As will be seen, the surface of the egg is covered with a layer of thin, flattened cells, while beneath are the entoderm cells. The cumulus itself is thicker, partly owing to the fact that its component cells are more columnar, and also to the fact that lower layer cells have been formed. The spot in the cumulus, which in surface views (Fig. 21) looks like a pit, is seen in sections to be produced by the greatly thickened centre of the cumulus.

¹ In a few instances I have seen reason to doubt this. In almost every instance I have seen all six appendages arise at the same time, but in two or three cases, (e.g. Fig. 28) but five appendages appear at first, the appendage 1 being noticeable at a later date. This may indicate that the corresponding segment may be correspondingly delayed; and that the above interpretation is not correct. On the other hand, these instances may belong to some of the many anomalies, which are found in examining a large series of *Limulus* embryos.

Sections of the embryos shown in Figs. 21-23 show but slight differences from those of Fig. 24, and hence a description of that will suffice. Fig. 37 represents a section through the anterior end of the streak at stage C. In the median line is the streak itself, which shows a median proliferation of cells extending some distance into the yolk, while on either side is a less conspicuous thickening of the blastoderm. All three of these elements enter into the formation of the appearance of a primitive streak as viewed from the surface, and all contribute to the formation of the middle germ layer. At the point of the section the median ridge extends below the others, and the nuclei at its inner extremity show a tendency to spread towards right and left. Farther back (Fig. 38) these same centres of proliferation may be traced, and here the lateral as well as the median band contributes to the mesoderm. From the primitive streak the mesoderm here extends right and left to the margin of the germinal area, where, apparently, it again connects with the ectoderm. In some sections, especially in later stages, other points of connection occur between ecto- and meso-derm, but I have not been able to trace any regularity in these.

This account accords well with that of Patten ('90), except that I have failed to trace, in surface view, the ring of mesoderm extending completely around the embryo to which he refers (p. 375). Probably this is represented by the marginal connection between ectoderm and mesoderm in my figure.

In this method of mesoderm formation a portion of the peripheral part of the yolk is cut off by the outgrowing middle layer, and comes to lie between it and the ectoderm (Fig. 39). This yolk is in such position that it can readily serve as food for the growing ectoderm, and although I have no evidence on this point, I believe that such is its fate.

The subsequent history of the mesoderm and its derivatives will be followed in detail in the next portion of these studies.

DEVELOPMENT OF EXTERNAL FORM.

Stage D. — The next step is the formation of the appendages. So far as my observations go this process would seem to take place nearly simultaneously on all of the cephalothoracic post-oral segments in the majority of eggs. Yet this is not the case

in all. Figs. 27 and 28 show two modifications which I have witnessed, the latter in two instances. In the first and more normal of these figures the cephalic region is small, and behind it come six somites, each with the outline of a pair of appendages. The sixth appendage is faint, and the segment which bears it is not yet differentiated from the abdominal region. The same state of affairs is shown in the slightly later stage represented in Fig. 29, made from an embryo peeled from the egg, and which also shows several other points to be described later. In this the mesodermic somites are obscured, while the abdominal region is more elongate. On the other hand, Fig. 28, also made from a transparent specimen, shows but *five* pairs of thoracic feet, while in other respects the embryo is much further advanced, as is shown by the existence of appendages VII and VIII (operculum and first gill-bearing appendage) in the abdominal region.

This variation in the time and order of the appearance of the appendages probably explains the difference between Dohrn ('71) and Packard, the former stating that appendage I appears later than the others. This is certainly true in some cases, but out of several hundred eggs examined at about the time of the appearance of the feet, I have seen but two instances, and in my former papers ('85 and '90) I took Packard's position, as at the times when those papers were written I had not seen a specimen without appendage I.

Professor H. L. Osborn ('85, p. 2) gives the following account of the appearance of the limbs in *Limulus*: "On July 28th, 11.30 A.M. [the eggs were fertilized July 23], a deep semicircular depression showed itself. On the 29th, in the space between the two lips of the depression of the day before, a pair of buds appeared—the beginnings of the anterior pairs of limbs. On the following day two more pairs are added, and in front of the first pair and behind the front lip of the fold a most important structure is for the first time seen: it is a slit elongated antero-posteriorly,—the definitive mouth opening. It is distinctly in front of the first pair of limbs. It is to be noted that the anal opening has not yet shown itself, according to my observations. The stomodæum and the three somites are now included in an area which is plainly marked off from the rest of the egg and surrounded by an oval elevation. On the following day,

July 31, there had appeared inside this rim the remaining pairs of cephalothoracic appendages."

Although I have looked carefully for the appearances thus described, I cannot confirm the description. Still, there are so many anomalies in the history of many eggs that it is possible that the conditions witnessed by Professor Osborn may sometimes occur. For instance, in some eggs, after the somites are partially outlined, a deep longitudinal groove appears, transverse to the somites and extending the whole length of the embryonic area. The lips of this groove sometimes even touch each other, and in the tube thus formed the limbs bud out. Again, in other eggs a deep invagination may take place in the abdominal region, carrying in with it the abdominal feet. Such eggs appear later to regain the normal appearance and to develop in the regular manner.

Concerning the later features of external development but little needs to be said. The figures given by Packard Dohrn, and myself are sufficient to indicate most of the features of the growth of body shape and the positions and changes of forms of the various appendages.

Stage E (= Kingsley, '85, Fig. 5; Packard, '72, Fig. 12).—In this stage the edge of the carapax has been differentiated, forming a clear-cut line marking off the ventral from the dorsal surface. The six pairs of cephalothoracic legs retain a post-oral position, while the first pair (operculum) of abdominal appendages is outlined.¹

Stage F (Packard, Fig. 12; Self, '85, Fig. 6; present article, Fig. 28).—In this stage the embryo is much as before, except that the second (first gill) appendage of the abdomen has made its appearance, while the series of sense (?) organs briefly mentioned by Patten ('89, p. 602) are prominent, especially in mounts peeled from the egg and in osmic acid preparations. These sense organs, to which I shall return later, are six in number on either side of the body. I earlier ('90) described their fates, which are as follows: The first pair give rise to the median ocelli of the adult; the second move to a position in front of the mouth, where near the median line they form a peculiar sense organ as yet undescribed; the third and sixth disappear at a very

¹ This is not well shown in Packard's figures.

early day; the fourth forms the structure called by Watase ('89 and '90^a) the "dorsal organ," which early reaches a large size and then disappears; while I believe that the fifth gives rise to the compound eye.¹ I now believe that this account will require serious modification. Of the existence of the organs there is no doubt, but their fate is in question.

Stage G (Fig. 32) is characterized by the relative change in position of mouth and the first pair of limbs. At first the mouth is distinctly pre-appendicular (*vide* Figs. 27, 28, 29). At this time its shape is approximately circular. Soon, however, the mouth becomes more elongate, its front margin becoming acute as if the right and left lips were coalescing (Figs. 30, 31). By this process a true ectodermal stomodæum is invaginated, and the mouth is carried backward, as I have already explained and diagrammatically illustrated ('85, Pl. XXXIX, p. 526, Figs. 40-43), so that as a result the first pair of appendages become distinctly post-oral. Other features are the budding of the curious appendix (flabellum Auct.; appendice lancéolé de la hanche, van der Hoeven) upon the basal joint of the sixth pair of appendages; and the outlining of the so-called metastoma upon the sixth body segment. I have already pointed out that this last cannot be regarded as an appendage of a metameric nature (Self, '85, p. 532), since it is borne on the same segment as the true sixth appendage.

In *Stage H*, Fig. 33 (Packard, Fig. 19; Kingsley, '85, Fig. 12), the distinction between cephalothorax and abdomen is evident; the legs are longer and show evident segmentation. (Fig. 33.)

In *Stage I* (Kingsley, '85, Fig. 14; Packard, Fig. 24) the appearance is quite like that of the adult. The body is now much more depressed, the legs are like those of the adult, and the cephalothorax is considerably larger than the abdomen. The abdomen exhibits traces of segmentation, while its margin bears the movable spines upon its margin which are characteristic of the adult. The telson as yet remains as a slight lobe of the middle of the hinder margin of the abdomen.

¹ This account varies from that of Patten, ('90) if I understand him correctly. According to him the median eye falls outside the category of these organs. The compound eye ("convex eye") "arises from three small sense organs near the third thoracic segment," while the "eye of the fourth segment" is very large, thus putting the compound eye in front of the 'dorsal organ.' Watase, on the other hand ('90^a) places the compound eye behind the dorsal organ. (See Postscript.)

Stage K, Figs. 34 and 35 (Packard, Fig. 25; Self, Figs. 16 and 17) is the last stage previous to the molt which results in the adult form. The abdomen is relatively much larger than before; the opercular lobes have nearly met in the median line, and the animal begins to burrow in the sand, although embryos of this stage are not infrequently taken in the towing net.

Stage L (Packard, Fig. 27) is produced from the last by a single molt. It is characterized by the presence of an elongate telson much like that of the adult. With this stage my studies end.

The following points may also be of interest. The Blastodermhaut is molted at about *Stage F*, the time varying with different eggs. It still persists as an embryonic envelope (vicarious chorion of Packard) until a late stage. Soon after it is shed from the parent cells a second embryonic cuticle is cast, and then the true chorion is shed, and the embryo, encased in the distended Blastodermhaut, escapes from the egg at about *Stage K* or *L*. The Blastodermhaut itself is ruptured, and the animal begins its free existence at the end of *Stage I*.

COMPARISONS.

A. With Previous Accounts. — H. L. Osborn ('85) and Brooks and Bruce ('85) have described some of the phases of segmentation, the latter studying sections. Their account so far as it goes is reconcilable with what I have described, including the pre-segmental movements. They have also noticed the primitive cumulus and interpret it as giving rise to the mesoderm, a point to be discussed later. Neither, however, traces the relationship of the cumulus to the embryo. According to the last quoted paper the blastoderm is to be regarded as ecto-mesoderm, the yolk as at least largely, if not wholly, entoderm.

Packard ('72) has apparently seen some of the phases of segmentation, but it is difficult to arrange his account in its proper order, as it is evident that some of his eggs were addled. In others he figures nuclei which had no actual existence. From segmentation until the appearance of the limbs Packard has seen nothing except the formation of the Blastodermhaut, which he in various papers has compared to the Hexapod amnion — a view which I ('84) showed to be untenable. H. L. Osborn's account of the formation of the limbs, etc., I have referred to above

(p. 50). Patten has incidentally described some of the early stages of *Limulus* ('90). Packard, Dohrn, Lockwood, *et al.* have described the later stages, and the foregoing brief *résumé* calls for no comparisons with their results. (See Postscript.)

B. With Other Arthropods.—Three types of segmentation of the egg may be recognized in the Arthropods.

In the first, examples of which are furnished by the lower Crustacea, *Lucifer*, (?) *Palæmon* (Bobretzky), *Phronima*, *Chelifer*, *Gammarus locusta* (Van Beneden and Bessels), Pycnogonids (Morgan), etc., the egg undergoes a regular or irregular total segmentation (holoblastic).

In the second the egg consists of a central nucleus and protoplasm with peripheral yolk. The central protoplasm segments, but until several or many blastomeres result, the yolk remains undivided. This is the type usually called centrolecithal, or endolecithal (Claus) and superficial. I have already pointed out with some detail ('86, pp. 112-138) that these terms are misleading, and would substitute *ectolecithal* therefor. 'Superficial segmentation' as usually described is characteristic only of late stages of ectolecithal or of meroblastic eggs. In these ectolecithal eggs two secondary modifications are noticeable. In the one the yolk is extracellular; it lies between the cells formed by the dividing protoplasm and nuclei, as in Phryganids (Patten, '85), Crangon (Kingsley, '86), and *Julus* (Heathcote, '86). In the other the yolk itself becomes divided, forming balls (true yolk cells), in the centre of each of which the nucleus and protoplasm occur (examples, most Hexapods).¹ Of these the second is structurally, if not phylogenetically, nearest to the meroblastic type.

In the third or meroblastic type the segmentation is, strictly speaking, superficial, and is at first confined to one side of the egg. Instances are less common among the Arthropods than of the other two, although several have been described; *e.g.* *Scorpio* (Metschnikoff, '71; Laurie, '90), *Mysis* (Van Beneden), *Oniscus*² (Bobretzky).

¹ Mereschowski ('82) has described what he regards as a fourth type, occurring in *Callianassa mediterranea*. It is plainly closely related to the second modification just mentioned.

² According to Reinhard's brief note ('87) it would appear as if in *Porcellio* the segmentation was of the ectolecithal type, and that the meroblastic conditions

Owing to my inability to find the segmentation nucleus, I am unable to say with certainty to which of the types the egg of *Limulus* should be referred, but all the facts point towards the second modification of the ectolecithal type. However, segmentation is at best an uncertain guide to affinities.

The matter of differentiation of the germ layers is more important. Until recently delamination was believed to be confined to the Cœlenterates and a few other forms.¹ It would appear, however, that delamination is of frequent occurrence in the Arachnid phylum. Morgan finds in the Pycnogonids ('90) a true multipolar delamination, and he uses this as one reason for assigning these forms to a position near the Arachnids. He refers to Chelifer as described by Metschnikoff and to Balfour's account of Agelena, and to these additional references may be given. Locy ('86, pp. 74-75) clearly confirms Balfour so far as Agelena is concerned; Henking ('86) describes a delaminate type of blastoderm formation in the Phalangids, while Faussek ('91), studying the same forms, is in full accord and expressly uses the term delamination in this connection. Schimkewitch ('84 and '87) also clearly describes delamination in Epeira, Pholcus, Agelena, and Lycosa.

On the other hand, the following forms have the yolk at one time free from nuclei, and hence, if delamination occur in connection with the primitive keel, it is not of that type which obtains in the cases mentioned above: Theridion (Morin, '87) at the 128-cell stage; a Japanese species of Agelena (Kishinouye, '90), Scorpion (Kowalewsky and Schulgin, '86; Laurie, '90).

So far as I know, nothing approaching delamination occurs in the Crustacea, while that in the Tracheates, already referred to, is of a character far different from that in the Arachnids. Hence *Limulus*, in the method of differentiation of entoderm from ecto-mesoderm, finds its closest analogues within the Arachnid phylum.

resulted from a migration of the blastomeres to one pole of the egg. Dr. McMurrich informs me that, according to his observations on both *Porcellio* and *Armadillidium*, the segmentation is as I have interpreted it in this note, — a fact which would tend to show that Bobretzky described a stage too late to decide the question.

¹ Balfour ('81), p. 278, compares the origin of the germ layers in most 'Tracheates' to a type which approaches delamination, but he expressly states that there are strong grounds for regarding it as "a secondary modification of an invaginate type."

There can be no question that delamination in these forms is not a direct derivative from delamination in the *Cœlenterates*. It has rather arisen in the *Arachnids* and probably from a true gastrulate type. The considerations which lead to this conclusion are these:—

It is at least probable that the *Arthropods* have had an annelidan ancestry, and in these latter forms delamination does not occur. Hence we must either regard it as having been lost in the segmented worms while it is retained in the *Arachnids*, or we must consider it as of cænogenetic character in the latter group. I believe that delamination, as it occurs in *Limulus* and the *Pycnogonids*, may be traced back to an ancestral invaginate condition; in fact, all stages between a regular embolic gastrula like that of *Lucifer* and the extreme delamination of the *Pycnogonids* can be found in the *Arthropod* phylum, although not in the *Arachnids* themselves.

The series between *Lucifer* (Brooks, '82) with an archenteric cavity of large size is easily traced through conditions like those of *Astacus* and *Palæmon*, to that presented by *Crangon*, where the invaginated entoderm is solid, but in which the blastopore is still readily recognized. *Crangon*, on the other hand, presents many similarities to *Theridion* (Morin, '87) and the Japanese species of *Agelena* studied by Kishenouye. In the forms just mentioned there is apparently¹ a time when every nucleus has reached the surface and has participated in the formation of the blastoderm, leaving the large central yolk in an anneliate condition. Later, the blastoderm thus formed becomes thickened by cell proliferation, and from the ridge thus formed cells pass "into the yolk and become scattered without definite arrangement through the entire yolk. These are the entoderm cells" (Kishenouye, p. 62; cf. Kingsley, '86, p. 110).

Now in forms like *Astacus*, *Palæmon*, and *Crangon* the mesoderm arises from the lips of the blastopore and from what may be regarded as its forward continuation in the median line, and from this fact we are justified in regarding the thickening which in the Japanese *Agelena* and in *Scorpio* (Laurie) gives rise to mesoderm and entoderm as an obsolescent blastopore homologous with the actual open blastopore in the other forms mentioned.

¹ Kishenouye could not "detect any nucleus at all in the yolk, thus confirming the views of Morin in opposition to Balfour's" ('90, p. 60).

The transition from the Japanese *Agelena* and *Scorpio* to a true delamination is greater than that already traced; and as yet, so far as the literature at hand enables me to decide, it cannot be traced without going outside the limited group of Arachnids. Still the successive stages are readily imagined.

In the ectolecithal egg the blastoderm arises by migration of the primitively central cells to the periphery, and in many forms every nucleus goes through this migration, leaving the yolk at one period entirely free from cells. In others only a portion of the cells reach the surface, the others remaining behind in the yolk. Concerning the fate of these latter, opinions differ. In some forms they are described as playing no part in the building up of the embryo, but rather acting as 'vitellophags,' the sole function of which is to gradually metabolize the deutoplasm, after which they disappear. On the other hand, instances are not wanting in which these yolk cells are to be regarded as true entoderm cells, from which later the epithelium of the mid-gut is to be built up. This is the case with *Limulus*, as I shall detail later, and apparently also in many *Araneina* and *Hexapods*.

With such conditions as are afforded by *Crangon*, *Theridion*, etc., it can readily be seen that any acceleration of development which would prevent certain of the central blastomeres from migrating to the surface, only to be immediately returned as entoderm, would be a distinct gain; and this, in my opinion, is the way the peculiar conditions in many *Hexapods* have been brought about. At least, this view has the merit of rendering intelligible many features of Arthropod ontogeny which otherwise are not readily understood.

A farther step in the same direction is afforded by *Limulus*, where a farther economy is seen in the cutting off of the peripheral from the deeper ends of the cells, thus at once differentiating an outer ecto-mesodermal layer from an inner entoderm rich in food yolk. The final stage, as we know it, is seen in *Tanystylum* and *Phoxichilidium* as described by Morgan ('90). Here the egg is much reduced in size, the blastomeres are fewer, and each cell is at once (apparently) differentiated into entodermal and ecto-mesodermal portions, the result being a condition which closely simulates the multipolar delamination found in *Geryonia*, made classic by the researches of Fol and

Metschnikoff, but of course without actual phyletic connection with it.

Our knowledge of mesoderm development in the Arthropods is far from complete, and at present it is not possible to point out the peculiarities which characterize the different groups. My account of mesoderm formation, as it occurs in *Limulus*, agrees well in its major features with the account of Patten ('90), except that he describes at the posterior end of the embryo a "slit-like" primitive streak, and he further regards the proliferated cells as both mesoderm and entoderm (p. 373). The lateral connection of mesoderm and ectoderm he compares with the Keimwall of the Vertebrates — a point upon which I would rather admit analogy than actual homology.

The accounts of mesoderm formation in *Scorpio* differ. Laurie ('90) describes the inpushing of a mes-entoderm from all parts of the upper (outer) surface of which the mesoderm is afterward proliferated. Patten ('90), on the other hand, describes a median posterior thickening from which cells grow forward and laterally, the median portion forming the sexual organs and botryoidal cord; the lateral, the mesoderm and entoderm.

In the Decapodous and Isopodous Crustacea the mesoderm would appear to grow forward as two bands from the anterior margin and sides of the blastopore. In some Cladocera and Copepods (Grobbsen, '79 and '81) somewhat similar conditions may be traced, except that the primitive mesoderm cells are *behind* the point of entodermal invagination. In *Cyclops*, on the other hand (Urbanowicz, '84), mesenchyme is described as budding from the blastoderm cells, and Ulianin ('81) describes the same in *Orchestia*.

In the Arachnids our knowledge of mesoderm formation is extremely scanty. All agree, so far as the Araneida are concerned, that the primitive cumulus and posterior cloud are concerned in the process, and some show that at first the mesoderm forms a continuous band across the embryo. A comparison of figures (*e.g.* Locy, '86, Fig. 49) of Arachnid embryos with my own of *Limulus* will, I think, show similarities which cannot be paralleled by similar resemblances between *Limulus* and the Crustacea.

In the differentiation of the germ the resemblances of *Limulus* to the Arachnids are striking. So far as I know primitive

cumulus and posterior cloud occur only in these forms; and the succeeding stages are almost equally close. As I correlative them, my figures of *Limulus* are to be compared with those of the true Arachnids as follows:—

LIMULUS.

ARACHNIDA.

- Fig. 21 Agelena, Locy, Fig. 1; Kishenouye, Fig. 5.
 Fig. 23 Agelena, Kishenouye, Fig. 5; Balfour, Fig. 1.
 Fig. 24 Agelena, Locy, Fig. 3; Scorpio, Metschnikoff, Pl. XVII, Fig. 2.
 Fig. 25 Scorpio, Metschnikoff, Pl. XVII, Fig. 3 (one less somite); Laurie, Fig. 17 (one more segment and lacks primitive groove).
 Fig. 26 Agelena, Schimkewitsch, Pl. XVIII, Fig. 1; Balfour, Fig. 3. Locy, Fig. 6; Scorpio, Metschnikoff, Pl. XVII, Fig. 6.

A slight comparison of these figures will show that previous to the appearance of the limbs there are a remarkable series of parallels. *Limulus* agrees with the Arachnids and differs from the Crustacea in the external appearance and growth of the germinal disc; in the considerable development of metamerism before the appearance of the appendages,¹ and in the simultaneous appearance of the anterior five or six pairs of appendages. When one of the six is lacking at first, it is apparently the anterior pair which forms later. This has been shown by Balfour, Schimkewitsch, and Kishenouye in *Agelena*; by Metschnikoff and Laurie in *Scorpio*, and by Dohrn and myself in the present paper. On the other hand, Claparède ('68) describes the sixth pair as lacking in *Myobia*, and Van Beneden ('51) gives the same account of *Atax*. *Limulus* agrees with the Arachnids and differs from the Crustacea in the total absence of a nauplius stage.

AUGUST, 1891.

 POSTSCRIPT.

Since the foregoing pages were in the printer's hands K. Kishenouye has published his complete paper on the development

¹ In *Chelifera* (Metschnikoff, '70), the chelicerae apparently are formed before the somites are outlined.

of the Japanese King Crab (*L. longispina*), which presents some points of difference from the *L. polyphemus* of the Atlantic coast. Some of these variations may be noticed here.

In the external development Kishenouye did not distinguish between primitive cunulus and posterior cloud. In the process of metamerism the first line of demarcation occurs between somites I and II, while the appearance which I have called the primitive streak does not occur until two somites are differentiated from the anterior and posterior areas. In the later stages he finds organs homologous with the flabellum of appendage VI, occurring as transitory rudiments on somites 2-5. These are clearly not homologous with the peculiar (sense?) organs mentioned on p. 49, since the latter occur outside the ventral disc, while the flabella of Kishenouye are all within that area.

In the internal development the discrepancies are more important. Thus Kishenouye describes the ectoderm as separating from lower-layer cells, and says that the mesoderm has three origins: (1) from the lower-layer cells, (2) from the edges of the primitive streak, which is confined to the posterior portion of the ventral disc, and (3) from cells in the dorsal region which migrate from the yolk. The primitive streak mesoderm is confined to the abdominal region, while that derived from the lower-layer cells gives rise to the tissues of the cephalothorax.

A still farther point of difference is with regard to the metastoma. This Kishenouye regards as a true appendage serially homologous with the other appendage of the body. In this I cannot agree with him. My observations show no metastomal somite and no corresponding neuromere.¹ On the other hand, it seems probable that there is here an error in interpretation, for a study of his figures inclines me to believe that his metastoma is in reality the operculum, and that the following appendages are to be correspondingly changed. The other points of difference will be discussed in the second part of this paper.

TUFTS COLLEGE, MASS., August, 1892.

¹ See Kingsley, '85, p. 532, Pl. XXXVIII, Fig. 22.

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EXPLANATION OF THE FIGURES.

REFERENCE LETTERS.

<i>ar.</i>	Artery.	<i>ov. e.</i>	Ovarian epithelium.
<i>bl.</i>	Blastema.	<i>pc.</i>	Primitive cumulus.
<i>bs.</i>	Blood sinus.	<i>pg.</i>	Polar globule?
<i>c.</i>	Cerebrum.	<i>pgv.</i>	Primitive groove.
<i>ec.</i>	Ectoderm.	<i>po.</i>	Primordial ovum.
<i>f.</i>	Flabellum.	<i>pr.</i>	Protoplasmic processes.
<i>g.</i>	Gill-bearing appendage.	<i>ss.</i>	Segmental structures (glands or sense organs?).
<i>I.</i>	Appendage I.	<i>x.</i>	Cell in process of delamination.
<i>l.</i>	Liver tubule.	<i>y.</i>	Yolk.
<i>me.</i>	Mesoderm.	<i>z.</i>	Junction of ectoderm and mesoderm at the margin of the germinal disc.
<i>mo.</i>	Mouth.		
<i>n.</i>	Neuromeres.		
<i>o.</i>	Ovum.		
<i>op.</i>	Operculum.		

DESCRIPTION OF PLATE V.

FIGS. 1, 2. Sections (longitudinal and transverse) through a portion of the liver and ovary of a *Limulus* four inches in length, showing the formation of the primordial ova and the intrusion of older ova between the ovarian epithelium and tunica propria.

FIG. 3. Section of an egg one hour after impregnation, showing a possible polar globule.

FIGS. 4, 5, 6. Surface views of eggs four hours after impregnation, showing the peculiar segmentation of the surface previous to true segmentation. Fig. 6 is a polar view of the egg shown in Fig. 4.

FIG. 7. Section through an egg of four hours, showing the peripheral columns, distinctly cut off in most regions from the central yolk.

FIG. 8. A portion of the egg in Fig. 7, more enlarged.

FIG. 9. Projection of an egg with eight nuclei.

FIGS. 10-14. Surface views of successive stages of surface division.

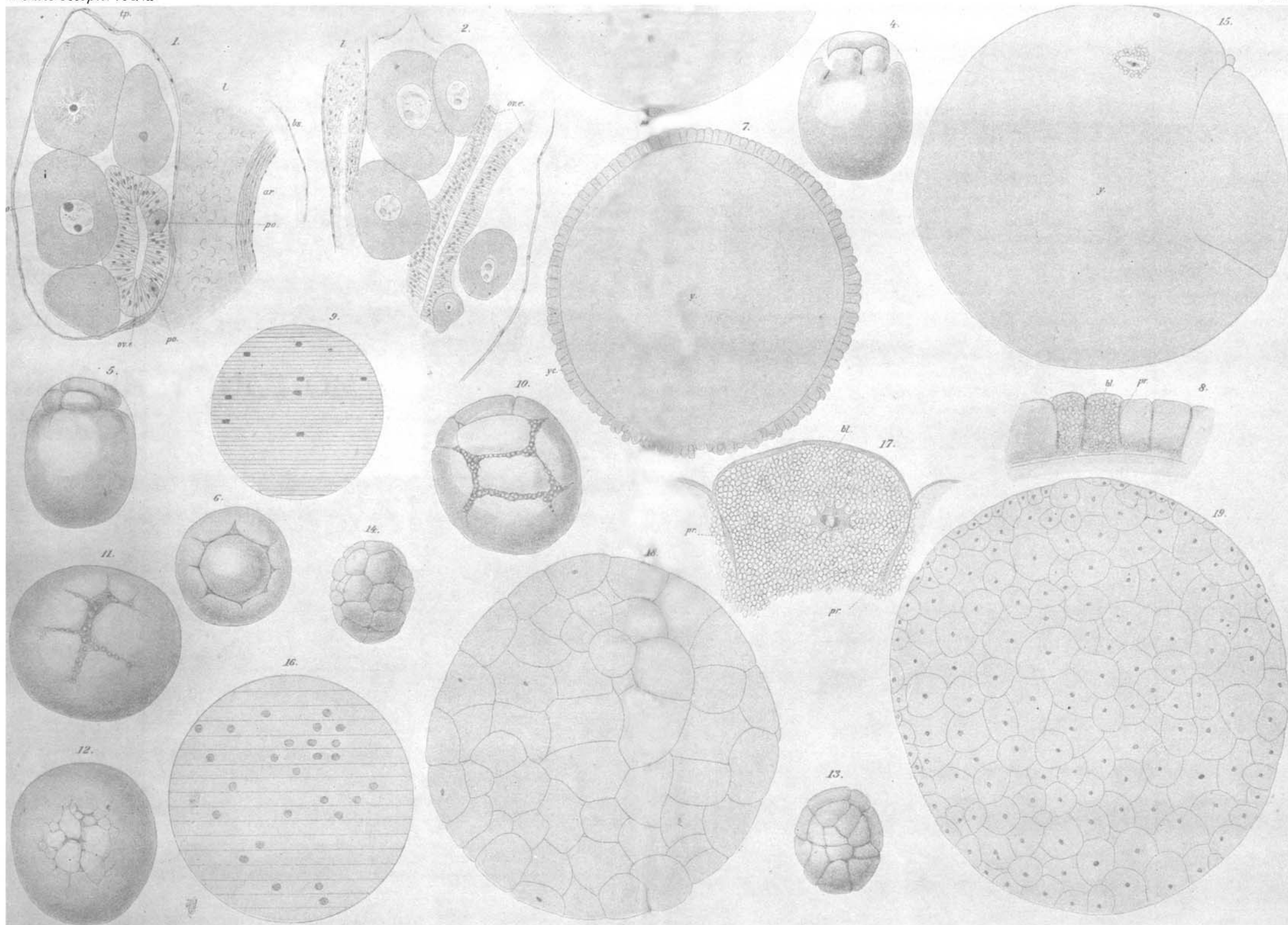
FIG. 15. Section of an egg in early segmentation showing cleavage planes at one pole of the egg.

FIG. 16. Projection of an egg with twenty-six nuclei.

FIG. 17. Enlarged view of a superficial cell in early segmentation showing the peripheral protoplasm (blastema) and protoplasmic processes extending down between the blastomeres.

FIG. 18. Egg at the close of early segmentation, before the differentiation of ecto-mesoderm.

FIG. 19. Section of an egg during the process of delamination.



DESCRIPTION OF PLATE VI.

FIG. 20. A part of Fig. 19 more enlarged, showing the process of delamination.

FIGS. 21-26. Successive stages of the germinal area previous to the formation of the appendages. See the text.

FIG. 27. Budding of the legs.

FIG. 28. An unusual form of embryo, appendage I. not yet formed.

FIG. 29. The germ viewed as a transparent object. Appendages I.-VI. present. The nervous system is covered by circularly arranged nuclei, the centres of rapid cell proliferation. Outside the germinal area are seen (*ss*) segmentally arranged structures of possibly glandular or sensory functions.

FIGS. 30, 31. Two surface views illustrating the transfer of the mouth backwards, accompanied by the formation of the stomodæum.

FIG. 32. Appearance of the embryo before the distinction of cephalothorax and abdomen is prominent.

FIG. 33. Side view of a late embryo, the abdomen differentiated.

FIGS. 34, 35. Dorsal and ventral views of the last larval stage before the appearance of the telson. R. Takano, del.

FIG. 36. Longitudinal section of a stage about like Fig. 21, showing the primitive cumulus and its central spot.

FIG. 37. Early stage of mesoderm formation.

FIG. 38. Late stage of same, showing primitive groove and lateral connection of mesoderm and ectoderm.

FIG. 39. More enlarged view of primitive groove.

