CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR.

No. 132.

THE EARLY DEVELOPMENT OF LEPAS. A STUDY OF CELL-LINEAGE AND GERM-LAYERS.

BY MAURICE A. BIGELOW.

WITH TWELVE PLATES.

FROM THE BULLETIN OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE, VOL. XL. NO. 2.

CAMBRIDGE, MASS., U. S. A.

JULY, 1902.

59.13.15 : 53.5

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR.

No. 132.

THE EARLY DEVELOPMENT OF LEPAS. A STUDY OF CELL-LINEAGE AND GERM-LAYERS.

By Maurice A. Bigelow.

With Twelve Plates.

From the Bulletin of the Museum of Comparative Zoölogy at Harvard College, Vol. XL. No. 2.

CAMBRIDGE, MASS., U. S. A.
July, 1902.

No. 2. — CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR. No. 132.

The Early Development of Lepas. A Study of Cell-Lineage and Germ-Layers.

By MAURICE A. BIGELOW.

TABLE OF CONTENTS.

I. Introduction.

In the inception of this work on the barnacles of the genus Lepas it was planned to make a careful investigation of the early development with reference to the origin and fate of the germ-layers. With this object in view the methods of workers on cell-lineage were adopted, because detailed studies seemed necessary in order to determine accurately the origin of the germ-layers. These studies were not undertaken with any expectation of extending or testing the accuracy of the generalizations which have come from the epoch-making investigations on cell-lineage in the eggs of annelids, mollusks, and other animals. Whatever opinion may be held regarding the fundamental importance of the generalizations growing out of such studies, it is usually conceded that the tracing of cell-lineage gives a basis for accurate description of the details of embryological development. Such accuracy in itself seems to furnish sufficient present justification for studies in cell-lineage, for no one can predict what interpretations may in the future grow out of any recorded facts of to-day.

A study of Lepas fascicularis was begun by me in June 1894. Late in that year there appeared an elaborate and important paper by T. T. Groom on the development of several Cirripedia. As stated in a preliminary note (Bigelow, '96), my independent studies of Lepas fascicularis partly confirmed Groom's results in the case of other species of this genus, but evidence in hand at the time of the publication of Groom's paper indicated that, so far as accurate description of cleavage and the formation of germ-layers is concerned, his account did not agree with the development as observed in L. fascicularis. The studies already begun by me were, therefore, continued and extended to Lepas anatifera and other species which Groom had described. The account given in this paper is based primarily upon studies of L. anatifera, and L. fascicularis.

I take this opportunity to express my great indebtedness to my former teacher, Prof. E. G. Conklin of the University of Pennsylvania, under whose guidance the general outlines of the work were developed.

The completion of the observational work was carried out during the year 1898–99 in the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College. To all the instructors of the department I am greatly indebted for stimulating interest, but especially do I owe acknowledgment to Dr. W. E. Castle, who continuously followed my work and gave me the benefit of his advice and criticism, and to

Prof. E. L. Mark, who has carefully examined and criticised all my results and given me many helpful suggestions during the arrangement of the results for publication.

During several summers the work has been carried on in the Marine Biological Laboratory and in the United States Fish Commission Station at Wood's Hole, Mass. I wish to express my appreciation of the assistance, in the line of facilities for work, which was extended to me by the officials of these two laboratories, particularly by their respective directors, Prof. C. O. Whitman and Prof. H. C. Bumpus.

II. Historical.

The history of the development of our knowledge of the Cirripedia has been so often written that for the purpose of this paper it is sufficient to give a mere outline. The now classical monograph of Darwin ('51, '54) reviewed so exhaustively the knowledge obtained by earlier observers, and added such a mass of original information on structure, metamorphosis, relationships, and natural history, that in these respects the Cirripedia have since ranked among well known groups of invertebrate animals. Since Darwin's time much of the investigation on the animals of the group has been concerned with embryological development, to which very little of Darwin's work was devoted. In the "Challenger" Reports Hoek ('83, '84) made important additions to our knowledge of the anatomy and relationships of many cirripedes, and gave a good historical sketch of the group. Gerstäcker's historical review in Bronn's Klassen u. Ordnungen is exhaustive.

The papers of Van Beneden ('70), Willemoes-Suhm ('76), Hoek ('76), Lang ('78), Nassonow ('85, '87), Nussbaum ('90), and Groom ('94) deal in more or less detail with embryonic development, and these papers include the most important existing contributions to our knowledge of cirripede embryology. Müller ('64), Filippi ('65), Münter und Buchholz ('69) and Bovallius ('75) have made contributions regarding certain points in the early development.

Our knowledge of the early development of species of Balanus is due principally to the studies of Münter und Buchholz ('69), Hoek ('76), Lang ('78), Nassonow ('85, '87), and Groom ('94).

The early development of species of Lepas is known through the investigations of Willemoes-Suhm ('76), Groom ('94), and Bigelow ('96).

The only recorded observations on the early development of Lepas fascicularis earlier than those of the present writer are the published

notes of Willemoes-Suhm ('76), who died during the voyage of the "Challenger" before his studies were completed. His paper gives a very complete account of the history of the above mentioned cirripede from the Nauplius to the sessile adult, but only a short and fragmentary description of embryonic development. In some of the later embryonic stages the observations are quite correct, but the few descriptions and figures of cleavage stages are very inaccurate.

The embryology of Pollicipes has been studied by Nussbaum ('90), but his account is somewhat fragmentary.

Among the Rhizocephalan Cirripedia the only description of a complete series of embryonic stages is Van Beneden's ('70) account of Sacculina.

Further, one or more of the investigators already mentioned has studied the early development of species of the following genera of Cirripedia: — Conchoderma, Scalpellum, Tetraclita, Dichelaspis, Chthamalus. However, much of this embryological work has been fragmentary, and often superficial.

The last, and by far the most important, paper on the early embryology of the Cirripedia was published by Groom in 1894. This contains a good résumé of the previous work on the subject, reviewing the contributions of the various investigators mentioned in the preceding paragraphs. Groom studied the embryology of five species, namely, Balanus perforatus, Lepas anatifera, L. pectinata, Chthamalus stellatus, and Conchoderma virgata. His observations on the later stages of embryonic development and on the larval stages were exhaustive. The study of the cleavage was undertaken secondarily, and was not investigated as accurately as were the later stages.

The accounts of the early embryology of cirripides which were given by observers before Groom do not as a rule contain records of detailed observation, which alone could be used comparatively in a paper from the standpoint of cell-lineage. Groom reviewed well the general accounts of previous investigators, and brought their results into line with his own observations. In reviewing the literature I must necessarily deal primarily with Groom's account, because he is the only investigator who has attempted detailed description of the early stages of cirripede development.

III. Materials and Methods.

The material upon which this paper is based was collected at Wood's Hole, Mass., in the summers of 1894, 1895, 1898, and 1899. Prof. Harold Heath of Stanford University, Cal., has collected and preserved

for me the eggs of Lepas hillii, Pollicipes polymerus and Sacculina, which have been used for comparative study.

In Vineyard Sound and Buzzard's Bay, groups of Lepas fascicularis, L. anatifera and L. pectinata have been found at various times between June and September. Any of these forms may appear at times when the prolonged south-east winds have carried the drifting material of the Gulf Stream in the direction of the Elizabeth Islands. So many elements of chance are involved in getting the animals that it has been found difficult to collect complete developmental series, and the work has been often delayed.

A very large majority of the animals of all species carry eggs in advanced stages of development when they arrive in the waters near Wood's Hole. This has been found especially true of the numerous specimens of L. fascicularis, hundreds of which have been found carrying eggs ready to hatch, but only a few dozen with eggs in early cleavage stages. In two different summers a few animals of this species have been found early in June with eggs in stages of maturation, but when large numbers of animals arrived in July, few cleavage stages could be found and in many cases Nauplii were escaping from the brood-lamellæ.

Much drifting timber carrying L. anatifera was obtained about the middle of August, 1898. The adult animals all carried eggs which were in advanced stages of development and were hatching rapidly. Many animals which were about half the adult size were laying eggs. The timbers were anchored in the harbor, and for several weeks it was possible to obtain an abundance of material in maturation and cleavage stages. The stages of living and preserved material thus secured for study represented the important phases of every mitotic division in the early development.

As is well known, the development from egg to Nauplius takes place in the mantle chamber. The eggs, each enclosed in a vitelline membrane, lie in the cavities of the egg-plates, or ovigerous lamellæ, which lie between the body and the mantle. In studying living ova it is easy to tear the lamellæ and thus free large numbers of eggs, but in preserving material it is more convenient to fix the lamellæ in large pieces.

Maturation and cleavage were studied first in the living eggs. It was found impossible to keep eggs developing normally under artificial conditions outside the mantle cavity longer than from five to ten hours. Other workers on Cirripedia have had the same experience. It was rarely possible to follow a single egg through the maturation phases to the close of the second cleavage, and fresh material, which had under-

gone the early cleavage while in the brood-lamellæ, was necessarily used for the study of later cleavages.

Many of the fixing reagents ordinarily employed in embryological work have been tried, but only solutions containing picric acid have proven entirely satisfactory. Kleinenberg's stronger fluid and a saturated solution of picric acid in 35% alcohol both gave excellent fixation, but a saturated solution of picric acid in 5% acetic acid gave results which were far superior to those obtained by any other fixing solution. This fluid penetrated rapidly, and eggs thus prepared were very transparent when stained and mounted entire. This transparency was a very important feature in the study of all cleavage stages. The picro-acetic mixture also gave the best results for material which was to be sectioned. It should be remarked that solutions with less acetic acid lack penetrating power.

Strong solutions of mercuric chloride in distilled water, in sea water, in alcohol, or combined with picric acid, gave some good results in the study of maturation and early cleavage stages by means of sections, but material thus fixed proved too opaque for preparations of entire eggs. Material fixed in the mercuric chloride solutions was especially valuable in determining the distribution of the yolk, which readily stained differentially after such fixation. In the study of all stages of development use was made both of sections and of entire eggs viewed as transparent objects. The method of preparing the latter will be described first. Small pieces of egg-lamellæ which had been fixed in the picro-acetic mixture were stained from one to three hours in a concentrated solution of borax-carmine in 35% alcohol (Grenacher's formula). They were then washed in alcohol and rapidly decolorized in 70% alcohol containing 0.3% hydrochloric acid. The decolorizing was watched with a compound microscope, and quickly checked when nuclei and cell-boundaries began to appear. The piece of egg-lamella was then dehydrated and, within two or three hours after staining, cleared.

All the ordinary clearing oils were tried, but no other one gave results comparable in excellence with those obtained by the use of clove oil. This oil renders the egg-lamellæ brittle, so that the eggs can easily be isolated by the use of needles. In practice the stained pieces of egg-lamellæ were placed in a drop of clove oil on a glass slide. Then, using a dissecting microscope, the lamellæ were cut with fine needles and the eggs set free, but they were still surrounded by the vitelline membrane. All attempts at removing this membrane proved unsuccessful. After the greater part of the clove oil had been drained away, the eggs were mounted in xylol-balsam.

Eggs prepared by the above method were so transparent that even in later stages the outlines of cells on either side of the embryo could be clearly seen by appropriate focussing. It was, therefore, easy to study and draw optical sections in any plane. The refractive index of clove oil[1] is such that the vitelline membrane becomes almost invisible.

By carefully moving the cover glass it is possible to roll eggs into any desired position, and for this purpose the balsam was for months kept semi-fluid by occasionally applying a drop of xylol to the edge of the cover glass.

It was found practicable, and in some cases profitable, after studying an egg in balsam, to remove the cover glass, dissolve the surrounding balsam with xylol, lift the egg by means of a capillary tube, transfer it to paraffine, imbed by the watch-glass method and section it. When imbedded near the surface of the block of paraffine, the long axis of the egg can be distinguished by the use of a lens, and hence sections can be cut longitudinally or transversely as desired. This method of sectioning single eggs was employed only for the purpose of gaining an idea of the appearance of sections of particular stages in known planes. As a rule, pieces of the egg-lamellæ rather than single eggs were imbedded and sectioned, the sections being stained on the slide. Since the eggs have no definite arrangement in the lamellæ, sections in all planes were thus obtained. By comparison with sections of single ova in which the orientation had been definitely established, it was possible to choose with certainty the sections representing any desired plane in any stage of development.

For staining sections on the slide Delafield's hæmatoxylin diluted with four or five times its volume of distilled water gave the best results. In the later cleavage stages and in embryonic stages orange G or eosin were used after the hæmatoxylin. By this means the entoblastic yolk-cells were sharply differentiated.

In the study of preparations of the entire eggs a sub-stage condenser with iris diaphragm was absolutely necessary. A $\frac{1}{12}$ inch homogeneous immersion objective with long working distance was of great service.

Most of the preparations upon which this paper is based are yet in good condition, and are therefore available as evidence in support of the following account of the development of Lepas.

[1] Since this paper was written I have found that oil of cassia for clearing gives results even superior to those obtained by the use of clove oil. It has also proved to be an excellent mounting medium, but probably the preparations will not retain stains permanently.

The methods employed have been given at length, because it is believed that the results obtained, which differ widely from those of earlier workers, are due largely to the successful making of transparent preparations of entire eggs. In examining the figures given by previous workers it is evident that none of them had the advantage of such preparations, and consequently none of them were able to follow accurately the history of the nuclei, which is very important for the determination of cell-lineage.

IV. Maturation and Fertilization. The Unsegmented Ovum.

In agreement with the observations of Weismann und Ischikawa ('88), eggs taken from the oviducts were found to contain the first maturation spindle. Owing to mutual pressure, there is great distortion of the eggs in the oviducts, but when artificially liberated into sea water they quickly assume a spherical form. The separation of the first polar cell takes place at about the time when the eggs leave the oviducts. Soon after this the formation of the vitelline membrane begins, so that it occupies a position between the first polar cell and the egg (Plate **11**, Fig. 95, *mb.vt.*). This is followed by the development of a second polar cell (Plate **2**, Fig. 17), which lies within the vitelline membrane (Plate **11**, Fig. 95, *cl.pol.*^{2}). From the time of assuming the spherical shape, soon after leaving the oviduct, the eggs retain this form, except when pressure of surrounding eggs in the egg-lamellæ distorts them. The egg represented in Figure 17 is an example of the influence of pressure in the egg-lamellæ; such a form at this stage has not been seen among eggs kept isolated in watch glasses. It should be noted here that the uniform distribution of yolk serves to distinguish such eggs, which are pressed into an elongated shape, from later stages in which the eggs are normally ellipsoidal even when isolated, but in which the yolk is collected at the vegetative pole.

Eggs which are isolated soon after oviposition retain the spherical condition and the uniform distribution of the yolk until about the time when the second polar cell is formed. Then the egg begins to elongate in the direction of the chief axis, and the protoplasmic materials begin to concentrate at the animal pole, where the polar cells are located; at the same time the yolk is removed to the lower half of the egg, being concentrated around the vegetative pole. This movement of protoplasm and yolk, towards animal and vegetative poles respectively, continues

and finally results in a telolecithal arrangement of the materials of the egg.

Eggs taken from the egg-lamellæ at all phases of the maturation have been carefully compared with the corresponding stages of isolated eggs which were kept in watch glasses. The distortions in form produced by pressure apparently do not disturb the normal course of cytological changes in the egg.

Figures 1–6 represent a series of camera sketches made from a living egg at intervals within a period of three hours. In Figure 1 the egg is represented just at the completion of the separation of the second polar cell. The egg is approximately spherical and closely surrounded by the vitelline membrane (*mb.vt.*). The yolk with its oil globules is in general uniformly distributed, but already some of the globules have been seen to move towards the vegetative pole. Figure 2 shows the well-marked beginning of elongation; the yolk is collecting at the vegetative pole and a mass of protoplasm, concentrating into the animal half of the egg, is dark and granular. Figure 3 represents a stage some minutes later. A circular depression has appeared around the egg at the equator constricting the egg into nearly equal lobes. The upper, protoplasmic lobe is dark and granular, especially near its centre, whereas the lower or yolk-lobe is relatively clear and transparent, as represented in Figure 18 (Plate **2**). The constriction now moves toward the vegetative pole of the egg, where the yolk is collecting (Fig. 4). Gradually the constricting furrow disappears (Fig. 5), and the egg becomes ellipsoidal, as shown in Figure 6. At the animal pole the egg continues to be bluntly rounded, while at the vegetative pole it becomes more pointed. The vitelline membrane, having taken on this shape, retains it throughout the development, and appears to be quite rigid from this stage onward. At the close of the elongation the upper, animal portion of the egg is largely composed of dark granular protoplasm containing some small granules of yolk, but no oil globules (Plate **2**, Figs. 19, 20). The lower vegetative part of the egg is more transparent and contains the mass of yolk granules. The oil globules are concentrated at the pointed end of the egg and for a time are arranged in strict radial symmetry with respect to the long (chief) axis of the egg. Protoplasmic strands extend throughout the vegetative half of the egg.

The elongation of the egg and the separation of yolk and protoplasm, which result in the telolecithal condition and the establishment of visible polarity, are entirely distinct from the first cleavage processes, with which Groom ('**94**) has confused them (see review of the literature on first

cleavage). They belong more properly to the maturation phases, and have many characteristics known for ova of other groups of animals. The polar axis thus established in the cirripede ovum has the same relation to polar cells, maturation spindles, and first segmentation spindle, as is found ordinarily in telolecithal ova.

The phenomena occurring during the elongation and distribution of the materials of the cirripede egg, especially the formation of a constriction which marks off a yolk-lobe at the vegetative pole, are apparently similar to conditions which obtain in some molluscan eggs; for example, in the gasteropods Nassa (Bobretzky, '76) and Ilyanassa (Crampton, '96). In these cases the formation of the yolk-lobe closely resembles that process in Lepas, but its later history is widely different. At one stage of the maturation, the eggs of Nassa and Ilyanassa have a form similar to that of the egg of Lepas as represented in Figure 3, a constriction marking off a yolk-lobe. Whereas in the cirripede the constriction disappears before the first cleavage, in the gasteropods the first cleavage plane forms so that in the unequal division a smaller cell (*ab*) is separated from a larger one (*cd*), which still retains the yolk-lobe. After cleavage the yolk-lobe gradually disappears and the cell *cd* becomes spheroidal in form. In Lepas, as in Nassa and Ilyanassa, the materials composing the yolk-lobe are after the first cleavage contained in the cell *cd*.

In my attempts to determine the precise time of penetration of the spermatozoön I have failed, as have all earlier investigators; but we may infer that it enters before the formation of the vitelline membrane, probably about the time when the first polar cell is separated. In sections similar to that represented in Plate 2, Figure 17 (formation of second polar cell) I have noted a darkly staining body near the vegetative pole of the egg. I am not certain of having identified the male pronucleus in a stage earlier than one corresponding in external form to Figures 3 and 18, in which, however, the pronuclei were widely separated, as shown in Figure 19. A further comparison of Figures 18 and 19 shows that there is not a constant relation between the relative positions of the pronuclei and the telolecithal distribution of the yolk and protoplasm. In external outline and in the presence of the constriction marking off the yolk-lobe, the egg represented in Figure 18, corresponding to Figure 3, is earlier than that shown in Figure 19, which corresponds to Figure 6. But in Figure 18 the size and contact of the pronuclei indicate an older stage than that of Figure 19.

After the disappearance of the yolk-lobe the pronuclei are usually

found in contact, as shown in Plate 2, Figure 20, which suggests that there is retardation in the approach of the pronuclei in cases similar to Figure 19. All my observations point to the conclusion that the pronuclei usually come into contact during the time when the yolk-lobe is disappearing, and the egg is assuming the ellipsoidal form, that is, in stages corresponding to Figures 4–6.

Review of Literature on Maturation and Fertilization.

A general review of the literature on these phases of cirripede development is given by Groom ('94), consequently reference will not be made in this connection to writings unless they have direct bearing upon observations recorded in this paper.

The formation of polar bodies and vitelline membrane have been observed and described by Weismann und Ischikawa ('88), Nussbaum ('89), Solger ('90), Groom ('94), and others. My observations on the formation of these structures are merely confirmatory of these earlier writers, and have been recorded simply to complete my account of associated phenomena.

The contractions of the egg during elongation and the segregation of protoplasm and yolk have been observed by Groom and others; but the process has, apparently, not been followed continuously, and has been confused with the first cleavage, as will be shown in the review of literature bearing on that stage.

Groom ('94, p. 133) states that in the unfertilized ovum of Lepas anatifera no difference can be distinguished between the two poles, and suggests that the ovum may become oriented only upon fertilization. Opposed to such conclusion is the fact that in eggs taken from the oviducts the first maturation spindle marks the chief axis of the egg, which thus seems to be determined long before fertilization. Nussbaum ('90) correctly observed that the axes of the embryo are established with the formation of the polar bodies.

Groom ('94, p. 136) states that "the axis of the spindle of the segmentation-nucleus is not at right angles to that of the second directive spindle." In the account of the first cleavage it will be shown that, in opposition to this view, the first cleavage spindle is formed in a plane perpendicular to the chief axis of the egg, with which the second maturation spindle coincides at the moment when the polar cell is separated. There is, therefore, in Lepas complete agreement with the usual condition in the eggs of other animals.

With regard to the male pronucleus Groom ('94, p. 134) states: "Sections made of ova of Lepas anatifera before or shortly after the formation of the first polar body show the first directive spindle or a small round nucleus with several chromatin elements." Having failed to find the male pronucleus, he concluded that it "must be exceedingly small and easily overlooked, otherwise it would be necessary to conclude that the fusion of the two pronuclei takes place immediately after the first polar body is formed (in which case it would be very rarely detected in ova which had given off the first polar body); but this seems improbable, though traces of a male pronucleus were never found in sections at any later phase even in ova where the second polar body was being or had just been given off."

Some of these observations by Groom are in accord with my statement that the male pronucleus has not been certainly identified in sections corresponding to a stage earlier than that represented in my Figure 3, although the spermatozoön is probably present at a stage earlier than that represented in Figure 1, in which the second polar cell has just been separated. Groom's supposition that the pronuclei fuse soon after the formation of the first polar cell is opposed by the evidence afforded by my Figures 17–21. It will be shown later that Groom probably saw the male pronucleus in these later stages, but misinterpreted it as one of the daughter nuclei resulting from the first division of the egg.

Groom says (p. 135), "The nucleus, which, during the period at which the ovum was undergoing contraction [yolk-lobe stages], was small and situated peripherally and anteriorly [at animal pole], and was invisible without special preparation, now becomes larger, and appears as a definite clear spot." He further states (p. 137) that, "the clear spot appearing with the separation of the protoplasm is almost certainly the segmentation-nucleus." I have seen this "clear spot," and sections show that it is the female pronucleus, or sometimes the two pronuclei so approximated that viewed through the opaque substance of the living egg the appearance is that of one transparent area. Groom's statements regarding these stages were apparently based upon studies of living eggs, which are so opaque as to render observation difficult and uncertain.

In a stage which Groom interpreted as that of the first cleavage, he found "two nuclei in the newly-formed [first] blastomere"; these were regarded as the daughter nuclei of the first segmentation nucleus (pp. 137, 142, 145). In the review of literature on first cleavage it will be pointed out that Groom apparently has mistaken for the first segmentation of the ovum a maturation phase, such as that represented in my

Figures 3 and 18; the two nuclei which he describes being evidently the pronuclei and not daughter nuclei sprung from the first segmentation nucleus. The figures in the present paper show that a segmentation nucleus does not exist during the separation of yolk and protoplasm. Two pronuclei are in the egg, but they do not appear to fuse completely until the nuclear membranes fade away at the beginning of division. My figures of the first cleavage show, as opposed to Groom's description, that the nuclei resulting from the first division are not at first both located in the upper half of the egg, where the protoplasm is more concentrated.

Nussbaum ('90) observed the two nuclei in Pollicipes as the waves of constriction passed over the egg during the separation of yolk and protoplasm, and interpreted them as pronuclei. He figured and described the pronuclei as approaching along a line nearly coinciding with the long axis of the egg; and he assumed that the plane of the first cleavage is perpendicular to the contact surface of the pronuclei. My Figures 18–20 confirm his observations on Pollicipes, for it is certain that there are two pronuclei in the protoplasmic mass at the animal pole of the egg in L. anatifera and L. fascicularis as the separation of yolk and protoplasm progresses. I have studied sections of Pollicipes which show similar conditions. Nussbaum's interpretation of these nuclei as pronuclei is certainly correct, as is likewise his description of their approach and contact.

V. General Sketch of Cleavage and Germ-Layers.

The cleavage of Lepas is total, unequal, and regular. Stages of 2, 4, 8, 16, 32 and 62 cells are normally formed. Cells of a given generation may anticipate their sister cells in division, but no second division of such cells takes place before all other cells have completed corresponding cleavages and reached the same generation.

The first cleavage plane is nearly parallel to the long axis of the ellipsoidal egg, which divides into a small anterior cell (micromere) and a large posterior yolk-bearing cell (macromere). The plane of the second cleavage is perpendicular to that of the first, a second micromere being cut off from the yolk-bearing macromere, while the first micromere divides into two of equal size. The plane of the third cleavage is essentially perpendicular to both the preceding ones. A third micromere is separated at this cleavage from the yolk-macromere, which is now purely mes-entoblastic. Thus by the first, second, and third cleavages three

micromeres are separated from the yolk-bearing macromere. These three cells contain all the ectoblast, and by their repeated division form the blastoderm. Certain cells of the blastoderm, which are derived from the first two micromeres, give rise to a portion of the mesoblast, hence these two micromeres are not purely ectoblastic. The third contains only ectoblast. In the fourth cleavage a mesoblast cell is separated from the yolk-macromere, which now represents entoblast alone.

The sixteen-cell stage, therefore, is composed of fourteen derivatives of the three micromeres, one mesoblast cell, and one entoblast cell (yolk-macromere). The entoblastic yolk-macromere is nearly enveloped by the fourteen smaller cells composing the blastoderm, only a small part of the entoblast cell being exposed at the blastopore. The single mesoblast cell lies at the posterior edge of the blastopore, and were its history not known would certainly be regarded as a cell of the blastoderm. At the fifth cleavage each of the sixteen cells divides, the two resulting mesoblastic cells still remaining at the surface. At the sixth cleavage all the cells except the two entoblast cells divide, thus producing a sixty-two-cell stage. During the sixth cleavage the two mesoblastic cells, before dividing, sink beneath the blastoderm, as this closes over the entoblast and obliterates the blastopore. At the same time four cells of the blastoderm, lying at the anterior and lateral edges of the blastopore, divide parallel to the surface. The four deep cells thus formed beneath the blastoderm constitute a part of the mesoblast. The mesoblast, then, is derived in part from one cell which is separated from the entoblast in the fourth cleavage (sixteen-cell stage) and in part from four other cells which are detached from the blastoderm during the sixth cleavage.

Gastrulation is of the epibolic type, and is the result of the extension of the blastoderm over the entoblastic yolk-macromere. During the sixth cleavage, which leads to the formation of a sixty-two-cell stage, the blastoderm usually closes over the blastopore, which marks the ventral and posterior part of the future embryo.

In the general features of the late development of the embryo the results of this investigation confirm those of some earlier workers.

VI. Nomenclature of Cleavage.

For convenience in describing the cell-lineage of Lepas and in making comparisons with the development of other forms, it is desirable that some system of cell-nomenclature should be applied.

The common systems, which have been developed with special refer-

ence to the conditions in the developing eggs of annelids and mollusks, are dominated by the conception of cells cleaving in sets of fours or quartets. The system of Blochmann ('81) and its successors have, with few exceptions, been applied to eggs in which a quartet of macromeres (in a morphological sense) is formed by the first two cleavages, and by later cleavages these give rise to successive quartets of micromeres. In all the annelids and mollusks in which the cell-lineage has been determined with certainty, the cells of the four quadrants (*a*, *b*, *c*, *d*) formed by the first two cleavages are equivalent, in that each cell contains a portion of the two primary germ-layers, ectoblast and entoblast. The mesoblast is not so distributed with reference to the quadrants. It will be shown in this paper that the four-cell stage of Lepas is not a quartet of equivalent cells so far as the two primary germ-layers are concerned. Whereas in the annelidan and molluskan eggs each cell of the four-cell stage contains both ectoblast and entoblast, in Lepas three of these cells (*a*, *b*, *c*) contain ectoblast but no entoblast; and the fourth cell (*d*) contains both ectoblast and *all* the entoblast. In the annelids and mollusks the cells of the first quartet of micromeres (eight-cell stage) contain the ectoblast which is first separated from the entoblastic macromeres; but in Lepas one of the cells of the two-cell stage is the first ectoblast to be separated from entoblast.

Enough has been said, in anticipation of the account of the cleavage, to make it evident that the well-known quartet systems of nomenclature would not have their usual significance as indexes of homologies, if applied to the cleavage of Lepas, for the cells of the four-cell stage in annelids and mollusks are apparently not comparable with the cells of the same stage of Lepas, which would be given the same designations. However, a quartet system has been employed for the purposes of this paper, for the reason that it is convenient and familiar. The above statements will show that the system has not been used here with a view to indicating by it homologies with which it has become associated in its application to the spiral cleavage of annelids and mollusks. As far as regards the cirripede egg, the known facts do not seem to me to warrant the interpretation that cleavage occurs in cells grouped as quartets in the sense in which the term is applied to spiral cleavage; and while the notation of a quartet system has been adapted to the purposes of this paper, the term "quartet" has not been applied in description as designating groups of cells in the cleaving egg of Lepas.[1]

[1] See Addendum by E. L. M. and W. E. C. (p. 136) following the General Summary.

The system devised by Kofoid ('94) — which Castle applied to the bilateral cleavage of tunicates, where the conditions of cleavage resemble those of Lepas — has with some necessary modifications been followed. The cells of the four-cell stage are designated *a*, *b*, *c* and *d* in the usual order, *a* being the left anterior cell. An exponent indicates the number of the generation, starting with the ovum as the first, e. g. a^3, b^3, etc. A second exponent is used to distinguish a cell from other cells of the same generation and derivation, e. g. $a^{4.1}$, $a^{4.2}$, $a^{4.3}$, etc. In assigning the second exponent I have followed in part suggestions made by Kofoid ('94) and put into practice by Castle ('96). In cases of equatorial division the *odd* numbers have been applied to the cells nearer the *vegetative* pole, and the even to those nearer the animal pole. Thus of the cells in the four-cell stage a^3 divides, forming $a^{4.1}$ which is nearer the vegetative, and $a^{4.2}$ which is nearer the animal pole, while its sister cell, b^3, forms $b^{4.1}$ and $b^{4.2}$ (see Plate 4, Figs. 34–38). In later stages, where cells do not divide equatorially, but parallel to the sagittal plane, the *odd* exponent has been applied to the cell lying *nearer* that plane. In cases where a cell lies in the sagittal plane and undergoes division in the same plane, the daughter cell on the *right* side of that plane is designated by the *odd* exponent. Whenever cells divide transversely to the chief axis of the embryo, the *anterior* cell is designated by the *odd* exponent.

In determining the designation of cells, the rules given by Kofoid are here applied to Lepas. The designation of any derivative of cells *a*, *b*, *c*, *d* being given, the designation of mother cell or daughter cells can be quickly determined. The first exponent indicating the generation of the mother cell will, of course, be one less than that of the daughter cell. The second exponent of the mother cell will be one-half of that of the daughter cell, if that be an even number, and one-half the sum of the second exponent plus one, if that be an odd number. Thus $a^{4.1}$ and $a^{4.2}$ are daughter cells of $a^{3.1}$. Likewise, to determine the first exponent of the daughter cells, add one to the first exponent of the mother cell; to determine the second exponent, multiply the second exponent of the mother cell by two and the product is the designation to be applied to the cell bearing the even number as exponent, while that product less one designates the sister cell. Thus $a^{5.6}$ dividing forms $a^{6.12}$ and $a^{6.11}$.

A summary of the important points in the cell-lineage of Lepas is given in a table in connection with the general summary.

VII. Cleavage.

1. Introductory.

The following description of the cleavage of the egg of Lepas applies particularly to L. anatifera, of which I obtained abundant material of all stages in 1898, being thus able to study the early development in considerable detail. An extensive series of the eggs of L. fascicularis was later obtained and its development has been carefully compared with that of L. anatifera. There is such close parallelism in the development of the two species that the following account will apply in all important respects to L. fascicularis as well as to L. anatifera. Figures 95–126 (Plates **11**, **12**) of L. fascicularis when compared with those of L. anatifera show how close is the similarity between the two species. At the close of this chapter (p. 117) there are some notes on the early development of L. fascicularis which supplement and correct a preliminary account of this species published by me in 1896.

The principal stages in the development of L. pectinata and L. hillii have also been examined, but their development does not appear to differ in any important respects from that of L. anatifera and L. fascicularis.

2. First Cleavage. Two Cells.

The first cleavage of the egg of all Lepadidæ and Balanidæ whose development has been heretofore described results in the formation of two unlike cells. The smaller cell, rich in protoplasm, is situated at the rounded end of the vitelline membrane; the other, laden with yolk, at its pointed end (Plate **1**, Fig. 16). In previous accounts the first cleavage plane has usually been described as being formed perpendicularly to the long axis (chief axis) of the egg. The first cleavage plane has, accordingly, been characterized as equatorial, and the long axis of the two-cell stage has been regarded as identical with the long axis (chief axis) of the unsegmented egg.

In the following account[1] it will be shown that the first cleavage furrow appears approximately in the long axis (chief axis) of the egg; and that, therefore, the first cleavage is meridional, not equatorial as was hitherto supposed. It will be shown, further, that the position of the cleavage plane in the two-cell stage is due to a rotation of the dividing

[1] Some notes on the first cleavage of L anatifera have already been published (Bigelow, '**99**).

egg as a whole through an arc of 90° within the vitelline membrane. The long axis of the two-cell stage is, therefore, at right angles to the chief axis, which has rotated 90° from its original position of coincidence with the long axis of the vitelline membrane. The chief axis, which is the longer axis of the unsegmented egg, becomes the shorter axis of the two-cell stage. An examination of Figures 1–16, which represent a series of camera lucida drawings made at intervals during cleavage, will make clear the changes in form and position which the egg of Lepas undergoes in the course of the first cleavage.

In a preceding chapter it has been shown that, after the formation of the second polar cell and at about the time of the union of the pronuclei, the yolk becomes partially separated from the protoplasm and becomes aggregated at the vegetative pole of the egg (Figs. 2–6, 18–20). Shortly afterwards it is shifted to one side of the polar area (Figs. 7, 8); this is the first indication that the egg is rapidly approaching cleavage. Soon a wide shallow groove appears, passing obliquely around the ovum from the animal pole (Fig. 8). The furrow rapidly deepens and the forming cells become spheroidal, causing the ovum to elongate perpendicularly to the plane of cleavage (Figs. 9, 10). The ovum *as a whole* at the same time gradually rotates within the vitelline membrane (Figs. 10–15); consequently the plane of cleavage rotates until, at the completion of cleavage, the furrow is usually transverse to the long axis of the vitelline membrane, still unchanged in form; that is, the cleavage furrow occupies a plane almost at right angles to that in which it at first appeared relative to the vitelline membrane (compare Figs. 8 and 15). These facts explain the conflict between the conclusions of earlier observers and the generally accepted idea that the first cleavage is meridional in the ova of nearly all animals.

The figures show that the second polar cell continues to lie in the cleavage furrow, and consequently has retained a fixed position with reference to the egg during its rotation within the vitelline membrane.

In some ova the rotation is through less than a quadrant, so that at the close of the first cleavage the plane of division is more or less oblique to the long axis of the vitelline membrane. In examining living ova taken at random, many oblique cleavage furrows are noticed, but continuous observation usually shows that the obliquity is the result of preparation for the second cleavage. Accordingly, it may be stated as a general rule that at the close of the first cleavage of the ova of Lepas the cleavage plane is transverse to the long axis of the vitelline membrane, and that only in comparatively few cases is it markedly oblique.

In those eggs in which it is oblique at the close of the first cleavage, the vitelline membrane appears relatively broader, and the divided ovum is easily adjusted to an oblique position within the membrane.

Fifteen or twenty minutes usually elapse between the first external appearances of division and the complete separation of the cells. From the cases which I followed continuously it appears that the cleavage begins within two to three hours after the formation of the second polar cell.

During this cleavage the ova are seen to undergo a series of marked contractions, as shown in Figures 11 and 14. Immediately following each contraction the cleavage furrow deepens and the ovum rotates through several degrees. These phenomena are probably due to the action of the astral fibres, which, as will be shown later, are a well-marked feature of the cleaving ovum. The external appearances would lead one to think that the internal contractions occur spasmodically rather than continuously. Similar appearances were many times noted also in the later cleavages.

Additional evidence in support of this observation concerning rotation of the dividing egg has been obtained from living eggs of L. fascicularis and a species of Balanus. In L. fascicularis (Plate 11, Figs. 95–97) the first polar cell has been observed to remain attached to the vitelline membrane at its blunter pole until after the close of the first cleavage, when the second polar cell, attached to the egg, has moved 90° from the blunt pole of the vitelline membrane. This observation is conclusive confirmation of my earlier observations on L. anatifera.

While no observations have as yet been made on the living ova of species of Cirripedia other than those already mentioned, the study of preserved material of other species indicates that in these the first cleavage takes place as in L. anatifera and in L. fascicularis. In L. hillii, L. pectinata, Pollicipes, and Balanus the chief axis coincides with the long axis of the unsegmented ovum and of the vitelline membrane. After the first cleavage, I find the polar cell in the cleavage furrow, which approximately coincides with a transverse plane of the vitelline membrane.

So far as known similar relations exist between the ovum and the vitelline membrane before and after cleavage in the ova of all Eucirripedia; therefore, it is very probable that cleavage takes place in the entire group as in L. anatifera. Van Beneden's ('70) figures of Sacculina suggest that the same may also be true for the ova of Rhizocephalan Cirripedia.

The internal phenomena connected with the cleavage could not be

accurately interpreted from observations on the opaque living egg, but sections of ova killed at various stages in the cleavage show some interesting conditions. About the time when the pronuclei come into contact, two clear areas are often seen near the pronuclei, as shown in Figure 20 (Plate **2**), but frequently in a plane more nearly transverse than that in which they are shown in the figure cited. In the same positions well-defined asters later make their appearance, and the first cleavage spindle begins to form with its axis oblique to that of the vitelline membrane (Fig. 21). In many cases the spindle begins to form in a plane almost perpendicular to the long axis of the ovum. This is true particularly in L. fascicularis (compare Plate **11**, Fig. 98).

In the metaphase of the mitosis the spindle is usually oblique to the long axis of the ovum (Fig. 22); sometimes it is almost transverse (Fig. 98), but never parallel to the long axis. In L. fascicularis it is most frequently perpendicular to the chief axis, as shown in Figure 98. In L. anatifera the spindle is usually almost as long as the transverse axis of the ovum. The astral radiations are very distinct, and appear to be continuous with the general protoplasmic reticulum of the cell (Fig. 22). In the stage of the living ovum corresponding to this the yolk has taken an eccentric position at the vegetative pole (Fig. 7). The relation seen to exist between the yolk and the aster nearest the vegetative pole (Fig. 22) suggests that the movement of the yolk to the eccentric position has some relation to the formation of the aster, for it is during the development of that structure that the yolk moves to the eccentric position.

In the next stage figured, an early anaphase (Plate **3**, Fig. 23), the spindle is still oblique and the cleavage furrow has not begun to form. The chromosomes have separated along a plane which is usually inclined to the plane in which the cleavage furrow later appears. This stage corresponds to a stage of the living ovum which is slightly later than that represented in Figure 7.

Figure 24 represents a stage in the anaphase after the cleavage furrow has become well developed, and the dividing ovum has begun to rotate. This is the condition in stages of the living egg corresponding to those shown in Figures 10–13. The central part of the spindle is almost perpendicular to the plane of cleavage, but there is a distinct bend in the spindle near either end. These bends may be regarded as evidence of torsion. Comparing Figures 23 and 24, it appears that during division there has been some shifting of the egg substance with reference to the spindle, which is at first somewhat oblique to the plane

in which the cleavage furrow will appear; but later, when the furrow begins to form, the spindle becomes perpendicular to the plane of cleavage. In L. fascicularis the spindle is usually from the very beginning of cleavage perpendicular to the chief axis, in which the cleavage furrow later appears. I have noticed the same conditions in the eggs of a species of Balanus. In living eggs of Lepas I have observed movements of the egg substances which lend support to the evidence afforded by sections. Figures 8–11 represent conditions between the stages corresponding to Figures 23 and 24, and they show that the egg undergoes great changes in form before rotation begins. It is probable that the turning of the spindle takes place at the time of contractions of the egg such as those represented in Figures 9–11.

The astrospheres are well-marked features of the anaphase (Fig. 24), and are distinctly visible as clearer regions in the living egg.

In a late anaphase the spindle has become straight again and is perpendicular to the cleavage plane (Fig. 26). The rotation of the ovum is now completed. In this stage the cells are still connected in the centre by a mass of cell-substance, surrounding the spindle (Fig. 26).

Finally, in the telophase the chromosomes swell into vesicles, and then fuse together to form the nuclei of the two daughter cells in a manner well known for other ova (Figs. 25–27). The cell plate is next completed, and then the separation of the cells (ab^2, cd^2) is accomplished. Remnants of the spindle may persist for some time, and a well-marked "Zwischenkörper" is often seen.

Figure 25 represents the condition in the comparatively rare cases in which the cleavage plane remains oblique in an early telophase.

In observing the living egg it was noted that at the close of the anaphase the protoplasm of the yolk-cell (cd^2) is centrally located and that the yolk remains in its original position in the vicinity of the pointed end of the vitelline membrane (Figs. 15, 26). The chief axis of the egg now coincides with the transverse axis of the oval vitelline membrane, the animal pole being marked by the second polar cell, which lies in the cleavage furrow. The formative and nutritive materials of the yolk-cell are not as yet arranged with reference to the chief axis, as they naturally would be if they kept their original relations to the chief axis during the rotation of the dividing ovum. It has been observed that in the living egg the yolk and the central mass of protoplasm move to their respective poles in from twenty to fifty minutes after the complete separation of the cells (Figs. 15, 16). It will be seen later that this can have nothing to do with the processes of the second cleavage,

which occur two to three hours later. Sections of ova which were fixed at intervals during the first hour after the close of the first cleavage show that the above mentioned movement of protoplasm and yolk occurs at about the time when the spindle and asters have disappeared (Fig. 27). These facts suggest that the spindle and asters may have in some way inhibited the movement of the yolk in its return to its original position at the vegetative pole of the chief axis, out of which it appears to have been forced during the rotation of the dividing egg. The relative positions of spindle, protoplasmic mass and yolk, as shown in Figures 22–27, seem to lend support to this suggestion. The spindle and astral radiations appear to be arranged so as to hold the cell-substances in the same relative positions which they occupied before the cleavage (Figs. 7, 22); with the disappearance of the spindle and asters the mass of protoplasm apparently became free to move toward the animal pole, while the yolk was moved to the vegetative pole (Plate **1**, Fig. 16; Plate **3**, Fig. 27). It seems that the formative and nutritive materials after having been displaced return to their respective poles of the egg as soon as the displacing and inhibiting cause is removed. In this case the tendency to return to the original polar relations seems to be related to the phenomenon of cell-polarity, the causes of which are thus far hidden.

Throughout cleavage the mass of protoplasm in the yolk-cell remains at the animal pole of the egg, which is marked by the second polar cell, and the successive blastomeres formed by the unequal division of the yolk-cell are cut off as near the animal pole as is consistent with the position of previously formed cells.

Conklin ('**97**) has pointed out for the egg of the gasteropod Crepidula a tendency of the protoplasmic mass in the macromeres to remain near the animal pole, while successive ectomeres are cut off as near that pole as the position of previously formed cells will allow. The condition in the egg of Lepas furnishes a parallel case, and the return of the protoplasmic mass to the polar position after displacement in the first cleavage indicates a strong tendency towards adherence to the original polarity of the unsegmented ovum.

The rotation of the dividing ovum appears to be dependent upon the cleavage processes, and capable of an explanation along mechanical lines. The cleavage furrow arises in an almost longitudinal position, passing through the animal pole (Plate **1**, Fig. 8). As the furrow deepens, the forming cells tend to become spheroidal and hence to lengthen the axis of the ovum perpendicular to the plane of cleavage

(Figs. 9–11). If no firm envelope confined the ovum, interfering with change in its form, the long axis of the two-cell stage would be perpendicular to the plane in which the cleavage begins; but the vitelline membrane evidently does interfere with extension in a direction perpendicular to that plane. Therefore, as the cleavage progresses and the resulting cells become more and more spheroidal (Figs. 10–13), a rotation of the ovum becomes necessary, for evidently the long axis of the two-cell stage must approximately coincide with the long axis of the vitelline membrane. An examination of the figures makes it appear that, as the forming blastomeres become more spheroidal and consequently increase the length of the axis of the ovum perpendicular to the plane of cleavage, pressure is obliquely applied to the vitelline membrane with the result that the ovum *as a whole* rotates, and gradually the dividing ovum adjusts itself to the form of the vitelline membrane. The cleavage plane becomes transverse or oblique, depending upon the amount of rotation necessary to meet adjustment. With a relatively wide vitelline membrane the rotation is less than 90°, for the divided ovum can then become adjusted to an oblique axis of the membrane, and the cleavage plane consequently remains oblique.

A rotation of the ovum as a result of cleavage has also been shown in the case of the rotifer Callidina, described by Zelinka ('91). Like that of Lepas, the ovum of Callidina is ellipsoidal and surrounded by a rigid membrane. The polar body is situated at one end of the ovum, and the cleavage plane passes through this point. Zelinka figures an oblique spindle, but no sections showing the relations in the various stages of mitosis. According to Zelinka the rotation of the ovum occurs after division, but the extent of the cleavage plane at the time of rotation was not determined by study of sections. It seems probable that, as in the cirripede ovum, the rotation may be found to take place during the division.

Jennings ('96, p. 20), commenting upon the rotation in Callidina, writes: — "It thus appears that in Callidina the direction of division itself is determined neither by the principle of Berthold [surface tension] nor that of Hertwig [spindle in long axis of protoplasmic mass], but that the later arrangement of the cells might be held to be due to the action of Berthold's principle." The conditions in Lepas appear to be similar to those in Callidina, and Jennings' conclusion is applicable in the case of the cirripede.

In the eggs of some nematodes there are conditions at the time of fertilization very similar to those existing in Lepas. The contiguous

surfaces of the pronuclei are in a plane which is perpendicular, or slightly oblique, to the long axis of the ellipsoidal egg, and the spindle often begins to form with its long axis in the same transverse plane. Several investigators, among whom may be cited Auerbach ('**74**, p. 212, Taf. 4) and Ziegler ('**95**, pp. 379–387), have observed that there occurs a turning of the pronuclei around each other so that their contiguous surfaces and the spindle axis come to coincide with the chief axis of the egg. This turning of the pronuclei and spindle appears to be brought about by streaming movements of the substances of the egg. In addition to these observations on the nuclei during their rotation, there is evidence in the two-cell stage of the nematode that the egg as a whole has not rotated, for the polar cell remains in the long axis of that stage 90° from the equatorial cleavage plane.

As a result of the turning of the pronuclei and the consequent longitudinal position of the spindle, the nematode egg divides in such a plane that the two-cell stage does not require readjustment in order to accommodate its long axis to that of the surrounding egg envelope. Thus the turning of the pronuclei and spindle in the nematode eggs affects the orientation of the two-cell stage as completely as does the rotation of the dividing egg *as a whole* in the case of Lepas. My observation that in L. anatifera the spindle often appears to begin its formation in a transverse plane and then becomes oblique, suggests that there is a tendency towards coincidence of the spindle axis with the long axis of the egg. If such a tendency really exists, it is inhibited by some unknown conditions, possibly the yolk-mass influencing the streaming of the protoplasm, and as a result the cleavage plane is formed in such a position that the two-cell stage must become readjusted to the vitelline membrane.

Summary of the First Cleavage.

It has been shown that in L. anatifera, L. fascicularis, and a species of Balanus, the cleavage plane lies at the beginning of cleavage approximately in the long axis of the unsegmented ovum as well as that of the vitelline membrane, and passes through the animal pole. During the division a rotation of the ovum *as a whole* through an arc of 90° takes place, so that at the close of the division the plane of cleavage coincides with the transverse axis of the vitelline membrane.

The evidence afforded by preserved material and published figures makes it probable that a rotation of the dividing ovum occurs in all

Cirripedia which have ellipsoidal eggs surrounded by a rigid vitelline membrane.

The rotation appears to be due to the mechanical relations existing between the dividing ovum and the vitelline membrane.

The first cleavage is a typical case of unequal cell division; this is widely at variance with the account given by Groom (see the following review of the literature).

3. Review of the Literature on the First Cleavage.

According to the accounts or figures of Fillippi ('65), Münter und Buchholz ('69), Hoek ('76), Lang ('78), Nassonow ('87), and Groom ('94), the first cleavage plane in all the species of Lepadidæ and Balanidæ, which have been studied by them, is generally transverse to the chief axis; but it has been sometimes described as occasionally more or less oblique owing to variation. These investigators noticed that the long axis (chief axis) of the unsegmented ovum coincides with the long axis of the vitelline membrane, and that in the two-cell stage the plane of separation is transverse to that axis. These positions of the egg with reference to the vitelline membrane before and after cleavage led to the view that the first cleavage plane is formed at right angles to the chief axis of the egg, i. e., that cleavage is equatorial. Had the position of the polar cell during and after cleavage been carefully observed, this view would not have gained acceptance. Of the above named authors Groom and Nassonow have figured the polar cell in the two-cell stage, and they represent it as situated in the original position near the rounded end of the vitelline membrane, 90° from the cleavage plane.

Nussbaum ('87, '90) observed in some ova of Pollicipes cleavage planes in various degrees of obliquity with reference to the vitelline membrane, from nearly longitudinal to transverse. He is the only author who has figured or described a polar cell as lying in the cleavage furrow of the two-cell stage of a cirripede egg. Nussbaum explained these varying positions of the cleavage plane and polar cell with reference to the long axis of the vitelline membrane by assuming that the ovum divides almost longitudinally, and that after division the egg turns within the vitelline membrane. The various positions of the first cleavage plane, which were observed by Nussbaum in different eggs, were assumed to represent phases in the turning of the egg as it rotated from the position in which the forming cleavage plane is nearly longitudinal to the final position, in which it is transverse. Nussbaum sug-

gested that the turn of the egg might be explained on the principle of least resistance, since the long axis of the divided egg can only be adjusted to the long axis of the vitelline membrane. He failed to study sections of stages in the first division and to follow continuously the cleavage of a living ovum. Groom ('94) expressed doubt concerning Nussbaum's identification of the body in the cleavage furrow as the polar cell, for it had not been followed continuously from its formation. Nussbaum's figures of three different ova with cleavage planes respectively in almost longitudinal, in oblique, and in transverse positions do not give conclusive evidence in support of his assumption that the egg rotates after cleavage. Groom has remarked that, if a rotation occurs, an ovum with oblique cleavage plane should show a correspondingly situated polar cell, and Nussbaum's figure of such a stage does not show this. So far as the evidence offered by Nussbaum is concerned, one might well accept Groom's view, that the various positions of the first cleavage plane in different ova indicate merely variation of the position in which it forms.

Although Nussbaum failed to support his assumption with conclusive evidence, he was certainly in the main correct, as the evidence offered in this paper proves. Studies of the preserved material have convinced me that the relations in Pollicipes agrees with those in Lepas. Nussbaum's assumption that the rotation takes place after division does not agree with the facts in the case of Lepas. I have shown that the rotation takes place not after, but during division, and have suggested that the forces concerned in cleavage, reacting upon the rigid vitelline membrane, are apparently the cause of the rotation of the dividing ovum.

Groom's account of the first cleavage is so involved with his description of the separation of the protoplasm from the yolk during maturation that no sharp line is drawn by him between the two processes. I quote from his paper ('94, pp. 135–136) the following description: —

"The polar bodies become pale and disintegrated, and the external one often gets washed away. The protoplasm is at last mainly collected at the anterior pole of the egg, and the yolk at the other (Figs. 6, 7). . . . The surface separating the protoplasmic half from the yolk commonly intersects the ovum in a perfect circle, and marks off what will form the first blastomere. . . . Very generally the line of separation of the protoplasm and yolk is almost accurately transverse, . . . I have frequently seen cases when the wall was accurately transverse, and the polar body situated apically (Figs. 6, 7). Lastly I have been able to watch the gradual formation of the protoplasmic half in a single ovum; the line of junction in these cases was transverse from the first."

It is evident that this account refers to the processes which I have described in the chapter on maturation of the ovum. They are phenomena concerned with the establishment of visible polarity in the egg, and not with the cleavage process, as Groom's account leads us to infer. The surface marking the boundary of yolk and protoplasm, as shown in Groom's Figures 6 and 7 (in this paper Figs. 3 and 18), does not "mark off what will be the first blastomere." Groom evidently mistook the constriction which I have described in the account of maturation (Fig. 3) for the forming cleavage plane; but I have shown the cleavage plane to be almost perpendicular to this transverse constriction, which merely marks off the yolk-lobe (see Figs. 3 and 18). Groom's misinterpretation explains the cases described by him, in which the cleavage plane appeared transverse and the polar cell apical in position; see his Figures 6 and 7, which evidently correspond to my Figures 3 and 18. Groom has interpreted his Figures 6, 7 and 8 (L. anatifera), and 45, 46 and 47 (L. pectinata) as representing successive stages in the formation of the first cleavage plane. As a matter of fact there intervene between the last two stages of each of these series all the stages which are shown in this paper by Figures 4–15. The identification by Groom of the transverse constricting furrow of the maturation period as the forming cleavage furrow has probably led to his erroneous interpretation of the position of the polar cell with reference to the first cleavage plane. It was natural that Groom, considering the three figures mentioned above (Figs. 6, 7, 8) as a continuous series, should expect to find the polar cell at the place of its formation, and should overlook it in the first cleavage furrow. The best of observers could easily have been misled, unless an opportunity came for following a single ovum uninterruptedly through the maturation and first cleavage stage. The polar cell lies deep in the cleavage furrow, and is easily overlooked in the living ovum, unless one's attention has been attracted to it in prepared ova, where it is clearly shown in the majority of cases. The rare cases observed by Groom of ova in which the polar cell retained its original position in undoubted two-cell stages are explained by my observation that the polar cell sometimes, but very rarely, fails to rotate with the ovum. That the polar cell is not soon lost, as Groom believed, is evident from many of my figures of later stages. In preparations it is as often seen in later stages of cleavage as in the unsegmented ovum.

Groom's Figure 101 (L. anatifera), showing a longitudinal position of the spindle, is certainly from a section taken in a plane oblique to the chief axis so as to show the spindle in the long axis of the sec-

tion. A spindle parallel with the chief axis would be in harmony with Groom's view that the first cleavage furrow is perpendicular to that axis. Numerous transparent preparations of entire eggs have convinced me that such is never the case.

In the review of literature on maturation and fertilization I have already referred to Groom's mistake in identifying the pronuclei as the daughter-nuclei of the segmentation nucleus. He speaks (p. 145) of two nuclei seen in "the first blastomere" (cell ab^2 of this paper). One of the two nuclei which he regards as the daughter-nuclei of the segmentation nucleus remains as the nucleus of "the first blastomere," the other passes into the "yolk hemisphere" (yolk-cell cd^2 in this account) just before the cell-plate is formed. This is certainly erroneous, and is apparently the result of his interpretation of the transverse furrow accompanying maturation as the cleavage furrow. In Groom's Figure 8 two distinct nuclei are represented in the "protoplasmic" part of the egg, which he considered "the first blastomere." It is evident from my figures that the daughter-nuclei of the segmentation nucleus could not normally get into such a position; but the pronuclei are often seen on one side of the constriction during maturation phases (see my Figure 18). I interpret Groom's Figure 8 as representing the pre-cleavage stage corresponding to my Figures 3 and 18, and the lower half of the egg as the yolk-lobe, not the yolk-cell cd^2. I have already stated that, unless eggs are kept under continuous observation, it is easy to confuse this stage with the two-cell stage, when only living eggs are examined. My series of figures shows that no such interpretation as that above quoted fits the facts. There are two nuclei (pronuclei) in the protoplasmic hemisphere during the later maturation phases (Figs. 18, 20); but in the "first blastomere" (cell ab^2 in my Figs. 26, 27) there are never two, one of which is destined to pass into the yolk. Groom's description of the "yolk" (cell cd^2) as at first without a nucleus, but receiving one from the "first formed blastomere" (first micromere ab^2), is erroneous. Neither cell can be said to receive a nucleus from the other, for the division of the segmentation nucleus, and the formation of the first cleavage plane is such as ordinarily takes place in unequal cell division.

The last statement applies also to all the later cleavages. The micromeres rich in protoplasm, which are later cut off from the yolk-macromere, cannot be said to give rise to a nucleus which migrates into the yolk before complete separation of the "protoplasmic" cell.

4. Second Cleavage. Four Cells.

The first cleavage results in the division of the ovum into two cells of unequal size; the smaller cell (first micromere ab^2), which is anterior in position, is largely protoplasmic, whereas the larger, posterior cell (cd^2) contains the yolk, and will be designated as "yolk-cell." For convenience in description this cell is regarded in the following account of cleavage as a macromere; it retains its individuality during three successive unequal cleavages, giving rise to three "protoplasmic" micromeres, the yolk after each cleavage remaining in the larger daughter-cell, which in each stage will be designated as "yolk-cell." The addition of the exponent indicating the cell generation will prevent the confusion which would arise from the use of the term "yolk-cell" alone, when applied to the cell d^3, $d^{4.1}$ or $d^{5.1}$, which are the yolk-bearing derivatives of the cell cd^2 of the two-cell stage. The micromeres are numbered in the order of their separation from the yolk-cell, ab^2 being the first and c^3 the second.

The nearly synchronous successive divisions of the first two cells (ab^2, cd^2), and afterwards of their derivatives, result in "resting" stages of the egg, which normally consist of 2, 4, 8, 16 and 32 cells, and it becomes easy to classify the successive cleavages of the egg as second, third, fourth and fifth. It will be noticed, however, that in the second and following cleavages the yolk-bearing cell tends to divide after the other cells, and that its division becomes more retarded at each successive generation. This seems to be correlated with the fact that at each division the protoplasm in the yolk-cell is diminished in proportion to the amount of yolk. In the fourth and fifth cleavages the yolk-cell usually completes its division just as the other cells prepare for the next cleavage. However, it is not until after the fifth cleavage (thirty-two cells) that it lags a full generation behind the other cells. The cleavages can, therefore, be classified naturally according to the resting stages, each stage containing twice as many cells as the preceding.

The second cleavage may take place in the cells ab^2 and cd^2 simultaneously (Fig. 28), but either cell may complete the cleavage slightly in advance of the other. In the majority of cases division of the anterior cell (ab^2) precedes (Fig. 99), but usually the differences in the phases of mitosis in the two cells are very slight.

In both cells the mitotic spindles for the second cleavage are formed perpendicularly both to the first cleavage spindle (compare Figs. 26 and 28) and to the chief axis of the egg. In the first micromere (ab^2) the spindle is centrally situated; the cleavage plane is formed at right angles

to the first cleavage plane, and passes through the animal pole of the egg (Figs. 29, 30).

The spindle in the yolk-cell cd^2 is eccentric in position, lying nearer the animal pole of the egg, and near the centre of the protoplasmic mass; it is nearly perpendicular to the chief axis (Fig. 29). As cleavage progresses the spindle becomes inclined so that one end dips into the yolk-mass, which lies at the vegetative pole of the yolk-cell (Figs. 31 and 99). From the point of view of a miniature observer occupying the chief axis of the ovum with his head directed toward the animal pole, the left end of the spindle is the one that is nearer the animal pole, that is, the spindle is læotropically oblique. Usually the spindle makes an angle of about 30° or 40° with the chief axis.

The yolk-cell cd^2 cleaves unequally, and the cleavage plane may be considered a modified meridional one. The cleavage planes of the "protoplasmic" cell ab^2 and of the yolk-cell meet in a line which passes through the animal pole, but does not coincide with the chief axis; it makes with this axis an angle of about 45°. To our imaginary observer the resulting smaller cell (c^3) lies to the left of and above the larger or yolk-cell d^3 (Fig. 31), and also this cell lies above the anterior cell b^3. The cell c^3 is the second micromere which is separated from the yolk.

At the close of the second cleavage a general tendency towards a læotropic arrangement of the cells is noticed (Figs. 32–34, 100–102). This arrangement in the case of the posterior cells (c^3, d^3) is apparently the result of the oblique position of the spindle in the yolk-cell cd^2. Whenever the anterior cell ab^2 (first micromere) divides in advance of the yolk-cell cd^2, there is no suggestion of a læotropic arrangement either in its spindle or in the position of the resulting cells (a^3, b^3, Fig. 99); but after cleavage of the yolk-cell, the right anterior cell b^3 is depressed by the higher lying cell c^3. This change can be seen in the living ovum as the cleavage of the yolk-cell cd^2 progresses.

Soon after the completion of the second cleavage the four cells tend to become rounded, and adjustments of position occur. Figures 32–35 and 102, 103 represent the arrangements which are usually seen, and in all of them a definite plan can be recognized. The axis of the future embryo can now be described as passing through the nuclei of the anterior cell, b^3, and of the yolk-cell, d^3 (Fig. 31). The anterior cell, b^3, always comes to lie nearer the vegetative pole than the cells a^3 and c^3, and it is usually more or less covered on the animal side by one or both of these cells (Figs. 34, 35). After examining the eight-cell stage, in which the bilateral symmetry is distinctly marked, it will be seen that

the arrangement of the cells in the four-cell stage and of the spindles for the next cleavage are such that the daughter cells invariably assume definite and constant positions in the eight-cell stage.

Summary of the Second Cleavage.

Both cells of the two-cell stage divide nearly or quite simultaneously. The second cleavage plane is meridional and perpendicular to that of the first cleavage. The first micromere (ab^2) divides equally, whereas the yolk-cell cd^2 divides unequally, giving rise to the second micromere, c^3.

After the second cleavage the four cells (a^3, b^3, c^3, d^3) become adjusted in a læotropic arrangement.

In the four-cell stage a plane passing through the second polar cell and the nuclei of cells b^3 and d^3 is apparently near the sagittal plane of the future embryo. In this stage, then, there is a suggestion of bilateral arrangement of the cells.

The yolk-cell undergoes ordinary unequal cleavage (see the following review of the literature).

5. Review of Literature on Second and Succeeding Cleavages.

In this connection it is necessary to give a general review of the literature bearing on all early cleavages after the first, because no previous worker has recognized definite stages into which the cleavages of the cirripede ovum can be grouped. It is therefore impossible to make any comparison of my account with that of others, except in a general way.

The division of the "protoplasmic" cell (ab^2) of the two-cell stage of the cirripede egg has been correctly described by most authors. The plane of cleavage has been generally described as perpendicular to the first cleavage plane, but Nussbaum ('90) has recognized that in Pollicipes it intersects the first cleavage plane at the polar cell and is, therefore, meridional.

No investigator of the early development of Cirripedia, except Groom, has shown that the yolk-cell, cd^2, of the two-cell stage divides and adds new cells to the blastoderm. All other observers, Buchholz ('69), Hoek ('76), Lang ('78), Nassonow ('87), and Nussbaum ('90), have described the yolk-cell cd^2 as remaining undivided while the other cell (ab^2) repeatedly divides and its products grow around the yolk-cell, forming the blastoderm. After completion of the blastoderm, and closing of the blastopore, the yolk-cell cd^2 was said to divide, separating the mesoblast from the entoblast. According to this view the cell ab^2, which forms

the blastoderm, contains only ectoblastic material. An exception is to be noted in the case of Nussbaum, who saw the mesoblast apparently proliferating from the edge of the blastoderm. The cell ab^2 according to his interpretation, then, contains all the ectoblast and the mesoblast.

The erroneous interpretations of the earlier observers are largely explained by the fact that their observations were almost exclusively confined to living eggs, in which the nuclear conditions are hidden. Without sections or transparent preparations divisions of the yolk-cell might be easily overlooked. Lang ('78) and Nassonow ('87) figured for Balanus, and Nussbaum ('90) for Pollicipes, distinct protoplasmic radiations in the yolk-cell, but failed to see their significance as indicating division. I am convinced that the structures seen were asters or archoplasmic radiations. Korschelt und Heider ('90) made the suggestion, based on Nassonow's figures, that the yolk-cell cd^2 divides and contributes cells to the blastoderm.

Groom ('94) described the yolk-cell cd^2 in the case of all cirripedes whose development he observed, as a macromere giving rise in succession to a number of "blastomeres," which are added to the blastoderm. He proved conclusively that the "protoplasmic" cell ab^2 (his "first blastomere," my "first micromere") does not give rise to all of the ectoblast, as supposed by all previous observers. According to his account several cells (estimated at nine or ten) are cut off from the yolk-cell after the first cleavage, and with the derivatives of the "first blastomere" form the blastoderm.

Several years ago, without knowledge of Groom's results, owing to the inaccessibility of the literature, I ('96) found that in Lepas fascicularis the yolk-macromere divides several times, practically synchronously with the divisions of the other cells, thus contributing to the formation of stages of 2, 4, 8, 16 and 32 cells. This confirmed Groom's results in general; but as to the order, method, and number of the divisions I was forced to dissent from his account.

According to Groom's description there is great variation in the number, order, and position of cleavages both in the yolk-cell and in the other cells of the cleaving egg. He concluded that the cleavage of the cirripede egg is decidedly irregular. He writes (p. 140), "there is no constancy in the mode of growth of the blastoderm over the yolk;" and mentions (pp. 139–140) many of the variations which occur.

Many of these supposed variations are certainly misinterpretations due to errors in orientation, and others are apparently based upon abnormal eggs. Mention may be made of several cases. Groom states

that the "second blastomere" (cell c^8, second micromere, in my figures) may be formed on either side of the yolk-cell d^8, and illustrates such conditions by his Figures 10 and 12 (L. anatifera). There is nothing in either his text or figures to prove that these are not entirely similar eggs viewed from almost opposite poles. They were certainly drawn from different points of view, and the apparently different positions occupied by the "second blastomere" are thus easily explained. Likewise, the "third blastomere" ($d^{4.2}$, third micromere, in this paper) is said to arise on either the right or left of the second. Groom's Figures 15 and 16 (L. anatifera), which illustrate this, are certainly views of two similar eggs, and apparently the cell considered the "second blastomere" is not the same in both cases. The position of the "third blastomere" shown as "emerging from the yolk," in one figure on the right and in the other on the left, I interpret as being near the animal pole of the egg. A number of other cases of such results based upon uncertain orientation of the egg might be drawn from Groom's paper; but enough has been said to show that his evidence is far from convincing, that there is much variation even in the earliest stages, and that the assumed variability of the later stages rests upon a very uncertain basis. In opposition to this view of the cleavage of the cirripede egg as variable and irregular, I shall give evidence supporting my interpretation of the cleavage of Lepas as normally regular and constant.

In this connection I wish to consider Groom's account of the method in which the yolk-cell divides. The discussion will apply to the second or any later cleavage by which blastoderm cells are cut off from the yolk-cell, for the method of division is the same in all.

The following quotations from Groom's paper give his interpretation of the method by which new cells are formed from the yolk-cell. On page 197 he writes: "As the first blastomere becomes cut off from the yolk the nucleus divides and one daughter-nucleus passes into the yolk half, and soon emerges accompanied by protoplasm to form a second blastomere and generally situated close to the first. As this becomes cut off from the yolk it gives off into the yolk a nucleus, which behaving similarly to the daughter-nucleus of the germinal vesicle, forms new protoplasm and emerges as a third blastomere. At each successive stage the yolk is in communication with one merocyte or newly-forming blastomere, and this, before becoming shut off as a blastomere, gives off a single nucleus into the yolk." A similar statement on page 145 of Groom's paper contains some other points to which it will be necessary to refer. One daughter-nucleus of the segmentation nucleus is said to

"pass into the yolk hemisphere, where it transforms yolk material into protoplasm; the second merocyte, formed partly in this way and partly from previously existing protoplasm, issues as the second blastomere, while the first becomes simultaneously cut off from the yolk . . . the nucleus of the third merocyte is derived from that of the second; the latter becomes spindle-shaped, and gives off a nucleus, which, accompanied by little or by no appreciable quantity of protoplasm, passes into the yolk. . . . The third merocyte, in similar manner, while emerging as a blastomere, divides and gives off a nucleus to the yolk, which in a similar manner gives rise to new merocytes and blastomeres."

It is evident, as indeed Groom distinctly states in another place, that he regards the yolk as non-nucleated and receiving nuclei from the successively formed blastomeres. In the discussion of the first cleavage I have pointed out that a nucleus from "the first blastomere" (the cell ab^2 in this paper) does not pass into the yolk-cell just before the separation of the two cells. This also applies to all succeeding cleavages. The yolk-cell does not derive its nucleus from successively formed "protoplasmic" cells ("blastomeres") — such a description is inaccurate and misleading. In no case can either "blastomere" or the yolk-cell be said to derive its nucleus from the other, for the micromeres are merely the result of ordinary unequal division, which differs from the division of cell ab^2 in the inequality of the products, but not in the method by which it is brought about.

The term "merocyte" conveys the idea that the protoplasm is more or less sharply distinct from the yolk, as in the case of eggs which undergo superficial cleavage. This is evidently the idea intended to be expressed in the above quotations from Groom. Neither living eggs nor stained sections support such an interpretation. A considerable part of the yolk-cell cd^2 is protoplasmic, the yolk and protoplasm being so mingled that there is no justification for the use of the term "merocyte." I cannot agree with Groom's statement that throughout the main portion of its mass the yolk-cell contains little protoplasm. Protoplasmic processes extend even among the oil droplets which lie near the periphery at the vegetative pole of the egg (Fig. 27). I cannot confirm the statement (p. 198) that there is little protoplasm left in the yolk-cell immediately after the separation of a new blastomere, and that the nucleus rapidly transforms yolk into protoplasm to form the new blastomere. The amount of yolk is not very much diminished before the sixth cleavage. This is in accord with the facts known in the case of the development of other animals, for rapid transforma-

tion of yolk during cleavage has rarely been described. The mass of protoplasm in the yolk-cell after the first cleavage is certainly nearly equal in volume to the next cell (second micromere c^2) which will be cut off (see Fig. 27). The same is true for the later cleavages. All these facts, together with those relating to the nucleus which were mentioned in the preceding paragraph, are opposed to the idea of an "emergence of merocytes from the yolk," and support the interpretation which I have given, viz., that all divisions of the yolk-cell are cases of unequal total cleavage. There is nothing to warrant the phrase "emergence of merocytes."

In concluding this general discussion of the method of cleavage of the yolk-cell, I wish to emphasize the statement that there appears to be no reason for regarding that cell in any of the cleavage stages as essentially different in its nature or in its method of division from such well-known examples of yolk-macromeres as are found in gasteropod eggs. So far as I have found, the division of such macromeres is described as differing essentially from that of other cells more rich in protoplasm only in the inequality of the products. Furthermore, I can see no essential difference between the process of cleavage in the yolk-cell of L. anatifera, where there is much yolk, and in that of L. fascicularis, in which there is relatively little yolk, and in which the division is clearly of the ordinary unequal type.

According to Groom's account ('94, p. 137) a forming or "emerging blastomere" is characterized by a radial arrangement of granules around a clear central space situated near the periphery of the yolk-cell. Groom's Figures 50, 86 and 88 represent this condition. He speaks of the nucleus of the forming blastomere as the centre of the radiation (see his Fig. 14). The clear area seen in a living egg at this stage is certainly not the nucleus, but the astrosphere, and the radiations represent an aster. Groom's description of the development of these structures (p. 137) is good. During the division well-marked protoplasmic movements give visible evidence of the differential distribution of the cell-substances. The nucleus itself is not easily seen in the living egg at any stage, and certainly is not vesicular at the time when the astrosphere is clearly defined. Figures 25, 26, and 30 represent sections of eggs in which, when living, the centres of the radiations presented much the appearance shown in Groom's Figures 10–15. The centres of the radiations are seen to be the astrospheres, and the nuclei are represented by the chromatin vesicles, which are certainly invisible in the living egg.

Groom correctly described the radial arrangement of the protoplasm as persisting for some time after cleavage. In my Figure 27 there is represented a radial arrangement of granules which is a persistence of the condition shown in Figure 26 as occurring at the close of the first cleavage. The astrospheres have disappeared, and the nuclei lie near the centres of the persisting radiations. This radial arrangement disappears as soon as the second cleavage spindle forms (Fig. 28), but the new radiations then formed may in turn persist after the cleavage until the formation of the spindles for the third cleavage (Fig. 30).

Groom ('94) states that two or more blastomeres may arise simultaneously from the yolk-cell! "Similar cells [blastomeres from the yolk-cell] are seen to arise in quite different positions at later stages, sometimes two or more at a time," (p. 138). Again, on page 140 he writes: "In the early as in the later stages the merocyte before emerging from the yolk may not uncommonly be seen to give rise by division to a second merocyte." Such conditions are represented in Groom's Figures 17a (L. anatifera), and also in his Figures 53 and 57 (Balanus). Certainly none of these figures really represents two blastomeres arising at once. The two sets of radiations (asters) which Groom wrongly interpreted as two "emerging merocytes" probably represent cases in which the spindle was in such a position that both asters were visible at the surface. Usually, however, only one aster is to be seen in the living egg, the other being closely connected with the yolk. Sometimes the spindle is long, so that the two asters are visible on opposite sides of the egg. I have frequently seen the two sets of radiations in the living egg, and sections show that the interpretation which I have just given is the correct one.

Sometimes multipolar spindles, which are probably the result of abnormal conditions, are seen in sections of the yolk-cell, and these may possibly result in a multiple cleavage.

Rarely the cell c^3 (Groom's "second blastomere") may be formed near the posterior end of the yolk-cell, as shown by Groom in his Figure 13.

Many other deviations from the regular course of cleavage have been seen, but they are comparatively rare, and are to be regarded as abnormalities. Certainly they should not be interpreted as showing great variability in the cleavage, as was done by Groom. I have noticed that such cases are much more common when the animals have been kept for some time in aquaria, but are rarely seen in eggs taken from animals which were recently removed from the open sea. I have attributed

these abnormalities to the action of chemical impurities and to lack of oxygen. The respiratory movements of the animals are more sluggish when they have been kept several hours in aquaria, and hence the eggs in the mantle chamber may fail to get a sufficient amount of oxygen. It is well known that such abnormal conditions may affect great modifications in otherwise regular cleavage.

Orientation of the Embryo.

It has already been stated that in the four-cell stage a line drawn through the nuclei of the cells b^3 and d^3 coincides with the longitudinal (antero-posterior) axis of the future embryo, the cell d^3 being posterior. This relation is shown in the orientation on the plate of Figure 31, from which it also appears that the first cleavage plane is oblique to the same axis. The chief axis of the egg coincides with the dorso-ventral axis of the future embryo, the second polar cell at the animal pole being dorsal. The spherules of yolk are at the opposite pole of the yolk-bearing cell, thus marking the vegetative pole and the ventral side of the embryo. The blastopore later appears on this surface near the posterior end of the egg.

The anterior end of the embryo lies, as several investigators have noted, at the rounded end of the vitelline membrane. In the four-cell and later stages the long axis of the vitelline membrane and that of the future embryo apparently coincide, but in the two-cell stage the long axis of the future embryo is oblique to that of the vitelline membrane. The long axis of the embryo is brought into coincidence with that of the vitelline membrane when the cells adjust themselves after the completion of the second cleavage (compare Figs. 31 and 32).

The animal and vegetative poles, which are marked respectively by the second polar cell and the mass of yolk spherules, have a constant relation to the blastomeres and to the planes of cleavage, and I have made use of them as a basis for orientation. Previous investigators of the cleavage of cirripede ova have recognized no definite and constant points of orientation. In 1896 I pointed them out in the cleaving ovum of L. fascicularis; since then I have found that the polar cell has exactly the same relations to the embryonic cells in all the stages of cleavage in four species of Lepas and in Pollicipes polymerus.

6. Third Cleavage. Eight Cells.

The third cleavage is essentially equatorial. The spindle figures arrange themselves approximately parallel with the chief axis, and therefore nearly perpendicular to the spindles of the preceding cleavages. The spindle in the median anterior cell (b^3) is somewhat exceptional, in that it is more or less inclined toward the horizontal plane (Plate **4**, Fig. 36). The spindle in the yolk-cell d^3 is generally more nearly parallel to the chief axis. The cells a^3, b^3 and c^3 often complete their division in advance of the yolk cell (Plate **11**, Fig. 103). Sometimes the spindle in the yolk-cell is just forming as the other cells divide, but the yolk-cell completes the cleavage while the other cells remain in the "resting" condition. Stages with five, six, or seven cells are seen when examining living ova, but after preparation of such ova the nuclei of some cells are found to be retarded in the third division. Such variations in the rhythm of cleavage are not uncommon in the synchronously cleaving ova of other animals. The normal "resting" stage following the third cleavage in Lepas is composed of eight cells as invariably as if the cleavage were perfectly synchronous in all of the cells.

The positions of the cells which result from the third cleavage are shown in Figures 37–40 (Plates **4**, **5**), and 104–106 (Plate **11**). The three "protoplasmic" cells (a^3, b^3, c^3) have divided equally, the yolk-cell unequally. The cell ($d^{4.2}$) which is cut off from the yolk-cell lies in the median plane near the animal pole (Fig. 37). This is the *third* micromere. The cells resulting from the division of a^3 occupy the left side, and are symmetrical with those derived from c^3, which occupy the right side of the egg (Fig. 37). The cell b^3 has given rise to two cells lying in the median plane, one ($b^{4.1}$) near the yolk-cell at the vegetative pole, the other ($b^{4.2}$) at the anterior end of the egg (Figs. 38, 40).

The seven "protoplasmic" cells have now begun to form the blastoderm (Plate **8**, Fig. 66), which will later enclose the yolk-entoblast. A very small space, which is the cleavage cavity (*cav. sq.*, Fig. 66), is often seen in sections, but it soon becomes filled with yolk, by the ingrowth of the yolk-cell.

The bilaterality in the arrangement of cells was indicated in the stage with four cells; it is well marked in the stage with eight. The characteristic arrangement of the cells, as shown in Figures 37–40, is visible in the great majority of living or prepared ova, if they are properly

oriented. The bilateral arrangement of cells when the egg is viewed from the animal pole and the position of the yolk near the vegetative pole (Figs. 38, 66) are features which aid in quickly identifying the individual cells when the egg is rolled into proper positions.

During the third cleavage the polar cell is usually crowded beneath the blastoderm, and comes to occupy in the cleavage cavity the position indicated in Figure 66 — a condition which has been described as occurring in the eggs of several other Entomostraca. Sometimes at the close of this cleavage it is found lodged between cells. Occasionally it becomes shifted in the earlier stages so that it no longer lies deep in the cleavage furrow; in such an event it is not forced beneath the blastoderm during the third cleavage, but may be found on the surface in later stages. I have noticed it on the outside of the embryo in stages as late as those of about five hundred cells. In such cases it is sometimes far from its normal position at the anterior dorsal side (animal pole) of the embryo. In its usual position beneath the blastoderm the polar cell is quite definitely situated until very late stages. In the eight-cell stage it is almost equidistant from the two poles of the chief axis of the egg; but it usually lies much nearer the animal pole after the fourth cleavage, and is a very useful "landmark" for orientation of the later stages. In good transparent preparations of entire eggs of any cleavage stage the polar cell is clearly visible, and it is often seen lying beneath the blastoderm in stages with over five hundred cells.

The yolk-cell of the eight-cell stage ($d^{4.1}$, Plate **5**, Fig. 40; Plate **8**, Fig. 66) contains only future mesoblast and entoblast, and will be referred to as mes-entoblast. The third micromere ($d^{4.2}$), separated from the yolk-cell in the third cleavage, is purely ectoblastic, and is the last cell containing ectoblast which is given off from the yolk-macromere. The ectoblast is, therefore, separated from the yolk-laden entoblast in the first three cleavages, being contained in the derivatives of the three micromeres, ab^2, c^3 and $d^{4.2}$, which are separated from the yolk-bearing macromere in the first, second and third cleavages respectively. A study of the cell-lineage through the later stages of cleavage shows that the cells ab^2 and c^3 are not purely ectoblastic, but contain a portion of the future mesoblast; they may, therefore, be called mes-ectoblasts. Of their descendants in the eight-cell stage, the cells at the animal pole ($a^{4.2}$, $b^{4.2}$, $c^{4.2}$) are purely ectoblastic, while the lower cells around the vegetative pole ($a^{4.1}$, $b^{4.1}$, $c^{4.1}$) contain future "secondary mesoblast" (ectoblastic mesoblast).

Summary of the Third Cleavage.

The spindles for the third cleavage are essentially perpendicular to those of the first two cleavages, the cleavage being practically equatorial. The three cells a^3, b^3 and c^3 divide equally and synchronously. The yolk-cell d^3, which is often slightly retarded, divides unequally, the smaller, more protoplasmic, product ($d^{4.2}$) of this division, being the *third* and last micromere containing ectoblast which is separated from the yolk-macromere.

The yolk-cell ($d^{4.1}$) is now mes-entoblastic, and bilaterality in cleavage is well marked.

The arrangement of the cells of this stage is definite and constant.

The second polar cell is crowded into the cleavage cavity during the third cleavage.

7. Fourth Cleavage. Sixteen Cells.

The mitotic spindles for the fourth cleavage, shown in Figures 39, 40 (Plate **5**), and 104–106 (Plate **11**), have a well-marked bilateral arrangement. The cell $b^{4.2}$, at the anterior end of the egg, and also the cell $d^{4.2}$ have their spindles perpendicular to the sagittal plane of the future embryo, and their cleavage planes coincide with that plane. In the yolk-cell $d^{4.1}$ the mitotic spindle approaches parallelism with the chief axis, as in the third cleavage. In all the other cells the spindles are parallel with the long axis of the egg.

The seven "protoplasmic" cells divide as a rule equally and quite synchronously. Division of the yolk-cell $d^{4.1}$ is delayed more than in the preceding cleavage, but is completed while the fourteen "protoplasmic" cells are in the "resting" phase following division (Plate **5**, Fig. 41; Plate **8**, Fig. 67; Plate **11**, Fig. 108). The stage with all cells in the "resting" phase is composed of sixteen cells (Figs. 42, 43). The yolk-cell, as in the preceding divisions, has divided unequally, and the smaller, "protoplasmic" cell ($d^{5.2}$) thus formed lies in the median plane on the dorsal side of the embryo (animal pole) and immediately posterior to the cells $d^{5.4}$ and $d^{5.3}$, which have resulted from the division of $d^{4.2}$, the third micromere (Figs. 42, 44, 45, 68). This cell ($d^{5.2}$), formed by division of the yolk-cell $d^{4.1}$ in the fourth cleavage, is the *primary mesoblast*, as will appear from the subsequent history of its descendants, which sink beneath the blastoderm in a later stage. The yolk-cell $d^{5.1}$ is now purely *entoblastic*. The cells $a^{5.2}$, $b^{5.2}$, and $c^{5.2}$, which touch the yolk-cell on the anterior and lateral boundaries of its uncov-

ered ventral portion (Fig. 43) are mes-ectoblasts, and the remaining eleven dorsally-lying cells contain only ectoblast.

Figures 42–46 (Plate **5**), and 107–113 (Plates **11**, **12**), show the positions of the cells in the sixteen-cell stage, regarding which it will be sufficient to call attention to their bilateral arrangement. All the cells of the eight-cell stage, with the exception of the cell $b^{4.1}$, which lies at the vegetative pole (Fig. 40), divide so that their daughter cells both lie either on the right or on the left of the median plane of the embryo. The exceptional cell, $b^{4.1}$, divides in a plane parallel to the plane of the preceding cleavage, and, consequently, the daughter cells ($b^{5.1}$ and $b^{5.2}$) are not separated by a plane coinciding with the median plane of the embryo (see Figs. 40 and 43).

The regular and definite arrangement of the cells represented in the figures of the sixteen-cell stage is quite noticeable. This first suggested to me that the arrangement had arisen from an equally definite one in the earlier stages. Figures of a similar stage accompany the accounts of other investigators, who seem to have observed a constant arrangement of the cells in this stage.

At the sixteen-cell stage the "protoplasmic" cells have become extended far over the yolk-cell (compare Plate **5**, Fig. 40 with Fig. 45, and Plate **8**, Fig. 66 with Fig. 68). This extension is due in part to the addition of a new cell (the primary mesoblast) from the yolk-cell, but more especially to the spreading of the blastoderm, which is caused by division of the derivatives of the three micromeres (ab^2, c^3, $d^{4.2}$).

The blastopore is marked by that portion of the entoblast cell ($d^{5.1}$), which is still exposed to the exterior (Figs. 45, 46, 68), and it is widely open. Eggs with a relatively small amount of yolk have the blastopore more nearly closed; but, as will be shown later, the number and order of cleavages are constant whether an egg contains a large or a small amount of yolk.

Summary of the Fourth Cleavage.

A sixteen-cell stage is regularly formed with cells of particular origins occupying definite and constant positions in relation to other cells.

The derivatives of the three micromeres (ab^2, c^3, $d^{4.2}$) divide synchronously. The yolk-cell $d^{4.1}$ (mes-entoblast) is delayed in cleavage.

The primary mesoblast ($d^{5.2}$) is separated from the yolk-cell $d^{5.1}$, which is now entoblast.

The blastoderm is greatly extended during the fourth cleavage.

8. Fifth Cleavage. Thirty-two Cells.

All of the sixteen cells of the previous stage are involved in the fifth cleavage, but the primary mesoblast cell ($d^{5.2}$) and the yolk-entoblast ($d^{5.1}$) are greatly retarded in division (Plate **5**, Figs. 44–46). The fourteen cells of the blastoderm divide about synchronously, but occasionally some of the anterior cells slightly precede in the cleavage (Plate **5**, Figs. 44, 45; Plate **6**, Fig. 47; Plate **12**, Figs. 112, 113). The nuclear spindles for this cleavage are arranged perpendicularly to those of the preceding cleavage, with the exception of those in the three mes-ectoblast cells ($a^{5.2}$, $b^{5.2}$, $c^{5.2}$), which touch the yolk-cell at the blastopore (Fig. 46). The spindles in the cells $a^{5.2}$ and $c^{5.2}$ are always somewhat oblique to those of the preceding cleavage (compare Figs. 40, 45, 46). They appear to be arranged more or less at right angles to the lines along which the greatest pressure would be exerted by the contiguous cells of the blastoderm (see Figs. 45, 46), and the arrangement therefore seems to be in accord with the principle that spindles tend to become arranged in the line of least resistance.

The spindle in the median cell $b^{5.2}$ is sometimes placed almost longitudinally (Figure 113), in which case the resulting cells ($b^{6.3}$, $b^{6.4}$, Fig. 46) are arranged as in Figures 48, 52 and 116. Sometimes the spindle in $b^{5.2}$ is almost transverse (Fig. 112) and the resulting arrangement of the daughter cells is shown in Figure 51. Many intermediate oblique positions of spindle and cleavage plane have been noted. This, too, is apparently a case of adjustment to least resistance. In the next stage these two cells ($b^{6.3}$, $b^{6.4}$) become so shifted in position that they lie one to the right and the other to the left of the sagittal plane, but usually one is more or less in front of its companion. In the sixty-two-cell stage their derivatives always form the anterior boundary of the blastopore, although in the thirty-two-cell stage one of the cells ($b^{6.3}$) may not be in immediate contact with the yolk-entoblast, a condition shown in Figures 48 and 52.

In Figure 70 (Plate **8**) it is noticeable that the cleavage planes which separate the mes-ectoblasts $a^{6.3}$, and $c^{6.3}$ from their sister cells ($a^{6.4}$, $c^{6.4}$) are markedly oblique, so that the latter overlap the former. Attention is here called to the tendency of cells around the blastopore to divide in this manner, for in the succeeding stage there is a similar oblique division of $a^{6.3}$ and $c^{6.3}$, and the inner derivatives are overgrown by the outer overlapping cells.

About the time that the fourteen blastoderm cells have completed their division, the primary mesoblast cell ($d^{5.2}$) prepares to divide, its spindle being transverse to the long axis of the egg (Plate **5**, Fig. 48). The cleavage plane coincides with the sagittal plane of the embryo, and the resulting cells form the posterior boundary of the blastopore (Fig. 52). The constant and definite position of these two mesoblast cells, their retarded division, which gives them distinctive nuclear phases, their tendency to stain less intensely than other cells, the definiteness of the position and cleavage direction of the surrounding cells — all these features make it possible to identify positively the derivatives of the primary mesoblast cell ($d^{5.2}$) in this and the following stages.

The yolk-cell (entoblast, $d^{5.1}$) is the last cell to undergo the fifth cleavage; it commonly divides about the time that the blastoderm cells prepare for the next (sixth) cleavage; but at times the cleavage of the entoblast is so delayed as to be nearly simultaneous with the sixth cleavage of the blastoderm cells. The nuclear spindle is usually almost perpendicular to the sagittal plane (Figs. 52, 116, 117). A cleavage plane, dividing the yolk nearly equally makes its appearance at this stage, but it becomes more clearly visible about the time that the next division takes place in the blastoderm cells, and it may therefore be described later, in connection with the figures which illustrate the account of the sixth cleavage.

The blastoderm has been greatly extended since the last stage, owing to the multiplication of its cells by division, and to the accompanying increase of surface produced by the flattening of the cells. The blastopore has become less extensive as the yolk-cell (entoblast) has become more completely covered (Plate **6**, Figs. 51, 54; Plate **8**, Fig. 69). It is filled by the protoplasmic portion of the yolk-entoblast, and is bounded posteriorly by the two primary mesoblast cells ($d^{6.3}$, $d^{6.4}$), anteriorly and laterally by the four mes-ectoblast cells ($a^{6.3}$, $b^{6.3}$, $b^{6.4}$, $c^{6.3}$). With the exception of these four cells, which are in contact with the yolk-entoblast at the blastopore, all other cells of the blastoderm are purely ectoblastic.

Figures 47–55 (Plate **6**), 69, 70 (Plate **8**), and 114–117 (Plate **12**), show the details of cell arrangement in the thirty-two-cell stage. There is slight variability in the adjustment of the cells to one another, but examination of the figures shows that the relative positions of the cells are the same in all cases. In good transparent preparations I have seen hundreds of eggs in the thirty-two-cell stage conforming to the conditions shown in the figures, very few in which the arrangement of

the cells could not have been harmonized with the general plan indicated by the direction of the spindles of the fifth cleavage as represented in Figures 44–47.

Summary of Fifth Cleavage.

The blastoderm cells of the sixteen-cell stage divide synchronously. The primary mesoblast ($d^{5.2}$) and yolk-entoblast ($d^{5.1}$) are greatly delayed in cleavage.

The blastoderm has extended far over the yolk-entoblast.

Regular arrangement of cells of definite origin is as characteristic of this as of preceding stages.

9. Sixth Cleavage. Sixty-two Cells. Closing of the Blastopore. The Germ-Layers.

The twenty-eight cells of the blastoderm of the thirty-two-cell stage are the first ones to undergo the sixth cleavage. Cases are often seen in which all of the blastoderm cells have spindles arranged approximately perpendicular to those of the preceding cleavage. About the time that the resulting fifty-six cells pass into the "resting" phase the two daughter cells of the primary mesoblast ($d^{6.3}$, $d^{6.4}$) are found to be in division. The two entoblast nuclei ($d^{6.1}$, $d^{6.2}$) remain undivided until a much later stage. The sixth cleavage, therefore, results in the formation of a sixty-two-cell stage.

A preliminary description of the sixty-two-cell stage resulting from the sixth cleavage will aid in the discussion of the details of that cleavage. Figure 56 (Plate **7**) represents an optical sagittal section of an egg with closed blastopore. All of the twenty-eight blastoderm cells of the preceding stage have divided. The two yolk-entoblasts ($d^{6.1}$, $d^{6.2}$) have not divided. The two mesoblast cells ($d^{6.3}$, $d^{6.4}$) are in the sixth cleavage. Two cells ($b^{7.5}$ and $c^{7.5}$) are represented between these mesoblasts and the blastoderm in the region of the closed blastopore. These two cells contribute to the mesoblast of the embryo, and for purposes of description they may be called the "secondary mesoblasts," to distinguish them from the mesoblasts, $d^{6.3}$ and $d^{6.4}$, which are derived from the primary mesoblast $d^{5.2}$ (Plate **5**, Figs. 44, 45), which was separated from the yolk-entoblast in the fourth cleavage. Referring to Figures 72 and 73 (Plate **8**), which represent transverse sections, it will be seen that there are two pairs of "secondary mesoblasts" (*ms'bl'.*), an anterior pair, $b^{7.5}$ and $b^{7.7}$ (compare Plate **7**, Fig. 62), and a posterior pair, $a^{7.5}$ and $c^{7.5}$. The series of sections represented by Figures 74–77 (Plate **9**) shows con-

clusively that there are, besides the four "secondary mesoblasts," two entoblasts and two dividing primary mesoblasts in the egg of this stage. The cells of the anterior pair of "secondary mesoblasts" ($b^{7.5}$, $b^{7.7}$) are always hemispherical in form (Fig. 73), while those of the posterior pair are flattened between the primary mesoblast cells ($d^{6.3}$, $d^{6.4}$) and the blastoderm (Fig. 72). It also appears from the figures that the two derivatives of the primary mesoblast ($d^{5.2}$), the two pairs of "secondary mesoblasts," and the two entoblasts, are arranged according to a plan of bilateral symmetry. The division plane in the yolk (Fig. 73) is the cleavage plane formed between the entoblast cells during the fifth cleavage. With this brief description of the sixty-two-cell stage we may now turn to a more detailed consideration of the sixth cleavage, which formed the stage.

The large number of small cells and the absence of "landmarks" makes rapid and certain identification of individual cells of the blastoderm on the dorsal surface impossible in the sixty-two-cell and later stages. By carefully comparing drawings of stages in which the cells of the blastoderm are in early and late stages of mitosis, it is often possible to identify all the individual blastoderm cells in the sixty-two-cell stage. But since it is impossible to follow the blastoderm cells to their fate in organs of the Nauplius, I have not attempted to give in this account the lineage of all cells after the thirty-two-cell stage. After that stage the most important cells concerned with the germ-layers are near the blastopore. These are followed easily and with certainty.

During the fourth and fifth cleavages the blastoderm was greatly extended by the flattening of its cells and by the increase of surface associated with cell-division. This is repeated during the sixth cleavage, and the result is that the blastoderm in the majority of cases is completed, the yolk-entoblast cells being no longer exposed to the exterior at the blastopore (see Plate **7**, Fig. 56, and Plate **8**, Fig. 71).

In most cases a very small opening between the blastoderm cells represents the remnant of the blastopore. In fact the cells bounding the blastopore rarely come so closely together in this stage as to completely obliterate the opening (see Plate **7**, Figs. 57, 60, 62; Plate **8**, Fig. 71; Plate **2**, Fig. 76). This persistence of the blastopore has been of great service in determining the origin of the "secondary mesoblasts" and in the orientation of succeeding stages.

Along with the growth of the blastoderm over the blastopore during

the sixth cleavage, the two primary mesoblast cells ($d^{6.3}$, $d^{6.4}$) are crowded into the yolk beneath the blastoderm, pushing the two entoblast nuclei deeper into the yolk (Plate **7**, Fig. 59). The primary mesoblast cells thus come to lie beneath the blastoderm at the posterior end of the embryo. As in the two preceding stages, they are easily identified by their distinguishing features, and furthermore the divisions of all surrounding cells are accounted for, so that there can be no doubt of the lineage of the primary mesoblast cells. In series of eggs in various phases of the sixth cleavage the primary mesoblast cells have been seen in their successive positions, from that of the thirty-two-cell stage to that of the sixty-two-cell stage. At a time when some ectoblastic cells are undivided and the blastoderm is not completed, the two primary mesoblast cells are seen filling the blastopore and in part exposed to the exterior, but as the blastopore becomes closed they sink into the yolk, and the blastoderm closes over them.

The primary mesoblast cells ($d^{6.3}$, $d^{6.4}$), before the sixth cleavage takes place in them, may be symmetrically placed with reference to the sagittal plane (Plate **7**, Fig. 64; Plate **8**, Fig. 72; Plate **12**, Fig. 120); but more often one ($d^{6.3}$) is found in a position dorsal or anterior to the other (Figs. 56, 59, 60, 71). In the majority of eggs the two cells appear to have undergone torsion as the blastoderm closed around and over them. In the thirty-two-cell stage they are usually symmetrically placed side by side, but even in this stage there may be some shifting, as shown in Figure 52 (Plate **6**). Figures 62 and 63 (Plate **7**) show a very common condition, in which they have been so turned that the cleavage plane between them no longer coincides with the sagittal plane. In all such cases they appear to retain their original positions with reference to the right and left sides of the embryo. The various positions occupied by these cells may be the result of shiftings in adjustment to least resistance at the time when the overgrowing blastoderm crowds them inwards.

The spindles concerned with the sixth cleavage of the two derivatives ($d^{6.3}$, $d^{6.4}$) of the primary mesoblast cell are more often about perpendicular to the long axis of the egg (Plate **7**, Fig. 56), but sometimes almost parallel to that axis; all intermediate conditions are seen. In Figures 65 (Plate **7**) and 121 (Plate **12**) the two cells are represented as having completed the sixth cleavage, so that there exists a stage with sixty-two cells. Immediately after division the four resulting cells ($d^{7.5-8}$) are rounded, as shown in Figure 65, but soon afterwards

they become flattened and massed together at the extreme posterior end of the egg (Fig. 121).

The amount of yolk in the entoblast cells is in some eggs so great that the blastoderm cannot completely close over the blastopore during the sixth cleavage. Eggs are sometimes seen in which all the blastoderm cells have undergone the sixth cleavage and the two primary mesoblasts, still in division, are seen lying in the blastopore, and projecting far into the yolk (Plate 7, Figs. 60, 61). The anterior pair of "secondary mesoblasts" ($b^{7.5}$, $b^{7.7}$) are seen in their usual place beneath the blastoderm immediately in front of the anterior edge of the blastopore; but the posterior pair ($a^{7.5}$, $c^{7.5}$), which originates from cells lying at the sides of the blastopore, are seen at the surface at the side of the primary mesoblasts (Fig. 60). As these primary mesoblast cells complete the sixth cleavage they move farther into the yolk. Their positions with reference to the surrounding blastoderm cells (Fig. 61) suggests that the change of form during cleavage results in a movement of the dividing cells into the yolk, in which direction there is, apparently, the least resistance. The posterior pair of "secondary mesoblasts" ($a^{7.5}$, $c^{7.5}$) sink below the level of the surface as the blastoderm closes over the blastopore. In many cases this closing is evidently brought about by the next (seventh) cleavage of the blastoderm cells. Certainly the blastopore is always closed and both the primary and "secondary mesoblasts" are completely covered by the blastoderm after the seventh cleavage.

The origin of the two pairs of the "secondary mesoblasts" now remains to be described. Careful study of the cleavage in numerous eggs gives evidence that these are the result of the sixth cleavage in the four blastoderm cells, $a^{6.3}$, $b^{6.3}$, $b^{6.4}$, $c^{6.3}$, which form the lateral and anterior boundaries of the blastopore in the thirty-two-cell stage (Plate 6, Figs. 51, 52). These four blastoderm cells have their spindles for the sixth cleavage arranged more or less perpendicular to the surface, as shown in Figures 58 and 59 (Plate 7). The anterior pair of "secondary mesoblasts" ($b^{7.5}$, $b^{7.7}$) lies in front of the anterior edge of the blastopore, as is shown in Figure 57, which represents a section through an egg with incompletely closed blastopore. This is exactly the position of the cells $b^{6.3}$ and $b^{6.4}$ in the thirty-two-cell stage (Fig. 51). In Figures 58 and 59 (Plate 7) these cells are shown with spindles (sixth cleavage) somewhat inclined from a perpendicular to the surface. Their relation to the blastopore leaves no doubt that they are the cells $b^{6.3}$ and $b^{6.4}$ of the thirty-two-cell stage.

It has been stated in the account of the preceding cleavage that the cell $b^{6.3}$ does not always touch the anterior edge of the blastopore (see Plate 6, Figs. 48 and 52), for the reason that the cleavage plane between $b^{6.3}$ and $b^{6.4}$ may vary in position from perpendicular to the long axis of the egg to coincidence with the sagittal plane of the embryo. In any event it seems certain that these two cells always form the anterior pair of "secondary mesoblasts." In cases like that represented in Figures 48 and 52, the cells become shifted during the sixth cleavage, so that the plane between them approaches coincidence with the sagittal plane of the embryo — the common position of these cells in the thirty-two-cell stage.

The position of the posterior pair of "secondary mesoblasts" with reference to the anterior pair and also to the blastopore leads to the unavoidable conclusion that they are cut off from the cells $a^{6.3}$ and $c^{6.3}$, which are at the sides of blastopore in the thirty-two-cell stage (Figs. 51, 52). These cells are represented in Figures 58 and 59 (Plate 7) as dividing. From their position later, I infer that as division progresses the extension of the blastoderm causes these cells to approach the median plane, where they meet and complete the closing of the blastopore. At the same time the primary mesoblasts $d^{6.3}$, $d^{6.4}$ are overgrown by the blastoderm, and the cells $a^{6.3}$ and $c^{6.3}$ complete their division into the outer cells ($a^{7.6}$, $c^{7.6}$), which remain in the blastoderm, and the inner cells ($a^{7.5}$, $c^{7.5}$), which constitute the posterior pair of "secondary mesoblasts," lie between the blastoderm and the primary mesoblasts (see Plate 7, Fig. 62; Plate 8, Fig. 72).

Cases like those illustrated by Figures 60 and 61 (Plate 7) give additional evidence in support of the above interpretation of the origin of the "secondary mesoblasts." In the egg represented in Figure 60 a remnant of the blastopore is present and at its anterior edge are the two blastoderm cells $b^{7.6}$, $b^{7.8}$. Immediately beneath them are the derivatives $b^{7.5}$ and $b^{7.7}$, the anterior pair of "secondary mesoblasts." In the egg represented in Figure 71 (Plate 8) the primary mesoblasts ($d^{6.3}$, $d^{6.4}$) have sunk beneath the blastoderm. The same relations exist between blastopore and anterior "secondary mesoblasts." Similarly in Figure 62 the posterior "secondary mesoblasts" lie beneath the cells $a^{7.6}$ and $c^{7.6}$, which bound the sides of the blastopore. These cells are contiguous to $b^{7.6}$ and $b^{7.8}$. The same relations hold in Figure 60 and in Figures 58 and 59 (Plate 7), which represent the divisions forming the "secondary mesoblasts." Comparison of the arrangement of the cells around the blastopore in the thirty-two-cell stage (Plate 6, Figs. 51, 52) with

the cell arrangement and spindles as shown in Figure 58, 60 and 62 gives evidence entirely in favor of the explanation given of the cell-lineage of the "secondary mesoblasts." They are certainly derived from the ectoblastic cells of the blastoderm, and the evidence completely supports the interpretation that they are derived directly from the cells bounding the blastopore laterally and anteriorly in the thirty-two-cell stage.

The cell-lineage of the "secondary mesoblasts" is, then, as shown in the following table (see also complete table of the cell-lineage on page 135).

$b^{6.3}$ < $b^{7.5}$ right anterior "secondary mesoblast" cell.
$b^{7.6}$ blastoderm cell (ectoblast).

$b^{6.4}$ < $b^{7.7}$ left anterior "secondary mesoblast" cell.
$b^{7.8}$ blastoderm cell (ectoblast).

$a^{6.3}$ < $a^{7.5}$ left posterior "secondary mesoblast" cell.
$a^{7.6}$ blastoderm cell (ectoblast).

$c^{6.3}$ < $c^{7.5}$ right posterior "secondary mesoblast" cell.
$c^{7.6}$ blastoderm cell (ectoblast).

It will be noticed that "secondary mesoblast" originates from the quadrants *a*, *b*, and *c*. One cell each is contributed by *a* and *c* but two cells come from *b*. Tracing the lineage to the three micromeres which are separated from the yolk-macromere in the first three cleavages, it is found that only the first (ab^2) and the second (c^3) contain "secondary mesoblast"; the third ($d^{4.2}$) is purely ectoblastic.

After the sixty-two-cell stage the derivatives of the "secondary mesoblasts" have not been distinguished from those cells which were derived from the primary mesoblast. The cells of the two origins become mingled together and there appear to be in Lepas no distinguishing characteristics. Hereafter the term mesoblast (*ms'bl.* in the figures) will be used in the description as including the mesoblast cells of the two origins.

The entoblast nuclei ($d^{6.1}$, $^{6.2}$) are always near the primary mesoblast cells, but, as shown in the figures, they occupy no constant position in relation to particular cells. They stain more intensely than the nuclei

of the mesoblast cells, and in good transparent preparations of the entire egg are easily recognizable. The cleavage plane separating the yolk-entoblast cells may occupy various positions at this stage. If the primary mesoblasts are symmetrically placed with reference to the median plane (Plate 7, Fig. 64), the cleavage plane in the yolk coincides approximately with the sagittal plane of the embryo; but when one of the primary mesoblasts is anterior or dorsal to its sister cell, the plane of separation between the entoblasts is inclined towards the horizontal, or, if vertical, is oblique to the long axis, as in Figure 63. In all cases it appears to extend from near the plane separating the right and left primary mesoblasts towards the antero-dorsal side of the embryo (Figs. 63, 64, 65, 73). This relation suggests that the horizontal and oblique positions are secondary and due to movement of the yolk when the primary mesoblast cells are forced beneath the blastoderm and adjusted to unsymmetrical positions. The fact that when the primary mesoblasts retain their original symmetrical relation, the cleavage plane in the yolk is found apparently coinciding with the sagittal plane, lends support to this view.

It may be of interest to notice that the cleavages involved in the segregation of the germ-layers are always the same, no matter whether the blastoderm is completed in the sixth or seventh cleavages. The cleavages separating from the yolk-cell the micromeres which form the blastoderm are not variable in number, but definite (three); and there is no variation in regard to the number of micromeres which produce the variable numbers of blastoderm cells required to cover the yolk. This conclusion is opposed to that of Groom ('**94**, p. 141). (See review of literature on late cleavage.) This relation is exactly what has been found in the case of the eggs of gasteropods and annelids, in which it has been shown (Conklin, '**97**, pp. 61–63) that the number of micromeres (ectoblasts) separated from the macromeres (mes-entoblast) is constant for all species which have been studied, although the macromeres in some cases are very large and require a large number of ectoblastic cells to complete the blastoderm; in such cases — precisely as in Lepas anatifera and L. fascicularis — there is more subdivision of the micromeres before the blastoderm is completed. It appears that the same relation exists in the case of the other species of Lepas.

Summary of Sixth Cleavage.

All derivatives of the three micromeres (ab^2, c^2 and $d^{1.2}$) and of the two primary mesoblasts ($d^{6.3}$, $d^{6\,4}$) undergo division. The two entoblast

cells remain undivided. The "resting" stage following the sixth cleavage normally consists of sixty-two cells.

By the extension of the blastoderm during the sixth cleavage the blastopore is usually closed. As to the method of closing the blastopore, this account completely disagrees with Groom ('94; see also review of literature on the closing of the blastopore).

During this cleavage the two primary mesoblasts sink beneath the blastoderm as it closes over the blastopore.

Four blastoderm cells, derived from cells a^3, b^3 and c^3 (the first and the second micromeres, ab^2 and c^3), are divided parallel with the surface, thus cutting off four cells which lie in the yolk beneath the blastoderm. These are designated "secondary mesoblasts."

The mesoblast is, then, derived from each of the four quadrants of the four-cell stage. In the cells a^3, b^3 and c^3 there is mesoblast in connection with ectoblast (ectoblastic mesoblast), whereas in the d quadrant the mesoblast arises directly from entoblast, and may be designated entoblastic mesoblast. The origin of the mesoblast in Cirripedia has not heretofore been traced accurately (see review of the literature on the germ-layers).

All cells sharing in the formation of the lip of the blastopore in the thirty-two-cell stage, as represented in Figure 51, contribute to the mesoblast.

The blastoderm is composed of derivatives of *three*, and only three, micromeres (ab^2, c^3, $d^{4.2}$), even when the size of the yolk-mass does not permit of the blastopore being closed until the following cleavage.

10. Seventh Cleavage. The Mesoblast.

The sixty-two-cell stage has been described as embracing fifty-two ectoblastic cells composing the blastoderm, which has usually grown over the blastopore; eight mesoblast cells, of which four have been designated as "secondary"; and two entoblast cells, resulting from the division of the yolk-macromere. All these, excepting the two entoblast cells, divide more or less synchronously and form a stage which may be estimated to consist of about one hundred and twenty-two cells. The planes of cleavage appear in most cases to be perpendicular to those of the sixth cleavage. For convenience in description this may be designated the seventh cleavage.

Figures 78–80 (Plate 9) represent a series of parasagittal sections through an egg of the 122-cell stage, but some of the cells have not completed the seventh cleavage. Figures 81–86 represent a series of

transverse sections of the same stage, of which 81 is the most posterior. In the blastoderm at this stage there is nothing worthy of note except the indentation which marks the former position of the blastopore. The cells in this region are rarely as closely arranged as in the other parts of the blastoderm.

The mesoblast cells are crowded together, and it is impossible to distinguish in all cases between those derived from the primary mesoblast and those from the "secondary mesoblast." As used in the description of later stages, the term mesoblast includes both the primary and "secondary mesoblast."

The possibility of origin of mesoblast cells from the blastoderm after the sixth cleavage has been kept in mind during the observations, but there is no evidence of such an origin. The cleavage spindles in all parts of the embryo have been seen, but not one perpendicular to the surface has been detected. Moreover, the mesoblast cells have been repeatedly counted in sections and their nuclei have also been counted in transparent preparations of the entire egg, and there have never been seen more cells than could be accounted for by the division of the eight mesoblast cells described in the sixty-two-cell stage.

It should be mentioned that by rapid decolorization of specimens stained in borax carmine it has often been found possible to draw the color from the nuclei of the blastoderm cells and stop the reaction while the mesoblast nuclei were still brilliantly stained. With such preparations it is easy to count the nuclei of the mesoblast cells in the entire egg. This method has been employed in all the stages with mesoblast.

The entoblast nuclei are stained brightly by this carmine method, and are easily identified in transparent preparations of entire eggs, as well as in sections. In all stages between that of thirty-two cells and that with about one hundred and twenty cells there is no evidence of division of these nuclei. In these stages only two "resting" nuclei are to be found in the yolk, as shown in Figures 78–80 and 81–86 (Plate 9). Usually in the 120-cell stage the two nuclei are enlarged, while the chromosomes are distinct. Evidently the nuclei are preparing for division, but the spindles are rarely seen until after the blastoderm cells have divided again. In the resulting stage, with about two hundred and fifty cells, four entoblast nuclei are often seen. It does not seem possible that there can have been an overlooked division of these nuclei. Moreover, the origin of the mesoblast cells has been determined to be independent of the two entoblast cells, which are seen in this and in the preceding stage.

Summary of the Seventh Cleavage.

All cells, except the two entoblasts, divide.

Derivatives of the two kinds of mesoblast have not been distinguished after the cells are crowded together at the posterior end.

There is no evidence that mesoblast originates otherwise than as described in the preceding account of the sixth cleavage. The entoblast nuclei have been traced from the sixteen-cell stage and there has been but one division. Hence, contrary to the assumption of earlier investigators, the entoblast nuclei cannot contribute to the mesoblast (see the following review of the literature).

11. Review of Literature on Late Stages of Cleavage, on Closing of the Blastopore, and on Differentiation of the Germ-Layers.

a. Late Cleavage. — Groom ('**94**) did not follow the later cleavages in detail, because his results showed so great variation in the early stages. He describes the later growth of the blastoderm over the yolk as "taking place in precisely the same manner as in the earlier stages, i. e., by the emergence of merocytes from the yolk and the division of blastoderm cells. . . . The variation is so great that the process may be said to be irregular. . . . I am unable to say how many merocytes take part in the formation of the blastoderm; but in all probability the number is variable, but not large. As the ovum is often half covered when four or five have emerged, some such number as nine or ten may not be far from the mark" (Groom, '**94**, pp. 140, 141).

The supposed variation in early stages of cleavage has already been discussed in the reviews of the literature on those stages. The later cleavage and growth of the blastoderm have been shown in this paper to be very regular, and the variations upon which Groom has placed much stress are comparatively rare. These variations can usually be ascribed with strong probability to unfavorable conditions in the environment of the developing egg. The number of "protoplasmic" cells (micromeres) formed from the yolk-cell has been shown to be not variable (nine or ten), as Groom supposed, but constant, viz. *four*, of which the first *three* — containing all the ectoblast and "secondary mesoblast" — are separated from the yolk by the first three cleavages, while the fourth cleavage differentiates the primary mesoblast from the yolk-entoblast. Groom's statement (p. 198) that epiblastic cells continue to be

formed at the expense of the yolk-cell until the blastopore closes, is completely disproved by the facts of cell-lineage.

b. Closing of Blastopore. — Groom did not see the closing of the blastopore in L. anatifera, but he ('94, p. 141) described it for other species as follows: "The end of the yolk projects out at one point as a small rounded elevation. . . . A merocyte appears in the centre of this, and fills the gap between the surrounding cells, and finally emerges from the yolk as the blastomere."

This description is far from being in harmony with the facts in the case of L. anatifera. The closing of the blastopore has been shown in this paper to be due to the repeated divisions of the ectoblastic derivatives of the three micromeres (ab^2, c^3, $d^{4\cdot2}$) which are separated from the yolk-macromere in the first three cleavages. The "merocyte" which Groom saw in the blastopore (see his Fig. 127) is represented by the protoplasmic mass concentrated around the nucleus of the entoblast cell, which is situated as shown in my Figure 54 (Plate 6). I have shown by tracing the cell-lineage that this cell divides (Fig. 52, fifth cleavage), usually before the closing of the blastopore, sometimes during the sixth cleavage of the ectoblastic cells, and that the resulting entoblast nuclei are later found deeper in the yolk. Nussbaum observed in Pollicipes a division of the yolk before the blastopore closed. Groom ('94, p. 147) states that this may rarely occur, a condition which is completely at variance with his account of the closing of the blastopore.

The evidence presented in the present account of the cell-lineage leads to the conclusion that no cell is cut off directly from the yolk to fill the blastopore. It has been shown that at the time of closing there are two nuclei in the yolk, not as Groom stated, a single one. Hence Groom's conclusion, that the "merocyte" which fills that blastopore "before becoming shut off as a blastomere, gives off a single nucleus into the yolk" ('94, p. 198), cannot be accepted. The evidence is completely opposed to such a view. It appears that in Groom's account of the closing of the blastopore, his view of "emerging merocytes" has led, as in the early stages, to an erroneous interpretation.

c. Differentiation of the Germ-Layers. — Groom's account of the "meso-hypoblast" agrees in general with the descriptions of all the earlier authors, who regarded this as represented by the yolk-cell, or cells, after the closing of the blastopore. Groom ('94, p. 146) writes: "The closing of the blastopore is almost immediately followed by the division of the yolk into two pyramids or segments; the formation of the mesoblast immediately commences by the successive cutting off and

sub-division of nucleated segments from the two yolk segments." According to Groom these yolk-segments after separation of the mesoblast divide and form endoderm cells.

In opposition to this it has been shown in the present paper that the mesoblast clearly does not originate directly from the yolk-cells after the closing of the blastopore; but from certain cells which have been designated in this account as primary and secondary mesoblasts. The origin of all these cells has been definitely traced. Moreover, evidence has been presented to show that the two yolk-entoblasts do not begin to divide after the thirty-two-cell stage until at least one hundred and twenty cells are present, of which more than a dozen are mesoblastic. Since the entoblast cells do not divide during these stages, they cannot be the direct progenitors of any of the mesoblast cells. All the evidence given seems conclusive and opposed to Groom's interpretation.

The figures of Groom fail to establish his conclusions regarding the origin of mesoblasts from yolk-entoblasts, for in no case are nuclear spindles, the only unimpeachable evidence of such origin, shown. His interpretation of the origin of mesoblast cells seems to be based upon their position. In numerous preparations I have seen all the conditions which Groom figures, but I have found no evidence opposed to my interpretation of the origin of the mesoblast. Groom did not have transparent preparations of entire eggs, and his account of the mesoblast is based entirely upon sections. His figures represent isolated sections, when in many cases only complete series of sections would be convincing. His erroneous conclusion, that the mesoblast is cut off in a series of divisions occurring in a pair of yolk-cells ("meso-hypoblast"), may have resulted from certain conditions which I have frequently noted. Sometimes in stained sections the cell-boundaries of the mesoblast cells are invisible, they appearing to be continuous with the yolk. Under such conditions the mitotic spindles of the mesoblast cells might easily be mistaken for division of the yolk-cells to form new mesoblast cells. I have seen many such cases which exactly simulated some of Groom's figures, but after removal of the cover glass and restaining, the cell-boundaries of the mesoblast cells and the nuclei of the yolk-entoblasts appeared as usual.

Nussbaum ('90) described the mesoblast in Pollicipes as formed by the division of blastoderm cells surrounding the blastopore before it closes. The mesoblast was said to grow inwards and anteriorly over the yolk. The account of the origin of mesoblast given in the present paper makes it probable that Nussbaum's description is in a general way correct. Had

not the details of the cell-lineage been traced in Lepas, I should be led to describe in similar general terms the origin of the mesoblast. I infer from Nussbaum's description that in Pollicipes the blastopore does not become closed as early as in Lepas. It seems probable that in Pollicipes the primary and secondary mesoblast cells may undergo some divisions before they are forced beneath the overgrowing blastoderm. Such a process would have the appearance of the production of mesoblast from the blastoderm cells at the edge of the blastopore.

In stages preceding gastrulation Nussbaum saw two large cells at the posterior pole, but he lacked material for following out their history. It seems probable that he saw the two primary mesoblasts which I have seen in the thirty-two-cell stage of Lepas.

12. Determinate Cleavage.

The small size and large number of cells make it impossible to determine the lineage of the individual cells of the embryo beyond the sixty-two-cell stage, and they cannot therefore be traced directly to particular organs of the Nauplius. However, the great regularity and constancy of preceding stages renders it extremely probable that the cells are destined for definite organs. Cells of definite origin have been traced to definite positions in the later cleavage stages. Careful observation has given no evidence of changes in position of cells taking place after the completed segregation of the germ-layers. Indeed the beginning of irregularity is scarcely to be expected in such late and well differentiated stages of development. The regions of the embryo from which particular organs arise have been definitely traced to groups of cells of known lineage. There seems to be no reasonable doubt that the cells of the late cleavage stages are destined to enter into the formation of particular organs. The cleavage of Lepas is, then, an example of what Conklin ('98) has termed "determinate cleavage."

The conclusions in the preceding paragraph on "determinate cleavage" are widely at variance with those of all previous writers on cirripede development. The early development of the ova of cirripedes has always been regarded as irregular and indeterminate. Great variations have been said to occur.

Groom ('94, p. 199) summarizes his study of the cleavage of various Cirripedia as follows: — "In describing the details of division of the cells of the blastoderm and yolk-endoderm much variation has been shown to occur, so much indeed that the process may be termed irregu-

lar. Such differences show well the morphological insignificance of the details of cell division in the present case, for the Nauplii vary proportionately much less; every one of the numerous, simple, or compound bristles or spines of the Nauplius has its definite character and position, which are maintained with surprising constancy throughout, although they must have been produced by epiblast cells having very different modes of origin and arrangement."

In the preceding account of the various stages of cleavage this supposed great variation in development has been discussed. It has been shown that the development is extremely regular, and that there is not the slightest foundation for views such as those above quoted.

In a preliminary paper on L. fascicularis (Bigelow, '96) the results were summarized as follows: — "In all important respects the cleavage of L. fascicularis is as regular as is ordinarily found in other Metazoa. All previous observers have failed to recognize any definite order in the cleavage of cirripede ova. It has always been described as exceedingly variable, irregular and *sui generis*. There is undoubtedly some irregularity and variation in the cleavage of the ova of those cirripedes where a great amount of yolk is present. However, as will be pointed out in a future paper, the cleavage of these forms, when interpreted by the cleavage of L. fascicularis, is seen to follow a much more regular order than has been supposed."

Later studies have completely supported this interpretation, and even the irregularity of development which I formerly believed to exist in the case of those cirripedes whose ova have much yolk, appears not to exist in the course of normal development. More extended study has shown that L. anatifera, one of the forms which I at first interpreted as somewhat variable in its development, is extremely regular. Studies now in progress on other genera support the conclusion which I have drawn from L. fascicularis and L. anatifera, namely, that the evidence derived from a study of cell-lineage indicates that *the development of Lepas is as regular as the well known cases among gasteropods and annelids.*

13. Notes on Cleavage and Germ-Layers in L. Fascicularis.

The early development of Lepas fascicularis is so closely like that already described in the case of L. anatifera that extensive special description is unnecessary, but some remarks are needed in order to correct and supplement a preliminary note on this species which I published in 1896.

Figures 95–121 (Plates **11**, **12**) show how close is the resemblance to the cleavage of L. anatifera. Except in size and some unimportant details, the various stages of the two species are indistinguishable, and the description of the figures of L. anatifera may be applied to those of L. fascicularis.

A renewed study of the few old preparations, supplemented by many new ones, shows that I ('**96**) was wrong in the conclusion that the ectoblast is detached from the yolk-macromere by means of four successive divisions ('**96**, ectomeres *A*, *B*, *C*, and *D*). The supposed fourth ectomere ('**96**, Figs. 6 and 7 *D*) is the primary mesoblast cell. In origin and position it corresponds exactly with the mesoblast cell ($d^{5.2}$) seen in the sixteen-cell stage of L. anatifera. I now interpret the spindle seen in the yolk during the fifth cleavage ('**96**, Fig. 7), which was then supposed to represent the separation of the mesoblast and the entoblast, as a rare case of precocious division of the entoblast. Study of the complete series, with all mitotic phases represented, shows that in L. fascicularis, as in L. anatifera, the first, second, and third cleavages form micromeres containing the ectoblast and "secondary mesoblast," while the fourth cleavage separates mesoblast and entoblast from each other.

With regard to the planes of cleavage and orientation, I find no important disagreement with L. anatifera. The descriptions of the first and second cleavages in the preliminary note were similar to those of L. anatifera given in this paper. The rotation during the first cleavage was not then known. The equatorial nature of the third cleavage was not clearly shown by the figure of a four-cell stage with inclined spindles in the preliminary note; Figures 100–103 (Plate **11**) in this paper better represent the four-cell stage and the third cleavage. The figure of the eight-cell stage ('**96**, Fig. 6) was drawn from an egg which is now known to have been incorrectly oriented. Eggs which give exactly such camera tracings will, when properly oriented by moving the cover glass, always show the same arrangement of cells as that seen in Figures 104–106 in this paper.

Figure 6 of the preliminary paper represented a separation of mesoblast and entoblast (fourth cleavage), and not as was incorrectly assumed, the formation of a "fourth ectomere." Figures 108–110 are the corresponding figures in this paper.

The primary mesoblast cell, shown in Figure 8 of the preliminary paper as filling the blastopore, represented the delayed fifth cleavage, which was in progress. The single entoblast nucleus was not yet undergoing the fifth cleavage. The inferred connection between the spindle

in the yolk-cell, in the sixteen-cell stage, and the separation of a mesoblast cell is now known to have been an erroneous interpretation. The series of stages is now so complete as to leave no doubt that the mesoblast cell is separated from the yolk-entoblast in the fourth and not in the fifth cleavage.

In the sixty-two-cell stage the origin and position of cells is certainly the same as in L. anatifera. The "secondary mesoblasts" were observed and figured during my earlier studies, but were interpreted as derivatives of the primary mesoblast, which seemed to divide more rapidly than did the other cells. It now appears from a study of all phases of the sixth cleavage that there are eight mesoblast cells in the sixty-two-cell stage, only four of which are derived directly from the ectoblast. Up to this stage the divisions of the primary mesoblast are the same as have been described in detail in the case of L. anatifera. In living eggs recently studied, and also in preparations of favorably preserved material, I have observed the cell-wall between the two entoblast nuclei of this stage, and it follows that — contrary to my former supposition — there is no exception to the rule that every nuclear division during the cleavage is associated with total cell division.

VIII. Extension of the Mesoblast and Entoblast. Later Development of the Germ-Layers.

The mesoblast in the 122-cell stage consists of a mass of cells at the posterior end of the embryo, near the former position of the blastopore (Plate **9**, Figs. 78–86). The arrangement of the cells leaves no doubt about the position of the blastopore, but orientation of the succeeding stage is more difficult and uncertain. During the next division the embryo begins to elongate posteriorly. A comparison of the blastoderm cells on the ventral surface of the 122-cell and 250-cell (estimated numbers) stages leads to the suggestion that the elongation is due to flattening of the ventral blastoderm cells, while those on the dorsal surface remain columnar in form. At any rate, this elongation appears to be confined mostly to the ventral region of the blastoderm, anterior to the former position of the blastopore. The result is that the cells which closed the blastopore and the adjoining mesoblast cells are moved from the ventral surface towards the extreme posterior end, where for a time the mesoblast consists of a conical mass of cells (compare Plate **9**, Fig. 80 with Plate **10**, Fig. 87). The rapid division of the mesoblast cells produces a plate, which grows forward on the dorsal side of the embryo

(Fig. 87). That this plate of mesoblast is on the side of the embryo opposite that on which the blastopore was situated, is supported to some extent by the facts above mentioned concerning the posterior growth of the blastoderm. Further evidence of this is found in the columnar shape of the cells, which is characteristic for those on the dorsal side; moreover many embryos long retain a slight depression marking the place of the blastopore, and the blastoderm (ectoblast) cells in this region are often delayed in division in late stages, as well as in the earlier stages, as may be seen when the position of the blastopore is definitely known. It should also be mentioned that the second polar cell, which lies dorsally (animal pole) in the yolk at the anterior end, is often visible near the anterior extension of the mesoblast both in sections and in transparent preparations of entire embryos corresponding to Figures 87 and 88 (Plate **10**). These facts all seem to favor the conclusion that the forward growing band of mesoblast (Figs. 87, 88) is on the side opposite that occupied by the blastopore in earlier stages, and consequently opposite that on which the mesoblast extends farthest forward at the time of the closing of the blastopore (Plate **8**, Fig. 71; Plate **9**, Fig. 80).

Examination of Figures 88, 89 and 90 (Plate **10**), representing longitudinal and transverse sections, will give some idea of the direction and extent of growth in the mesoblast. A solid, conical mass of cells lies at the extreme posterior end and extends anteriorly as a broad band on the dorsal side (Fig. 88); this grows laterally towards the ventral side (Fig. 90). The mesoblast at first consists of a single layer of cells, which divide rapidly; the layer becomes many cells in thickness on the dorsal side, but gradually thinner towards the ventral edges of the band (Figs. 90, 92). At the same time that the extension of the mesoblast has been in progress, the entoblast cells have been dividing. Their cell-boundaries are often well defined, and the nuclei do not migrate far from the positions where they are formed by division (Figs. 91, 92).

The blastoderm has remained a single cell in thickness, as shown in the Figures 87–94.

As shown in the preceding chapter, Groom's (**94**) view of the origin of the mesoblast is erroneous, but the account which I have given of the extension of the mesoblast is, in essentials, entirely confirmatory of Groom's description of the same process. Groom has given many good figures of entire eggs, showing the appearance of the entoblast yolk-cells in living eggs of Lepas and Balanus. All my observations on these

stages agree essentially with his account. His figures showing the extension of the mesoblast closely correspond with those which I have given and described, not with an idea of contributing new facts, but in order to connect these stages with my account of the early development.

Groom interpreted the anterior growth of the mesoblast as taking place on the dorsal side, and I shall later give confirmation of this opinion, which rests on an orientation that I have used thus far without adequate proof.

IX. Formation of the Appendages of the Nauplius, and Development of the Organs.

With regard to these phases of the development, my observations are quite in harmony with the account by Groom ('94, pp. 151–154). A few figures have been placed in this paper in order to show relations to the early stages, but since there is such close agreement with Groom, it is unnecessary to give a detailed description and numerous figures.

Groom's important observation, that the appendages first appear on the side which has the band of mesoblast, and that this is dorsal, is supported by my Figures 91–94 (Plate **10**) and 122–126 (Plate **12**). All earlier writers on cirripede development had considered the mesoblast band as ventral (see review of literature in Groom's paper).

Figures 91 and 122 represent the first indication of the segmentation of the embryo. Two transverse furrows (*1*, *2*) appear on the dorsal side, and extend around towards, but do not reach, the ventral surface. The limit of extension of the transverse furrows corresponds closely with that of the underlying mesoblast. The body is divided by the two furrows into three regions, corresponding to the three segments of the Nauplius.

Soon after the appearance of the transverse furrows there appears a median longitudinal furrow on the same side (dorsal) of the embryo. This is shown in transverse section in Figure 92 and in dorsal view in Figure 125. This furrow intersects the two transverse furrows, but does not extend to the extreme end of the embryo. Two new transverse furrows now appear (3, 4, Figs. 93, 123–125), superficially dividing the anterior and posterior segments of the Nauplius. Earlier writers have published many drawings of these stages, and it seems unnecessary to insert similar ones in this paper.

The transverse furrows and the median longitudinal one deepen rapidly, and cut off the three pairs of appendages, as has been correctly described by Groom and earlier workers. The extension of the floor of

the longitudinal furrow laterally and ventrally is shown in Figure 94, which also shows the ectoblast and mesoblast composing the appendages. The deepening of the furrows progresses and the appendages are folded off commencing at their dorsal distal ends until finally their attachment is to the ventral side of the embryo, as determined by the position of the mouth and labrum (Figs. 124, 126). It will be seen that my account confirms Groom in that the mesoblast band and the furrows are dorsal, and that the appendages are folded off from dorsal to ventral, the free ends of the appendages remaining directed dorsally until about the time of hatching. Investigators before Groom gave good descriptions and figures of the formation of appendages, but considered that the mesoblastic band and the furrows were ventral instead of dorsal.

Many of my preparations and unpublished figures of later stages confirm Groom's account regarding the formation of the stomodæum and proctodæum, and the development of the mesenteron from the yolk-entoblast cells.

It is to be noted that many of Groom's minor observations on later stages were confirmatory of earlier writers, whose work he has reviewed, and it has, therefore, for my purposes been sufficient to refer directly to Groom's paper. For the details of late development of organs of the Nauplius, reference must be made to Groom and earlier workers, for this paper is concerned, primarily, with cleavage and germ-layer formation.

The fate of the germ-layers, which were identified in the sixty-two-cell stage, may be summarized as follows : — The ectoblast forms the outer covering of the body and appendages, the stomodæum, proctodæum, and the nervous system. The yolk-entoblast forms the mesenteron. The mesoblast forms the muscles and connective tissues of the appendages, and of the body of the Nauplius.

So far it has not been possible to distinguish between the fate of the primary and secondary mesoblasts. It can only be stated that at least a part of the muscular and mesenchymatous tissues of the Nauplius come from the ecto-mesoblast ("secondary mesoblast"). In other genera of Cirripedia an attempt is now being made at tracing the two kinds of mesoblast farther than has been possible in Lepas.

X. General Considerations on Cleavage and Cell-Lineage.

Korschelt und Heider ('90–91) have classed the cleavage of the cirripede ovum with their type II of crustacean cleavage — a type beginning with total cleavage, but soon changing to superficial. This classification

was evidently based upon Nassonow's figures of Balanus; but is shown to be erroneous by subsequent investigations. It is controverted in the case of Balanus, by the account of Groom, as well as by unpublished observations of my own; and in the case of Lepas it is clearly inapplicable. In both these genera cleavage is total and unequal.

Knipowitsch ('92) described the cleavage of the Ascothoracidan genus Laura as superficial from the very beginning of development. His figures do not warrant such a conclusion, for cell-boundaries appear to form after every nuclear division. The few figures of segmentating eggs in Knipowitsch's paper resemble the figures which other authors have drawn from the eggs of parasitic copepods; for example, Pedaschenko's ('93) figures of Lernæa. The latter is evidently a case of total, but very unequal, cleavage, and the cleavage of Laura is apparently to be interpreted in the same way.

Van Beneden's ('70) figures illustrating his account of the development of Sacculina indicate to my mind that the cleavage of Rhizocephalan Cirripedia is also of the unequal total type. Even the fact that in late stages the four yolk-macromeres appear to fuse does not support the interpretation that the cleavage is in later stages superficial. In no stage of the development is there nuclear division which is not associated with total cell division, and we are led to the conclusion that the cleavage of Sacculina cannot be correctly characterized as superficial in any stage.

Regarding the type of cleavage of cirripede ova, the conclusion is that, so far as present knowledge extends, the eggs undergo unequal total cleavage, and with respect to the cleavage processes there is no close resemblance to the superficial cleavage of the higher Crustacea; rather is the resemblance to that of the yolk-laden eggs of gasteropods.

In the order of the cleavages involved in the establishment of the germ-layers there are in Lepas some interesting resemblances to the annelids and mollusks. As is well known, studies of the cell-lineage of annelids, gasteropods, lamellibranchs, and chitons have shown that in all of these forms the ectoblast is separated from the mes-entoblast by three successive cleavages, while a fourth cleavage separates the primary mesoblast from the entoblast. Moreover, it has been shown in the cases of some gasteropods and lamellibranch mollusks, that the mesoblast is derived from both primary germ-layers; in addition to the primary mesoblast (*entoblastic* mesoblast) there are mesoblast cells which come from the ectoblast (*ectoblastic* mesoblast). This has been designated "secondary mesoblast" or "larval mesenchyme" (Lillie, '95, p. 24; Conklin, '97, p. 150).

So far it has not been shown conclusively that the mesoblast of annelids has a like double origin, but the studies of Wilson ('98) make it appear probable that in the annelid egg there is mesoblast of ectoblastic origin, which is comparable to the "secondary mesoblast" or "larval mesenchyme" of mollusks.[1]

It must be understood that, in offering the following suggestions of some resemblances between the cleavage of Lepas and the forms above mentioned, it is not here claimed that any cell homologies exist. Our knowledge of this subject is not as yet sufficiently extensive to warrant any decision for or against such a conclusion.

The fact that in Lepas the ectoblast is separated from the mes-entoblast by three successive cleavages, while the fourth separates the primary mesoblast from the entoblast is, at least, an interesting coincidence. The double origin of mesoblast is another point of resemblance, for in Lepas, as in gasteropods, lamellibranchs and probably annelids also, the ectoblast is a second source of mesoblastic cells.

In one important respect there seems to be a wide difference between the cleavage of Lepas and that of annelids and mollusks; for in these latter groups there are three quartets of ectoblastic micromeres formed by as many successive cleavages of four macromeres, whereas in Lepas there are not three quartets of cells but three cells formed in the same order of cleavage. In the annelids and mollusks the first segregation of ectoblast from entoblast is represented by the upper four cells (first quartet of micromeres) of the eight-cell stage, formed by the third cleavage, whereas in Lepas the first segregated ectoblast is one of the two cells formed by the first cleavage. Stated in other terms, in annelids and mollusks, unlike Lepas, the first and second cleavages are not directly concerned with the segregation of ectoblast from entoblast, but they divide the egg into a quartet of macromeres, each containing entoblast, from which in succession three quartets of ectoblastic micromeres are separated. In Lepas the segregation of ectoblast begins, as it were precociously, without the previous division of the entoblast into a quartet of cells. As a result of this there is in Lepas one entoblastic macromere instead of four, as in annelids and mollusks, and single micromeres appear to represent quartets. So far as the order of cleavage involved in the segregation of the primary germ-layers is concerned, the first micromere (ab^2) of Lepas apparently corresponds to the first quartet of

[1] Since this paragraph was written, several investigators have given support to the suggestion that there is a double origin of the mesoblast in annelids. See Treadwell (: 01, p. 427), Wilson (: 01, p. 891) and Torrey (: 02, p. 576).

ectoblastic micromeres seen in the eight-cell stage of such eggs as have four macromeres resulting from the quartet-forming (first and second) cleavages. The micromeres of Lepas are, then, according to this view, to be regarded as equivalent to quartets of micromeres, while the single yolk-macromere equals a quartet of macromeres. It must be recognized that there are great, perhaps irreconcilable, differences between the development of the cirripedes and that of annelids and mollusks, and that consequently, the above comparisons might be extreme, if they were to be used as evidence of the existence of cell-homologies. At present it is possible simply to compare the order of cleavages involved in segregating the germ-layers.

A similar relation in cleavage occurs within the group of the Cirripedia. Van Beneden ('70) showed that in the Rhizocephalan genus Sacculina, the first and second cleavages divide the egg into a quartet of yolk-bearing macromeres, all containing entoblast, from which a quartet of ectoblastic micromeres is separated by the third cleavage in the formation of the eight-cell stage. This is exactly the order of cleavages in the eggs of annelids and mollusks. In Sacculina, then, the first segregation of ectoblast occurs two cleavages later than in Lepas, in which there is precocious segregation of ectoblast. In Sacculina the first and second cleavages divide the egg into four yolk-bearing macromeres, each containing entoblast and ectoblast, and the segregation of the primary germ-layers begins at the third cleavage; but in Lepas the segregation begins at the first cleavage without subdivision of the egg into four quadrants. Comparing the four-cell stage of the two genera, the entoblast in Lepas is all concentrated into one of the four cells each of which in Sacculina contains entoblast. According to this view the first cleavage of Lepas corresponds to the third of Sacculina so far as the first segregation of ectoblast is concerned. Whether the first micromere of Lepas is homologous with the quartet of micromeres in Sacculina cannot be determined until the fate of those cells is traced in the latter genus. There is reason for inferring that in Sacculina other quartets of ectomeres are cut off from the yolk-macromeres and added to the ectoblast. This must be settled before any further conclusions can be drawn. The final result of the development — the Nauplius — is similar in Lepas and in Sacculina. A comparison of the cell-lineage of the two genera may be expected to yield some results bearing on the suggestion that possibly the micromeres (ab^2, c^3, $d^{4.2}$) of Lepas may be equivalent to quartets of ectoblastic micromeres in Sacculina, and possibly to those in more distantly related forms. These are

merely suggestions which have grown out of comparison of the order of the cleavages involved in segregating the germ-layers.

The segregation of the ectoblast as three micromeres is apparently not peculiar to Lepas among Entomostraca. The cleavage of certain parasitic Copepoda has close resemblances to that of Lepas as regards number of cleavages involved in the segregation of the germ-layers. In Lernæa, according to Pedaschenko ('93), the ectoblast and mesoblast are separated from the yolk-macromere (entoblast) by means of four cleavages. It will appear in the discussion of the germ-layers in the following section of this paper, that in the instance just cited the first three micromeres probably contain all the ectoblast with the "secondary mesoblast," while the fourth is the primary mesoblast; in this case, then, the number and order of cleavages involved in germ-layer segregation would agree with my observations on Lepas.

In the figures and accounts of the cleavages of various phyllopods and copepods, in which the germ-layers appear to be established as early as the thirty-two-cell stage, there are found many suggestions that further investigations may show a close resemblance to the cell-lineage of Lepas. Some examples of such suggestive papers are those of Grobben ('79, '81) on Moina and Cetochilus, Urbanowicz ('86) and Häcker ('92, '97) on Cyclops, and Pedaschenko ('93) on Lernæa; but in none of these genera are the facts as yet sufficiently well known to warrant close comparison with Lepas, especially since there is much disagreement between the observations of these investigators. At present this mention of a possible resemblance to the cleavage of Lepas can have only the value of a suggestion, which may possibly stimulate comparative study of the cleavage of those Entomostraca in which the early segregation of the germ-layers makes it possible to trace the lineage of the cells to the complete separation of the germ-layers.

The cleavage of Lepas has some general resemblances to that of the nematodes. Particularly is there resemblance in the early segregation of the germ-layers; but, as to the order of cleavage involved in this process, there are great and at present irreconcilable differences. The first cleavage in Nematoda begins the separation of the germ-layers. Thus the cell ab^2 contains ectoblast in the nematodes as in the cirripede, and cd^2 contains ectoblast and mes-entoblast. The second cleavage in the nematodes completes the segregation of the mes-entoblast from ectoblast, whereas this is accomplished by the third cleavage in Lepas. It is obviously impossible to make any comparison of the details of the early development.

In certain respects the cell-lineage of Lepas recalls that of some rotifers, as described by Zelinka ('91) and especially by Jennings ('96). In the rotifers, as in Lepas, the separation of the primary germ-layers begins with the first cleavage, the cell ab^2 being ectoblastic, and cd^2 containing ectoblast in addition to all the entoblast. Still more remarkable is the resemblance in that the entoblast is derived from the cell $d^{5\cdot1}$ both in Asplanchna and in Lepas. This cell is purely entoblastic in Lepas, and probably so in Asplanchna; its two minute derivatives $d^{6\cdot2}$ and $d^{7\cdot2}$ are regarded by Jennings as belonging to this germ-layer. The macromere d^3 in this rotifer, as in Lepas, gives rise to $d^{4\cdot2}$ in the third cleavage and $d^{5\cdot2}$ in the fourth. In both $d^{4\cdot2}$ is purely ectoblastic. In Lepas $d^{5\cdot2}$ is the primary mesoblast, but in Asplanchna it is ectoblast. However, the exact origin of the mesoblast in the rotifers is unknown. It is evident that the number and order of cleavages which are involved in the segregation of the entoblast from the ectoblast are the same in the rotifer as in the cirripede.

XI. Comparisons of the Germ-Layers of Lepas with those of other Crustacea.

The account here given of the development of Lepas agrees with the published descriptions of the development of the majority of Crustacea, in that the blastopore is posterior and ventral, and apparently near the position of the future anal aperture. This similarity in the relation of the blastopore appears at first to be without significance, if one compares the embryo of Lepas, which has the mesoblastic band on its dorsal side, with crustacean embryos containing much yolk and having the mesoblastic plate ventral in position, as it is in decapods. However, the facts appear to allow of the following interpretations: In crustacean eggs which are heavily laden with yolk, the embryonic disk is at first confined to the ventral surface, but gradually extends dorsally over the yolk-mass. The mesoblast is formed while the embryonic disk is ventral. In Lepas, and some other Crustacea in which there is a relatively small amount of yolk, the embryonic disk is not confined to the ventral surface, but from the close of cleavage it is extensive enough to surround the yolk completely. In consequence of this the mesoblast, which in higher Crustacea forms bands on either side of the median ventral line, in Lepas extends along the dorsal line. If one imagines an ordinary decapod egg deprived of the greater part of its yolk until, at the close of cleavage, the edges of the embryonic disk meet on the dor-

sal surface, the conditions in Lepas would be closely imitated. The mesoblast bands would in such a case come to lie more and more dorsally, in proportion as the loss of yolk allowed the embryonic disk to cover the whole surface. In Lepas these bands in their position near the median-dorsal line, where the distal ends of the appendages later appear, may be considered as representing the outer edge of the embryonic disk of eggs having so much yolk that the disk is spread out over the ventral surface only, not being folded completely around the yolk as in the case of Lepas. It appears, then, that, though the mesoblast of Lepas is dorsal and that of yolk-laden eggs of higher Crustacea ventral, the two may be regarded as having homologous positions. In comparing Lepas with most other Crustacea the blastopore may be considered as having the same relative position, and the germ-layers may be compared with reference to their method of formation at the blastopore and their extension from that region.

Groom ('94, p. 199), who regarded the mesoblast and entoblast as originating from a single yolk-cell after the blastopore is closed, was necessarily led to the conclusion that "with respect to the origin of the mesoblast and hypoblast of the Nauplius, the cirripedes occupy an isolated position among Crustacea." This statement is based upon his view that the yolk-cells after the closing of the blastopore constitute the mes-entoblast. This view is at variance with the conditions in other Crustacea, for the mesoblast commonly originates from the blastoderm and not from yolk-cells lying beneath that structure. In this paper it has been shown that, in general terms, the mesoblast in Lepas originates from the blastoderm, and that, consequently, Groom's view is incorrect.

The accounts of most earlier workers on cirripede embryology lead to conclusions practically the same as Groom's. In opposition to such conclusions it will be pointed out in the following discussion that in the formation of the germ-layers there are many fundamental resemblances between Lepas and other Crustacea.

Among all Crustacea whose embryology is at present known, the closest resemblance to the development of Cirripedia appears to be found among the Phyllopoda and Copepoda, especially the latter. In the preceding chapter reference has been made to similarity of cleavage in these three groups of Entomostraca, but here the comparison between the germ-layers is to be emphasized.

Urbanowicz ('86) has studied the germ-layers of the copepod Cyclops and has found only one entoblast cell, over which the ectoblast grows

closing the blastopore. Ectoblastic cells around the blastopore give rise to mesenchyme ("secondary mesoblast"), which forms most of the mesoblastic structures of the Nauplius. The mesoblast proper probably originates from the entoblast, as does the primary mesoblast of Lepas. It is evident that there is in Cyclops, according to Urbanowicz, a condition closely resembling that of Lepas.

In close agreement with Urbanowicz's account of Cyclops and my own of Lepas, is Pedaschenko's ('93) description of the formation of the germ-layers of the parisitic copepod Lernæa. In this genus the mesoblast and ectoblast are separated from the yolk-entoblast in the first four divisions, as in Lepas. The four micromeres thus produced subdivide and form the blastoderm, which grows over the entoblast. At the margin of the growing blastoderm (blastopore) some cells (apparently ectoblastic) divide parallel to the surface and form migrating mesenchyme cells. These apparently correspond to the "secondary mesoblast" of Lepas. On the ventral side four of the cells sink beneath the ectoblast and constitute the primitive mesoblast cells. The lineage of these cells has not been definitely traced, but from their position I infer that they are probably the direct descendants of the fourth micromere, in which case the primary mesoblast originates directly from the entoblast, as in Lepas.

Häcker's ('92, '97) studies of Cyclops led to results widely different from those of Urbanowicz. According to Häcker, a cell lying in the blastopore divides into a genital cell and a primitive mesoderm cell. The cells surrounding the blastopore divide, giving rise to the primitive endoderm cells; this is in line with Grobben's account of Cetochilus, to which reference will be made later, and opposed to Urbanowicz, who found mesenchyme cells originating from cells bounding the blastopore.

Grobben's ('81) views of the formation of the germ-layers in the copepod Cetochilus do not agree with the account of Cyclops given by Urbanowicz, and only in part is there agreement with Häcker's account of Cyclops. His description of the thirty-two-cell stage of Cetochilus forms the best starting-point for purposes of comparison. In this stage, viewed from the vegetative pole, there is noticed a distinct bilateral symmetry in arrangement of the cells. A "central entoderm" cell and one small "anterior entoderm" cell lie in the median plane. Four cells placed symmetrically on either side of the "central entoderm" cell will by the next division form "entoderm" and ectoderm. The cell in the median line and posterior to the "central entoderm" cell forms in later division four cells, of which the two nearer the "central

entoderm" are said to be the primitive mesoblast cells, and the two posterior products ectodermal.

It appears that the "central entoderm" cell of Grobben is probably the single entoblast cell to which Urbanowicz refers. The blastoderm cells lying laterally and anterior to the entoderm cell in Cyclops are said by Urbanowicz to give rise to mesenchyme, while Grobben in Cetochilus and Häcker in Cyclops find entoderm originating from cells in corresponding positions. It is probable that this contradiction arose from failure to follow the germ-layers into the ultimate organs. The figures of Cetochilus by Grobben and those of Cyclops by Häcker do not give conclusive proof regarding the fate of the cells which they consider endoderm. I have not seen the original figures by Urbanowicz. The differences between these authors will probably be adjusted when the later history of the mesoblast and entoblast is more accurately traced.

The cell posterior to the "central endoderm" cell in the thirty-two-cell stage of Cetochilus is said by Grobben to form the mesoblast and also to contain some ectoblast. This latter point must still be regarded as problematical, for Grobben's figures do not give convincing proof. It is possible that the cell in question may be wholly mesoblastic, instead of only partly so. However, the important point is that this cell appears to originate in connection with the "central endoderm" cell. Accordingly mesoblast in Cetochilus originates from entoblast; a condition certainly existing in the case of the barnacle Lepas, and the studies of Urbanowicz make it appear probable that such is also the case in Cyclops.

Grobben's ('79) account of the development of the phyllopod Moina agrees with Urbanowicz's account of Cyclops and my own account of Lepas as to the formation of ectoblastic mesoblast from blastoderm cells bounding the blastopore laterally and anteriorly. But in a position corresponding to that of the entoblast cell of Lepas and Cyclops there is in Moina a "primitive genital cell," and the entoblast is said to be developed from a cell lying immediately posterior to it. It should be mentioned here that Samassa ('93), while agreeing essentially with Grobben's description of cleavage stages, failed to find evidence of such early differentiation. With respect to this result it must be considered improbable that the visible peculiarities of the cells in the region of the blastopore in cleavage stages are without significance. It seems more probable that the peculiar features of certain cells do represent early differentiations, as Grobben claimed. The results of Samassa and

others render doubtful the early differentiation of a genital cell in Moina; but Häcker ('92, '97) has contributed some important cytological evidence favorable to Grobben's conclusions.

To summarize the comparison of Lepas with the Copepoda and Phyllopoda, it has been pointed out that —

1. In Lepas, in Moina (Grobben), in Cyclops (Urbanowicz), and probably in the parasitic copepod Lernæa (Pedaschenko) mesoblast originates from ectoblastic cells of the blastoderm around the blastopore. In Cetochilus (Grobben) and in Cyclops (Häcker) there is a disagreement with Lepas, in that the *entoblast* cells are said to originate from cells whose origin and position is similar to those which in the above mentioned forms produce mesoblast.

2. In Lepas, Cyclops (Urbanowicz) and Lernæa a single entoblast cell, in Cetochilus (Grobben) the "central entoblast" cell, at first lies in the blastopore and it, or its derivatives, are overgrown by the blastoderm.

3. In Lepas, Cyclops (Urbanowicz), Cetochilus (Grobben) and Lernæa (?) (Pedaschenko) some mesoblast originates directly from the entoblast cell which lies in the blastopore, that is to say, the yolk-macromere is mes-entoblastic. In all of these except Cetochilus (Grobben) mesoblast also originates from ectoblastic cells around the blastopore.

The foregoing comparisons of the germ-layer formation in Lepas and other Entomostraca in which early differentiation takes place, brings out many points of resemblance. But in some cases there are differences apparently irreconcilable. One can scarcely believe that such contradictory statements as have been summarized in the preceding paragraphs are based upon observations all equally reliable. Renewed investigation of the uncertain points is much needed. The numerous resemblances even from the beginning of development, make it very desirable that the cell-lineage should in these cases be carefully studied so as to give a basis for accurate comparisons. Until such data are accessible it is unsafe to draw conclusions respecting homologies of cells or even of the germ-layers.

In many Crustacea there is at the blastopore an immigration of many cells into the cleavage cavity. In some of these cases the cavity is up to that time filled with yolk. The cell-mass thus formed by immigration into the cleavage cavity is mes-entoblastic, and the mesoblast and entoblast are at first indistinguishable, or at any rate investigators have failed to find distinguishing marks. As examples of

such conditions may be cited Daphnia, according to Lebedinsky ('91); Moina and Daphnia, according to Samassa ('93); and many higher Crustacea.

Such an origin of mesoblast and entoblast is not necessarily opposed to the account which I have given of the germ-layer formation of Lepas, for differentiation, though not observable, may yet occur in the cases mentioned. Were there not in Lepas peculiarities by which the cells can be distinguished at an early stage, the immigrating mass of cells, composed of entoblast, and of primary and secondary mesoblast, would be correctly described as mes-entoblast, out of which the two layers become later visibly differentiated. If the entoblast cells of Lepas were completely separated from the yolk-mass, as is the case in many other Crustacea, it would perhaps be impossible, in the absence of the easily recognized yolk-laden entoblast, to trace the lineage of the mesoblast independently of the entoblast, and in such conditions it would be necessary to consider the immigrating mass of cells as mes-entoblastic. It is probable that some such conditions obtain in some of the Crustacea in which a mes-entoblastic immigration is said to occur. At any rate, germ-layer formation in such cases agrees in essentials with that observed in Lepas. Grobben's ('79) study of Moina suggests that in this genus, at least, the immigrating mass of mes-entoblast may be not entirely undifferentiated as Samassa ('93) supposed.

There is some evidence that the comparison between Lepas and certain higher Crustacea may be carried still farther than the suggestions offered in the preceding paragraph. In Astacus, according to Reichenbach ('86), the mesoblast originates at the anterior margin of the blastopore, where the ectoblast joins the entoblast. Reichenbach distinguished in the invagination both yolk-absorbing cells (vitellophags), which enter into the yolk-pyramids, and also the cells forming the entoderm plate. All these cells are said to enter into the mesenteron and liver lobes, and hence the invagination is entoblastic. However, McMurrich ('95, pp. 135, 136) reviews the evidence and suggests that the yolk-pyramids give rise to some mesoblast. If this proves true, the invagination is to be regarded as mes-entoblastic; but, in addition to mesoblast so formed from entoblast, other mesoblast cells certainly originate from the blastoderm in front of the invagination. It follows that there are, as regards origin, two kinds of mesoblast — ectoblastic and entoblastic.

In other accounts of development of the higher Crustacea there are suggestions of such a double origin of mesoblast, but there is as yet

lack of a definiteness of statement sufficient to afford basis for comparisons of any value.

Comparing the development of Astacus with that of Lepas, the ectoblastic mesoblast at the anterior edge of the blastopore appears to be equivalent to the "secondary mesoblast" of Lepas. If the suggestion, that the invagination is mes-entoblastic, proves true, it may be possible to regard the mes-entoblastic cell $d^{4.1}$ of Lepas as representing the invaginated cells of the higher Crustacea; the primary mesoblast and entoblast of Lepas would then be comparable with the germ-layers derived from the invagination in the higher forms. In such a case there would be further agreement with Lepas in that the mesoblast originates from both ectoblast and entoblast.

Summary.

1. Lepas resembles most other Crustacea (*a*) in respect to position of the blastopore, which is ventral and posterior, (*b*) in extension of the entoblast and mesoblast from the blastopore as a starting-point, (*c*) in the mode of formation of the organs of the larva.

2. In Lepas, as in most other Crustacea, the mesoblast and entoblast originate in the region of the blastopore from cells which, speaking in general terms, at first lie in the blastoderm and later migrate into the cleavage cavity.

3. Among the migrating mes-entoblastic cells one can distinguish in Lepas the individual cells of entoblast and of two varieties of mesoblast. Representatives, if not precise homologues, of these kinds of cells are probably present both in other Entomostraca and in the higher Crustacea.

XII. General Summary with Table of Cell-Lineage of Lepas.

The results which are of special interest in relation to the development of Cirripedia have already been summarized in connection with the accounts of the several stages of development. Only results of more general interest are again summarized here.

The cleavage of Lepas is throughout total and unequal.

Stages with 2, 4, 8, 16, 32, and 62 "resting" cells are regularly formed.

In the eight-cell stage and thereafter there is a well-marked bilateral arrangement of the cells.

In the first three cleavages three "protoplasmic" micromeres are

separated from the yolk-bearing macromere, and the fourth cleavage separates the primary mesoblast from the yolk-entoblast. Thus, in the sixteen-cell stage the entoblast is completely separated from the other germ-layers.

Mesoblast originates both from entoblast (fourth cleavage) and from ectoblast (sixth cleavage). The mesoblast derived from ectoblast ("secondary mesoblast") forms a large part at least of the mesenchyme of the Nauplius. The fate of the primary mesoblast (entoblastic mesoblast) has not been distinguished from that of the "secondary mesoblast" (ectoblastic mesoblast).

The blastoderm grows over the yolk-bearing entoblast, usually closing the blastopore after the sixth cleavage. In cases where the yolk-mass is very large, the closing of the blastopore may not occur until the succeeding cleavage. But in all cases the blastoderm is formed from derivatives of *three* and only three micromeres (ab^2, c^2, $d^{4.2}$), which are cut off in the first three cleavages.

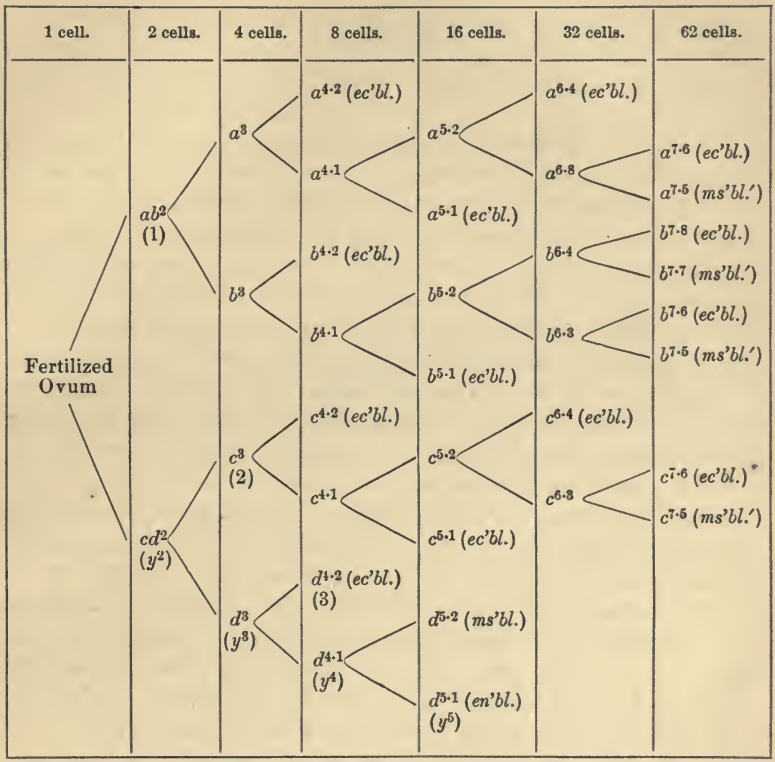
The yolk-macromere of the sixteen-cell stage has been traced to the mesenteron. All the evidence supports entirely the interpretation that after the fourth cleavage the yolk-macromere is purely entoblastic.

The irregularity and variability which authors have ascribed to the cleavage of cirripedes do not normally exist in the case of Lepas. The origin, relative position, and fate of all cells of all cleavage stages have been shown to be constant, definite, and "determinate" so far as the formation of germ-layers is concerned. In later stages specific areas of cells, known to be of definite origin, enter into the formation of particular organs. It is therefore probable that the cells in cleavage stages bear a definite and constant relation to future organs.

The chief points in the cell-lineage and their relation to the formation of the germ-layers are summarized in the accompanying table.

Describing the formation of the germ-layers of Lepas in general terms, there is no conflict with most existing accounts of the development of other Crustacea; in the absence of complete records of the cell-lineage in other Crustacea, it is not possible to compare the details with certainty (see *Summary*, p. 133).

TABLE OF THE CELL-LINEAGE OF LEPAS.



y^2, y^3, y^4, y^5 designate the yolk-bearing macromere; (1), (2), (3), the three micromeres containing ectoblast; *ec'bl.*, ectoblast; *en'bl.*, entoblast; *ms'bl.*, primary mesoblast, *ms'bl.'*, "secondary mesoblast."

ADDENDUM.

By E. L. Mark and W. E. Castle.

To avoid any misunderstanding we wish to state that the opinions expressed by Dr. Bigelow regarding "quartet" cleavage are not wholly shared by us. Lepas seems to us a good example of modified "quartet" cleavage, and for that reason we think the quartet nomenclature has more than mere convenience in its favor. To be sure, the quadrants in Lepas are not symmetrical, but perfect symmetry is rarely met with in quartet cleavage. So far as we recall, complete symmetry of the quadrants is found only in platodes. The condition there realized may be considered primitive, all four quadrants sharing equally in the production of ectoblast, mesoblast, and endoblast (see Wilson, '98). *One* modification of this primitive symmetry is found in annelids and mollusks, *another* in rotifers and cirripedes.

In the first-named groups the *mesoblast* is segregated, more or less completely, in quadrant *d*, while the endoblast remains distributed among all four quadrants. In the rotifers (see Jennings, '96) the *endoblast* is segregated in quadrant *d*, precisely as in Lepas, yet the cleavage progresses in perfect quadrant symmetry through at least the first eight cell-generations, even though, to realize this symmetry, so-called "mechanical laws of cleavage" are repeatedly transgressed. The origin of the mesoblast in rotifers remains uncertain, but in Lepas, as Dr. Bigelow clearly shows, the mesoblast arises *from all four quadrants*. An examination of his table of cell-lineage (p. 135) shows other unmistakable evidences of quadrant symmetry in Lepas.

1. The first-formed definitive ectomeres — which are also the first cells to be differentiated for a particular germ-layer — arise symmetrically and synchronously from all four quadrants. They are the four dorsal cells of the eight-cell stage, namely, $a^{4.2}$, $b^{4.2}$, $c^{4.2}$, and $d^{4.2}$. They correspond with what in polyclads, annelids, and mollusks have been called the "first quartet of micromeres," which in these forms, as in Lepas, are always the first ectomeres to be differentiated.

2. At the sixteen-cell stage, in Lepas, the mesoblast is included in corresponding blastomeres ($a^{5.2}$, $b^{5.2}$, $c^{5.2}$, $d^{5.2}$) in all four quadrants.

The only essential difference among the quadrants in the mode of separation of the mesoblast is this: In quadrant *d*, cell $d^{5.2}$ is *purely* mesoblastic; but the corresponding cells in each of the other quadrants contain mesoblast associated as yet with ectoblast, and the two are not separated until the second later generation, that is, in the sixty-four-cell stage. The earlier separation of the mesoblast in quadrant *d*, as compared with the other quadrants, may be due to the relatively *greater bulk* of the mesoblast in quadrant *d*. The mesoblast is really *partially segregated* in quadrant *d*, — since that quadrant contains a greater portion of mesoblast than any of the three remaining quadrants, — while the endoblast is *completely* segregated in that quadrant. The segregation of the mesoblast in quadrant *d* finds a parallel repeatedly in mollusks and annelids; that of the endoblast in the same quadrant is paralleled in rotifers.

Notwithstanding these coenogenetic modifications, the primitive quadrant-symmetry finds frequent expression in the cleavage of Lepas, a fact to which the quadrant nomenclature clearly directs attention.

It is true that in Lepas radial symmetry is replaced by bilateral symmetry considerably earlier than is the case in most annelids and mollusks, and much earlier than in rotifers, but the difference is one of degree rather than of kind. Cleavage in Lepas, as truly as in the other forms mentioned, is at first radial, and only gradually becomes bilateral.

BIBLIOGRAPHY.[1]

Auerbach, L.
'74. Organologische Studien. Breslau. 262 pp., 4 Taf.

Beneden, E. van.
'70. Recherches sur l'embryogénie des Crustacés. III. Développement de l'œuf et de l'embryon des Sacculines (Sacculini carcini, Thomps.). Bull. Acad. Roy. Belgique, sér. 2, Tom. 29, pp. 99–112, 1 pl.

Bigelow, M. A.
'96. On the early Development of Lepas fascicularis. (A preliminary note.) Anat. Anz., Bd. 12, pp. 263–269, 9 figs.

Bigelow, M. A.
'99. Notes on the First Cleavage of Lepas. Zoöl. Bull., Vol. 2, pp. 173–177, 7 figs.

Blochmann, F.
'81. Über die Entwickelung der Neritina fluviatilis Müll. Zeit. f. wiss. Zool., Bd. 36, pp. 125–174, Taf. 6–8.

Bobretzky, N.
'76. Studien über die embryonale Entwickelung der Gastropoden. Arch. f. mikr. Anat., Bd. 13, pp. 95–169, Taf. 8–13.

Bovallius, C.
'75. Embryologiska Studier. I. Om Balanidernas Utveckling. Stockholm. 44 pp., 5 pls.

Castle, W. E.
'96. The Early Embryology of Ciona intestinalis. Bull. Mus. Comp. Zoöl., Harvard Coll., Vol. 27, pp. 201–280, 13 pls.

Conklin, E. G.
'97. The Embryology of Crepidula, a Contribution to the Cell Lineage and early Development of some Marine Gasteropods. Jour. Morph., Vol. 13, pp. 1–226, pls. 1–9.

[1] No attempt has been made to cite a complete bibliography on cirripede embryology. Groom ('94) has given a tolerably complete list of the literature on the structure and development of the Cirripedia.

Conklin, E. G.
'98. Cleavage and Differentiation. Biol. Lect., Wood's Holl, 1896–97, pp. 17–43.

Crampton, H. E.
'96. Experimental Studies on Gasteropod Development. Arch. f. Entwickelungsmech. d. Organismen, Bd. 3, pp. 1–19, Taf. 1–4.

Darwin, C.
'51. A Monograph on the Sub-class Cirripedia. Lepadidæ. Ray Society, London. xi + 400 pp., 10 pls.

Darwin, C.
'54. A Monograph on the Sub-class Cirripedia. (Balanidæ, Verrucidæ, etc.). Ray Society, London. viii + 684 pp., 30 pls.

Filippi, F.
'65. Ueber die Entwicklung von Dichelaspis Darwinii. Untersuchungen zur Naturlehre (Moleschott), Bd. 9, pp. 113–120, 2 Taf.

Grobben, C.
'79. Die Entwicklungsgeschichte der Moina rectirostris, u. s. w. Arb. Zool. Inst. Wien, Tom. 2, pp. 203–268, Taf. 11–17.

Grobben, C.
'81. Die Entwicklungsgeschichte von Cetochilus septentrionalis. Arb. Zool. Inst. Wien, Tom. 3, pp. 243–282, Taf. 19–22.

Groom, T. T.
'92. On the Early Development of Cirripedia. (Abstract.) Proc. Roy. Soc. London, Vol. 52, pp. 158–162.

Groom, T. T.
'94. On the Early Development of Cirripedia. Phil. Trans. Roy. Soc. London, Vol. 185, B, pp. 119–232, pls. 14–28.

Häcker, V.
'92. Die Kerntheilungsvorgänge bei der Mesoderm- und Entodermbildung von Cyclops. Arch. f. mikr. Anat., Bd. 39, pp. 556–581, Taf. 24, 25.

Häcker, V.
'97. Die Keimbahn von Cyclops, u. s. w. Arch. f. mikr. Anat., Bd. 49, pp. 35–91, Taf. 4, 5.

Hoek, P. P. C.
'76. Zur Entwickelungsgeschichte der Entomostraken. (I. Embryologie von Balanus.) Niederl. Arch. f. Zool., Bd. 3, Heft 1, pp. 47–82, Taf. 3, 4.

Hoek, P. P. C.
'83. Report on the Cirripedia collected by H. M. S. Challenger. (Historical and Systematic.) Vol. 8, Part 25, Zoöl. Serics, 169 pp., 13 pls.

Hoek, P. P. C.
'84. Report on the Cirripedia collected by H. M. S. Challenger. (Anatomical.) Vol. 10, Part 28, Zoöl. Series, 47 pp., 6 pls.

Jennings, H. S.

'96. The Early Development of Asplanchna Herrickii de Guerne. A Contribution to Developmental Mechanics. Bull. Mus. Comp. Zoöl., Harvard Coll., Vol. 30, pp. 1–116, 10 pls.

Knipowitsch, N.

'92. Beiträge zur Kenntniss der Gruppe Ascothoracida. Trav. de la Soc. Nat. St. Petersburg, Tom. 23, pp. 82–155, 3 Taf. (Russian with abstract in German.)

Kofoid, C. A.

'94. On some Laws of Cleavage in Limax. A Preliminary Notice. Proc. Am. Acad. Arts and Sci., Vol. 29, pp. 180–203, 2 pls.

Kofoid, C. A.

'95. On the Early Development of Limax. Bull. Mus. Comp. Zoöl., Harvard Coll., Vol. 27, pp. 35–118, 8 pls.

Korschelt, E., und Heider, K.

'90–91. Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Thiere. Jena. xii + 908 pp.

Lang, A.

'78. Die Dotterfurchung von Balanus. Jena. Zeit. f. Naturw., Bd. 12, pp. 671–674, Taf. 20, 21.

Lebedinsky, J.

'91. Die Entwicklung der Daphnia aus dem Sommereie. Zool. Anz., Jahrg. 14, pp. 149–152.

Lillie, F. R.

'95. The Embryology of the Unionidæ. A Study in Cell-Lineage. Jour. Morph., Vol. 10, pp. 1–100, pls. 1–6.

McMurrich, J. P.

'95. Embryology of the Isopod Crustacea. Jour. Morph., Vol. 11, pp. 63–154, pls. 5–9.

Müller, F.

'64. Für Darwin. Leipzig. 91 pp., 67 figs.

Münter, J., und Buchholz, R.

'69. Über Balanus improvisus Darw., var. Gryphicus Münt., Beitrag zur carcinologischen Fauna Deutschlands. Mitth. aus dem Naturw. Verein von Neu-Vorpommern und Rügen. Bd. 1, pp. 1–40, Taf. 1, 2.

Review by Gerstäcker in Arch. f. Naturg. Jahrg. 1871, Bd. 2, pp. 364, 365.

Nassonow, [W.] N.

'85. Zur embryonalen Entwicklung von Balanus. Zool. Anz., Jahrg. 8, pp. 44–47.

Nassonow, [W.] N.
'87. On the Ontogeny of the Crustaceans Balanus and Artemia. Izvyest. imp. Obshch. Ljubit. Estestv. Antrop. i Ethnog. Moscow. Tom. 52, pp. 1–14, 35 figs. (Russian.)

Nussbaum, M.
'87. Vorläufige Bericht über die Ergebnisse einer mit Unterstützung der Königlichen Akademie ausgeführte Reise nach Californien. Sitzungsb. d. k. preuss. Akad. der Wiss. zu Berlin, pp. 1051–1055.

Nussbaum, M.
'89. Bildung und Anzahl der Richtungskörper bei Cirripedien. Zool. Anz., Jahrg. 12, p. 122.

Nussbaum, M.
'90. Anatomische Studien an Californischen Cirripedien. Bonn. 97 pp., 12 Taf.

Pedaschenko, D.
'93. Sur la segmentation de l'œuf et la formation des feuillets embryonnaires chez la Lernæa branchialis L. Rev. des. Sc. Nat. St. Petersburg, Tom. 4, pp. 186–199, 11 figs. (Russian with abstract in French.)

Reichenbach, H.
'86. Studien zur Entwicklungsgeschichte des Flusskrebses. Abhandl. Senckenberg. Naturf. Gesellsch. Frankfurt. Bd. 14, pp. 1–137, Taf. 1–14.

Samassa, P.
'93. Die Keimblätterbildung bei den Cladoceren. I. Moina rectirostris Baird. [II. Daphnella und Daphnia.] Arch. f. mikr. Anat., Bd. 41, pp. 339–366, Taf. 20–22; pp. 650–688, Taf. 36–39.

Solger, B.
'90. Die Richtungskörperchen von Balanus. Zool. Anz., Jahrg. 13, pp. 607–609.

Torrey, J. C.
:02. The Cell-Lineage of the Mesoblast-Bands and Mesenchyme in Thalassema. Science, n. s., Vol. 15, No. 380, pp. 576, 577.

Treadwell, A. L.
:01. The Cytogeny of Podarke obscura (Verrill). Jour. Morph., Vol. 17, No. 3, pp. 399–486, pls. 36–40.

Urbanowicz, F.
'84. Zur Entwicklungsgeschichte der Cyclopiden. (Vorl. Mitth.) Zool. Anz., Jahrg. 7, pp. 615–619.

Urbanowicz, F.
'86. Contributions to the Embryology of Copepods. Kosmos, Lemberg, Jahrg. 10, pp. 239–259, 300–314. (Polish.)

Urbanowicz, F.

'86[a]. Contributions à l'embryologie des Copépodes. Arch. Slav. Biol., Tom. 1, fasc. 3, pp. 663–667; et Mém. Univers. Varsovie. (Abstract of preceding paper.)

Van Beneden, E. See Beneden, E. van.

Weismann [A.] und Ischikawa [C.]

'88. Weitere Untersuchungen zum Zahlengesetz der Richtungskörper. Zool. Jahrb., Abth. f. Anat. u. Ontog., Bd. 3, pp. 575–610, Taf. 25–28.

Willemoes-Suhm, R. von.

'76. On the Development of Lepas fascicularis and the "Archizoëa" of Cirripedia. Phil. Trans. Roy. Soc. London, Vol. 166, pt. 1, pp. 131–154, pls. 10–15.

Wilson, E. B.

'98. Considerations on Cell-Lineage and Ancestral Reminiscence, etc. Annals N. Y. Acad. Sci., Vol. 11, pp. 1–27, 7 figs.

Wilson, E. B.

:01. Studien über den Körperbau der Anneliden, V. By Eduard Meyer. (Review.) Science, n. s., Vol. 14, No. 362, pp. 389–91.

Zelinka, C.

'91. Studien über Räderthiere. III. Zur Entwicklungsgeschichte der Räderthiere nebst Bemerkungen über ihre Anatomie und Biologie. Zeit. f. wiss. Zool., Bd. 53, pp. 1–159, Taf. 1–6.
Also in: Arbeiten a. d. Zool. Inst. Graz. Bd. 4, pp. 323–481, Taf. 1–6.

Ziegler, H. E.

'95. Untersuchungen über die ersten Entwicklungsvorgänge der Nematoden. Zugleich ein Beitrag zur Zellenlehre. Zeit. f. wiss. Zool., Bd. 60, pp. 351–410, Taf. 17–19.

EXPLANATION OF PLATES.

The figures of Plates **1–10** were drawn from the eggs of *Lepas anatifera*, and those of Plates **11** and **12** from *L. fascicularis*.

An Abbé camera lucida was in every case used in sketching the eggs. The figures of Plate **1**, and Figures 57, 63–65, 74–77 were drawn at a magnification of about 220 diameters; all others in Plates **1–10** at about 365 diameters. The figures of Plates **11** and **12** are magnified about 210 diameters.

All figures, except those of transverse sections, are so arranged that the *posterior* end of the embryo, or the more pointed end of the vitelline membrane, is directed toward the bottom of the Plate; in transverse sections the ventral side is toward the bottom.

Double-headed arrows are used in some of the figures to connect two cells of common origin.

The vitelline membrane has not been represented, except in Figures 1–17 and 94–97.

Figures 1–30 and 95–99 are oriented by the axis of the vitelline membrane; all others by the axis of the embryo.

The small circles without stippling indicate the positions of the oil spherules in the yolk. Nuclei are distinguished by wavy lines, or by stippling, to represent chromosomes.

In Plates **2** and **3** a pale yellowish buff tint has been used to represent the more finely granular and more "protoplasmic" portion of the egg and blastomeres.

Plates **1**, **4**, **11**, and **12** have been printed without tint. To aid in quickly distinguishing between the derivatives of quadrants *a*, *b*, and *c*, all the blastomeres of quadrant *b* in Figures 38–59, 61 are printed in *stipple without tint*, and in Figures 60 and 65 (Plate **7**) the same method of designation has been employed to indicate the cells ($b^{7.5}$–$b^{7.8}$) of this quadrant concerned in the formation of the secondary mesoblast.

In Plates **5–10** the pale yellowish buff tint has been employed to indicate the blastomeres derived from quadrant *d*, the *primary mesoblast* ($d^{5.2}$ and its descendants) being distinguished from the other derivatives by receiving a *stippling in addition to the tint*. In Plates **8–10** the tint has been restricted to $d^{4.1}$ (entoblast) and its derivatives.

ABBREVIATIONS.

For explanation of the letters and exponents designating blastomeres, see explanation of the nomenclature of cleavage (pp. 74–76).

ast'cœl.	Astrocœl.	*ec'bl.*	Ectoblast.
app.	Appendage.	*en'bl.*	Entoblast.
at 1.	First antenna.	*lbr.*	Labrum.
at 2.	Second antenna.	*mb.vt.*	Vitelline membrane.
bl'po.	Blastopore.	*md.*	Mandible.
bl'drm.	Blastoderm.	*ms'bl.*	Mesoblast of double origin.
cav.sg.	Cleavage cavity.	*ms'bl'.*	" Secondary mesoblast " (ectoblastic mesoblast).
cl.pol 1.	First polar cell.		
cl.pol 2.	Second polar cell.	*pr'nl.* ♂	Male pronucleus.
d.	Dorsal.	*pr'nl.* ♀	Female pronucleus.

The Roman numerals I. II. (Figs. 28, 30) indicate the position of the first and second cleavage planes, respectively; the Arabic numerals 1–4 (Figs. 91, 93, 122–126), the sequence in which the transverse furrows marking off the Nauplius appendages make their appearance.

Plate **1.**	Figs. 1–16.	Plate **7.**	Figs. 56–65.
Plate **2.**	Figs. 17–22.	Plate **8.**	Figs. 66–73.
Plate **3.**	Figs. 23–30.	Plate **9.**	Figs. 74–86.
Plate **4.**	Figs. 31–38.	Plate **10.**	Figs. 87–94.
Plate **5.**	Figs. 39–46.	Plate **11.**	Figs. 95–110.
Plate **6.**	Figs. 47–55.	Plate **12.**	Figs. 111–126.

PLATE 1.

Figures in this plate are all from living eggs, and represent stages between oviposition and the close of the first cleavage. The small circles represent the oil spherules which are embedded in the yolk.

Fig. 1. Egg about thirty minutes after oviposition. Vitelline membrane and second polar cell have appeared. Yolk uniformly distributed in the egg.

Figs. 2–5. Egg elongating. Protoplasm concentrating in upper half of the egg. Yolk becomes aggregated at the vegetative pole. Development of yolk-lobe.

Fig. 6. Yolk-lobe has disappeared. Yolk radially symmetrical with reference to chief axis of egg. Vitelline membrane has assumed its definitive form.

Fig. 7. Yolk moves to eccentric position with reference to the chief axis.

Figs. 8–15. First cleavage. Time thirty minutes. Drawings made at intervals of about four minutes. Rotation of the dividing egg within the vitelline membrane.

Fig. 16. One hour after close of first cleavage (Fig. 15). Yolk has returned somewhat toward the vegetative pole.

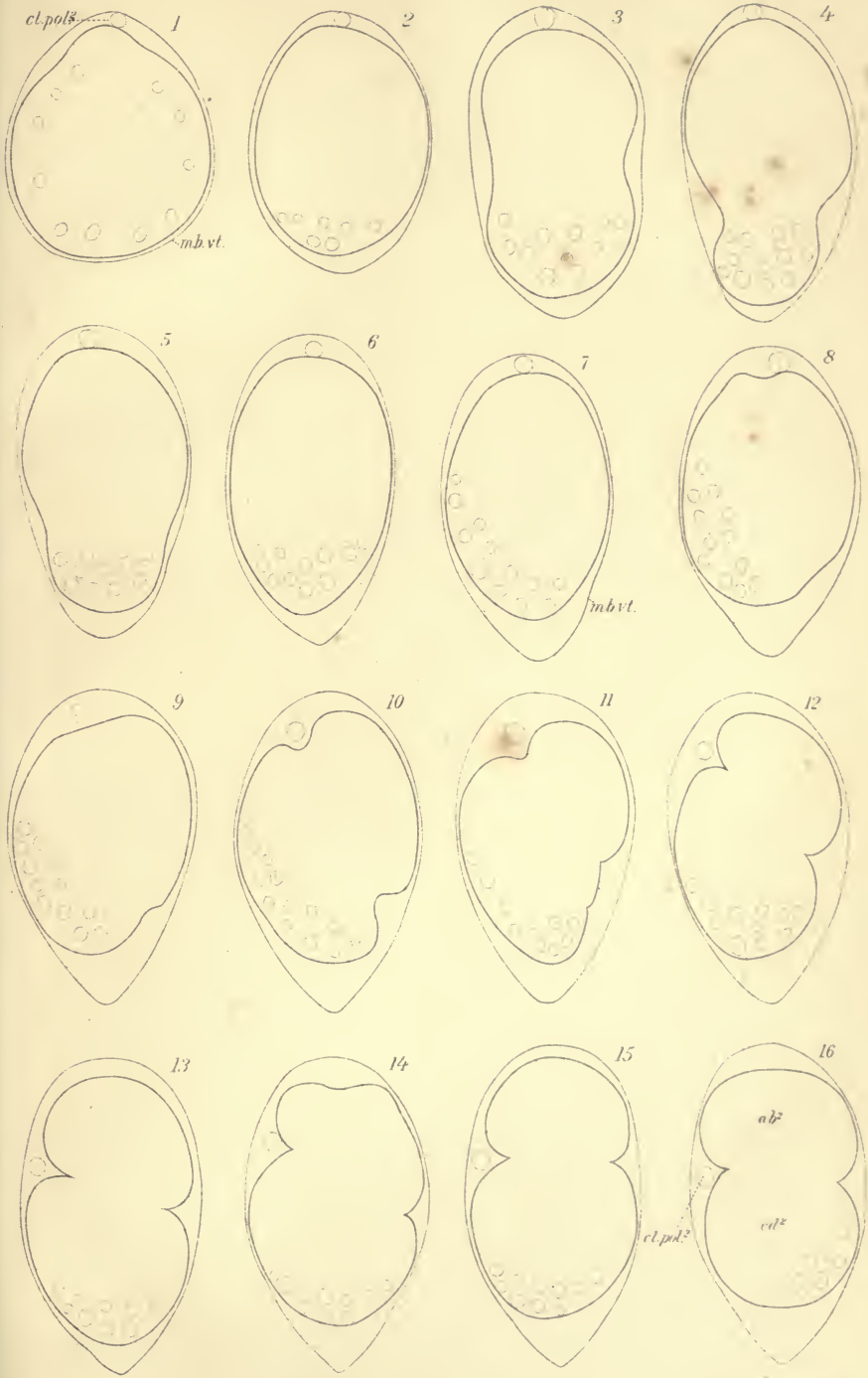

cl.pol²
1
mb.vt.
2
3
4
5
6
7
mb.vt.
8
9
10
11
12
13
14
15
16
ab²
cd²
cl.pol²

PLATE 2.

Sections of eggs representing stages shown in Plate 1.
The vitelline membrane is represented in Figure 17 only.

Fig. 17. Formation of second polar cell. Yolk uniformly distributed in the egg, which is somewhat distorted into a form more than normally elongated, owing to pressure in the egg-lamella.

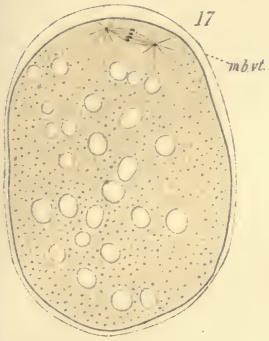
Fig. 18. Same stage as that represented in Plate 1, Figure 4. Male and female pronuclei in contact. Yolk collecting at the vegetative pole. The pronuclei in this stage, which is characterized by the presence of a yolk-lobe, are often separated as in Figure 19.

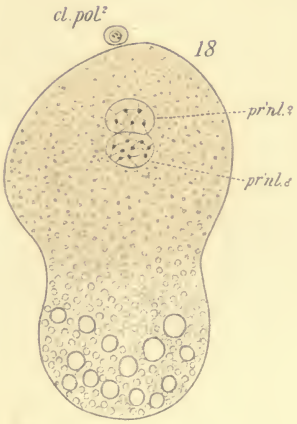
Fig. 19. Same stage as that shown in Figure 6. Pronuclei approaching; they are usually in contact in this stage, as in Figure 20.

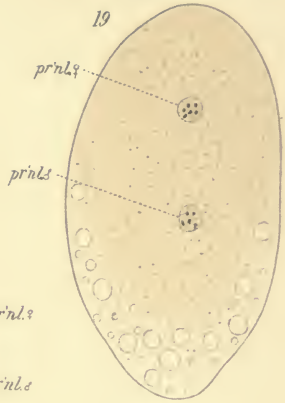
Fig. 20. From an egg fixed in mercuric chloride, showing the distribution and relative amount of the yolk. Early appearance of the asters (?). Pronuclei in contact. Same stage as that shown in Figure 6.

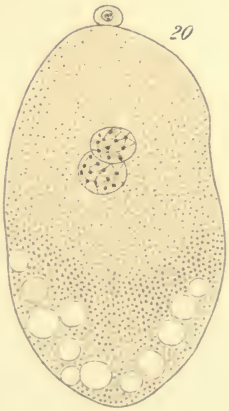
Fig. 21. Formation of first cleavage spindle. Yolk becomes eccentric, as shown in Figure 7.

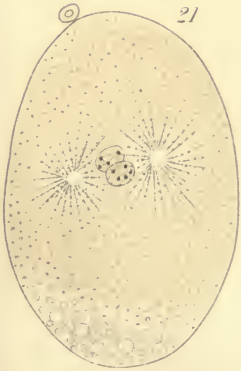
Fig. 22. Beginning of metaphase of first cleavage.


17
mb vt.


cl. pol²
18
pr'nl.♀
pr'nl.♂


19
pr'nl.♀
pr'nl.♂


20


21

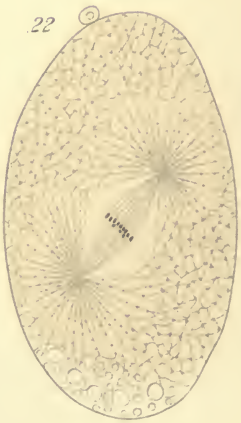

22

PLATE 3.

All Figures drawn from sections.

Fig. 23. Early anaphase of first cleavage.

Fig. 24. Late anaphase. Dividing egg in rotation. Second polar cell in cleavage furrow.

Fig. 25. Telophase of egg, which has not yet rotated through a complete quadrant.

Fig. 26. Rotation completed. Cleavage plane developing. Spindle disappearing. Chromosomes vesicular.

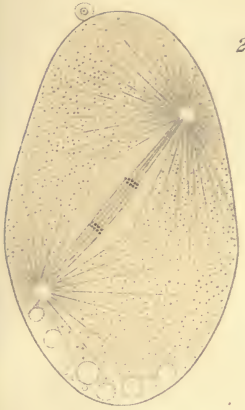
Fig. 27. Two-cell stage. Vesicular chromosomes unite to form the nuclei. Yolk has approached the vegetative pole, as in Figure 16.

Fig. 28. Second cleavage at beginning of metaphase, viewed from animal pole.

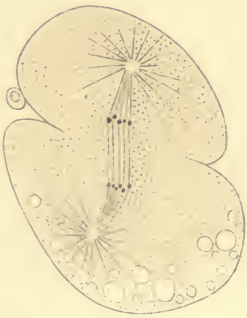
Fig. 29. Equatorial-plate stage of second cleavage; same egg as Figure 28. Lateral view.

Fig. 30. Second cleavage in late anaphase, viewed from animal pole. *I, I,* indicate first cleavage plane, *II, II,* second cleavage plane.
The long arrow falls in the projection of the sagittal plane of the embryo.

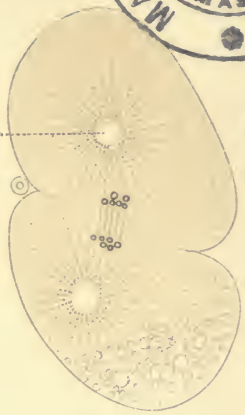
23.



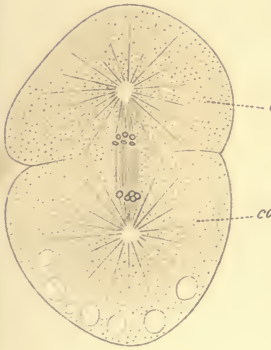
24.



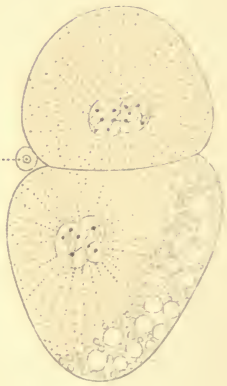
25.



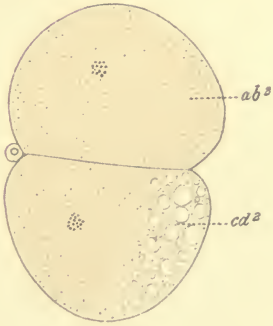
26.



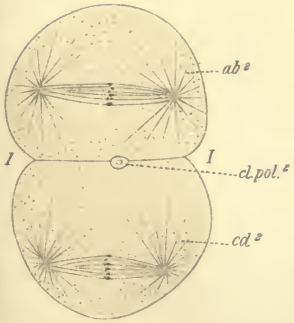
27.



29.



28.



30.

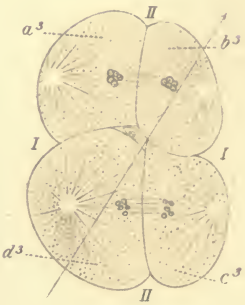


PLATE 4.

Figures drawn from transparent preparations of entire eggs. Vegetative pole at the *left* in lateral views.

Fig. 31. Egg viewed from animal pole. Late anaphase of second cleavage.

Fig. 32. Four-cell stage. Nuclei in "resting" phase. Egg viewed from animal pole.

Fig. 33. Same egg viewed laterally. Yolk at vegetative pole of cell d^3.

Fig. 34. Four-cell stage during third cleavage. Viewed from animal pole.

Fig. 35. Same egg from vegetative pole. Oil spherules of the yolk near the surface.

Fig. 36. Same egg in lateral view.

Fig. 37. Eight-cell stage from animal pole. All nuclei are in "resting" phase. Second polar cell covered in by the meeting of $a^{4\cdot2}$ and $c^{4\cdot2}$.

Fig. 38. Same egg from vegetative pole. Oil spherules near lower surface of yolk-cell. Cells of quadrant b ($b^{4\cdot1}$, $b^{4\cdot2}$) stippled.

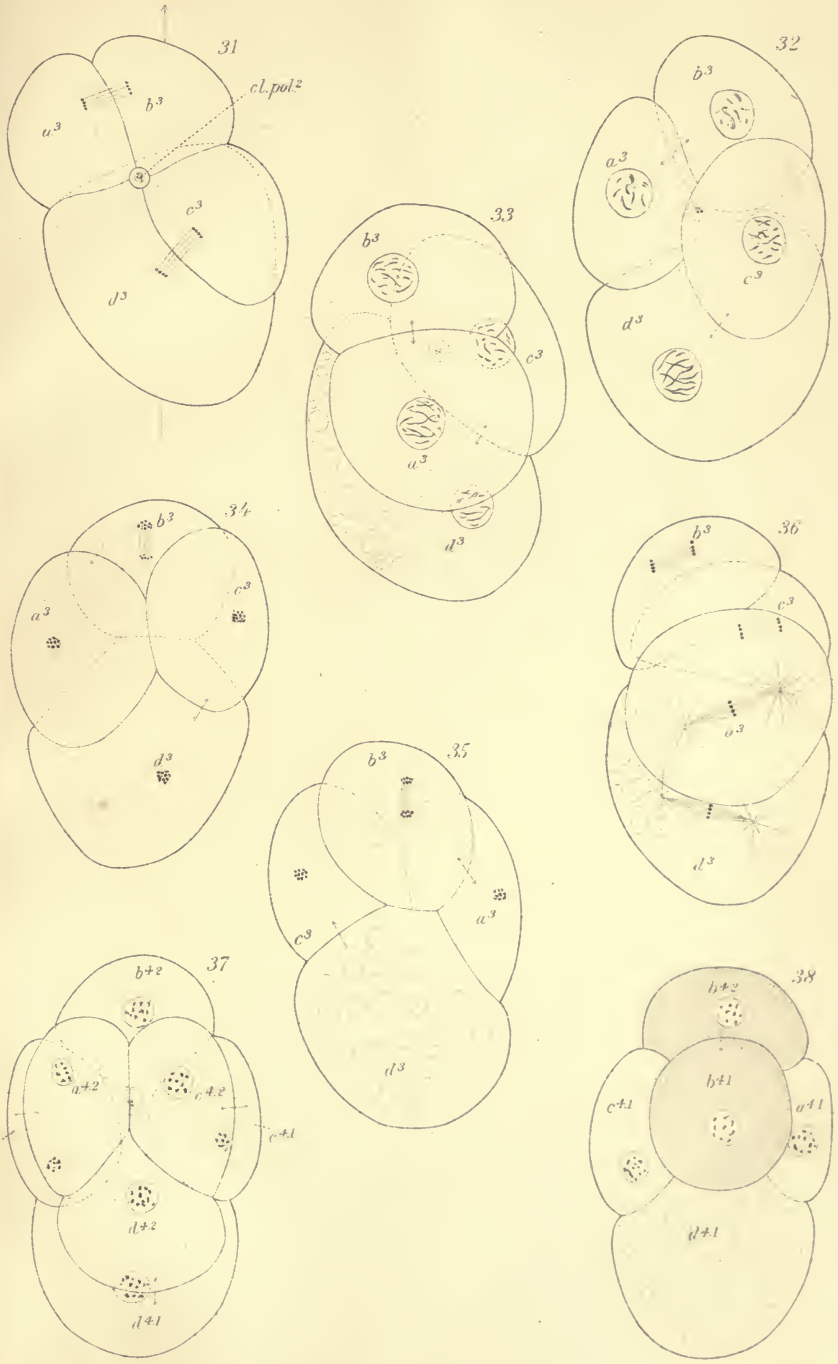

31
cl.pol²
a³
b³
c³
d³
32
b³
a³
c³
d³
33
b³
c³
a³
d³
34
b³
c³
a³
d³
35
b³
a³
c³
d³
36
b³
c³
a³
d³
37
b+2
a+2
c+2
a+1
c+1
d+2
d+1
38
b+2
b+1
c+1
a+1
d+1

PLATE 5.

Figures from transparent preparations of entire eggs. Vegetative pole at the *left* in figures which represent lateral views.

Fig. 39. Eight-cell stage from animal pole. The seven "protoplasmic" cells are in the fourth cleavage; the nucleus of yolk-cell ($d^{4\cdot1}$) is preparing for division.

Fig. 40. Same egg in lateral view. Yolk at vegetative pole of cell $d^{4\cdot1}$.

Fig. 41. Fifteen "protoplasmic" cells; the yolk-cell ($d^{4\cdot1}$, mes-entoblast) dividing. Lateral view.

Fig. 42. Sixteen-cell stage from animal pole. Nuclei of all cells are in "resting" phase. Primary mesoblast ($d^{5\cdot2}$) separated from entoblast ($d^{5\cdot1}$).

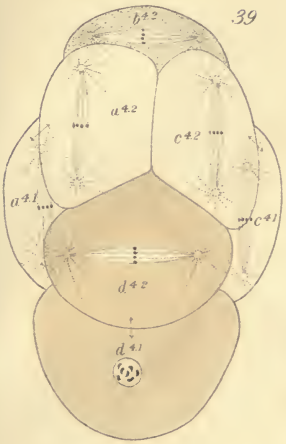
Fig. 43. Same egg viewed from vegetative pole. Oil spherules near lower surface of the yolk-cell.

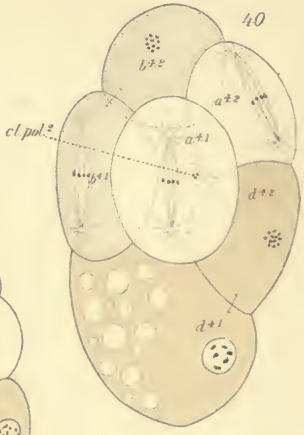
Fig. 44. Sixteen-cell stage from animal pole. All cells, except yolk-cell (entoblast $d^{5\cdot1}$) and the primary mesoblast cell ($d^{5\cdot2}$), are undergoing the fifth cleavage.

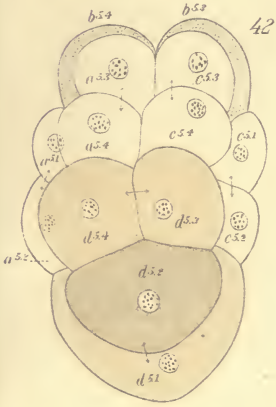
Fig. 45. Same egg in lateral view.

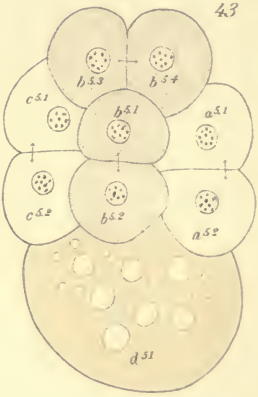
Fig. 46. Same stage from vegetative pole. The three mes-ectoblasts (compare Fig. 43, $a^{5\cdot2}$, $b^{5\cdot2}$, $c^{5\cdot2}$) contiguous to yolk-cell.

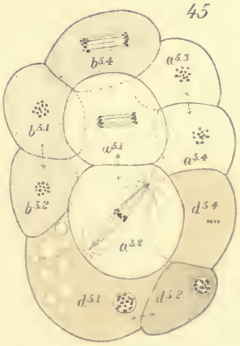
Note. — Cell $a^{5\cdot2}$ is represented as divided, and its derivatives should have been labelled $a^{6\cdot3}$, $a^{6\cdot4}$.

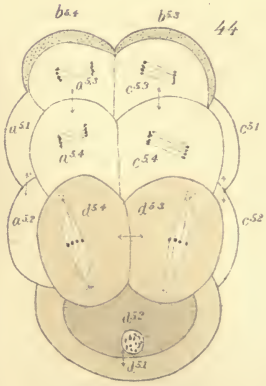












M.A.B. del.

B. Mensel, lith. Boston

PLATE 6.

Figures from transparent preparations of entire eggs. Vegetative pole at the *right* in figures representing lateral views.

Fig. 47. Sixteen-cell stage with all cells of the blastoderm in fifth cleavage. Primary mesoblast ($d^{5\cdot2}$) and entoblast ($d^{5\cdot1}$) with enlarging nuclei. Lateral view.

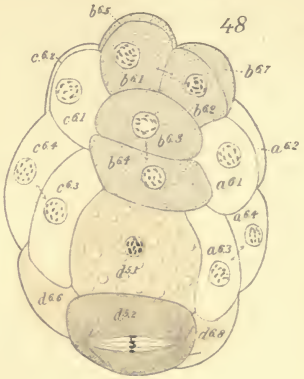
Figs. 48 and 51. Eggs with thirty cells, but the primary mesoblast cell ($d^{5\cdot2}$) has not yet completed the fifth cleavage. Nucleus of entoblast cell ($d^{5\cdot1}$) still in "resting" phase, but chromosomes preparing for fifth cleavage. Entoblast (blastopore) bounded anteriorly and laterally by mes-ectoblasts ($a^{6\cdot3}$, $b^{6\cdot3}$, $b^{6\cdot4}$, $c^{6\cdot3}$). Viewed from vegetative pole.

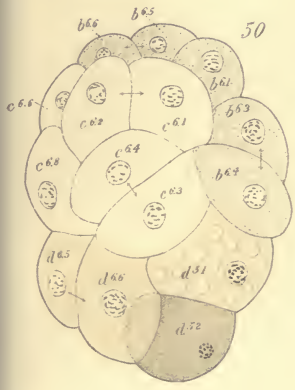
Figs. 49, 50 and 53. Same stage seen in lateral view. In Figure 53 more of the dorsal than of the ventral side is seen. Comparison shows that the cells have essentially the same positions in the three eggs.

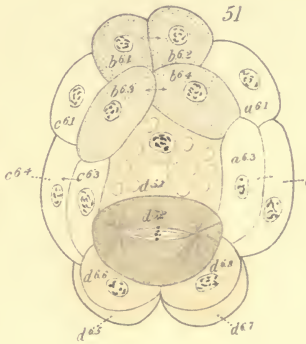
Fig. 52. Egg with thirty-two cells, reckoning the dividing yolk-entoblast as two cells. Derivatives ($d^{6\cdot3}$, $d^{6\cdot4}$) of the primary mesoblast at the posterior edge of entoblast (blastopore). Viewed from vegetative pole.

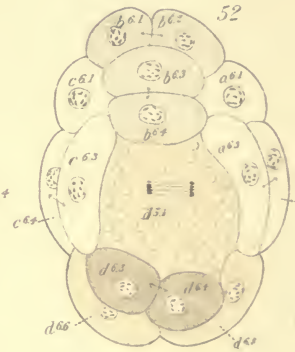
Fig. 54. Optical section in sagittal plane of egg similar to one represented in Figure 50. Cleavage cavity occupied by the yolk-entoblast, which is uncovered at the blastopore only.

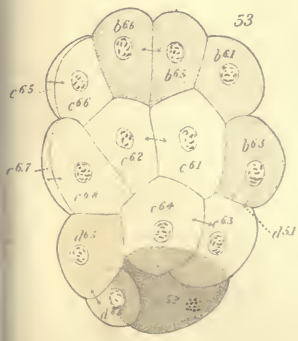
Fig. 55. View from animal pole of egg represented in lateral view in Figure 53.

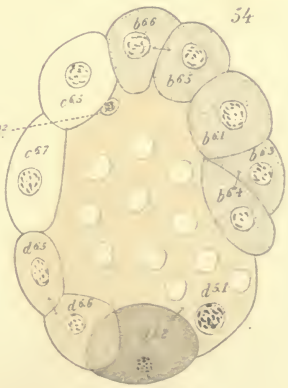


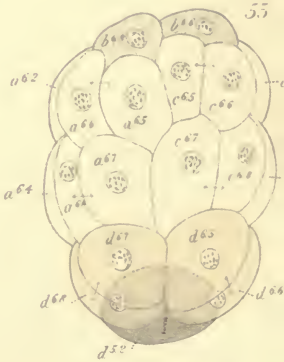












MAB del

B. Meisel, lith. Boston

PLATE 7.

Figures drawn from transparent preparations of entire eggs. Vegetative pole and blastopore at the *right* side in figures seen in lateral view.

Fig. 56. Optical section in sagittal plane. Sixty-two cells, counting the dividing primary mesoblasts ($d^{6\cdot3}$, $d^{6\cdot4}$) as four cells.

Fig. 57. Same stage. Actual section. Blastopore not completely closed.

Fig. 58. View from vegetative pole. The mes-ectoblasts ($a^{6\cdot3}$, $b^{6\cdot3}$, $b^{6\cdot4}$, $c^{6\cdot3}$) in sixth cleavage, which results in forming the "secondary mesoblasts." Blastopore slightly open.

Fig. 59. Same egg in optical section in parasagittal plane. The primary mesoblasts ($d^{6\cdot3}$, $d^{6\cdot4}$) not yet in sixth cleavage. Two entoblastic nuclei ($d^{6\cdot1}$, $d^{6\cdot2}$). Mes-ectoblast cells $b^{6\cdot3}$ and $c^{6\cdot3}$ dividing parallel to the surface of blastoderm, to form "secondary mesoblasts."

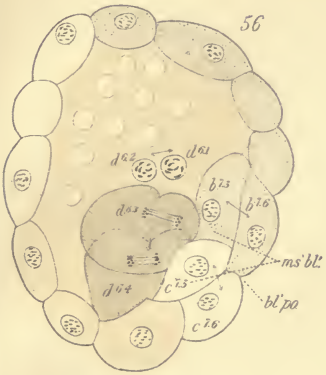
Fig. 60. View from vegetative pole of egg in which the primary mesoblasts ($d^{6\cdot3}$, $d^{6\cdot4}$) have not been overgrown by the blastoderm during the sixth cleavage. These cells nearly fill the blastopore; the posterior pair of "secondary mesoblasts" ($a^{7\cdot5}$, $c^{7\cdot5}$) lie at the sides of the primary mesoblasts.

Fig. 61. Optical section near sagittal plane of same egg, showing anterior pair of "secondary mesoblasts" ($b^{7\cdot5}$ and $b^{7\cdot7}$) and two entoblast nuclei.

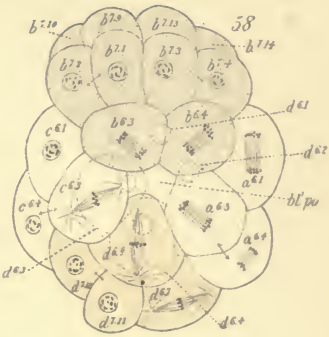
Fig. 62. View from vegetative pole of egg with fifty-six blastoderm cells, four "secondary mesoblasts" ($a^{7\cdot5}$, $b^{7\cdot7}$, $b^{7\cdot5}$, $c^{7\cdot5}$, represented by broken lines), two dividing primary mesoblasts ($d^{6\cdot3}$, $d^{6\cdot4}$, outlines shown by fine continuous line), and two entoblast nuclei (seen at deeper level but not figured).

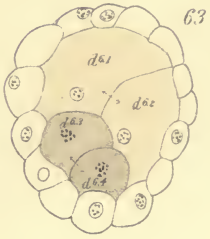
Figs. 63, 64. Optical sections in horizontal plane of different eggs, viewed from vegetative pole. Same stage as Figure 56. Figure 63 represents a common condition in which mesoblasts and entoblasts are not separated by the sagittal plane.

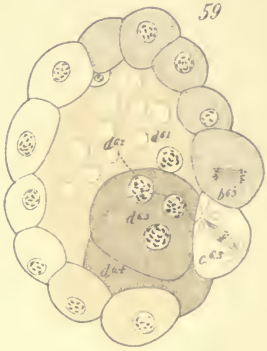
Fig. 65. Optical section in sagittal plane of egg with sixty-two cells. The primary mesoblasts have completed the sixth cleavage, forming $d^{7\cdot5\text{-}8}$.

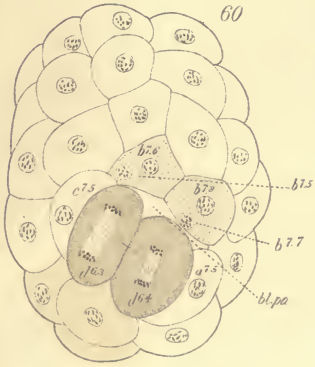

56
d6.2
d6.1
b7.5
b7.6
d6.3
ms'bl'
d6.4
c7.5
bl'po
c7.6

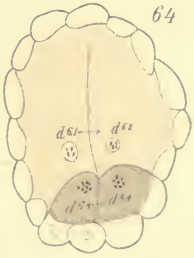

57
b7.5
b7.6
d6.4
bl'po

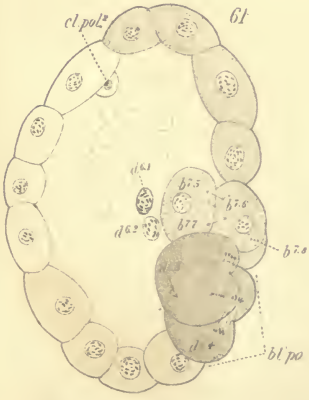

58
b7.10
b7.9
b7.13
b7.14
b7.1
b7.3
b7.2
b7.4
d6.1
b6.4
b6.3
c6.1
d6.2
c6.3
a6.1
bl'po
c6.4
a6.3
a6.4
d6.3
d7.8
d7.11
d6.4

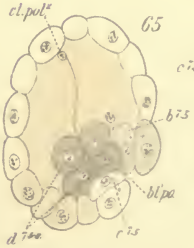

63
d6.1
d6.2
d6.3
d6.4


59
d6.1
d6.2
b6.3
d6.3
c6.3
d6.4


60
b7.6
c7.5
b7.5
b7.9
b7.7
d6.3
a7.5
d6.4
bl.po


64
d6.1
d6.2
d7.1
d6.4


cl.pol.²
61
d6.1
b7.5
b7.6
d6.2
b7.7
b7.8
d6.4
bl'po


cl.pol.²
65
b7.5
bl'po
d7.6
c7.5


62
b7.5
b7.7
c7.5
b7.6
bl'po
a7.5
d6.3
a7.6
d6.4
d6.3

PLATE 8.

All figures drawn from sections ten micra thick. Vegetative (ventral) pole and blastopore at the *left* in views of sagittal sections.

Fig. 66. Parasagittal section of eight-cell stage, a little to the left of the sagittal plane, and corresponding to the stage shown in Figure 40 (Plate 5).

Fig. 67. Section, in same plane, of stage with fifteen blastoderm cells; the yolk-cell still in the stage of fourth cleavage. This stage corresponds to that of Figure 41.

Fig. 68. Parasagittal section of sixteen-cell stage, corresponding to that shown in Figure 45.

Fig. 69. Sagittal section of egg with twenty-eight cells in blastoderm; primary mesoblast cell ($d^{5\cdot2}$) in division; entoblast nucleus preparing to divide. Compare with Figures 49, 50 (Plate 6).

Fig. 70. Horizontal section of same stage, seen from vegetative pole.

Fig. 71. Sagittal section of sixty-two-cell stage, counting two dividing primary mesoblasts ($d^{6\cdot3}$, $d^{6\cdot4}$) as four cells. Same age as Figure 56 (Plate 7).

Fig. 72. Transverse section of egg in similar stage cut through the primary mesoblasts and the posterior pair of "secondary mesoblasts" ($a^{7\cdot5}$, $c^{7\cdot5}$).

Fig. 73. Section immediately anterior to the one represented in the preceding figure. The anterior "secondary mesoblasts" ($b^{7\cdot5}$, $b^{7\cdot7}$) and the two entoblast cells ($d^{6\cdot1}$, $d^{6\cdot2}$) are represented.

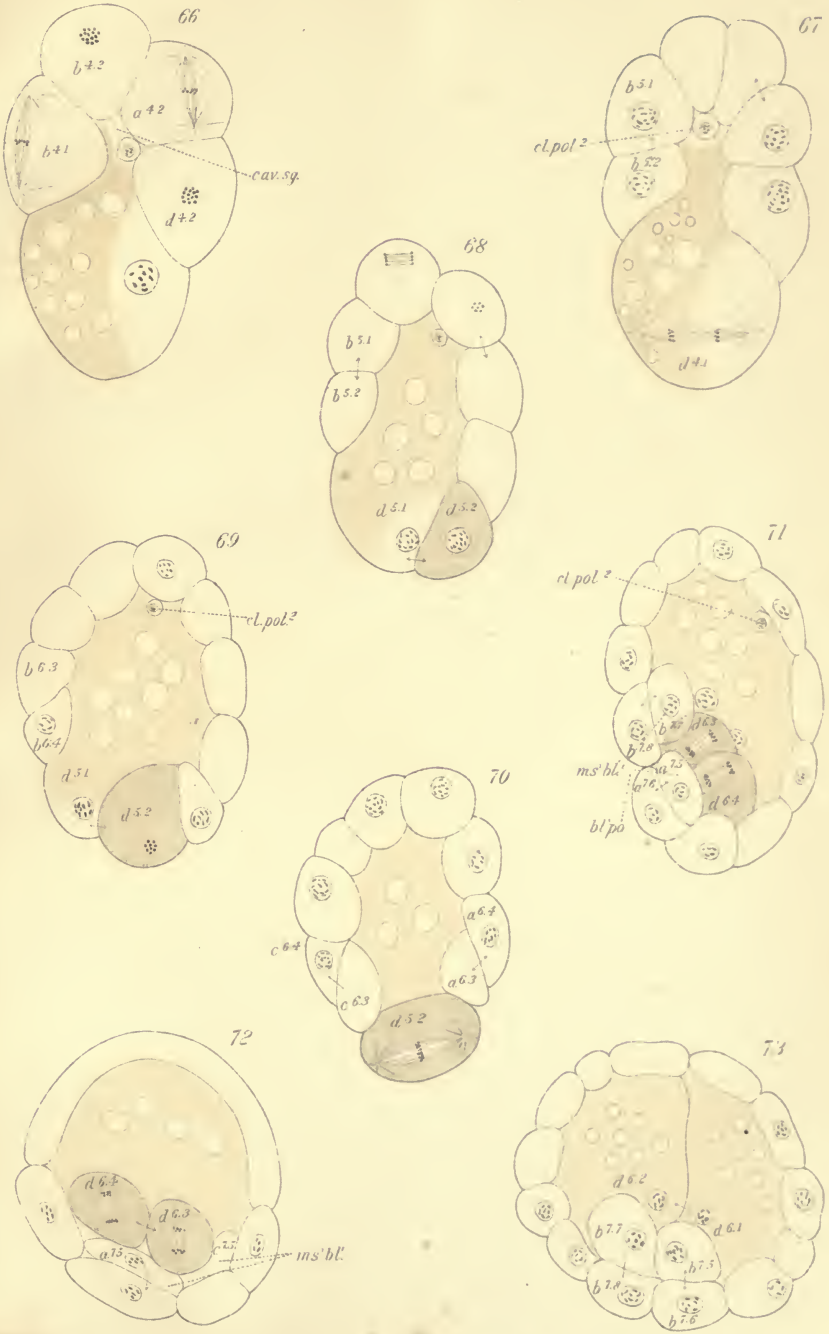

66
b4.2
a4.2
b4.1
cav.sg.
d4.2
67
b5.1
cl.pol.2
b5.2
d4.1
68
b5.1
b5.2
d5.1
d5.2
69
cl.pol.2
b6.3
b6.4
d5.1
d5.2
70
a6.4
c6.4
a6.3
c6.3
d5.2
71
cl.pol.2
b6.2
d6.3
b7.8
ms'bl'
a7.5
a7.6
d6.4
bl'pa
72
d6.4
d6.3
a7.5
ms'bl'
73
d6.2
d6.1
b7.7
b7.5
b7.8
b7.6

PLATE 9.

Figures from three sets of consecutive serial sections. Vegetative (ventral) pole and blastopore are at the *left* in Figures 74–80 and at the *lower* side in Figures 81–86. Blastoderm one cell in thickness.

Figs. 74–77. Series of consecutive sections parallel to sagittal plane from an egg in sixty-two-cell stage, counting two dividing primary mesoblasts as four cells. The first and sixth sections of this series contained only blastoderm cells and have not been figured.

Figs. 78–80. Series of consecutive sections parallel to sagittal plane through egg in a stage with about one hundred and twenty cells. The first and last sections of the series are not figured.

Figs. 81–86. Series of consecutive transverse sections (viewed from their posterior faces) from an egg in same stage as that of last series. Figure 81 shows the most posterior of the sections represented. The first and last sections of the series, containing only blastoderm cells, and three anterior to and similar to Figure 86 have not been figured.

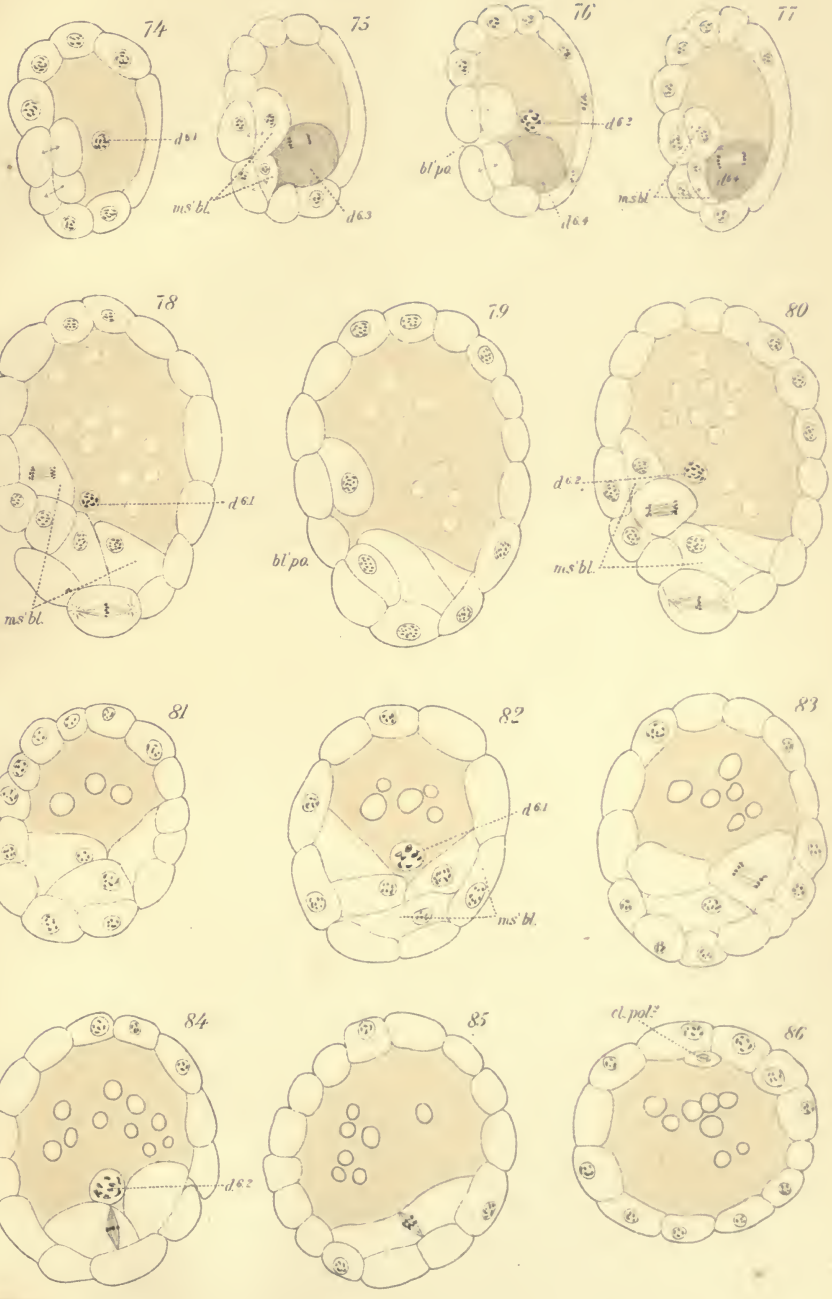

74
d6.1
75
ms'bl.
d6.3
76
d6.2
bl'pa.
d6.4
77
d6.4
ms.bl.
78
d6.1
ms'bl.
79
bl'pa.
80
d6.2
ms'bl.
81
82
d6.1
ms'bl.
83
84
d6.2
85
86
cl.pol.2

PLATE 10.

Figures from sections. Ventral side (blastopore) at the *left* in figures of sagittal sections, and at the *lower* side in figures of transverse sections. Blastoderm one cell in thickness.

Fig. 87. Sagittal section of a stage with two hundred and fifty cells (estimated). The mesoblast band (*ms'bl.*) is extending anteriorly along the *dorsal* side.

Fig. 88. Sagittal section of a later succeeding stage. Egg has elongated posteriorly. Continued extension of the mesoblast.

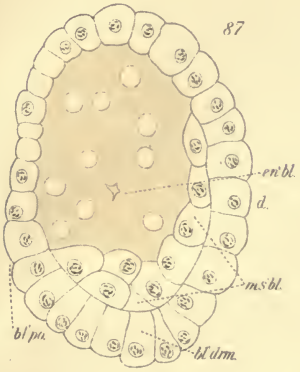
Figs. 89, 90. Transverse sections through an egg similar to the one represented in Figure 88 and made at the levels indicated in that figure by the numbers *89* and *90*. Mesoblast dorsal in Figure 90.

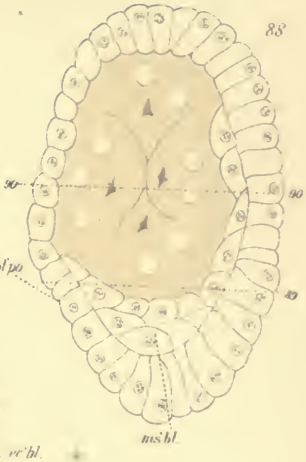
Fig. 91. Sagittal section of later stage. Two transverse dorsal furrows (*1*, *2*) mark off the three metameres. Compare with Figure 122.

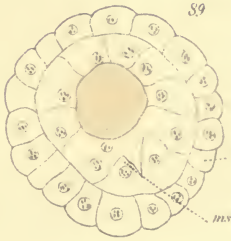
Fig. 92. Transverse section of egg in same stage as that of Figure 91, showing the median dorsal longitudinal furrow. The mesoblast has greatly thickened and extended ventrally on either side of the entoblast. Compare with Figure 90.

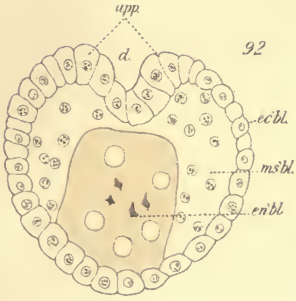
Fig. 93. Sagittal section of still later stage. Two new transverse furrows (*3*, *4*) partially subdivide the first and third metameres of the previous stage. Compare with Figures 123–125.

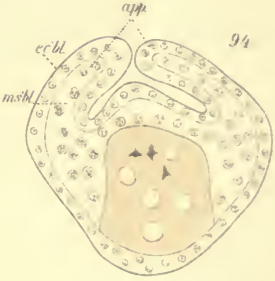
Fig. 94. Transverse section of stage similar to that shown in Figure 93. Longitudinal furrow extending laterally and ventrally folding off the appendages, in which process the transverse furrows 1–4 share.

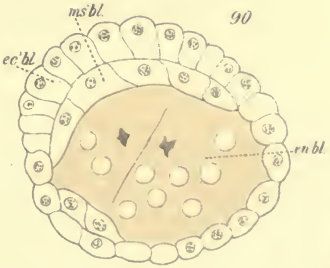

87
en'bl.
d.
ms'bl.
bl'po.
bl'dm.

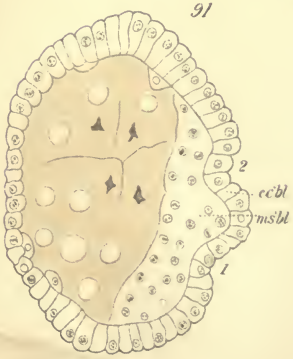

88
90
90
bl'po
ms'bl.


89
ec'bl.
ms'bl.


upp.
d.
92
ec'bl.
ms'bl.
en'bl.


upp.
ec'bl.
94
ms'bl.


ms'bl.
90
ec'bl.
en'bl.


91
2
ec'bl
ms'bl
1

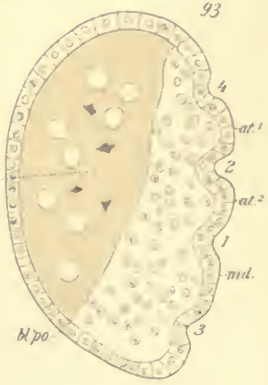

93
4
at.¹
2
at.²
1
mid.
3
bl'po
en'bl.

PLATE 11.

Lepas fascicularis.

The figures in parenthesis following the descriptions refer to corresponding stages of *L. anatifera*.

Fig. 95–97. Outlines of a living egg, showing its rotation within the vitelline membrane during the first cleavage. (Figs. 6–16.)

Figs. 98–110. Drawn from transparent preparations of entire eggs.

Fig. 98. First cleavage, spindle arranged transversely to chief axis of egg. (Figs. 21–23.)

Fig. 99. Second cleavage. View from animal pole. (Fig. 31.)

Figs. 100, 101. Four-cell stage from animal pole. (Figs. 32, 34.)

Fig. 102. Same from vegetative pole. (Fig. 35.)

Fig. 103. Same seen from the *left* side. "Protoplasmic" cells already in third cleavage. (Fig. 36.)

Fig. 104. Eight cells. View from animal pole. Seven "protoplasmic" cells in fourth cleavage. Yolk-cell ($d^{4.1}$) retarded in division. (Fig. 39.)

Fig. 105. Same stage from *left* side. (Fig. 40.)

Fig. 106. Same stage viewed from vegetative pole.

Fig. 107. The divisions shown in Figure 104 as beginning are now completed. View from animal pole. (Compare with Figs. 41, 42.)

Fig. 108. Same stage viewed from *left* side. Yolk-cell ($d^{4.1}$ mes-entoblast) in fourth cleavage. (Fig. 41.)

Fig 109. Optical sagittal section of egg in same stage viewed from *left* side. (Fig. 67.)

Fig. 110. Optical sagittal section of sixteen-cell stage. Left lateral view. (Fig. 68.)

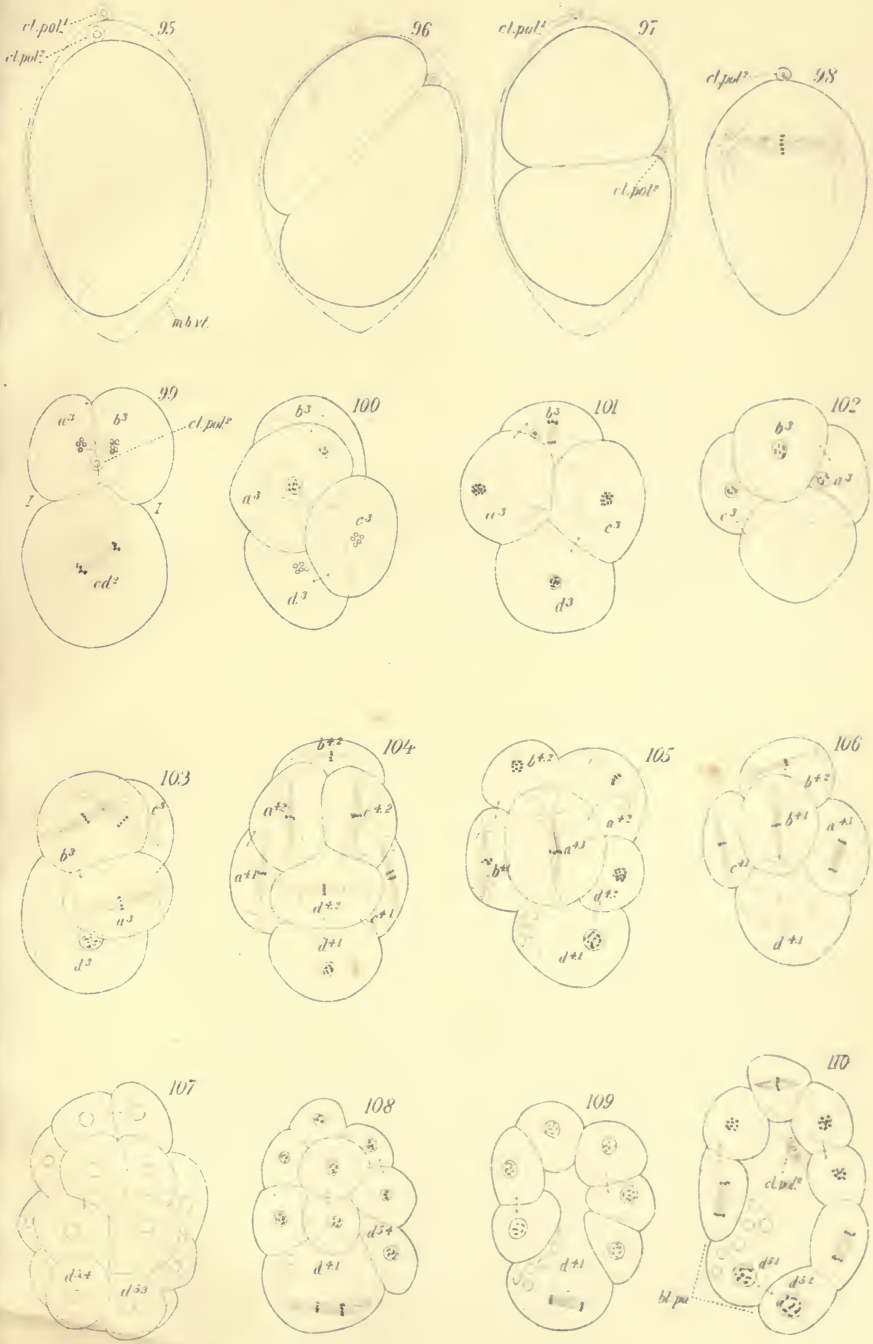
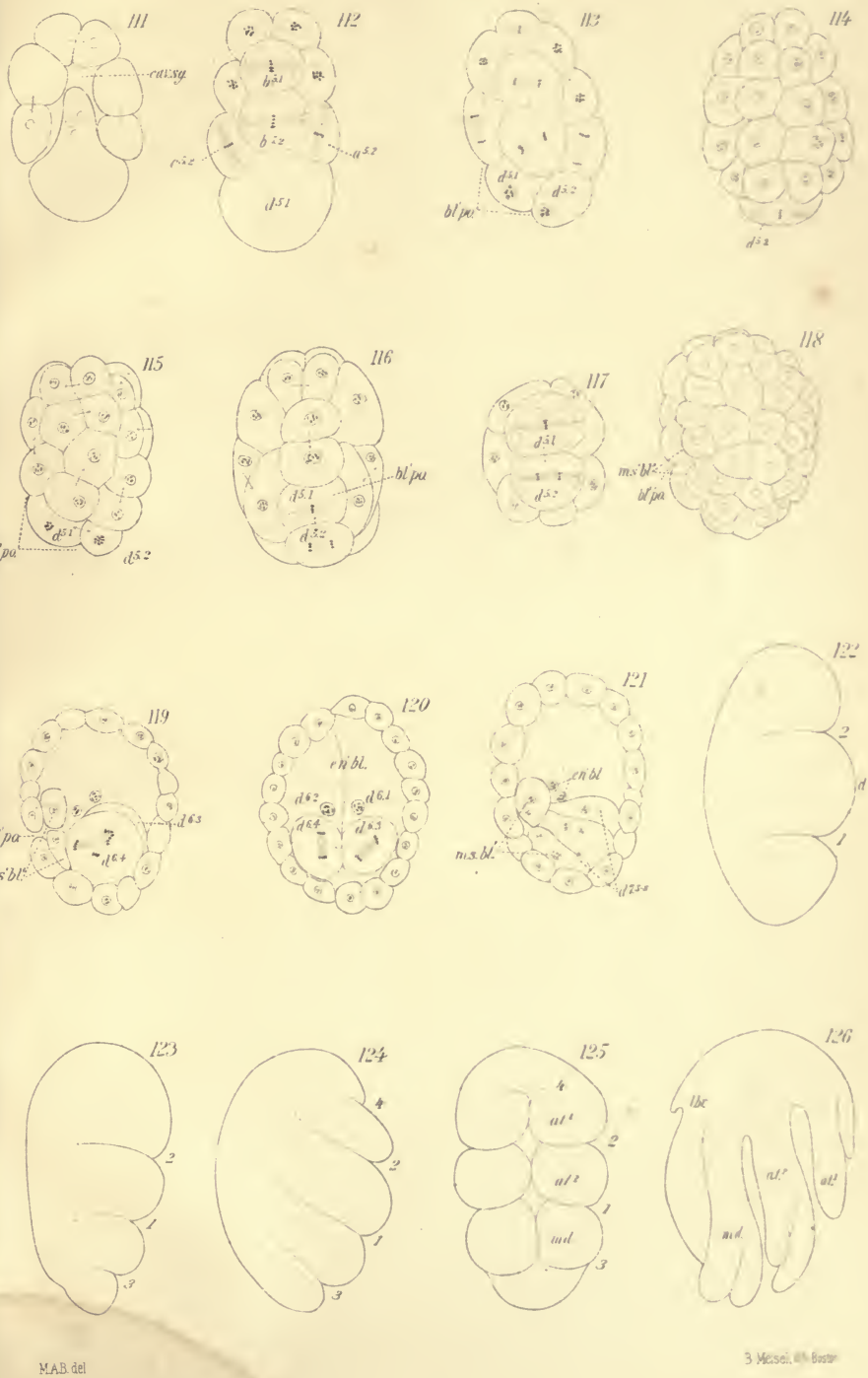

95
cl.pol¹
cl.pol²
m b vl.
96
97
cl.pol²
cl.pol²
98
cl.pol²
99
a³
b³
cl.pol²
cd²
100
b³
a³
c³
d³
101
b³
a³
c³
d³
102
b³
a³
c³
103
c³
b³
a³
d³
104
b+2
a+2
c+2
a+1
d+2
c+1
d+1
105
b+2
a+2
a+1
b+1
d+2
d+1
106
b+2
b+1
a+1
c+1
d+1
107
d+4
d+3
108
d+2
d+1
109
d+1
110
cl.pol²
d+1
d+2
bl pa

PLATE 12.

Lepas fascicularis.

The figures in parenthesis following the descriptions refer to corresponding stages of *L. anatifera.*

Fig. 111. Horizontal section of sixteen-cell stage. (Compare with Fig. 43.)
Fig. 112. Sixteen-cell stage viewed from vegetative pole. Fifth cleavage. (Fig. 46.)
Fig. 113. Same stage, seen from *left* side. (Fig. 45.)
Fig. 114. Thirty-two-cell stage viewed from animal pole. (Fig. 55.)
Fig. 115. Same stage seen from *left* side. (Fig. 53.)
Fig. 116. Same stage viewed from the vegetative pole. Primary mesoblast ($d^{5\cdot2}$) and entoblast ($d^{5\cdot1}$) in fifth cleavage. (Fig. 48.)
Fig. 117. Egg in same stage, looking upon the posterior pole.
Fig. 118. Sixty-two-cell stage seen from *left* side.
Fig. 119. Same stage. Sagittal optical section seen from *left* side. Primary mesoblasts still in sixth cleavage. (Fig. 56.)
Fig. 120. Same stage. Horizontal optical section seen from animal pole. (Fig. 64.)
Fig. 121. Sixty-two cells. Primary mesoblasts have completed sixth cleavage, being now four in number ($d^{7\cdot5}$-$d^{7\cdot8}$). Two entoblasts.
Fig. 122. Profile of late stage. Formation of dorsal transverse furrows (*1*, *2*), which mark off the three metameres. Seen from *left* side. (Fig. 91.)
Fig. 123. Somewhat later stage seen from *left* side. Appearance of a third furrow superficially subdividing the posterior (mandibular) metamere.
Fig. 124. Still later stage seen from *left* side. Another furrow subdivides the anterior (first antennary) metamere. (Fig. 93.)
Fig. 125. Dorsal view of same stage showing the longitudinal and transverse furrows, which, growing ventrally, fold off the appendages.
Fig. 126. Nauplius after development of paired appendages and beginning of the labrum. Seen from the *left* side, ventral being up.



CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE.

E. L. Mark, *Director*.

*** Abbreviations used:—

B. M. C. Z. . . . . for Bull. Mus. Comp. Zoöl.
P. A. A. . . . . for Proceed. Amer. Acad. Arts and Sci.
P. B. S. N. H. . . . . for Proceed. Bost. Soc. Nat. Hist.

1. Barnes, W. — On the Development of the Posterior Fissure of the Spinal Cord, and the Reduction of the Central Canal, in the Pig. P. A. A. **19**: 97–110. 3 pls. 1884.
2. Tuttle, A. H. — The Relation of the External Meatus, Tympanum, and Eustachian Tube to the First Visceral Cleft. P. A. A. **19**: 111–132. 2 pls. 1884.
3. Ayers, H. — On the Development of Oecanthus niveus and its Parasite, Teleas. Mem. Bost. Soc. Nat. Hist. **3**: 225–281. 8 pls. Jan., 1884.
4. Whitman, C. O. — The External Morphology of the Leech. P. A. A. **20**: 76–87. 1 pl. Sept., 1884.
5. Patten, W. — The Development of Phryganids, with a Preliminary Note on the Development of Blatta Germanica. Quart. Journ. Micr. Sci. **24**: 549–602. 3 pls. 1884.
6. Reighard, J. — On the Anatomy and Histology of Aulophorus vagus. P. A. A. **20**: 88–106. 3 pls. Oct., 1884.
7. Faxon, W. — Descriptions of New Species of Cambarus; to which is added a Synonymical List of the Known Species of Cambarus and Astacus. P. A. A. **20**: 107–158. Dec., 1884.
8. Locy, W. — Observations on the Development of Agelena naevia. B. M. C. Z. **12**: 63–103. 12 pls. Jan., 1886.
9. Fewkes, J. W. — Report on the Medusae collected by the U. S. Fish Commission Steamer Albatross in the Region of the Gulf Stream in 1883–'84. Ann. Rep. Commr. Fish and Fisheries for 1884, 927–980, 10 pls., 1886.
10. Ayers, H. — On the Carapax and Sternum of Decapod Crustacea. Bull. Essex Inst. **17**: 49–59. 2 pls. 1886.
11. Mark, E. L. — Simple Eyes in Arthropods. B. M. C. Z. **13**: 49–105. 5 pls. Feb., 1887.
12. Parker, G. H. — The Eyes in Scorpions. B. M. C. Z. **13**: 173–208. 4 pls. Dec., 1887.
13. Mayo, Florence. — The Superior Incisors and Canine Teeth of Sheep. B. M. C. Z. **13**: 247–258. 2 pls. Jun., 1888.
14. Platt, Julia B. — Studies on the Primitive Axial Segmentation of the Chick. B. M. C. Z. **17**: 171–190. 2 pls. Jul., 1889.
15. Mark, E. L. — Studies on Lepidosteus. Part 1. B. M. C. Z. **19**: 1–127. 9 pls. Feb., 1890.
16. Eigenmann, C. H. — On the Egg Membranes and Micropyle of some Osseous Fishes. B. M. C. Z. **19**: 129–154. 3 pls. Mar., 1890.
17. Parker, G. H. — The Histology and Development of the Eye in the Lobster. B. M. C. Z. **20**: 1–60. 4 pls. May, 1890.
18. Ayers, H. — The Morphology of the Carotids, based on a Study of the Blood-vessels of Chlamydoselachus anguineus, Garman. B. M. C. Z. **17**: 191–223. 1 pl. Oct., 1889.
19. Davenport, C. B. — Cristatella: The Origin and Development of the Individual in the Colony. B. M. C. Z. **20**: 101–151. 11 pls. Nov., 1890.
20. Parker, G. H. — The Eyes in Blind Crayfishes. B. M. C. Z. **20**: 153–162. 1 pl. Nov., 1890.

21. Henchman, Annie P. — The Origin and Development of the Central Nervous System in Limax maximus. B. M. C. Z. **20**: 169–208. 10 pls. Dec., 1890.

22. Ritter, W. E. — The Parietal Eye in some Lizards from the Western United States. B. M. C. Z. **20**: 209–228. 4 pls. Jan., 1891.

23. Davenport, C. B. — Preliminary Notice on Budding in Bryozoa. P. A. A. **25**: 278–282. Mar., 1891.

24. Woodworth, W. M. — Contributions to the Morphology of the Turbellaria. — I. On the Structure of Phagocata gracilis, Leidy. B. M. C. Z. **21**: 1–44. 4 pls. Apr., 1891.

25. Parker, G. H. — The Compound Eyes in Crustaceans. B. M. C. Z. **21**: 45–142. 10 pls. May, 1891.

26. Ward, H. B. — On some Points in the Anatomy and Histology of Sipunculus nudus, L. B. M. C. Z. **21**: 143–184. May, 1891.

27. Field, H. H. — The Development of the Pronephros and Segmental Duct in Amphibia. B. M. C. Z. **21**: 201–342. 8 pls. Jun., 1891.

28. Davenport, C. B. — Observations on Budding in Paludicella and some other Bryozoa. B. M. C. Z. **22**: 1–114. 12 pls. Dec., 1891.

29. Smith, F. — The Gastrulation of Aurelia flavidula, Pér. and Les. B. M. C. Z. **22**: 115–126. 2 pls. Dec., 1891.

30. Johnson, H. P. — Amitosis in the Embryonal Envelopes of the Scorpion. B. M. C. Z. **22**: 127–162. 3 pls. Jan., 1892.

31. Boyer, E. R. — The Mesoderm in Teleosts: especially its Share in the Formation of the Pectoral Fin. B. M. C. Z. **23**: 91–134. 8 pls. Apr., 1892.

32. Ward, H. B. — On Nectonema agile. B. M. C. Z. **23**: 135–188. 8 pls. Jun., 1892.

33. Davenport, C. B. — On Urnatella gracilis. B. M. C. Z. **24**: 1–44. 6 pls. Jan., 1893.

34. Davenport, C. B. — Note on the Carotids and the Ductus Botalli of the Alligator. B. M. C. Z. **24**: 45–50. 1 pl. Jan., 1893.

35. Ritter, W. E. — On the Eyes, the Integumentary Sense Papillae, and the Integument of the San Diego Blind Fish (Typhlogobius californiensis, Steindachner). B. M. C. Z. **24**: 51–102. 4 pls. Apr., 1893.

36. Nickerson, W. S. — The Development of the Scales in Lepidosteus. B. M. C. Z. **24**: 115–140. 4 pls. Jul., 1893.

37. Davenport, C. B. — Studies in Morphogenesis. — I. On the Development of the Cerata in Æolis. B. M. C. Z. **24**: 141–148. 2 pls. Jul., 1893.

38. Woodworth, W. McM. — A Method of Orienting small Objects for the Microtome. B. M. C. Z. **25**: 45–47. Dec., 1893.

39. Kofoid, C. A. — On some Laws of Cleavage in Limax. P. A. A. **29**: 180–203. 2 pls. 1894.

40. Davenport, C. B. — Studies, etc. — II. Regeneration in Obelia and its Bearing on Differentiation in the Germ-Plasma. Anat. Anz. **9**: 283–294. 6 figs. Feb. 15, 1894.

41. Holbrook, A. T. — The Origin of the Endocardium in Bony Fishes. B. M. C. Z. **25**: 75–97. 5 pls. Aug., 1894.

42. Castle, W. E. — On the Cell Lineage of the Ascidian Egg. A Preliminary Notice. P. A. A. **30**: 200–216. 2 pls. Oct., 1894.

43. Weysse, A. W. — On the Blastodermic Vesicle of Sus scrofa domesticus. P. A. A. **30**: 283–323. 4 pls. Dec., 1894.

44. Wilcox, E. V. — Spermatogenesis of Caloptenus femur-rubrum. Preliminary Notice. Anat. Anz. **10**: 303, 304. Dec. 19, 1894.

45. Miller, Gerrit S., Jr. — On the Introitus Vaginæ of certain Muridæ. P. B. S. N. H. **26**: 459–468. 1 pl. Feb., 1895.

46. Davenport, C. B., and Castle, W. E. — Studies, etc. — III. On the Acclimatization of Organisms to High Temperatures. Arch. f. Entwickelungsmechanik **2**: 227–249. Jul. 23, 1895.

47. Wilcox, E. V. — Spermatogenesis of Caloptenus femur-rubrum and Cicada tibicen. B. M. C. Z. **27**: 1–32. 5 pls. May, 1895.

48. Kofoid, C. A. — On the Early Development of Limax. B. M. C. Z. **27**: 33–118. 8 pls. Aug., 1895.

49. Nickerson, W. S. — On Stichocotyle nephropis Cunningham, a Parasite of the American Lobster. Zool. Jahrb., Abth. f. Anat. **8**: 447–480. 3 pls. 1895.

50. Davenport, C. B. — Studies, etc. — IV. A preliminary Catalogue of the Processes concerned in Ontogeny. B. M. C. Z. **27**: 171–199. 31 figs. in text. Nov., 1895.

51. Parker, G. H., and Floyd, R. — The Preservation of Mammalian Brains by Means of Formol and Alcohol. Anat. Anz. **11**: 156–158. Sept. 28, 1895.

52. Castle, W. E. — The Early Embryology of Ciona intestinalis, Flemming (L.). B. M. C. Z. **27**: 201–280. 13 pls. Jan., 1896.

53. Davenport, C. B., and Neal, H. V. — Studies, etc. — V. On the Acclimatization of Organisms to Poisonous Chemical Substances. Arch. f. Entwickelungsmechanik **2**: 564–583. 3 figs. Jan. 28, 1896.

54. Parker, G. H., and Floyd, R. — Formaldehyde, Formaline, Formol, and Formalose. Anat. Anz. **11**: 567, 568. Feb. 14, 1896.

55. Parker, G. H. — The Reactions of Metridium to Food and other Substances. B. M. C. Z. **29**: 105–119. Mar., 1896.

56. Gerould, J. H. — The Anatomy and Histology of Caudina arenata Gould. P. B. S. N. H. **27**: 7–74. 8 pls. *and* B. M. C. Z. **29**: 121–190. 8 pls. Apr., 1896.

57. Parker, G. H. — Variations in the Vertebral Column of Necturus. Anat. Anz. **11**: 711–717. 2 figs. Mar. 29, 1896.

58. Wilcox, E. V. — Further Studies on the Spermatogenesis of Caloptenus femur-rubrum. B. M. C. Z. **29**: 191–206. 3 pls. Jun., 1896.

59. Mayer, A. G. — The Development of the Wing Scales and their Pigment in Butterflies and Moths. B. M. C. Z. **29**: 207–236. 7 pls. Jun., 1896.

60. Folsom, J. W. — Neelus murinus, representing a new Thysanuran Family. Psyche, **7**: 391, 392. 1 pl. Jun., 1896

61. Goto, S. — Vorläufige Mittheilung über die Entwicklung des Seesternes Asterias pallida. Zool. Anz. **19**: 271–273. Jun. 15, 1896.

62. Parker, G. H. — Pigment Migration in the Eyes of Palaemonetes. A Preliminary Notice. Zool. Anz. **19**: 281–284. 2 figs. Jun. 29, 1896.

63. Woodworth, W. McM. — Preliminary Report on Collections of Turbellaria from Lake St. Clair and Charlevoix, Michigan. Bull. Michigan Fish Commission, No. **6**: 94, 95. 1896.

64. Goto, S. — Preliminary Notes on the Embryology of the Starfish (Asterias pallida). P. A. A. **31**: 333–335. Jul., 1896.

65. Woodworth, W. McM. — Report on the Turbellaria collected by the Michigan State Fish Commission during the Summers of 1893 and 1894. B. M. C. Z. **29**: 237–244. 1 pl. Jun., 1896.

66. Tower, W. L. — On the Nervous System of Cestodes. Zool. Anz. **19**: 323–327. 2 figs. Jul. 20, 1896.

67. Davenport, Gertrude C. — The Primitive Streak and Notochordal Canal in Chelonia. Radcliffe Coll. Monographs, No. **8**, 54 pp. 11 pls. [Sept.], 1896.

68. Lewis, Margaret. — Centrosome and Sphere in Certain of the Nerve Cells of an Invertebrate. Anat. Anz. **12**: 291–299. 11 figs. Sept. 2, 1896.

69. Judd, S. D. — Description of three Species of Sand Fleas (Amphipods) collected at Newport, Rhode Island. Proc. U. S. Nat. Mus. **18**: 593–603. 11 figs. Aug., 1896.

70. Jennings, H. S. — The Early Development of Asplanchna Herrickii de Guerne. A Contribution to Developmental Mechanics. B. M. C. Z. **30**: 1–118. 10 pls. Oct., 1896.

71. Neal, H. V. — A Summary of Studies on the Segmentation of the Nervous System in Squalus acanthias. A Preliminary Notice. Anat. Anz. **12**: 377–391. 6 figs. Oct. 20, 1896.

72. Davenport, C. B., and Cannon, W. B. — On the Determination of the Direction and Rate of Movement of Organisms by Light. Jour. of Physiol. **21**: 22–32. 1 fig. Feb. 5, 1897.

73. Davenport, C. B., and Bullard, C. — Studies, etc. — VI. A Contribution to the Quantitative Study of Correlated Variation and the Comparative Variability of the Sexes. P. A. A. **32**: 87–97. Dec., 1896.

74. Mayer, A. G. — On the Color and Color-Patterns of Moths and Butterflies. B. M. C. Z. **30**: 167–256. 10 pls. Feb. [Mar.], 1897 *and* P. B. S. N. H. **27**: 243–330. 10 pls. Mar., 1897.

75. Parker, G. H. — The Mesenteries and Siphonoglyphs in Metridium marginatum Milne-Edwards. B. M. C. Z. **30**: 257–272. 1 pl. Mar., 1897.

76. Parker, G. H. — Photomechanical Changes in the Retinal Pigment Cells of Palaemonetes, and their Relation to the Central Nervous System. B. M. C. Z. **30**: 273–300. 1 pl. Apr., 1897.

77. Bunker, F. S. — On the Structure of the Sensory Organs of the Lateral Line of Ameiurus nebulosus Le Sueur. Anat. Anz. **13**: 256–260. Mar. 3, 1897.

78. Woodworth, W. McM. — On a Method of Graphic Reconstruction from Serial Sections. Zeit. f. wiss. Mikr. **14**: 15–18. Jul., 1897.

79. Brewster, E. T. — A Measure of Variability, and the Relation of Individual Variations to Specific Differences. P. A. A. **32**: 269–280. May, 1897.

80. Davenport, C. B. — The Rôle of Water in Growth. P. B. S. N. H. **28**: 73–84. Jun., 1897.

81. Lewis, Margaret. — Clymene producta sp. nov. P. B. S. N. H. **28**: 111–115, 2 pls. Aug., 1897.

82. Porter, J. F. — Two new Gregarinida. Jour. Morph. **14**: 1–20. 3 pls. Jun., 1897.

83. Woodworth, W. McM. — Contributions, etc. — II. On some Turbellaria from Illinois. B. M. C. Z. **31**: 1–16. 1 pl. Oct., 1897.

84. Porter, J. F. — Trichonympha, and other Parasites of Termes flavipes. B. M. C. Z. **31**: 45–68. 6 pls. Oct., 1897.

85. Waite, F. C. — Variations in the Brachial and Lumbo-Sacral Plexi of Necturus maculosus Rafinesque. B. M. C. Z. **31**: 69–92. 2 pls. Nov., 1897.

86. Davenport, C. B., and Perkins, Helen. — A Contribution to the Study of Geotaxis in the Higher Animals. Jour. of Physiol. **22**: 99–110. Sept. 1, 1897.

87. Parker, G. H., and Tozier, C. H. — The Thoracic Derivatives of the Postcardinal Veins in Swine. B. M. C. Z. **31**: 131–144. 5 figs. Mar., 1898.

88. Goto, S. — The Metamorphosis of Asterias pallida, with Special Reference to the Fate of the Body Cavities. Jour. Coll. Sci., Tokyo, **10**: 239–278. 6 pls. 1898.

89. Neal, H. V. — The Segmentation of the Nervous System in Squalus acanthias. A Contribution to the Morphology of the Vertebrate Head. B. M. C. Z. **31**: 145–294. 9 pls. May, 1898.

90. Lewis, Margaret. — Studies on the Central and Peripheral Nervous Systems of two Polychaete Annelids. P. A. A. **33**: 223–268. 8 pls. Apr., 1898.

91. Hamaker, J. I. — The Nervous System of Nereis virens Sars. A Study in Comparative Neurology. B. M. C. Z. **32**: 87–124. 5 pls. Jun., 1898.

92. Field, W. L. W. — A Contribution to the Study of Individual Variation in the Wings of Lepidoptera. P. A. A. **33**: 389–396. 5 figs. Jun., 1898.

93. Mark, E. L. — Preliminary Report on Branchiocerianthus urceolus, A new Type of Actinian. B. M. C. Z. **32**: 145–154. 3 pls. Aug., 1898.

94. Sargent, P. E. — The Giant Ganglion Cells in the Spinal Cord of Ctenolabrus coeruleus. Anat. Anz. **15**; 212–225. 10 figs. Dec. 20, 1898.

95. Rand, H. W. — Regeneration and Regulation in Hydra viridis. Arch. Entwickelungsmechanik, **8**: 1–34. 4 pls. Feb. 21, 1899.

96. Folsom, J. W. — The Anatomy and Physiology of the Mouth-Parts of the Collembolan, Orchesella cincta L. B. M. C. Z. **35**: 5–39. Jul., 1899.

97. Mark, E. L. — "Branchiocerianthus," a Correction. Zool. Anz. **22**: 274, 275. Jun. 26, 1899.

98. Bancroft, F. W. — Ovogenesis in Distaplia occidentalis Ritter (MS.), with Remarks on Other Species. B. M. C. Z. **35**: 57–112. 6 pls. Oct., 1899.

99. Galloway, T. W. — Observations on Non-sexual Reproduction in Dero vaga. B. M. C. Z. **35**: 113–140. 5 pls. Oct., 1899.

100. Parker, G. H. — The Photomechanical Changes in the Retinal Pigment of Gammarus. B. M. C. Z. **35**: 141–148. 1 pl. Oct., 1899.

101. PARKER, G. H., AND DAVIS, FREDERICA K. — The Blood Vessels of the Heart in Carcharias, Raja, and Amia. P. B. S. N. H. **29**(8): 163–178. 3 pls. Oct., 1899.

102. RAND, H. W. — The Regulation of Graft Abnormalities in Hydra. Arch. f. Entwickelungsmechanik, **9**(2): 161–214. Pls. 5–7. Dec., 1899.

103. YERKES, R. M. — Reaction of Entomostraca to Stimulation by Light. Amer. Jour. Physiol. **3**(4): 157–182. Nov., 1899.

104. TOWER, W. L. — The Nervous System of the Cestode Moniezia expansa. Zool. Jahrb., Abth. f. Anat. **13**(3): 359–384. Pls. 21–26. Apr. 10, 1900.

105. WAITE, F. C. — The Structure and Development of the Antennal Glands in Homarus americanus Milne-Edwards. B. M. C. Z. **35**(7): 149–210. 6 pls. Dec., 1899.

106. SARGENT, P. E. — Reissner's Fibre in the Canalis Centralis of Vertebrates. Anat. Anz. **17**(2–3): 33–44. 3 pls. Jan. 15, 1900.

107. WILLIAMS, S. R. — The Specific Gravity of Some Fresh-Water Animals in Relation to their Habits, Development, and Composition. Amer. Nat. **34**(398): 95–108. 3 figs. Feb., 1900.

108. CASTLE, W. E. — The Metamerism of the Hirudinea. P. A. A. **35**(15): 283–303. 8 figs. Feb., 1900.

109. LINVILLE, H. R. — Maturation and Fertilization in Pulmonate Gasteropods. B. M. C. Z. **35**(8): 211–248. 4 pls. May, 1900.

110. PARKER, G. H. — Note on the Blood Vessels of the Heart in the Sunfish (Orthagoriscus mola Linn.). Anat. Anz. **17**(16–17): 313–316. 1 fig. Mar. 31, 1900.

111. PRATT, H. S. — The Embryonic History of Imaginal Discs in Melophagus ovinus L., etc. P. B. S. N. H. **29**(13): 241–272. 7 pls. June, 1900.

112. CASTLE, W. E. — Some North American Fresh-Water Rhynchobdellidae, and their Parasites. B. M. C. Z. **36**(2): 15–64. 8 pls. Aug., 1900.

113. BOWERS, MARY A. — Peripheral Distribution of the Cranial Nerves of Spelerpes bilineatus. P. A. A. **36**(11): 177–193. 2 pls. Oct., 1900.

114. FOLSOM, J. W. — The Development of the Mouth-Parts of Anurida maritima Guér. B. M. C. Z. **36**(5): 85–157. 8 pls. Oct., 1900.

115. PARKER, G. H., and BURNETT, F. L. — The Reactions of Planarians, with and without Eyes, to Light. Amer. Jour. Physiol. **4**(8): 373–385. 4 figs. Dec., 1900.

116. YERKES, R. M. — Reaction of Entomostraca, etc. II. Reactions of Daphnia and Cypris. Amer. Jour. Physiol. **4**(8): 405–422. 6 figs. Dec., 1900.

117. GALLOWAY, T. W. — Studies on the Cause of the Accelerating Effect of Heat upon Growth. Amer. Nat. **34**(408): 949–957. 6 figs. Dec., 1900.

118. PARKER, G. H. — Correlated Abnormalities in the Scutes and Bony Plates of the Carapace of the Sculptured Tortoise. Amer. Nat. **35** (409): 17–24. 5 figs. Jan., 1901.

119. YERKES, R. M. — A Study of Variation in the Fiddler Crab Gelasimus pugilator Latr. P. A. A. **36**(24): 415–442. 3 figs. Apr., 1901.

120. PARKER, G. H., AND ARKIN, L. — The Directive Influence of Light on the Earthworm Allolobophora fœtida (Sav.). Amer. Jour. Physiol. **5**(3): 151–157. 1 fig. Apr., 1901.

121. STRONG, R. M. — A Quantitative Study of Variation in the Smaller North-American Shrikes. Amer. Nat. **35** (412): 271–298. 8 figs. Apr., 1901.

122. SARGENT, P. E. — The Development and Function of Reissner's Fibre, and its Cellular Connections. P. A. A. **36**(25): 443–452. 2 pls. Apr., 1901.

123. PRENTISS, C. W. — The Otocyst of Decapod Crustacea: Its Structure, Development, and Functions. B. M. C. Z. **36**(7): 165–251. 10 pls. July, 1901.

124. PETERS, A. W. — Some Methods for Use in the Study of Infusoria. Amer. Nat. **35**(415): 553–559. 2 figs. July, 1901.

125. PRENTISS, C. W. — A Case of Incomplete Duplication of Parts and Apparent Regulation in Nereis virens Sars. Amer. Nat. **35**(415): 563–574. 6 figs. July, 1901.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. (*Continued.*)

126. Rand, H. W. — The Regenerating Nervous System of Lumbricidæ and the Centrosome of its Nerve Cells. B. M. C. Z. **37**(3): 83–164. 8 pls. Sept., 1901.

127. Frandsen, P. — Studies on the Reactions of Limax maximus to Directive Stimuli. P. A. A. **37**(8): 183–227. 22 figs. Oct., 1901.

128. Yerkes, R. M. — A Contribution to the Nervous System of Gonionemus murbachii. Pt. I. Amer. Jour. of Physiol. **6**(6): 434–449. Feb., 1902.

129. Oppenheimer, A. — Certain Sense Organs of the Proboscis of the Polychaetous Annelid Rhynchobolus dibranchiatus. P. A. A. **37**(21): 551–569. 6 pls. Apr., 1902.

130. Williams, S. R. — Changes Accompanying the Migration of the Eye and Observations on the Tractus opticus and Tectum opticum in Pseudopleuronectes americanus. B. M. Z. C. **40**(1): 1–57. 5 pls., 7 figs. May, 1902.

131. Yerkes, R. M. — A Contribution to the Nervous System of Gonionema murbachii. Pt. II. Amer. Jour. of Physiol. **7**(2): 181–198. May, 1902.

132. Bigelow, M. A. — The Early Development of Lepas. A Study of Cell-Lineage and Germ-Layers. B. M. C. Z. **40**(2): 59–144. 12 pls. July, 1902.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. (*Continued.*)

126. Rand, H. W. — The Regenerating Nervous System of Lumbricidæ and the Centrosome of its Nerve Cells. B. M. C. Z. **37**(3): 83–164. 8 pls. Sept., 1901.

127. Frandsen, P. — Studies on the Reactions of Limax maximus to Directive Stimuli. P. A. A. **37**(8): 183–227. 22 figs. Oct., 1901.

128. Yerkes, R. M. — A Contribution to the Nervous System of Gonionemus murbachii. Pt. I. Amer. Jour. of Physiol. **6**(6): 434–449. Feb., 1902.

129. Oppenheimer, A. — Certain Sense Organs of the Proboscis of the Polychaetous Annelid Rhynchobolus dibranchiatus. P. A. A. **37**(21): 551–569. 6 pls. Apr., 1902.

130. Williams, S. R. — Changes Accompanying the Migration of the Eye and Observations on the Tractus opticus and Tectum opticum in Pseudopleuronectes americanus. B. M. Z. C. **40**(1): 1–57. 5 pls., 7 figs. May, 1902.

131. Yerkes, R. M. — A Contribution to the Nervous System of Gonionema murbachii. Pt. II. Amer. Jour. of Physiol. **7**(2): 181–198. May, 1902.

132. Bigelow, M. A. — The Early Development of Lepas. A Study of Cell-Lineage and Germ-Layers. B. M. C. Z. **40**(2): 59–144. 12 pls. July, 1902.