embryo, and therefore when first formed divided the ovum into an anterior and a posterior half; while the second furrow, the one in which the polar furrow bends to the left, coincides with the median plane of the embryo, and hence divided the first two blastomeres into two right and two left macromeres.[1] While it is thus easy to determine from the earliest appearance of the first cleavage what the antero-posterior axis of the future embryo is to be, it is not possible to distinguish the anterior end from the posterior until the stage with twenty ectoderm cells, Fig. 22, when the mesentoblast is formed. A similar relation of the first cleavage plane to the embryonic axes is also found in Teredo (Hatschek, '80), Umbrella (Heymons, '93), and Nereis (Wilson, '92). In Crepidula I believe it has no causal relation to the bilateral symmetry of the embryo. The egg itself is not bilateral with respect to the first or second cleavage plane, as has been pointed out (p.), but is from the first up to the time when the mesentoblast is formed radially symmetrical. So far as the entoderm cells are concerned, the second furrow lies nearly in the median plane of the bilateral embryo, and the first furrow nearly at right angles to this; but among the ectoderm and mesoderm cells such shiftings of position occur that the final plane of bilateral symmetry in no way corresponds with either of the first two cleavage planes. This conclusion will be treated more fully after the facts upon which it is based have been taken up in their regular order.

II. THE SEGREGATION OF THE ECTOBLAST.

1. *Formation of the First Quartette of Micromeres. Figs. 12, 13, Diagram 3 (p. 60).*

The third cleavage separates four protoplasmic micromeres from the four yolk-containing macromeres. The karyokinetic

[1] It will be seen that in all the figures except those of the first plate the first furrow runs from right to left on the plate; for the sake of appearance merely, the figures of the first plate are arranged so that the first furrow runs up and down. In the first plate, therefore, the antero-posterior axis runs from right to left as the figures are arranged on the page, while in all the other plates it runs up and down.

spindles which introduce this cleavage have their inner ends at a higher level than the outer ends, and are usually very nearly radial in position, though they are frequently slightly inclined in a right spiral direction, and occasionally even in a left spiral. Whatever may be the direction of the spindles, however, the third cleavage itself is always a dexiotropic one. In the early stages of the formation of these spindles their axes may be radial or even laeotropic; but usually before the nuclear division is completed, and always before the cell division takes place, the cleavage becomes dexiotropic. Thus in Fig. 12 the spindle in the macromere C is most advanced, while those in D, A, and B show progressively earlier stages in the nuclear division. Now if we consider the outer ends of the spindles as remaining stationary, and the inner and upper ends as being movable, it will be seen that in C the inner end has been rotated slightly in a clockwise direction around the chief axis of the ovum as a centre; there may be slight indications of this rotation in D and A, though certainly it is not present in B. In other words, *the more advanced the cleavage is, the more pronounced the rotation becomes*, and what is true in this instance is true in every one that has come under my observation. *After the division wall between the dividing cells has appeared, the rotation still continues; in the formation of both the first and second quartettes there is an actual rotation of these cells*, and not merely an oblique cleavage, as is the case in Unio and in some of the cleavages of Nereis.

In some ova the formation of the micromeres of each quartette takes place in regular succession, as is shown by the successive stages of karyokinesis in the four macromeres of Fig. 12; in other ova no such regular succession can be determined. After the micromeres have been separated they continue to rotate until they come to lie in the furrows between the macromeres and alternate with them in position, Figs. 13 and 14. The outer cell walls of the micromeres are at first rounded, as shown in Fig. 13; but after they have taken their positions between the macromeres they become pressed down into the furrows so that their outer

border becomes pointed, as shown in Fig. 14. *Thus it is seen that the shape of the cell depends, in part at least, upon the position which it holds,* i.e., *the outlines of the cell are the result of the pressure to which it is subjected.* These micromeres at first meet each other in a point immediately under the polar bodies, though afterward, as the result of pressure, two of them may meet in a line or secondary polar furrow, as shown in Fig. 17. This secondary polar furrow is not a part of the original polar furrow, but is a new feature caused by the shifting of the cells of the first group of micromeres after they have been formed. Moreover, it bears no constant relation to the original polar furrow; in Figs. 17, 29, 31, 33, 42, 44, 46 this secondary polar furrow is almost parallel with the original one; in Figs. 32, 35, 36, 38, 41, 49, 64 it is nearly at right angles to it, and there is evidence that in the same egg it may change its relations at different periods. Among small cells very actively dividing polar furrows, or rather pressure surfaces, do not long preserve definite axial relations. The original polar furrow preserves its fixed position because it lies between macromeres, which in spite of numerous divisions still remain very large; the position of the polar furrow could not here be changed without profound changes in the positions of all the other cells and in the shape of the whole egg.

In Crepidula the dorsal portion of the polar furrow does not lie between any of the ectoblast cells, since in all cases the cells of the first quartette meet in a point when first formed; the polar furrow lies wholly and entirely between the macromeres, and its dorsal portion can be seen just beneath the cap of ectoblast cells.

Kofoid ('95) has found that in Limax both the dorsal and ventral polar furrows preserve their identity even up to an advanced stage of the cleavage, and here the axial relations of both furrows are also preserved. Kofoid says (p. 55): "With the completion of the sixteen-cell stage and the fifth generation, the dorsal and ventral cross furrows are restored to the conditions of the four-cell stage, *i.e.*, they cross each other at approximately right angles. A similar restoration to the conditions of the four-cell stage occurs in Nereis; also in Umbrella

at the twelve-cell stage, and probably in Neritina. In Planorbis, however, according to Rabl's interpretation, the cross furrow of the animal pole is not restored to the position of the four-cell stage, but is turned 90° from it (see his Taf. XXXII, Figs. 10 A, 11 A). To accomplish this it is necessary for each of the cells of the apical quartette to be shifted 90° to the left, and thus completely out of their own quadrants over upon the adjoining quadrants. It seems very probable that Rabl is in error in this matter, and that in Planorbis, as in the other forms, the division of the generations results in the restoration of the cross furrows to the conditions of the four-cell stage." It is possible that in Planorbis, as in Crepidula, there is no part of the original polar furrow between the cells of the apical quartette, and that the pressure surface, formed later, may lie in any direction with reference to the real polar furrow.

2. *Formation of the Second Quartette of Micromeres. Figs. 14–16, Diagram 3 (p. 60).*

When the first four micromeres have taken a position alternating with the macromeres, the nuclei of the latter again divide, as shown in Fig. 14. All the nuclei divide at nearly the same time, as in the preceding cleavage, but the spindles do not lie radially as before, but run transversely or tangentially in each macromere. One end of each spindle lies on the mid line of each macromere, the other end lies to the left, very near the furrow, between contiguous spheres ; the former is at a lower level than the latter, and hence the spindles are arranged in a left wound or anti-clockwise direction. Again, considering the deeper or central end of each spindle as fixed and the other as movable, it will be seen that as division advances the outer end swings inward toward the centre of the formative pole, and at the same time comes to lie at a considerably higher level, Figs. 14 and 15. As the four cells of the second quartette are being cut off from the macromeres, they rotate in an anti-clockwise direction until they occupy the furrows between the macromeres, and by this rotation they turn the cells of the first quartette back to their original positions over the centre of

each macromere, Diagram 8. These micromeres do not again shift their position to any considerable extent until the general rotation of the ectoblastic cap in the 52-cell stage.

The fact that the micromeres are more firmly bound to each other than to the macromeres is shown hereafter at almost every stage; it is first plainly indicated, however, in such stages as Figs. 15 and 16, where the second quartette of micromeres, in rotating in an anti-clockwise direction, carries with it the first quartette, as if the whole formed a rigid plate lying upon the macromeres. Evidence of this same fact is farther shown by Fig. 16, in which the micromere 2b does not lie in the furrow between A and B, though the other micromeres of this quartette, 2a, 2d, and 2c, lie in the other furrows. This is due to the fact that because of a very long polar furrow between macromeres B and D, the first and second cleavages are not at right angles to each other. Instead, therefore, of shoving past 1a into the furrow between A and B, the cell 2b remains in its proper position relative to the other micromeres, although by so doing it cannot come into the proper position relative to the macromeres.

This fact that the micromeres are more loosely connected with the macromeres than with each other may be in part accounted for by the presence of a small rectangular segmentation cavity lying just over the polar furrow and under the first set of micromeres. It is most clearly marked at the moment when the first quartette is separated from the macromeres, and it entirely disappears after the second quartette is formed.

3. *Division of the First Quartette of Micromeres and Formation of the Turret Cells (Trochoblasts). Figs. 16, 17, Diagram 4 (p. 60).*

Before the third and last quartette of micromeres is formed the first quartette divides in a laeotropic direction, as shown in Fig. 16. Division occurs at nearly the same time in each of the cells, and the central moieties ($1a^1$–$1d^1$) remain considerably larger than the peripheral ones ($1a^2$–$1d^2$). The smaller outer portions do not again divide until very late in the cleavage,

and they therefore form a valuable landmark for orientation. From their peculiar position and shape I shall call them the "turret cells"; their further history will be considered in another place.

After the division of the nuclei, and even after the cell body has divided, the turret cells continue to rotate in a clockwise direction until they lie at the ends of the furrows separating the four apical cells. *In this case, therefore, as in every other which I have observed, the spiral character of the cleavage is much more pronounced after the nuclear division than during that division. It seems to be a phenomenon belonging to and caused by the cytoplasm rather than the nucleus.*

In Discocoelis (Lang, '84), Nereis (Wilson, '92), and Limax (Kofoid, '95) the first quartette divides at the time the second is being formed, and before the third quartette is formed the first has divided twice. In Planorbis (Rabl, '79), Neritina (Blochmann, '81), Unio (Lillie, '95), and Crepidula the first quartette divides once before the third is formed; while in Umbrella (Heymons, '93) and Urosalpinx the first does not divide at all before the third is formed. In general the rate of development of the upper hemisphere is indicated by these facts ; in Nereis the development of the upper hemisphere is very precocious; it is very tardy in Umbrella and Urosalpinx; while Planorbis, Neritina, Unio, and Crepidula occupy an intermediate position in this respect.

In most gasteropods so far studied the turret cells have essentially the same peculiarities of size and position as in Crepidula, so that during the early stages of cleavage they can be recognized at a glance. In Nereis Wilson has found that these cells form the prototroch, and he therefore calls them the *trochoblasts*. Mead ('94) also has found that they form a part of the prototroch in Amphitrite and Clymenella. In Crepidula at least two of these cells, probably all four, form a portion of the velum; but because I am not certain as to the destiny of the two posterior ones ($1c^2$ and $1d^2$), I prefer to call the group for the present by a non-committal name. Their destiny has not been determined in any other form.

4. *Formation of the Third and Last Quartette of Micromeres and Complete Segregation of the Ectoblast. Figs. 17–19, Diagram 4.*

The last quartette of ectomeres is formed by dexiotropic cleavage. The axis of each spindle lies transverse to the median plane of each macromere, and nearer the right side than the left. The right end of the spindle is higher than the left, and lies on the right side of the macromere near the furrow between contiguous spheres, and in the space between successive micromeres of the second quartette. The left and

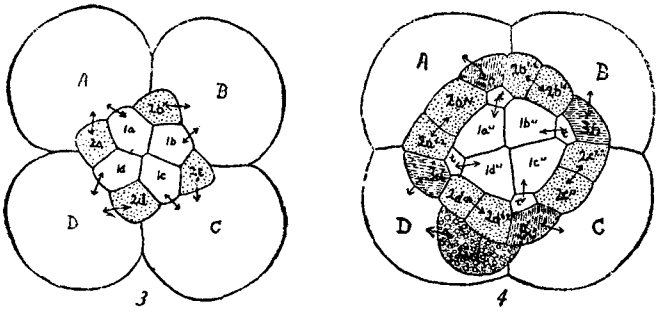

DIAGRAM 3.— Crepidula, twelve-cell stage.

DIAGRAM 4.— Crepidula, twenty-five cells; *t*, turret cells (trochoblasts). In these and some of the following diagrams the macromeres and first quartette are unshaded; the second quartette is stippled; the third quartette is shaded with lines; and the fourth quartette (4d) with dots and circles. The direction of the various cleavages is shown by means of arrows.

lower end of the spindle lies near the mid line of the macromere, and usually beneath the second group of micromeres, Fig. 17. As division advances, the right and upper end of each spindle is lifted to a higher level and swung inward toward the apical pole. When the cells of this quartette are separated they do not rotate in a clockwise direction, carrying the whole plate of micromeres with them, as in the formation of the first and second quartettes, but the ectoblastic plate remains fixed and the third group of micromeres is merely pressed into the spaces between the micromeres of the second group, Figs. 18, 19, 20 and Diagram 4.

Into these three quartettes of micromeres is gathered the entire ectoblast of the developing embryo; and from these

twelve cells (increased to sixteen by the division of the first quartette) comes the whole outer covering of the body, the shell gland and ciliated locomotor apparatus, the larval excretory cells and stomodaeum, the nervous system and sense organs.

The material of the macromeres is not homogeneous as yet, since one of them, the left posterior, contains most of the future mesoblast ; but at this early stage we have two layers, ectoblast and mesentoblast, perfectly differentiated.

It is a most remarkable fact that in all annelid and molluscan eggs with holoblastic segmentation the ectoblast is segregated in just three groups or quartettes of cells — no more and no less. The evidence for this remarkable fact has been accumulating until at present it is known to be true of at least a score of forms, and not a single trustworthy observation can be urged against it. A few apparent exceptions have been recorded among prosobranchiate gasteropods. Bobretzky ('77), after describing the formation of the first two groups of micromeres in Nassa, says : "The large spheres continue to bud off new cells around the circumference of those already formed, while the latter continue to divide." Of Fusus he says that after the first two cleavages the segmentation goes on as in Nassa. Of these statements it need only be said that the work was done at a time (1874–75) when little attention was paid to the details of cleavage, and confessedly the number of quartettes of ectomeres was not known. I have worked over the cleavage of Illyonassa and Urosalpinx, which are the nearest representatives of Nassa accessible to me, and, although the form of cleavage is very similar to that given for Nassa, there is no departure from the rule that three, and only three, quartettes of ectomeres are formed.

Another exception is recorded by McMurrich ('86) for Fulgur. After describing the formation of the first two groups of micromeres, he says : "The succeeding stages of segmentation I did not follow in detail, but can state that they result in the increase of the number of micromeres, partly by the division of those already formed, and partly by the separation of new ones from the macromeres. . . . It would seem that in Fulgur spherules

continue to be budded off from the macromeres for a much longer period than in some other forms ; thus, for instance, Blochmann describes only three generations of spherules from the macromeres in Neritina. In Nassa, according to Bobretzky, a greater number are formed ; he describes twenty spherules as arising in this manner, and it is possible that more arise in the same way in later stages. Probably the amount of yolk present influences the number of spherules so formed ; in other words, the greater the number of spherules required to surround the macromeres, the more frequently are generations formed from the macromeres." Lillie ('95) comments upon this passage, and justly remarks that "the important point is to determine how many of these generations are ectomeres," not all micromeres being ectomeres, as Wilson ('92) has shown in the case of Polymnia and Aricia.

In all four species of the genus Crepidula which I have studied, but three quartettes of micromeres are separated from the macromeres, and this in spite of the fact that the egg of C. adunca is twenty-seven times as large as the egg of C. plana ; in this case, therefore, the size of the egg has no influence on the number of quartettes separated from the macromeres, although a count of the nuclei shows that about five times as many ectoderm cells are present in C. adunca at the time of the closure of the blastopore as are found in C. plana. This increased number of ectoderm cells in the large egg is due entirely to the more rapid division of the three quartettes already formed, and not to the formation of additional quartettes.

McMurrich's conclusions are so much in conflict with my observations on Crepidula that I have taken the pains to briefly study the cleavage of Fulgur. The result of this study shows that in this case also three, and only three, quartettes of micromeres are separated from the macromeres. The cleavage is marvellously like that of Crepidula, though the eggs are from fifty to one hundred and forty times as large.

Two other somewhat doubtful exceptions to this rule have been recorded; *e.g.*, Salensky ('87) believed that more than three quartettes of micromeres were formed in Vermetus. More

recently Erlanger ('92) has reached the same view concerning Bythinia. Neither of these cases, however, is conclusive, and I have little doubt that a careful reëxamination would show that here also three, and only three, quartettes of *ectomeres* are formed.

No phenomenon in the whole history of cleavage seems to me more remarkable than this. As just said, it occurs almost universally among mollusks and annelids, in equal or unequal cleavage, and in eggs varying in size from a few microns to more than a millimeter in diameter. Associated with it is the formation of the mesoblast and entoblast in all these forms in the fourth quartette. *The cause of this remarkable phenomenon is to be found in the fact,* as I believe, *that each of these quartettes of ectomeres is the protoblast of definite regions and organs of the larva.* In all cases in which three quartettes of ectomeres are formed, the first quartette gives rise to all the umbrella region and at least a portion of the prototroch ; the second quartette gives rise to the median anterior, posterior, right and left portions of the body ; while the third quartette gives rise to the regions intermediate between those formed by the second quartette.

In Umbrella the different quartettes are successively larger, the first being smallest and the fourth largest. In Crepidula the difference in size between the first three quartettes is very slight, though the second quartette is perhaps somewhat larger than either the first or third ; the fourth quartette, owing chiefly to the amount of yolk which it contains, is very much larger than either of the preceding ones. In general the relative size of the different quartettes of ectomeres depends upon the relative size of the regions and organs of the larva to which they give rise, and also upon the relative time at which these organs are formed.

5. *Division of the Second Quartette of Micromeres. Figs. 18, 19, Diagram 4.*

Although it is not my purpose to take up the history of the micromeres until after I have described the complete segregation of the layers, it seems best in this section to trace the

history of the whole egg up to the point, Fig. 22, where the segregation of the layers is practically complete, and then to deal separately with the history of each of these layers ; accordingly, I shall describe here the first division of the second quartette, which occurs before the separation of the mesoblast.

Very soon after the formation of the third quartette the second quartette divides, Figs. 18 and 19. It is not possible during the nuclear division to tell which end of the spindle lies at the higher level, though the right end lies nearer the mid line of each macromere, Fig. 19, and after the cell division it is seen that the right moiety overlaps the left, Fig. 20. The spindles are, therefore, arranged in a right-wound spiral, and the division is dexiotropic. The two moieties are about equal in size, though the right one seems the larger because it overlaps to a certain extent the left.

At this stage there are twenty micromeres and four macromeres. The micromeres are arranged in a plate, the rounded corners of which lie in the furrows between the macromeres, Fig. 19, Diagrams 4 and 5. The centre of the plate is formed of four *apical cells* and four *turret cells*, which are the derivatives of the first quartette. These eight cells form a rectangular plate with its corners in the furrows between the macromeres. Around this central plate of eight cells is a belt of twelve cells, consisting of eight cells derived from the second quartette and four cells of the third quartette ; these cells we shall call the *belt cells*. In Fig. 20 it is seen that the apical and turret cells overlap the belt cells, so that the micromeres are arranged like the shingles on a roof. The apical cells do not overlap the turret cells ; in the division of the second quartette, as has been explained, the right moiety overlaps the left; while underlying all of these is the third quartette.

The first division of the second quartette occurs in essentially the same way, though subject to certain variations in time, in all cases in which the cleavage has been carefully studied, with the single exception of Neritina. Blochmann ('81) asserts that in this animal the cleavage is not dexiotropic, as is true elsewhere, but is laeotropic. This difference in itself might seem to be of little importance, but since it profoundly modifies

the interpretation of later stages, it demands a careful consideration.

Heymons ('93) called attention to the difference between Neritina and Umbrella in this cleavage, and he ascribed it to the difference in the axial relations of the "cross" of ectoblast cells in those two animals. In this he was certainly in error, as we shall see when we come to consider the cross in a subsequent section.

More recently Kofoid ('94) has discussed this unusual cleavage in Neritina and has presented strong evidence for believing that Blochmann was mistaken in his interpretation of it, and still more recently Lillie ('95) cites Kofoid's criticism with approval. Kofoid suggests a possible correction of Blochmann's interpretation (the nature of which is shown in the accompanying diagram, 5c), which would bring the cleavage of Neritina into conformity with the "law of alternating cleavages," but curiously enough, he just misses the true explanation. The modification suggested by Kofoid does meet the requirements of his law of alternating cleavages, but it does not harmonize with Blochmann's oft-repeated statement that the terminal cells in the transverse arms of the cross (his "Urvelarzellen") come from two cells, 2a and 2c, of the second quartette. Even before cleavage begins two masses of granules can be recognized on opposite sides of the animal pole, and in the formation of the second quartette these granules pass into the cells 2a and 2c, and finally they appear in the "Urvelarzellen," as soon as these are formed. Owing to the presence of these peculiar granules, it seems very improbable that Blochmann could have been mistaken in the derivation of the "Urvelarzellen." Kofoid recognizes this difficulty and attempts to meet it by suggesting that the granules originally present in the cells 2a and 2c may disappear, and that new granules may appear in the corresponding cells of the third quartette (3a and 3c), which are in turn handed over to the "Urvelarzellen." This suggestion seems to me as improbable as it is unnecessary. In Crepidula the terminal cells of the cross ("Urvelarzellen") are derived exactly as Blochmann asserts is the case in Neritina, and the same thing is

true of several other gasteropods which I have studied. I think, therefore, that Blochmann's derivation of these cells can no longer be called in question. But it is evident, as Kofoid points out, that he has made a mistake in the derivation of the

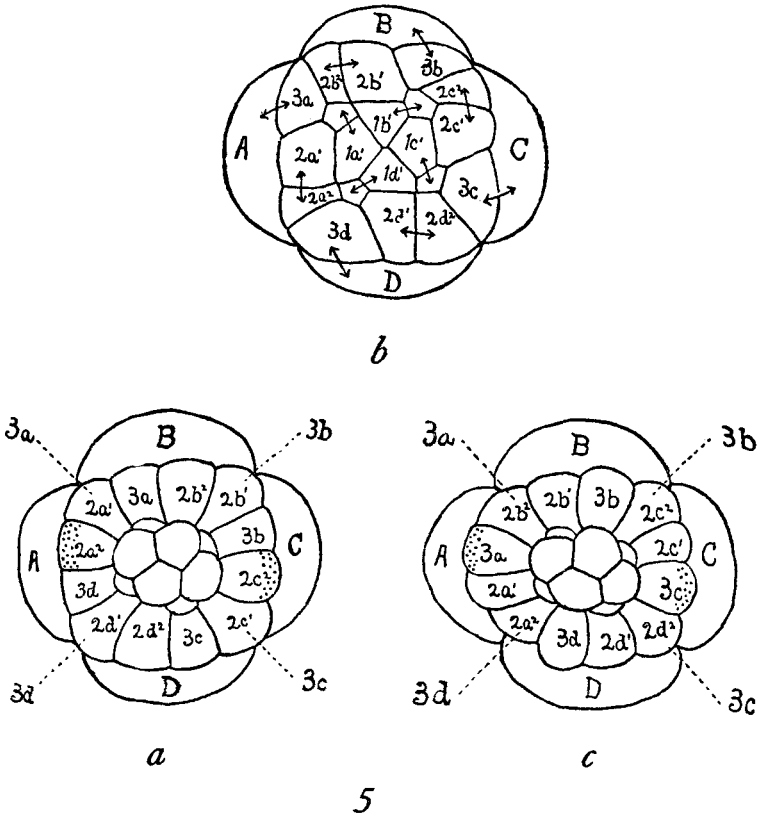

DIAGRAM 5. — The 24-cell stage in Neritina and Crepidula. The nomenclature is the one used throughout this paper. — *a*, The derivation of the belt cells of Neritina according to Blochmann. The stippled cells give rise to the "Urvelarzellen." — *c*, Kofoid's modification of Blochmann's account. — *b*, The derivation of these cells in Crepidula. The reference lines and letters in *a* and *c* indicate the modifications necessary to bring Neritina into agreement with Crepidula.

belt cells. In the accompanying diagrams I give Blochmann's Fig. 48, Kofoid's modification of this, and a corresponding stage in Crepidula; the nomenclature in each case has been reduced to the system used in the present paper.

It will be seen at once that both Blochmann and Kofoid fail to identify the cells of the third quartette with the corners of the ectoblastic plate, and consequently mislabel the whole of the belt, carrying the proper designations one cell too far to the right in the one case, and one cell too far to the left in the other. This proposed correction of Blochmann's account and of Kofoid's modification is further supported by a figure of the egg of Neritina of this same stage, given by Bütschli ('77), Pl. XVII, Fig. 3a, in which the position of the cells plainly shows that the angles of the ectoblastic plate are formed by the third quartette, while the two cells on each side between the angles have evidently come by division from a single cell.

With this slight modification of Blochmann's account Neritina is made to agree in the matter of the belt cells with Nereis, Umbrella, Limax, Unio, four species of Crepidula, Urosalpinx, Fulgur, Sycotypus, and Illyonassa, and at the same time Blochmann's statement as to the derivation of the "Urvelarzellen" is confirmed, and Kofoid's contention for the alternation of cleavages is satisfied.

III. The Segregation of the Mesoblast and Entoblast.

1. *Formation of the Mesentoblast. Figs. 21, 22, Diagram 4 (p. 60).*

At the stage just described, with twenty micromeres and four macromeres, the left posterior macromere divides in a laeotropic direction, as shown in Fig. 21. The cell thus formed is very much larger than any of the micromeres, and, unlike them, contains a considerable quantity of yolk. This cell, although formed by a laeotropic division, remains in nearly the same position in which it was first separated from the macromere until a much later stage, Fig. 33. Like the belt cells it is partly overlapped by the micromeres which lie nearer the apical pole, but a considerable part of it is exposed on the surface. In a strict use of the term, therefore, it cannot be said at this stage to form the *middle layer* any more than the belt cells form a middle layer. In fact, it is neither a "layer" nor is it "middle," and yet from a part of this cell most of the