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On the Formation of the Germinal Layers in Gastropoda.

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With 3 plates.

In spite of the fact that within recent years the morphogenesis of the Mollusca has received a great deal of attention from embryologists, there is still much disagreement among authors with regard even to some of the fundamental points in the early phases of development. This is no doubt due to actual differences in developmental processes found in the various animals employed for investigation, and this makes it highly desirable to extend our researches to as many different forms as possible, if we are to arrive at any comprehensive results in these vexed problems. With this aim in view I first paid a great deal of attention to the early development of the Gastoropoda, *Siphonaria lepida* GOULD. A preliminary note on that subject was published in the Tokyo Zoological Magazine, Vol. VII., No. 79, 1895. A few years later I undertook a similar investigation on another form, *Aplysia* sp. The conclusions reached in the latter study were

almost the same as those obtained from *Siphonaria*, there being only slight differences in minor details.

The present paper embodies the more essential points brought out in the investigation on these two animals.

THE EGGS.

The eggs of both species here to be considered are deposited on rocks between the tide-marks from the early part of March till late in June. Each form presents so many characteristic differences in egg masses, that it is convenient to describe them under separate headings.

Eggs of Siphonaria :—The eggs of *Siphonaria* are almost always found in masses, the shape of which may be described as an elliptical loop (Fig. 1, Plate I), the last laid portion usually resting on the portion laid first. When oviposition is completed, the head of the animal is pointed in the direction of the arrow in Fig. 1. Pl. I. When magnified the mass is seen to be formed of an innumerable number of oblong vesicles, which are linked one to another by a fine thread, the whole being cemented together by a gelatinous coating. Each vesicle is enveloped in two membranes, very thin and transparent, which are closely applied to each other, and separated only at the poles of the longer axis (Fig. 4). The egg (Figs. 2 and 4 *e*) found in each of these vesicles which must be regarded as the chorion lies rather eccentrically in the portion furthest removed from the genital opening. It is perfectly spherical in form, and is 0.1 *mm.* in diameter. When freshly deposited it is very slightly tinged with yellow, but in course of development it gradually becomes white. In far advanced stages especially in the larval form its color

becomes completely changed to dusky brown, owing to the presence of the shell. Besides the egg there is, within the chorion, an abundant supply of albuminous material uniformly distributed in the form of rather coarse spherules. Fig. 2 shows the general view of the egg in the chorion, which is seen to be enveloped in a thin layer of gelatine not yet coagulated. The spherules just cited are homogenous and translucent; but at about the time of the expulsion of the second polar body, vacuoles are produced in them (Fig. 3). Subsequently, all of the spherules gradually disintegrate, leaving behind only very fine almost imperceptible granules. They, however, can be easily coagulated, and therefore may be brought into view by the application of hardening reagents. By the time this coagulation stage is reached, it is found that the distribution of the albuminous material is no longer uniform throughout as was the case before disintegration. There is almost always a smaller quantity towards the side, which is uppermost in Fig. 4, *i.e.* the future dorso-anterior end of the egg, than on the opposite side.

Eggs of Aplysia:—In this species too, the eggs are deposited in a mass; but here it is in the form of a very long and intricately and loosely entangled cord which is about 1–1½ *mm.* in diameter. In this cord there is recognizable, even with the naked eye an innumerable number of yellow capsules, which are, as in the case of the former species, connected by a thin thread and enveloped in gelatine. The capsule represents the chorion, and enclosed therein we find eggs usually more than thirty in number (Fig. 37, Pl. II). The egg has a spherical contour, and is about 0.12 *mm.* in diameter. As is seen in Fig. 38 coarse granules of deutoplasm are conspicuous in that part of the egg which becomes the anterior portion of the future embryo. Their color is very

bright, particularly when fresh, varying from light red to yellow. In advanced stages, however, the color changes completely to dull brown just as in the case of *Siphonaria*.

THE BLASTOMERE STAGE.

Siphonaria:—In three or four hours after deposition, the egg gradually begins to elongate in the antero-posterior direction, *i.e.* almost parallel to the long axis of the chorion. Subsequently a portion becomes somewhat flattened or even depressed, and the cytoplasm thereabout becomes translucent in the form of an inverted cone. This portion begins to bulge out, and fifteen minutes later it finally assumes a spherical shape. This eventually separates from the mother egg, forming the first polar body. After the complete detachment of this body, the egg returns to its normal spherical form, and loses its transparency throughout its entire mass. Then ensues an interval of about thirty minutes; after which the egg again behaves as above described. The new spherical structure or the second polar body is thrown out here very close to the first. After the completion of the maturation processes, the restoration of the egg to the normal spherical form is again effected. Generally fifteen minutes are required for each of the two processes:—the formation of the second polar body, and the restoration of the spherical form. Then follows a pause of one or more hours, after which the egg elongates for the third time. This is the preparation for the first cleavage. If we carefully observe the exact contour of the egg at this stage, we shall find that it is not strictly elliptical in shape. The portion which becomes the future posterior end is almost always slightly pointed. Hence, using an exaggerated expression, it may

be said to have an ovoid shape. The same state of affairs has been observed in *Limax* by MARK[32]. Thus, before the cleavage process commences, it already shows a tendency to present inequalities in its two halves. After the elongation, a depression begins to appear in the animal pole near the polar body. It gradually extends downwards until it almost reaches the center of the egg. At about the same time a similar groove with a much sharper angle appears in the vegetative pole which proceeds upwards towards the center of the egg. (Fig. 5). The depression from the animal pole, however, proceeds much the more rapidly of the two until it reaches about the center of the egg. There it seems to stop, and to meet the approaching groove from the vegetative pole. Thus these two grooves encircle the egg leaving a bridge which for the time being connects the two halves. In the section, this bridge is proved to be the body of the spindle. This first cleavage plane is often described as moving directly from the animal pole to the vegetative in other species of Mollusca, for example, in *Umbrella* (HEYMONS)[23], *Unio* (LILLIE)[31], *Succinea* (SCHMIDT)[38], *Limax* (MEISENHEIMER)[33], etc. In either case the cleavage results in the total division of the egg into two halves or blastomeres. The plane itself in *Siphonaria* stands almost transverse to the longitudinal axis of the future adult. The blastomeres thus formed seem at first sight similar in size; but a careful observation shows that in reality that carrying the polar body is a little larger than its complement. This difference in the size of the two halves is the consequence of the original inequality existing in the natural configuration of the egg before the cleavage. When the final constriction of the blastomeres takes place each of them has a spherical outline, not departing from it even at the point where they come in contact with each

other. When we observe a fresh specimen in such a stage, we notice the existence of a period, although very short, in which the two blastomeres are completely separated from each other by a narrow space. In *Limax*, KOFOID[28] claims to have found at the spot "a rather vague, transparent, and unstained protoplasmic connection." In my specimens, both fresh and preserved, I could not finds any instance of such connection, although KOFOID's statement seems to be a highly reasonable one. The large blastomere thus formed contributes mostly to the formation of the anterior portion of the adult, while the smaller contributes to the posterior. Therefore, they will hereafter be designated respectively as the anterior and the posterior, blastomeres. The eggs of various Molluscan species which have been thus far studied are not alike in the size of the two blastomeres. For example, in *Unio* the posterior blastomere is larger, while in *Neritina* (BLOCHMANN)[1], *Umbrella*, etc. it is smaller than the anterior one.

Soon, the two blastomeres again come gradually in contact with each other, the surfaces by which they touch, becoming flattened. Simultaneously their general configuration begins to alter, and becomes somewhat elongated in the direction parallel to the plane of contact. During this period of apposition the anterior blastomere slightly shifts its position obliquely upwards in the direction of the animal pole. In about one hour, the blastomeres begin to stretch further in the direction already elongated and finally divide in a plane which is at right angles to the plane of contact or that of the first cleavage. This phenomenon appears usually first in the anterior one (Fig. 6), and is soon followed by the posterior complement. In the blastomeres thus transformed, the furrow causing the second cleavage commences almost simultaneously at the animal and the

vegetative poles. It stands almost at right angles to the first cleavage plane and coincides with the future median axis of the body. As a consequense the egg is subdivided into the right, and the left halves (Fig. 7). Concerning the priority of the cleavage in the first two blastomeres just described, there is much controversy. In *Umbrella*, *Aplysia*, and others the posterior blastomere divides first. On the contrary, in *Neritina*, *Unio*. and others the anterior precedes the other. Thus it is easy to see that in Molluscan eggs the priority of cleavage is quite independent of the amount of the deutoplasm and its distribution in the egg.

The four cells formed by the second cleavage take a spherical form just as in the case of the first blastomeres, but soon become flattened on one side as the result of mutual contact. They, as a whole, constitute the first quartet and are commonly designated as *A*, *B*, *C*, and *D*, (Fig. 8) of which *A* and *B* come from the anterior, and *C* and *D* from the posterior, blastomeres of the two cell stage. During the formation, and especially during the accommodation of this quartet the cell *C* shifts its position obliquely upwards. At length, it comes to stand in the same level with the two anterior blastomeres leaving its complement cell *D* in the original level lower than the others. By this shifting it also happens that the two diagonally opposite cells *B* and *D* are brought in direct contact at the animal, as well as at the vegetative, poles, forming what has been called the cross furrow. The cells forming this furrow differ in different species, and sometimes the furrow is formed by different cells at the two poles. Moreover, there are many cases, in which the length and the direction of the furrow presents marked differences in these two poles. However, in the species under consideration, the furrow is formed by the juxtaposition of the same cells both in the

dorsal and the ventral views, and it has also the same length on both surfaces.

Aplysia :—The egg of this species when freshly deposited is at almost the same stage as that of *Siphonaria*. It has not yet discharged the polar bodies. The process of fertilization, however, shows no material difference from that of the preceding species. It is worthy of note that the polar bodies appear in the small cytoplasmic portion of the egg. After the polar bodies are completely formed the egg begins gradually to elongate and at last assumes an ovoid shape, the pointed end being toward the cytoplasmic portion. The first cleavage plane appears just as in *Siphonaria* from both the animal and the vegetative poles (Fig. 39). The result of this division is the formation of two blastomeres totally different in size as well as in quality (Fig. 40). The larger blastomere is heavily laden with deutoplasm ; while the smaller one is almost wholly devoid of it. Such a striking difference in the two blastomeres is observed in only a few cases in Gastropoda even in the so called yolk-laden eggs, of *Umbrella*, *Planorbis*, etc. Of these two blastomeres the larger half represents the future anterior portion of the body, and the smaller half its posterior portion, these corresponding to the smaller and the larger blastomeres of *Siphonaria*. The further changes accompanying the first cleavage, such as the gradual and mutual accommodation of the blastomeres take the same course as in *Siphonaria* already fully described above. The second cleavage in this species takes place first in the posterior half (Fig. 41, Pl. II). The new cells thus generated are, as in the case of *Siphonaria*, designated as *A*, *B*, *C*, and *D* (Fig. 42). Their relation to the future axis of the body coincides with that of the former species and therefore requires no further explanation.

After complete accommodation it will be seen that here again the cross furrow lies between the cells *B*, and *D*, both at the animal and the vegetative poles. The same phase is observable in *A. limacina*,[2] and also with only a slight difference in *Umbrella*. The hypothesis advanced by KOFOID,[28] as to the relation existing between the cross furrow and the presence of an abundant deutoplasm can not be accepted in these cases.

THE FIRST QUARTET OF ECTOMERES.

Siphonaria (a_1, b_1, c_1, d_1, Figs. 9–11, Pl. I):—About two hours after the complete formation of the original quartet, the spindles appear simultaneously in all the blastomeres; but actual cleavage begins always at the left posterior one *D*. The axes of the spindles in all the cells are directed obliquely upwards (Fig. 10), and when seen from the animal pole they seem to converge towards one another (Fig. 9). The cleavage proceeds in one cell after another in a certain regular course. In this species the course of this cleavege is in a right-handed spiral or in the direction of the motion of clock-hands as shown in Fig. 9, the apical view, and also in Fig. 10, the posterior side-view. In the former figure we see that the blastomere *D* precedes all others in development, presenting already its small daughter-cell. This priority of cleavage and the further course of development is made clear by the next figure. Here the first ectomere cell, d_1 from the blastomere *D* is seen to be already well formed, while the formation of a_1 from the blastomere *A* is not as much advanced. In the other two blastomeres *B* and *C*, the division is indicated only by a spindle. It is stated by KOFOID, that in *Limax* the

spindles gradually shift their positions during this cleavage. Such does not seem to be the case in *Siphonaria :* the axis of the spindle, as may be partly gathered from the figures cited above, remains unchanged in direction throughout the whole of this phase. The newly formed cells, however, moves lightly when they come to adjust themselves to one another. Four daughter cells a_1, b_1, c_1 and d_1 (Fig. 11) thus propagated form the first quartet of ectomere cells, lie, when completely detached, on the dorsal side of the mother-blastomeres. They are small in size and are at first merely an aggregation of spherical bodies, which however soon begin to be applied one against another. When finally settled, they seem to have been shoved somewhat towards the anterior end (Fig. 11). Now in this new quartet the cross furrow is again formed by a juxtaposition of the ectomere-cells b_1 and d_1 just as it was in the preceding stage by their mother-blastomeres *B*, and *D* (Fig. 11). Thus the furrow of the ectomere-cells and that of the blastomeres are formed by the cells genetically related to one another. The lines of these two furrows form a small angle with each other, *i.e.*, neither runs parallel with, nor stands at right angles to, the other as is the case in many other species.

Aplysia (a_1, b_1, c_1, d_1, Figs. 43–44, Pl. III) :—The form tion of the first quartet of ectomeres here also commences first in the posterior blastomere (Fig. 44), most usually from its left component *D*, and is followed immediately by the cells *C*, *B*, and *A* in the order named (Fig. 43). Thus the cleavage proceeds in a left-handed spiral, *i. e.* in the direction just the reverse of that of *Siphonaria*. This direction is contrary to that ascertained by Blochmann in *A. limacina*, and also in other forms of yolk-laden egg noted by various authors. The first quartet of ectomeres formed are much smaller than the mother blastomeres ; and when

finally settled, the position they occupy is on the posterior dorsal portion of the egg, instead of on the anterior dorsal as in *Siphonaria*. On the other hand the cross furrow keeps its relative position exactly as in the case of the preceding species (Fig. 45).

THE SECOND QUARTET OF ECTOMERES.

Siphonaria (a_2, b_2, c_2, d_2, Fig. 12–13, Pl. I) :—After a pause of about one hour, the second generation of ectomeres originates again from the original blastomeres. They arise first in the posterior blastomeres as in the case of the first generation, but this time from the right cell *C*. Although spindles often appear in all the blastomeres at the same time as shown in Fig. 13, yet the cell *C* is always ahead of all the others in actual cleavage. The order of division here is just the reverse of that in the first quartet as shown in Fig. 12, in which we notice that of the second quartet cells c_2, b_2, and a_2 have already been produced, while *D* is just preparing to bud off d_2. These ectomere-cells are of the same size as those of the first quartet, and roughly speaking occupy positions alternate with these latter.

Aplysia (a_2, b_2, c_2, d_2, Figs. 45–47, Pl. III.) :—In this species too, the second quartet of ectomere-cells arise from the blastomeres, the process begining as in *Siphonaria* from the right posterior one *C*. Cleavage, however, proceeds in a right-handed spiral, the reverse of the order in which the first generation was budded off. This point as well as the relative position of the newly formed generation are clearly illustrated in Figs. 45, and 46, which give respectively the a pical and the left lateral, views of the egg. In Fig. 45 the spindles are seen in all the blastomeres except *B*, and that of *C* is most advanced. In the next

figure also, the order of cleavage is clearly indicated inasmuch as the blastomere C has already almost detached its new daughter cell C_2; and D is about to divide. In this species the second quartet of ectomere-cells are much larger than the first set and occupy positions alternate with these as in *Siphonaria*.

THE THIRD QUARTET OF ECTOMERES.

Siphonaria ($a_{1.1}$, $b_{1.1}$, $c_{1.1}$, $d_{1.1}$, Figs. 14–15, Pl. I) :—In a majority of the Molluscan eggs direct segmentation from the blastomeres stops temporarily with the second quartet of ectomeres, the third being derived mostly from the first quartet of ectomere-cells. This is also the case in the present species. In my preliminary note on the development of the present species communicated to the Zoological Magazine already referred to, I noted that this generation also originates from the blastomeres. A renewed investigation has shown that this view was a mistaken one, having been caused by the abnormal condition of the specimens then accessible. In reality, this new generation arises from the first quartet of ectomere-cells. Cleavage begins from the first descendant (d_1) of the left posterior blastomere and proceeds in a right-handed spiral. In Fig. 14, the apical view of this stage, $d_{1.1}$ alone has been budded off; while all the others show the spindle in a more or less similar state. These ectomere-cells of the third generation or trochoblast cells, as they are usually called, are smaller than any of the preceding generations. Nevertheless, they are rather conspicuous, coloring deeply when stained. They lie in the same level and in alternation with the ectomere-cells of the first quartet, pushing the cells of the second quartet (a_2, b_2 etc) to the left, in the order which indicates a

right-handed spiral. Such a displacement of the cells is recognizable when we compare Figs. 12 and 15.

Aplysia ($a_{2.1}$, $b_{2.1}$, $c_{2.1}$, $d_{2.1}$, Figs. 48–50, Pl. III):—In this species as well as in *A. limacina* the ectomere-cells of the third generation are not the descendants of the first quartet of ectomeres. Here it is the ectomere-cells of the second quartet, which present the spindles in a left-handed spiral, the right posterior cell c_2 taking the lead in cleavage. Fig. 48, the apical view, shows the priority of cleavage in the cell c_2, although sometimes we meet with abnormal cases as represented in Fig. 49, in which the cell a_2 precedes the others in cleavage. The new daughter cells are similar in size to any of the preceding generations, and do not take a bright stain like those of *Siphonaria*. Again as they are the descendants of the second generation, they stand in alternation with the latter, and their positions with regard to the first generation are different from what is seen in the preceding species.

THE FOURTH QUARTET OF ECTOMERES.

Siphonaria (a_3, b_3, c_3, d_3, Figs. 15–16, Pl. I):—An hour or so after the last stage, the blastomeres again exhibit the spindles. This time the spindle appears first in the right posterior blastomere *C*, and the cleavage proceeds in a right-handed spiral. The period in which this generation arises seems to differ with species, as will be seen directly in the case of *Aplysia*. In the present species its formation commences somewhat later so that it appears concomitantly with that of the next generation. This is easily proved by referring to Fig. 15, the apical view, and Fig. 16, the sagittal section seen from the left side. In the former,

all of the blastomeres except *D* are shown to have budded off their daughter cells c_3 *etc.*; while in the latter which represents a slightly earlier stage the blastomeres of which *A* and *D* are seen show their spindles in an early phase. It is moreover evident in both the figures, that the formation of this generation is immediately followed by that of the fifth quartet ($a_{2.1}$, *etc*). The cells of the fourth generation are, as a rule, rather larger than any of the foregoing ones. They occupy a position on the ventral aspect of the egg, just under the third generation ($a_{1.1}$, etc). By the formation of the fifth generation they, however, shift their position more to the left side; and eventually come to interpose themselves together with the fifth quartet between the cells of the second (a_2, etc) generation (Fig. 15).

After throwing off three series of ectomeres, *i.e.*, at the formation of this fourth generation of ectomeres the blastomeres are completely differentiated, and make no further contribution to the formation of the ectomere-cells. The cleavage which occurs later in the blastomeres gives rise to the generations of entomere-cells. Hence, the blastomeres receive the name of entomeres with the single exception of the left posterior one *D*. This latter ultimately gives birth, beside the entomere, to the mesomere, so that it is generally designated as the ento-mesomere.

Aplysia (a_3, b_3, c_3, d_3, Figs. 48–50, Pl. III) :—The fourth generation arises as in *Siphonaria* from the left posterior blastomere, and proceeds in a right-handed spiral. In this species too, as was mentioned in *Siphonaria*, the formation of this generation very often takes place simultaneously with that of another quartet, but here it is with the preceding (c_2, etc), and not with the following, generation. This fact is well illustrated in Figs. 48 and 50, respectively showing the apical

view, and the sagittal section seen from the right side. The ectomere-cells of this generation are a little larger than any of the preceding. When fully adjusted (Fig. 51), they take their positions, as in the first species interposing themselves between the cells of the second (a_2, etc) and the third ($a_{2.1}$, etc) quartets. In this species also, this generation is the final ectomere product from the blastomeres. Henceforth, the blastomeres are called entomeres or ento-mesomere according to their characters just as in *Siphonaria*.

At about this time a noticeable change occurs with regard to the relative positions of the anterior and the posterior entomeres. By the repeated cleavage already gone through, it is seen that the posterior half is gradually brought upwards toward the apical pole. This shifting increases with development and is combined with a slight movement toward the right side. If we refer to Fig. 44 a right lateral view of the blastomere stage, and to Figs. 46 and 49, representing the posterior views of the different stages, one before the formation of the second generation, and the other after the formation of the third generation, we can easily comprehend the above mentioned change in the cell arrangement. At the outset both the anterior and the posterior, halves of the egg stand almost on the same level (Fig. 44). By two consecutive cleavages of the first and the second quartets of ectomeres, the posterior half has shifted its position almost half way up the anterior components (Fig. 46). Finally the former seems to stand high up, and to surmount, the latter. That there is at the same time a slight shifting toward the right is seen by comparing Figs. 46 and 49. In the former the plane of contact of A and B is seen through the right portion of D and near the contact plane of D and C. In Fig. 49 the latter contact plane

is much further to the right. Moreover, it is to be observed that the difference in the amount of cytoplasm contained in the anterior and the posterior halves of the egg causes the gradual displacement of the ectomeres in general toward the posterior end. This seems to be due partly to the fact that during all the cleavage processes the axes of the spindles in the anterior blastomeres always have a tendency to be inclined toward the posterior end, so that an ectomere-cell budded off generally lies dorsally and *posteriorly* to its mother cell. The posterior position of ectomeres is marked even from the stage of the first ectomere generation; thus in Fig. 45 which shows the first quartet of ectomeres in their proper position we see how they lie more on the posterior blastomeres. The same arrangement is also continued up to quite late stages. Although this tendency is noticeable in other yolk-laden Molluscan eggs it does not seem as pronounced as in *Aplysia*, for their blastomeres shows no such marked difference in nature as there is in this species.

THE FIFTH QUARTET OF ECTOMERES.

Siphonaria ($a_{2.1}$, $b_{2.1}$, $c_{2.1}$, $d_{2.1}$, Figs. 15–16, Pl. I) :—As I have already stated the formation of this generation takes place in conjunction with the preceding one. It arises from the second quartet of ectomeres. As is seen in Fig. 15 the cleavage makes its first start from the right posterior cell c_2 and its course is in a right-handed spiral. The daughter cells $c_{2.1}$, etc thus formed are as large as those of the fourth generation. They are situated more ventrally than the mother cell and finally interpose themselves between the cells of the preceding generation, pushing the latter more to the left along the right spiral. From this time

onward it gradually becomes evident that all the generations of the ectomere-cell, as a whole, make but a slight displacement compared with what is generally seen in other species. They do not strictly overlay the entomeres; but lie toward the anterior, and not toward the posterior, end as was the case in *Aplysia* already alluded to.

Aplysia ($a_{1.1}$, $b_{1.1}$, $c_{1.1}$, $d_{1.1}$, Figs. 51–52, Pl. III) :—In *A. limacina* this generation is said to be propagated from the third quartet of ectomeres; but in this species it is descended from the first set of ectomeres. Cleavage begins with the cell c_1 and its course is in a left-handed spiral. In Fig. 51, representing the apical view of such a stage, the new ectomere-cell $c_{1.1}$ is seen to have been completely budded off from its mother-cell c_1 and to have already assumed the normal form; while in all of the other quadrants the spindle remains in the amphiaster stage. The same phase in the posterior two cells of the first ectomere-quartet is also recognizable in the optical transverse section seen from the posterior side (Fig. 52). The new ectomere-cell or the trochoblast is very much smaller than any of the foregoing ectomeres as in the case of *Siphonaria*. However in its general outline and in its reaction toward the staining fluids it bears a great similarity to the third quartet of ectomeres in *Siphonaria*, so that its recognition is a matter of considerable ease. The resemblance of these two generations of ectomeres in the two species is intelligible when we remember that they are both the first direct descendants of the first generation of ectomeres and thus have the same genealogical history. The fifth quartet of ectomeres occupies a position always ventral to its mother-cell and lies almost exactly on the ectomere-cells of the third generation. It also interposes itself between the ectomere-cells of the second generation.

THE SIXTH QUARTET OF ECTOMERES.

Siphonaria ($a_{2.2}$, $b_{2.2}$, $c_{2.2}$, $d_{2.2}$, Figs. 17–19, Pl. I):—The origin of this generation presents much variation according to the species studied. In *Siphonaria* it again arises from the second quartet of ectomeres. Here the cleavage takes place first in the right posterior cell c_2 followed immediately by the cell b_2, a_2, etc,, thus taking the course of a left-handed spiral. Fig. 17 illustrates a horizontal section of this stage, in which all the ectomere-cells of the second generation show their spindles. By the segmentation of this stage, the ectomere-cells of the second quartet are divided into two cells of unequal sizes. The newly formed daughter-cell $a_{2.2}$, etc. is smaller than its mother-cell; but it comes to occupy the position originally occupied by the latter. As a consequence, the mother-cell shifts its position ventrally toward the right side, and at length rests on the cells of the fourth and the fifth generations. In this stage or sometimes somewhat later, there takes place a noteworthy event, viz., the cleavage of the ento-mesomere D (Fig. 18). The spindle lies in the lower anterior part of the cell so that it is easily seen in the horizontal and the sagittal sections, Figs. 18 and 19. The cleavage is soon followed by the division into two cells, the entomere-cell D_1, and the mesomere-cell M (Fig. 20). They present considerable differences not merely in size, but also in general character. The entomere-cell D_1 is of about the same size as the ectomere-cell of the third quartet, and moreover like the latter has a comparatively large nucleus. It is situated anteriorly, ventrally to the mesomere-cell M, (Fig. 21) and maintains its direct contact with the right anterior entomere B, with the cross furrow between as before.

(Fig. 20). The mesomere-cell, on the other hand, is large, and translucent, and now comes to occupy a position almost in the median axis of the egg.

Aplysia ($a_{2.2}$, $b_{2.2}$, $c_{2.2}$, $d_{2.2}$, Figs. 53–55, Pl. III) :—Yolk-laden eggs of Mollusca present much difference in the origin of the present quartet of ectomeres. In *Umbrella* it arises from the third generation and in *Neritina* from the fifth. In the species of *Aplysia* other than the one studied in the present investigation, this generation is described as arising from the fourth quartet of ectomeres. In the present species, however, it is the outcome of the second generation just as in *Siphonaria;* and the course of cleavage is likewise quite identical in both cases. The spindle in the ectomere-cells of the second quartet lies almost horizontal, so that the daughter-cells $a_{2.2}$, etc. stand at the same level with their mother-cells; and when fully accomodated they push the ectomere-cells of the fifth generation to the left, finally interposing themselves between these latter and the mother-cells. Fig. 53, the optical horizontal section from the animal pole shows that the right posterior cell c_2 of the second generation of ectomere-cells has already propagated its new daughter-cell $c_{2.2}$; while its left component d_2 still presents a spindle. From this the course of cleavage and the relative position of this new series of daughter-cells will be easily comprehended. In this figure it will also be seen at a glance that the formation of the ectomere generations no longer follows with a strict regularity as in earlier stages, and spindles are present simultaneously in cells other than the second quartet of ectomeres. Such an irregularity in cleavage is also seen in Fig. 54, the optical sagittal section near the median line, the division of two consecutive sets of ectomeres here arising almost at the same time.

THE SEVENTH QUARTET OF ECTOMERES AND THE CHANGE FROM THE RADIAL TO THE BILATERAL, SYMMETRY.

Siphonaria ($a_{2.1.1}$, $b_{2.1.1}$, $c_{2.1.1}$, $d_{2.1.1}$, Fig. 20, Pl. I, and Fig. 21, Pl. II) :—The seventh quartet of ectomeres is the first descedant of the fifth quartet ($a_{2.1}$, etc.). In Fig. 20, a horizontal section near the vegetative pole, the first cell $d_{2.1.1}$ of the new generation is quite detached from the left posterior cell $d_{2.1}$ of the fifth generation, which, however, is not seen in this figure being situated more dorsally. Cleavage proceeds in a right-handed spiral. The daughter-cell is quite similar in its general character to the cells of the third, and the sixth generations. It lies ventral to the mother-cell, and ultimately assumes a position alternate with the cells of the fourth generation a_3, etc. (Fig. 24). From this time on until the differentiation of the three germinal layers none of the cells at the vegetative pole of the egg presents any marked change. The vegetative pole is occupied by the entomeres A, B, C, D_1, and the mesomere M surrounded by the ectomere-cells of the fourth (a_3, etc.) and the seventh ($a_{2.1.1}$, etc.) generations (Figs. 20 and 24).

Up to the present stage each of the successive generations of ectomeres has invariably been formed of a quartet, the cells of which are produced in a certain regular way, and keep their relative positions in a spiral form. Such a disposition of cells is generally known as the spiral symmetry. This form of symmetry is, however, generally not retained after the formation of the mesomere-cell M, and is eventually changed into a bilateral symmetry. This, so far as I am aware, is said by investigators to be caused, in most of the Molluscan eggs simply by the new posi-

tions taken up by that quartet of ectomeres which are produced as the first descendant of the fourth generation. Subsequently, all the cells come by degrees to shift their positions so as to accommodate themselves to this new generation. Thus the egg has been said to ultimately assume a bilateral symmetry, and as this takes place only very gradually, it is said that it is sometimes quite impossible to point out with exactness the transitional stage. In *Siphonaria* this phenomenon happens at, or a little after, the stage in which the seventh generation of ectomere-cells has been budded off. The alteration of the body-form is in this species also induced by the same generation of ectomeres that causes the change in the other species alluded to above (*viz.* the first descendant of the fourth generation); but what I make out respecting its formation and disposition is totally different from what has hitherto been stated. *The two posterior quadrants of the fourth ectomere-quartet* (c_3 *and* d_3), *adjoining laterally the mesomere-cell M begin to present the spindles* (*Figs.* 20 *and* 21), *the left component* (d_3) *taking the lead. Unlike all others these spindles lie in a strictly radial and horizontal direction. Consequently the daughter-cells,* lc and rc *when divided, come each to occupy a position more median than that of the mother-cell, close to the sides of the mesomere-cell M and also they are in exact bilateral symmetry with regard to each other* (Figs. 24 and 25). They are quite like the ectomere-cells of the seventh quartet in size as well as in general appearance. The two anterior quadrants a_3 and b_3 of this fourth generation, which are so often described as dividing almost synchronously with, or a little later than, their posterior component cells, never present in this species any trace of a spindle until after the complete differentiation of the germinal layers. On account of

this highly specialized method of the formation and characteristic disposition of the cells, such as has not yet been described in other species, I think it is better to denominate these as the *bilateral* cells, thus distinguishing them from other ectomere-cells. With the birth of the bilateral cells the spiral symmetry is materially and abruptly disturbed; and the configuration of the egg is at length completely transformed into a bilateral symmetry. Thus it is seen that the change of the symmetry takes place in this species at a° period more definite than in any others thus far known to us.

Aplysia ($a_{2.1.1}$, $b_{2.1.1}$, $c_{2.1.1}$, $d_{2.1.1}$, Fig. 54–55, Pl. III) :—In *Aplysia* the seventh quartet arises from one different in order, but of strictly the same genealogical derivation as the corresponding one of the foregoing species. Here it arises from the third generation, and indeed first from its left posterior-cell $d_{2.1}$, thence following the left-handed spiral. This state of things will be fairly understood when we compare Figs. 54 and 55, illustrating the sagittal, and the horizontal, optical sections respectively. In these two figures the ectomere-cell $d_{2.1}$ alone is represented. It is noteworthy that while the spindles of the cells heretofore described as well as of the other quadrants of this same quartet ($a_{2.1}$, *etc.*) lie almost horizontally the spindle in this cell is seen to take a somewhat vertical position. Consequently, when the new daughter-cell $d_{2.1.1}$ is budded off, it lies more ventrally than the other quadrants $a_{2.1.1}$, $b_{2.1.1}$, etc. The daughter-cells thus generated have a great similarity in general outline to those of either the fifth, or the sixth generations. They generally border the ventral side of the egg, lying almost under the cells of the second generation.

Concomittantly with this stage or a little earlier (Figs. 53

and 54), there takes place the differentiation of the ento-mesomere cell *D*. This often happens as early as the stage of the sixth quartet, as is shown in Fig. 54. The details of this event are quite like those of *Siphonaria*, and therefore need no further explanation. Even the entomere-cell D_1 itself thus formed, is like that of the former species. It lies anteriorly and ventrally to its mother-cell or mesomere M, and of course has the cross furrow in its original relation.

Besides this notable phenomenon there always ensues still another remarkable change in the process of cleavage, namely the formation of the bilateral cells similar to those described above in *Siphonaria*. The division of these new daughter-cells is quite similar to what takes place in the species just cited. The two posterior ectomere-cells of the fourth generation c_3 and d_3 adjoining the right and the left sides of the mesomere-cell M now begin to divide quite independently of their two anterior companions. As is well shown in Fig. 53 an apical view of the animal pole, the spindles in these two cells lie almost horizontally and are directed, unlike all the preceding ones, toward the median axis, that is, in a somewhat radial direction. The spindle usually appears first in the right cells c_3 (Fig. 55), and produces a small daughter-cell *rc* (Fig. 56) which resembles an ectomere-cell of the seventh generation, especially $d_{2.1.1}$ in its general appearance. The division of the left cell d_3 soon follows, producing the cell *lc*. Although the two bilateral cells *lc* and *rc* are propagated from the cells of the same generation they behave in a slightly different manner. The left bilateral cell *lc* at the beginning of its detachment lies very close to, or even in contact with, the entomere-cell D_1 (Fig. 56). Subsequently, by the cleavage of the mesomere-cell it is at last brought more toward

the posterior portion of the body so as to lie almost on the same level with its complementary cell *rc* on the opposite side. By comparing Figs. 56 and 57, the optical horizontal sections of two different stages, we are able to perceive this change in the position of the left bilateral cell. Thus it seems that the final position of the bilateral cells in the two species under consideration coincides in almost every respect. I am not in position to contradict the views expressed by previous authors, concerning the transition from the radial to the bilateral symmetry; but at least in the species that I have studied I am convinced that the bilateral cells are the main, if not the sole, cause of the transformation of the body symmetry. I should like to make a remark here on the same stage in *A. limacina* BLOCHMANN makes no allusion to the phenomenon mentioned above. But a close inspection of his figures shows us the occurrence of the same events in that species. His Fig. 13 has an intimate relation to the stage of the seventh ectomere generation, although it is introduced to show an earlier stage. There it is seen that c_2 has given rise to a small cell. This does not seem to form a quadrant of a quartet, but corresponds in its general character to the left bilateral cell. Again in his Figs. 14 and 15 the cell c_2 as well as the first entomere-cells are seen to occupy the same position with those noted above by me. Although it is to be gathered from the author's interpretation that the change of the body symmetry occurs as early as the stage of about 24 cells, yet his figures incline me to believe that this phenomenon takes place much later, just as in the case of the present species.

Immediately after the formation of the bilateral cells or sometimes simultaneously with it there takes place the cleavage of the mesomere M. A spindle appears lying in the transverse

direction (Fig. 56), and subsequently divides the cell into two almost equal halves. The daughter-cells (*m*, Figs. 57 and 58) are ovate in form and are characterized byt heir less granular contents. Their position is in a strict bilateral symmetry with respect to each other, and the plane in which they come in contact almost coincides with the future median axis of the body.

Previous to this stage the nuclei of the entomere-cells *A*, and *B* lie usually near the posterior end in the middle line of each cell as is shown in Fig. 55. They, however, have commenced to shift their position toward the right (Fig. 56). This goes so far in A that the nucleus reaches the ventral side of the anterior end as will become intelligible by a reference to Figs. 56 and 58. It would seem that such a shifting of the nucleus in the two entomeres could have no other purpose than to prepare for the formation of the future entoderm-cell. This latter event arises shortly after the bilateral symmetry becomes well pronounced by the cleavage of the mesomere-cell M. The formation of the entoderm-cells has its begining in the posterior cells; and indeed in the right component cell *C* (Fig. 57). In the formation of this generation the spindle lies almost horizontally, and the daughter-cells detached which are known as the entoderm-cells (A_1, B_1, C_1, Figs. 59 and 60) are rather small and situated alternately with, and outside of their mother-cells (*A*, *B*, *C*).

THE EIGHTH QUARTET.

Siphonaria ($a_{1.2}$, $b_{1.2}$, $c_{1.2}$, $d_{1.2}$, Figs. 22-24, Pl. II):—With the differentiation of the entomere-cell D_1 the behaviour of individual cells becomes hardly ascertainable in a surface view.

The formation of this generation was therefore made out in *Siphonaria* from sections. In this species the first ectomere generation gives birth to the eighth quartet of ectomeres (Fig. 22), the right posterior cell c_1 taking precedence over the others. The spindles lie in rather a horizontal plane (Fig. 23), and the course of the division is in a left-handed spindle. The newly formed cells $a_{1.2}$, $b_{1.2}$, etc., which are shown in the next Fig. 24, are much larger than their mother-cells a_1, b_1, etc., with which they lie on the same level and the same radii. After this cleavage the first ectomere generation takes the shape of a rosette, the cross furrow being almost obliterated. At this stage the normal and typical form of the egg is rather oval in a horizontal view, narrowing toward the posterior side (Fig. 27). In a side view, however, it takes a wedge shape with the thicker end turned also toward the posterior side. About this time the ectomere-cells lose their spherical contour, and losing their individuality become more or less flattened, altogether presenting the appearance of a layer (Fig. 26). Hence they will hereafter be called the ectoderm-layer. The ectoderm is, moreover, disposed more or less in different zones. Fig. 24 illustrates the egg in this stage, which is seen from the apical pole showing clearly the exact positions as well as the mutual relations of the cells. The highest or dorsal zone is occupied by the first (a_1, b_1, etc.), the third ($a_{1.1}$, $b_{1.1}$, etc.), and the eighth ($a_{1.2}$, $b_{1.2}$, etc.) generations of ectoderm-cells, and the ventral zone (Fig. 25) is formed of the fourth (a_3, b_3, etc.), and the seventh ($a_{2.1.1}$, $b_{2.1.1}$, etc.), generations of ectoderm-cells and the entomere-cells (A, B, C, D_1), together with the bilateral cells (*rc* and *lc*) and the mesomere-cell M; while the lateral zone is bordered with the ectoderm-cells of the second (a_2, b_2, etc.), the fifth ($a_{2.1}$, $b_{2.1}$, etc.), and the sixth ($a_{2.2}$, $b_{2.2}$, etc.), generations.

A similar but slightly advanced stage in *Aplysia* is also shown in Fig. 59. In this it is seen that unlike *Siphonaria* the first (a_1, b_1, etc.), the second (a_2, b_2, etc.), the fifth ($a_{1.1}$, $b_{1.1}$, etc.), and the sixth ($a_{2.2}$, $b_{2.2}$, etc.), generations of ectoderm-cells cover the dorsal apex of the egg. Futhermore the ectoderm-cells, as a whole, are aggregated in this species in a more posterior portion of the egg than in *Siphonaria*. Again, owing to the large size of the anterior entomeres the egg of *Aplysia* is generally thicker in that portion than is the case in the preceding species (see Fig. 54). Although not as marked as in other species, the first Anlage of the trochoblast-cells can be distinctly made out in the two species under consideration. As will be seen clearly in Figs. 24 and 59 the apical quartet of the first ectomeres is encircled by a girdle of eight cells, of which four ($a_{1.1}$, $b_{1.1}$, etc.), *i.e.* the first descendants of the first ectomere-quartet form the trochoblast. While the apical quartet gives rise to the "arms of the cross" its further development does not take place in these two species until after three germinal layers are firmly established.

In *Siphonaria*, after such a disposition of cells has been completed, the cleavage of the mesomere M and of the entomeres *A*, *B*, and *C* occurs synchronously (Fig. 25). Usually, however, the mesomere-cell M precedes the other three, as shown in the horizontal section (Fig. 25). The spindle in this cell is directed almost exactly horizontally (Fig. 26) and the cleavage plane thus formed comes to correspond with the median axis of the egg. The resultant cells (*m*) are in their nature quite identical with those of *Aplysia* to which allusion has already made (p. 25). It suffices here only to call attention to figures mentioned above, and to Fig. 27, which shows the horizontal section through the

middle portion of the egg. This latter is intended to illustrate the mesomere after its complete division into two daughter-cells and after their full accommodation. The three entomere-cells *A*, *B*, and *C* now commence to divide as in Fig. 25. In this case their nuclei do not show any shifting, such as was seen in *Aplysia*. The entomere-cells are divided into two halves almost equal in size; and all are arranged on the same level. From this time onward the entomere-cells dispose themselves in a layer and may now be called the entoderm-layer.

The further developmental course of the germ-layers has been studied with some degree of exactness only in *Siphonaria*. The ectoderm-cells gradually begin to divide indiscriminately; and hence it is wholly impossible to trace them in surface views beyond the tenth generation. The ninth quartet ($a_{2.2.1}$, $b_{2.2.1}$, etc.), arises from the sixth generation of ectoderm-cells ($a_{2.2}$, $b_{2.2}$, etc.), and the tenth ($a_{2.1.1.1}$, $b_{2.1.1.1}$, etc.), from the seventh ($a_{2.1.1}$, $b_{2.1.1}$, etc.), (Fig. 30). In what quadrant they first appear, and how the courses of cleavage run are quite uncertain. The new daughter-cells are always as small as their mother-cells.

The entoderm-cells go through their second cleavage, at the same time with the second division of the mesoderm-cells. This process begins with the cell A_1 and proceeds in a right-handed spiral, as seen in Fig. 34, a horizontal section of this stage, in which A_1 and B_1 have given off respectridy $A_{1.1}$, and $B_{1.1}$, and C_1 shows a spindle. The small daughter-cells thus produced exactly alternate in position with their original mother-cells. The subsequent growth of the entoderm-cells seems very slow, and I have not traced it beyond this stage. I will only add here that the first entoderm-cells retain their original relative positions for a long time as indicated by the persistence of the cross furrow.

In fact it remains unchanged until the cells enter into the permanent portion of the alimentary canal. Hence it is of great use in determining the orientation of the body.

As to the mesoderm, its formation may be followed up to a certain stage with some exactness. After the eighth ectomere stage each of the two daughter mesomere-cells m come to present the spindle. It is directed obliquely forward and upward (Fig. 28), and appears first most frequently in the left component (Fig. 29). The daughter-cell or the first mesoderm-cell m_1 is very small; but its presence is easily recognizable owing to its large nucleus (Fig. 30). The second mesoderm-cell originates from the mesomere-cell as did the first. The fact is shown in the horizontal section Fig. 32, and the sagittal section Fig. 33. This, however, happens much later in time when there has already been much increase in the number of the ectoderm-cells. The new mesoderm-cell m_2 is much like the first one but it takes a position more ventral than the latter. The exact seat of these cells is clearly indicated in Fig. 36, a sagittal section of the egg. The third mesoderm-cell m_3 is now derived from m_1 beginning with the right component. It is the most dorsally situated of all as is shown in Fig. 35, a transverse section through the posterior side of the egg. The first mesoderm-cell soon again subdivides, thus giving rise to the formation of the fourth one m_4. Fig. 36 just mentioned above also shows this fact, the spindle in the first mesoderm-cell m_1 being in the amphiaster stage. The mesoderm-cells, henceforth, seem to segment rather rapidly with no apparent regular order. At first they all aggregate as a mass in the posterior median line of the body. Gradually, however, they begin to spread toward the lateral parts, where they soon arrange themselves in distinct layers. In some other species of Mollusca the so called "larval

or secondary mesoblast" is often described as being formed from one or other of the ectoderm-cells, and it is supposed to contribute toward the formation of the mesenchyme. In the present species I was not able to find any trace of it so far as the investigation extended.

Several years have elapsed since I carried out the above investigation, and during that period, the investigations by WIERZEJSKI, MEISENHEIMER, HEATH, HOLMES, CARAZZI, GEORGEWITCH etc. on similar subjects have been published. Generally speaking, they have tended to confirm and verify the propositions that had already been made known by previous authors. I consider it advisable to refer to some of these works which bear directly on what I have described above.

In January 1900 there appeared a paper on the development of *Aplysia limacina* by CARAZZI, and again eight months after another paper by GEORGEWITCH on *A. depilans*.

CARAZZI has carefully traced out all the developmental processes up to the formation of the mesoderm-layer noting the time exactly. When we compare his results with those given above, it will be seen that they agree in the main. Nevertheless, there are discrepancies on some important points which cannot be passed without a word. The differentiation of the germ-layers in general is reached a little earlier in our species than in that studied by the Italian anthor, excepting the formation of the entomere D_1, which is far earlier in his species. In his Fig. 2 we see the illustration of an egg from its apical pole, which coincides with Fig. 53 of the present paper. But one of

the drawings in his Fig. 3 (illustrating the vegetative pole of the egg) which is like my Fig. 57, is interpreted in a different way. The bilateral cell, to which I have called attention, is in our species produced at about this time from the posterior half c_3 and d_3 of the fourth generation of ectomere-cells. Such a cleavage really occur also in *A. limacina;* but it is said to be accompanied ordinarily with the simultaneous division in its anterior components. Hence, in that species the new generation is formed of a quartet like all the preceding ectomeres and does not influence in any way the transformation of the cell-arrangement. Nevertheless the bilateral form of cleavage occurs also in the ectomere-cells $3c^1$ and $3d^1$, as may be gathered from the author's own words as "subito dopo, alla 28ª ora $3c^1$ e $3d^1$ si dividond con fusi transversali, cioè con divisione bilaterale, mentre $3a^1$ e $3b^1$ rimangò in riposo." However, this is the second division of those ectomeres, and indeed after the bilateral symmetry is fairly established by the formation of the mesomere-cell. Thus the transformation of the body symmetry here seems to be effected by the gradual and renewed disposition of already formed ectomeres rather than by a single cleavage of the posterior half of the fourth ectomeres. Such a method of transformation is seen in various species as I have already noted.

Concerning GEORGEWITCH's paper on *A. depilans* the author, it appears to me, has fallen into some confusion on important points. At all events, his ideas on the orientation of the egg are exastly contrary to those usually held, the portion called by him the posterior, being really the anterior, so that his "Urmesodermzell" is in fact derived from the anterior blastomere. And it is very strange that he describes the posterior (really anterior) half of the second quartet of ectomeres as the "Ur-

mesomere." By my investigation given above as well as by those of others it is known that this generation is the most active in its growth, at least before the establishment of a bilateral symmetry. They ought to have given rise to daughter-cells before the true mesoderm "Kleine Zelle *m*," was first perceived by the author. By a careful study, however, I have at last found that his description and figures do not harmonize. In his description not a word is said as to the fate of his anterior blastomeres *A* and *B* *i. e.* really the posterior *C* and *D* of authors. But we can ascertain from his Fig. 20 that these cells segment almost at the same time. In short, according to his paper we must finally arrive at the very embarrassing conclusion that he has put three totally different kinds of cells under one and the same name of "the mesoderm" viz., (1) the descendants of the second ectomere-cells or according to the author 2*c* and 2*d* (Fig. 10), and (2) the descendants of the entomere *C* or according to the author *m*, in addition to (3) the descendants of the proper mesomere, which seems to have been entirely overlooked by the author. Yet these cells, as they represent the ectomere-, the entomere-, and the mesomere-cells ought not of course to be confounded. This and some other conclusions of the author, which I can not help considering as too hasty are derived from his assumption of the homology existing in the formation of the original mesomere-cells in the species in question and in Polyclads. The fact is clearly seen in his own term—"Der Ursprung und die Lage dieser 2 Zelle *m* und m_1 stimmt so auffallend mit denjenigen bei Polycladen überein,....."

In the same year a paper entitled "The Early Development of Planorbis" was made public by HOLMES. By a careful study of this author new light has been thrown on many points,

especially concerning the fate of the cells. Nevertheless the alteration of the body-form in this species seems to be not so pronounced as in those I have studied. HOLMES seems to think that the ultimate cause of such a transformation lies in the different behaviour of the posterior cells of the third ectomere generation. Thus he goes on to say:- "we may view the earlier division of the cell 3*a* and 3*b* as the first foreshadowing of bilateral cleavage." By this expression it is quite evident that there is no special cell formation as in the cases described in the present paper. Such a gradual modification as is elucidated by the author is of a wide occurrence in the Molluscan egg and the change of symmetry by the cell formation near the posterior end of the body is demonstrated at present only in the species studied by myself. Nevertheless I believe that the same fact will hereafter be confirmed in other forms.

It may not be useless to summarize here the main points brought out in the present investigation.

1. Throughout the whole process of cleavage it is observable, that there is no fixed regularity in the course such as is expressed in the so-called law of alternation of spirals as stated by WILSON, KOFOID *et al.* Even the corresponding daughter-cells from the same blastomeres or ectomere-cells are propagated differently in different forms. The first generation of ectomere-cells is produced in *Siphonaria* and in *Aplysia* in spirals of opposite directions and even in the same species the cleavage sometimes takes place consecutively in the same direction, and not alternately to right and to left as has been observed in other forms.

Nevertheless, it should be borne in mind, that cleavage commences invariably from one or the other of the posterior blastomeres or its descendants.

2. After the second cleavage the opposite quadrants of the blastomeres usually come in jaxtaposition forming the cross furrow between. KOFOID has made the statement that the quadrants forming the furrow at the two opposite poles differ with species according to the amount of the deutoplasm in the egg. This does not hold good at least in the present cases. *Siphonaria* and *Aplysia* perhaps represent two extremes with regard to the quantity of the deutoplasm. Nevertheless, as has been seen, the cross furrow in these two species is formed at both poles by the same quadrants.

3. During the cleavage of the egg, the daughter-cells are disposed in a spiral form, that is, the individual cells shift their respective positions either toward the right or the left of their original positions. Such a spiral arrangement or symmetry is retained for some time. It is then abruptly transformed into a bilateral symmetry. This important phenomenon, so far as I am aware, has been interpreted as due to the rearrangement of the component cells. It is true that in an advanced stage the disposition of the cells becomes altered; but there is another important factor which necessitates such a transformation of the body symmetry. The factor is the existence of certain new cells propagated from the fourth generation of ectomere-cells. Of the quartet which forms this generation, the two posterior cells give rise in a peculiar way to the daughter cells which I have called the *bilateral* cells. These play an important rôle in bringing about the change of the body symmetry, which seems to take place in a comparatively short space of time. The period of

his change is, as WILSON remarks, after the entomere-cell differentiates itself from the left posterior ento-mesomere-cell, and takes its seat along the median axis. In other words, the bilateral symmetry appears just after the cells of the three germ-layers are distinguished. This corresponds with the stage when the sixth or seventh quartet of ectomeres is formed and the egg is composed of 29–33 cells as is shown in the tabulated form below. The exact period of the occurrence could not be expressed as it varies with the species, and even in the same species with the environment.

4. The cleavage of the mesomere-cell takes place after the bilateral symmetry is completely established. It is then subdivided into two equal halves, which stand in a strict bilateral symmetry with regard to the median axis. Shortly afterwards from each of these cells mesoderm-cells are propagated, which by the further division eventually form a layer. Hence, it is clear in these species that the mesoderm-cells take their first origin from the posterior quadrants of the blastomere as in many other species. The differentiation of the three germ-layers strictly speaking dates from the formation of the mesoderm-cells. It is at about the stage of the eighth generation of ectomere-cells in *Siphonaria* and of about the tenth generation in *Aplysia*. It is therefore the 42-cells stage in the former, while it is the 50-cells stage in the latter. Thus, the differentiation of the mesoderm-cells varies in time not only in the species named above, but also in all those forms before thoroughly investigated. The results of the present investigation as well as of those of other authors are compiled below in the form of tables to facilitate a comparative study of the Molluscan development:—

Tables showing the cell-lineage in Molluscan Egg.

b.c.....bilateral-cell
d.....entomere-cell
ent.....entomere-cells excepted
M.....mesomere-cell
m.....mesomere-cell segmented
m_1, m_2, etc.....mesoderm-cells
n.....generation of ectomere-cells

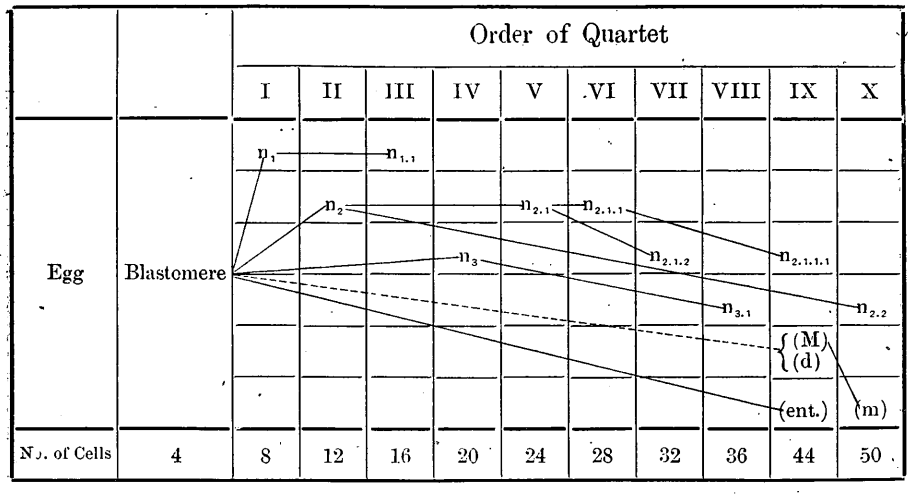
I Siphonaria

		Order of Quartet									
		I	II	III	IV	V	VI	VII	VIII	XI	X
Egg	Blastomere	n_1	$n_{1.1}$						$n_{1.2}$		
		n_2				$n_{2.1}$					
				n_3			$n_{2.2}$	$n_{2.1.1}$			
								(b.c)			$n_{2.1.1.1}$
							(M) (d)		(m) + (m_1) (ent.)	$n_{2.2.1}$	
											(m_2)
No. of Cells	4	8	12	16	20	24	29	35	43	47	51
Time	7 hours	8	9–10	11–12	13–15	18–19	20	21–22	22	24	26

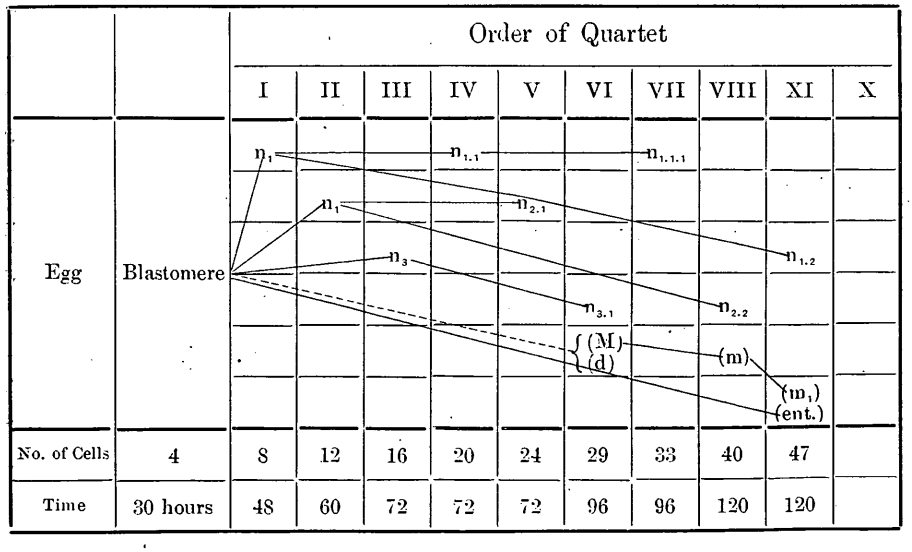
II Aplysia

		Order of Quartet									
		I	II	III	IV	V	VI	VII	VIII	IX	X
Egg	Blastomere	n_1				$n_{1.1}$					
		n_2	$n_{2.1}$					$n_{2.1.1}$			
				n_3			$n_{2.2}$				
							(b.c)				
							(M) (d)	(m)			
								(ent.)			(m_1)
No. of Cells	4	8	12	16	20	24	31	39	43	47	51

III Neritina (Blochmann)



IV Umbrella (Heymons)



V Limax (Meisenheimer)

		Order of Quartet									
		I	II	III	IV	V	VI	VII	VIII	IX	X
Egg	Blastomere	n_1		$n_{1.1}$			$n_{1.1.1}$			$n_{1.2}$	
			n_2			$n_{2.1}$			$n_{2.1.1}$		
					n_3			$n_{3.1}$		$n_{2.2}$	
										(d) (M) + (m) (ent.)	(m_1)
No. of Cells	4	8	12	16	20	24	28	32	36	45	51

These tables show, that in most cases the mesoderm formation arises in the stage of the tenth generation of ectomere-cells and at about the period when the cells number 50 or more without any regard to the duration of time consumed in their development. Further we learn that the genealogy of the ectomere-cells up to the stage of the sixth generation follows almost the same course, although the order of development sometimes varies with to species. And at the stage named, the differentiation of the germ-layers is first attained in some species. By referring to the table it is furthermore evident that the amount of deutoplasm present in the egg seems not to have any causal effect on the rate of differentiation. In this respect, *Neritina*, an egg rich in deutoplasm, forms the germ-layers in the same stage as *Limax*, and *Siphonaria*, which are scantily supplied with food-yolk.

Before closing I wish to express here my deepest obligations to Professors K. Mitsukuri and S. Watasé for their kind supervision of the present work.

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Explanation of Figures.

All the figures are drawn with a camera lucida, and are, unless otherwise designated, magnified with Zeiss D × 4.

Reference letters used.

a.	anterior blastomere.	M	mesomere-cell.
e.	egg.	*p.*	posterior blastomere.
lc.	left bilateral cell.	*pb.*	polar body.
m.	mesomere-cell segmented.	*rc.*	right bilateral cell.

The arrow under the ectomere-cell indicates its course of cleavage and the Roman numeral shows the order of the quartet to be formed.

Figs. 1–36 illustrate the development of *Siphonaria*.

Figs. 37–60 illustrate that of *Aplysia*.

T. FUJITA.

ON THE FORMATION OF THE GERMINAL LAYERS IN GASTROPODA.

PLATE I.

Plate I.

Fig. 1. Egg mass of *Siphonaria* deposited on rock. Natural size. Arrow indicates the orientation of the animal during egg-deposition.

Fig. 2. Three eggs magnified to show their manner of connection. Fresh. A × 2.

Fig. 3. Yolk spherules with vacuoles before disintegration. Fresh. E × 2.

Fig. 4. Egg after total disintegration of yolk spherules.

Fig. 5. First cleavage stage, two blastomeres being connected only by a narrow bridge. Fresh.

Fig. 6. Second cleavage stage. Its commencement, from above. Fresh.

Fig. 7. Same stage, further advanced.

Fig. 8. Completion of the same stage. Blastomeres are now designated as *A*, the left anterior, *B*, the right anterior, *C*, the right posterior, and *D*, the left posterior.

Fig. 9. Cleavage of the first quartet of ectomere-cells. From apical pole.

Fig. 10. Same stage. From posterior side.

Fig. 11. Completion of the same stage, to show the position of cells. From apical pole.

Fig. 12. Second ectomere-quartet stage, showing its formation. Apical view.

Fig. 13. Same stage. Posterior view.

Fig. 14. Commencement of third ectomere-quartet. Apical view.

Fig. 15. Cleavage of fourth and fifth ectomere-quartets. Horizontal section.

Fig. 16. Fifth ectomere-quartet stage with formation of entomere-cell D_1. Sagittal section.

Fig. 17. Sixth ectomere-quartet stage. Horizontal section.

Fig. 18. Formation of entomere-cell D_1. Horizontal section through vegetative pole.

Fig. 19. Same stage. Sagittal section.

Fig. 20. Cleavage of seventh ectomere-quartet. Horizontal section through vegetative pole.

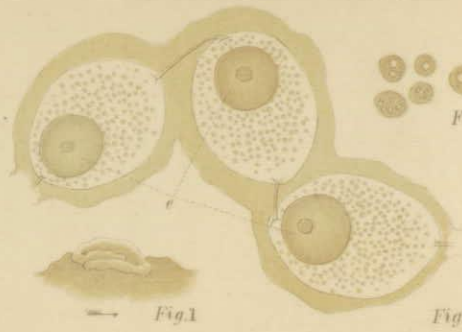
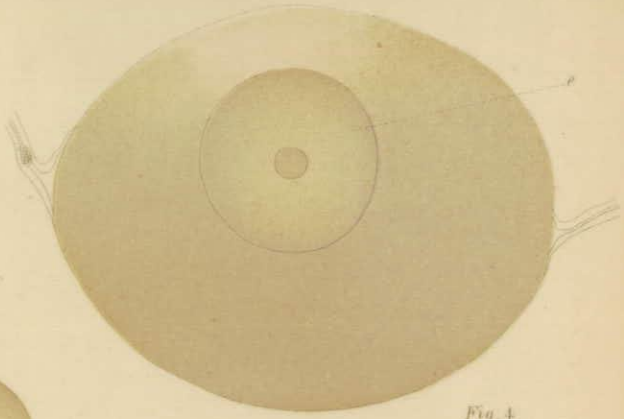


Fig. 1

Fig. 3

Fig. 2



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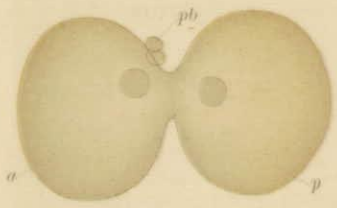

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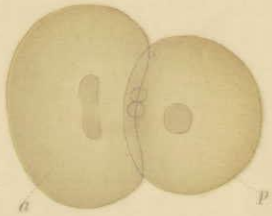

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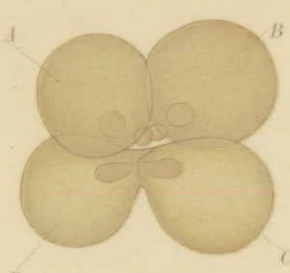


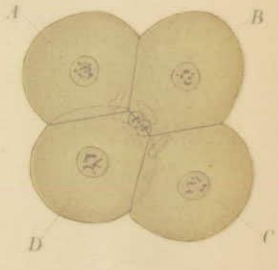
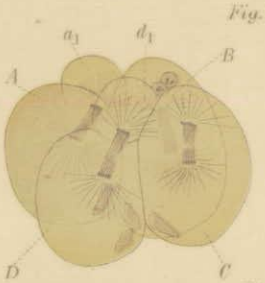


Fig. 7

Fig. 8

Fig. 9



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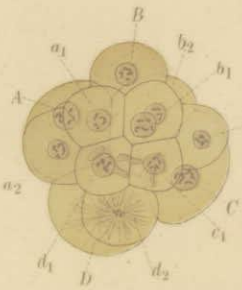

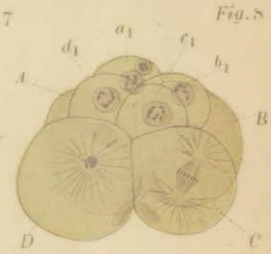

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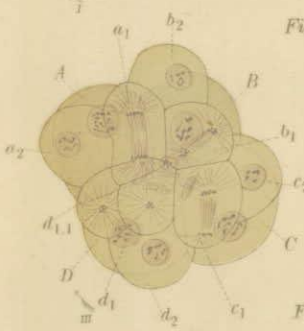
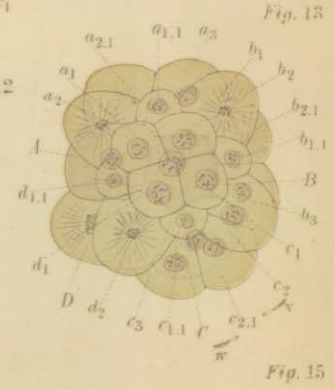


Fig. 14

Fig. 11

Fig. 12



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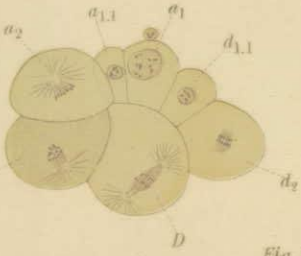

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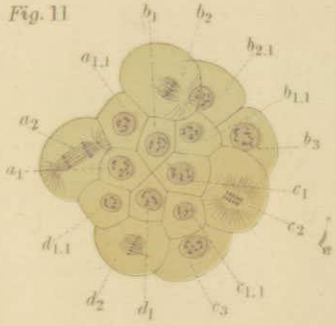

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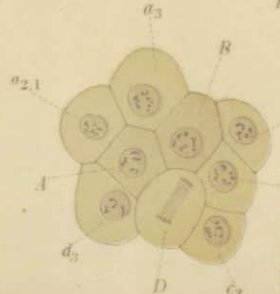
Fig. 20



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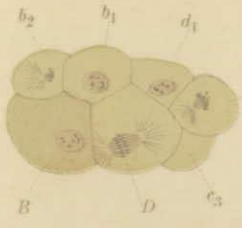

Fig. 19

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PLATE II.

Plate II.

Fig. 21. Formation of bilateral cells. Transverse section of a stage slightly more advanced than in Fig. 20.

Fig. 22. Formation of eighth ectomere-quartet. Horizontal section through apical pole.

Fig. 23. Same stage. Transverse section.

Fig. 24. Stage before cleavage of mesomere-cell; constructed from sections so as to show the mutual relative position of cells. Cells forming the ventral side of the egg are colored blue.

Fig. 25. Cleavage of mesomere-, and entomere-cells. Horizontal section through vegetative pole.

Fig. 26. Cleavage of mesomere-cell M. Transverse section.

Fig. 27. Completion of same stage, showing the relative position of ectomere-quartets. Horizontal section.

Fig. 28. Formation of first mesoderm-cells. Sagittal section.

Fig. 29. Same stage. Transverse section.

Fig. 30. Stage after formation of tenth ectomere-quartet. Horizontal section near vegetative pole.

Fig. 31. Horizontal section through vegetative pole, showing complete enclosure of mesomere-cells by epibolic growth of entoderm-cells.

Fig. 32. Cleavage of second mesoderm-cells. Horizontal section.

Fig. 33. Same stage. Sagittal section.

Fig. 34. Formation of second entoderm-cells. Horizontal section.

Fig. 35. Cleavage of third mesoderm-cells. Transverse section of the posterior side.

Fig. 36. Formation of fourth mesoderm-cells. Sagittal section.

Fig. 37. Eggs of *Aplysia*. Fresh.

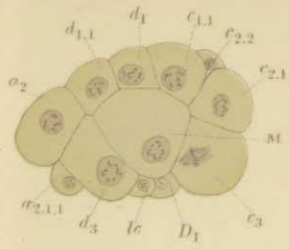
Fig. 38. Same, highly magnified. Fresh.

Fig. 39. Commencement of first cleavage stage. Side view. Fresh.

Fig. 40. Formation of blastomere-cells. Side view.

Fig. 41. A slightly more advanced stage of Fig. 40. Apical view. Fresh.

Fig. 42. Completion of same stage, showing the proper positions of blastomeres. Apical view. Fresh.



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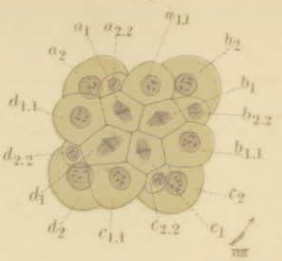

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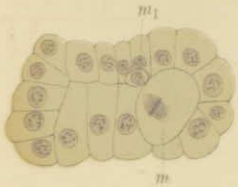

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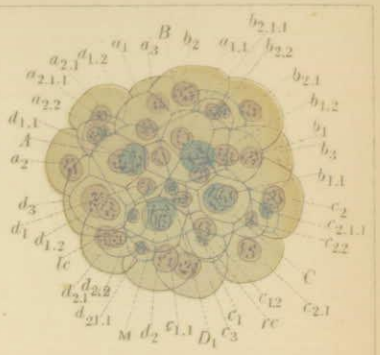

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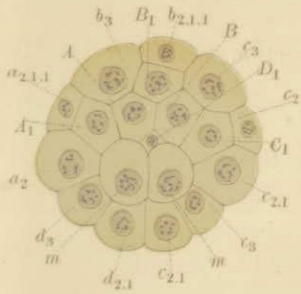

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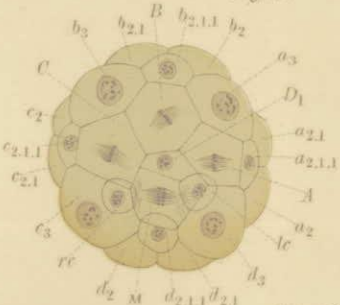

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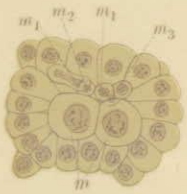

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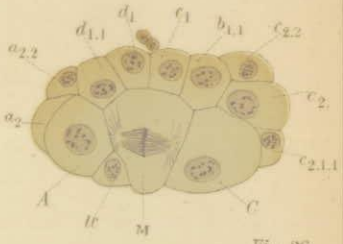

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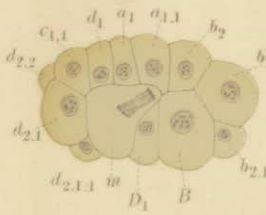

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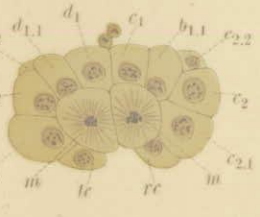

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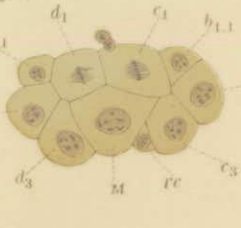

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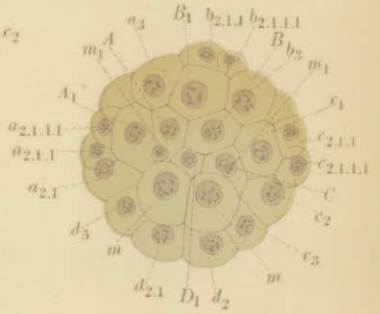

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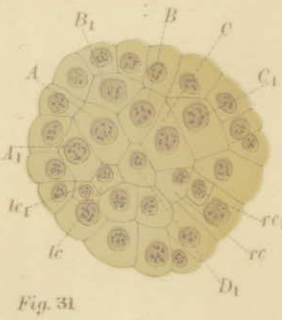
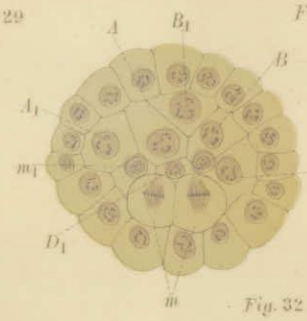


Fig. 31



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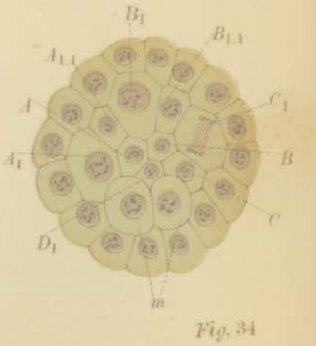

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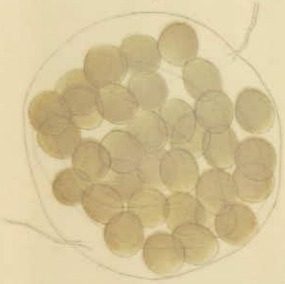

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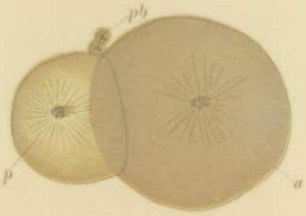

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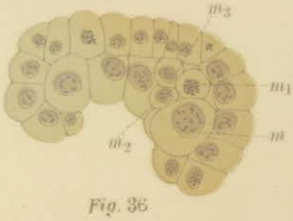

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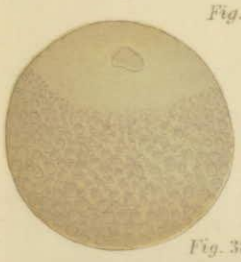

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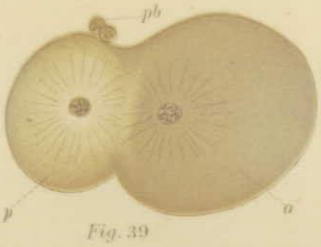

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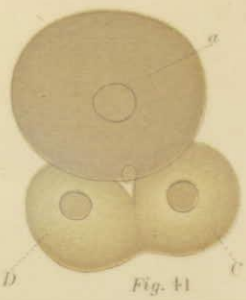

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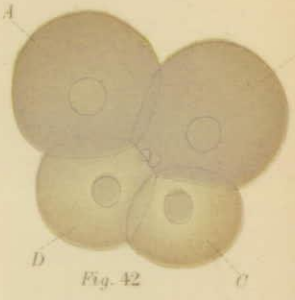

Fig. 42

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PLATE III.

Plate III.

Fig. 43. Formation of first ectomere-quartet. Apical view.
Fig. 44. Same stage. Right side view.
Fig. 45. Formation of second ectomere-quartet. Apical view.
Fig. 46. Same stage. Posterior view.
Fig. 47. Same stage. Left side view.
Fig. 48. Simultaneous formation of third and fourth ectomere-quartets stages. Apical view.
Fig. 49. Same stage. Posterior view.
Fig. 50. Same stage. Optical sagittal section.
Fig. 51. Formation of fifth ectomere-quartet stage. Apical view.
Fig. 52. Same stage. Optical transverse section.
Fig. 53. Cleavage of sixth ectomere-quartet, and formation of bilateral cells. Optical horizontal section.
Fig. 54. Same stage, with formation of seventh ectomere-quartet, and entomere-cell D_1. Sagittal section.
Fig. 55. Same stage, with formation of bilateral cells. Horizontal section.
Fig. 56. Cleavage of mesomere-cell. Optical horizontal section.
Fig. 57. Formation of first entoderm-cells. Optical horizontal section.
Fig. 58. Completion of same stage. Optical transverse section.
Fig. 59. Stage before formation of mesoderm-cell m_1 showing the arrangement of cells. Cells on ventral side are colored blue.
Fig. 60. Formation of first mesoderm-cell stage. Optical horizontal section.

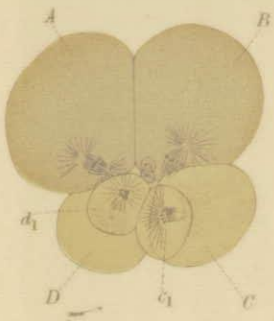
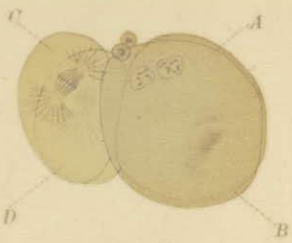


Fig. 44



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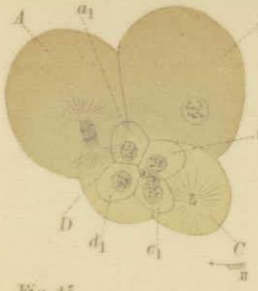

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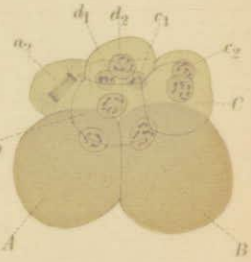

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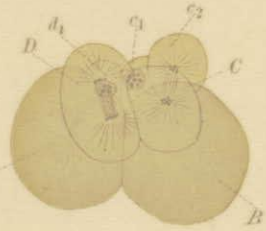

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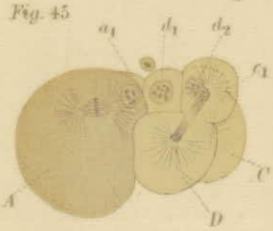

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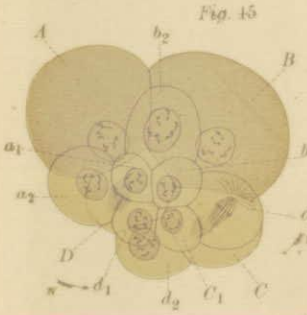

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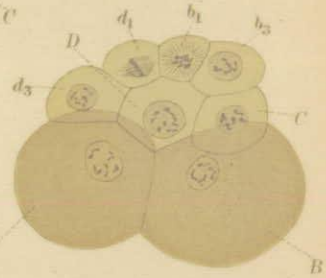

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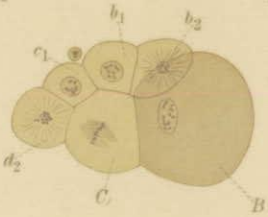

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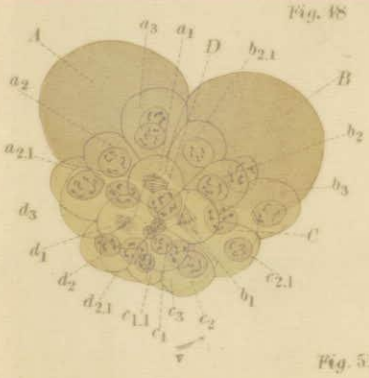

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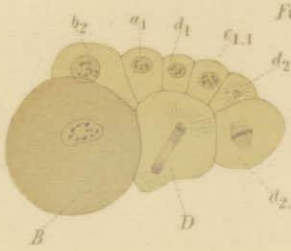

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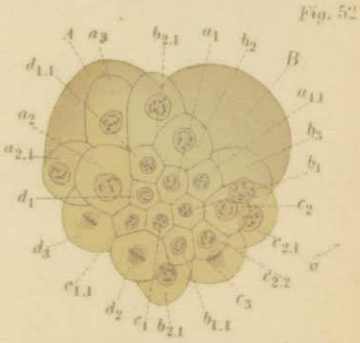

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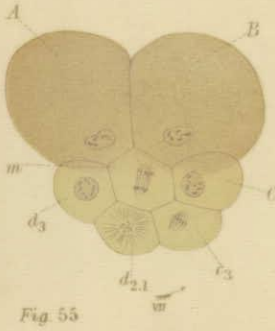
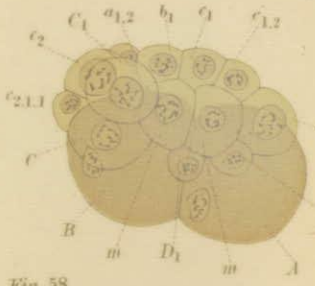


Fig. 56

Fig. 58



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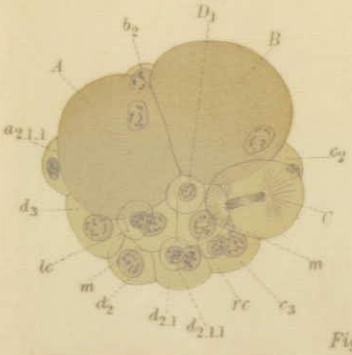

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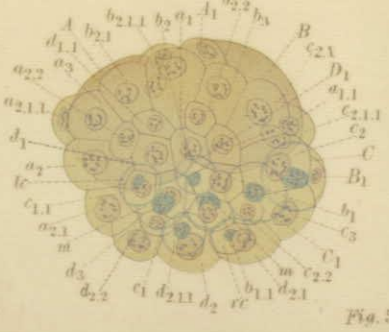

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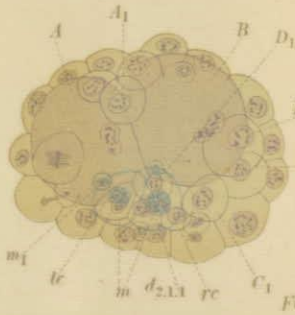

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