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Observations and Experiments on the Ctenophore Egg:

I. The Structure of the Egg and Experiments on Cell-division.

By

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Introduction.

The ctenophore egg has become classic in experimental embryology through the invaluable papers of CHUN ('92), DRIESCH and MORGAN ('95), ZIEGLER ('98, '03) and FISCHEL ('97, '98 and '03). Singularly enough, however, since the appearance of these works no further detailed analytical studies on the interesting egg have been undertaken. At the suggestion of Professor E. B. WILSON, the writer made observations and experiments upon the egg of four common species of ctenophore in the spring of 1906, at the Naples Zoological Station.[1]

The present paper deals first with the structure of the egg with a note on the polocytes and on fertilization; secondly, with observation on the process of cell-division, mainly that of the first cleavage; and thirdly with experiments performed upon the egg of *Beroë ovata*. It will be followed by two other papers; one on cytogeny and experiments on cleavage physiology, and the other on germinal localization.

As to experimentation, I wish to lay especial stress upon the following points. Great care was taken to secure good water quite

1 This study was made with the aid of a grant from the CARNEGIE Institution at Washington, for which I take this opportunity to express my gratitude. I also have pleasure in acknowledging my indebtedness to the staff of the Naples Zoological Station.

far from the shore. The water taken near the city of Naples was so polluted that it was unfit for use in developing egg-fragments into embryos. This is the indispensable condition for ctenophore experiments. The high mortality in DRIESCH and MORGAN'S work seems to have been due to the neglect of this precaution ('95 p. 217). To obtain eggs two or three animals were kept in a rather small cylindrical jar, so that they stirred the water more or less when swimming and kept the eggs they laid constantly in motion. If, on the contray, the ctenophores be put in a large jar, the eggs are liable to stay near the surface; there they become weak and give rise to less lively larvae or fail to develop at all.

I. Structure of the Egg.

The eggs of the following four common species of ctenophores were studied; namely *Beroë ovata*, *B. forskålii*, *Callianira bialata* and *Eucharis multicornis*. The relative sizes of the eggs of these forms are shown in Fig. I (*cf.* CHUN '80 p. 100). The egg of *Beroë ovata* was the one most carefully studied and exclusively used in experiments, being peculiarly suited for the purpose on account of its large size (1–1.2 mm) and of its consistency.

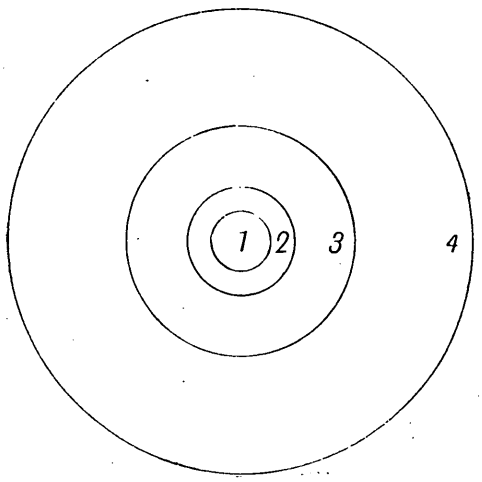

FIG. I.

Diagram showing the relative sizes of the eggs of *Callianira bialata* (1), *Eucharis multicornis* (2), *Beroë forskålii* (3) and *B.ovata* (4). ×6)

When the eggs are laid, they are found entangled in a string-like mass of jelly. Close to the egg is a thin gelatinous covering that turns into a thick layer of jelly after fertilization.[1] The egg has three visible con-

1 The eggs of *Eucharis multicornis* can be more easily taken out of the jelly than those of *Beroë*.

centric differentiations; namely (a) the extremely thin homogeneous outer layer, (b) the ectoplasm and (c) the entoplasm.

The outer "membrane" is a very thin semifluid layer free from granules. It can hardly be called membrane. It is difficult, if not impossible, to detect it. When the egg is compressed or wherever accumulation of the ectoplasm takes place, one can see it fairly well. I should not hesitate to homologize this with the ectosarc described by ANDREWS in the egg of *Hydra* ('98) and with a similar layer found in the sea-urchin egg, to which ZIEGLER has attached so much importance ('03, '04). It can not be looked upon as identical with the hypothetical cell-membrane of RHUMBLER ('99). What *rôle* this thin layer actually plays during cleavage is problematical. I am rather inclined to think that it has very little to do with that process.

The ectoplasm is, as has been described by many authors, a rather thick layer of finely alveolar plasm. It is of uniform thickness until fertilized. This layer is, contrary to ZIEGLER's view, not at all of the same nature as the "ectoplasm" of the sea-urchin egg. It is fluorescent and looks green under reflected light, reminding one of a piece of uramium glass (*cf.* CHUN '80 p. 100). In stimulating the egg with a weak electric current this layer alone seems to emit a beautiful greenish light. It should be mentioned, that, as the development advances, the ectoderm comes to monopolize this property. In a Wood's Holl species, *Mnemiopsis*, the egg before cleavage, according to PETERS ('05), was not phosphorescent.

It need hardly be mentioned that the entoplasm is a coarse alveolor structure. The alveoles of the *Callianira* egg are much fewer in number than those of the *Beroë* egg, the individual alveole of the former being much larger than that of the latter (Pl. II, Figs. 27 and 28). On crushing the *Callianira* egg I could count in one case 67, in another 64, and in still another 54 alveoles. ZIEGLER calls the alveoler substance "transparent yolk granules" ('98 p. 36). This seems to have given RHUMBLER the impression that it was made up of a rather highly viscous substance. But as a matter of fact, this is not so. CHUN ('80) has called it "Zellsaft." Though this term is not happily chosen, yet it is far better

than "yolk," In the material fixed with FLEMMING's fluid the alveolar substance is completely dissolved, being represented by holes. Whatever its chemical nature may be, it is certainly not similar to what commonly goes under the term yolk. In the entoplasm no axial differentiation can be detected; the size and nature of the alveoles are the same throughout the egg.[1]

II. Polocytes.

I have nothing to add about the formation of the polocytes, excepting that in one case a maturation spindle with no pole-rays was met with in an ectoplasmic accumulation (FLEMMING's fluid material).

In the living egg it was not difficult to see the polocytes. Pseudopodia are seen on both the polocytes. The first polocyte loses them and invariably divides in two; each part has a smooth surface. The pseudopodia, however, remain on the second polocyte (Pl. I, Fig. 4). As to whether or no the polocytes perform an amoeboid locomotion by means of the pseudopodia I have had no means of determining. Yet I am rather inclined to believe that they do not (*cf.* CHUN '80 p 101). As is shown in Figs. 1, 2 and 3 (Pl. I) a thickened portion of the ectoplasm is found beneath the polocytes. The egg nucleus undoubtedly lies in this. The accumulation lasts for some time after the formation of the second polocytes. But it soon disappears.

III. Entrance of the Spermatozoon into the Egg.

As already mentioned, when the eggs are discharged, they have a thin gelatinous covering about them. When fertilized this membrane changes into a thick layer of jelly. I could not ascertain how the process took place. It is highly probable that the change is of the same nature as that of echinoderm eggs. In any

1 In this connection it may be of some interest to note that FEWKES saw in the egg of *Agalma* a mass of rosy entoplasm at one pole ('85 p. 247).

event this jelly layer gives a splendid criterion for distinguishing at a glance fertilized egg from unfertilized.

One other phenomenon accompanying fertilization is that the oöplasm suddenly acquires greater consistency. This is especially frappant after the formation of an ectoplasmic thickening around the spermatozoon. One can hardly fail to notice this change when experimenting upon various stages of the egg.

The entrance of the spermatozoon can readily be seen in the egg of *Beroë*. Fig 5 (Pl. I) shows a surface view soon after its penetration into the egg. A refringent body (acrosome?) is seen a little apart from the head. Behind the head is a dark body, sperm-centre, provided with long rays. Soon, however, the rays disappear. In a side view one sees an entrance-cone consisting of a thickened external homogeneous layer and also considerable accumulated ectoplasm. The entoplasmic alveoles are arranged radially (Pl. I, Fig. 6). In them no rays are seen in the living egg. In section, however, distinct long rays come into view, which extend from the straightened alveolar walls of the ectoplasm into those of the entoplasm. As is seen in Figs. 7 and 8 (Pl. I) the ectoplasmic accumulations remain for some time, so that by them one can tell at once how many spermatozoa have entered the egg. On one occasion I saw an egg with as many as five of them in it. Polyspermy in this form is not at all a physiological phenomenon. It usually takes place when eggs are kept too crowded in a jar. I know nothing about the fate of those sperm-nuclei which fail to unite with the egg-nucleus. Yet judging from the fact that in many cases polyspermy does not lead to abnormal cleavage; those solitary sperm-nuclei seem to degenerate *in situ*.

IV. Cell-division.

a) *The First Cleavage.*

I could not make out how or where the germ-nuclei

meet.[1] At any rate prior to the first cleavage, there takes place a change in the distribution of the ectoplasm: it thickens considerably near the macromere pole,[2] while at the opposite pole it thins out a great deal (Pl. I, Figs. 9 and 10). Cleavage goes on, as has been observed by a good many investigators. Sometimes the cleavage furrow is bent slightly to one side near its completion (Pl. I, Fig. 11). It should here be especially mentioned that the cleavage is not strictly unilateral, contrary to ZIEGLER's observation ('98. p. 41; '03 p. 159 and his diagram Fig. 7), and also to RHUMBLER's opinion based upon ZIEGLER's results. A shallow depression is always present at the micromere pole, as is seen in the sketches drawn one upon another at different periods (Pl. I, Fig. 11). And it will be also noted that the top of each blastomere becomes more rounded. The lateral elongation is almost nil. The rate of the cleavage from the macromere pole is 8–19μ per minute, 17μ on an average, at a room temperature of 65°–67°F. The rate of cleavage from the opposite pole is a little slower, 13μ per minute on an average.

To supplement ZIEGLER's observations, the process of cleavage and especially the "cleavage head" (Furchenkopf) will be described in some detail. In the beginning a slight depression appears near the polocytes. Its optical section is shown in Fig. 12 (Pl. II). Here is a pair of prominences in the outer homogeneous layer (*cf.* ANDREWS '98). Sometimes they are continuous, forming a bridge over the now deepening furrow.[3] Sometimes there is one process on one side and two on the other (Pl. II, Fig. 12) (*cf.* KLEINENBERG '72 p. 49., Taf. 4, Fig 4; WAGER '09 p. 23, Pl. III, Fig. 23a). Around the process a fine display of spinning activity is visible. At the bottom of the cleavage depression are rays in the homogeneous layer.

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1 As is seen in Figs. 10 and 11, the polocytes are usually situated at some distance from the cleavage furrow. Whether the egg-nucleus or cleavage-nucleus moves a little from the spot where the polocytes have been formed, or whether the polocytes are transported by some means, is not certain. But the latter alternative seems to be the more probable one.

2 Macromere pole = vegetative pole of HATSCHEK (KORSCHELT and HEIDER '03 p. 24). Throughout the plates the macromere pole is above, and the micromere pole below.

3 The bridge is not so distinct as was observed by TANNREUTHER in the *Hydra* egg, where yolk granules were seen to pass from one blastomere to the other ('08 p. 267).

As a digession, the results from the study of sections of this stage may here be given. Cleavage begins at the telophase of the first mitosis. Rays (pole-rays) of a considerable length extend into the entoplasm of both blastomeres, centering about the newly formed nuclei (the centres are in all probability situated very close to the nuclei). Besides, something like sheath-rays are found between two asters. They are evidently cut apart by the growing "cleavage head." A similar condition has been observed in the *Hydra* egg by BRAUER ('91 Taf. IX, Figs. 16 and 17).

Now coming to the next stage (Pl. II, Fig. 13). The bottom of the depression has been carried farther down. Usually the clear protuberances at the entrance of the furrow are drawn in. Fine spinning is seen. The protoplasmic threads are not parallel to one another. Often they decussate. At the "head" are radiations as in the foregoing stage.

A fully formed "cleavage head" is shown in Fig. 14 (Pl. II). By this time protoplasmic spinning is restricted to the entrance and bottom of the furrow. The "head" is a thickened ectoplasm. Here one notices that the outer homogeneous layer also has increased in thickness. Refringent alveoles in the ectoplasm are arranged radially as extensions of fine radiations of the homogeneous layer. Towards the entoplasm are processes (Zacken) as has been rightly observed by ZIEGLER ('98, '03). I tried hard to detect rays extending from the tips of the processes into the entoplasm, but contrary to RHUMBLER's assumption ('99 p. 203 Fig. 12 and p. 205 Fig. 13), there were no such things; here the ectoplasm simply comes in contact with the entoplasm. The alveoles of entoplasm here show a peculiar arrangement worth noting. Those along the walls of the cleavage furrow seem to have been carried down with it and those found at the tip of the "head" are somewhat flattened (*cf.* Pl. II, Fig. 28). This undoubtedly shows that the "cleavage head" pushes downward instead of being pulled by the contraction of rays, stretching between the cleavage head and the micromere pole. At the next stage the alveoles recede from the median plane as is shown in Fig. 15 (Pl. II). It will be of some interest here to

examine two cleavage stages of the egg of *Beroë forskålii* (Pl. II, Figs. 19 and 20). In this particular egg the second cleavage has begun before the first has come down nearly two thirds of this entire course. By the precocious second division the typical alveolar arrangement has been considerably disturbed, a flow-figure having been formed in each blastomere. For all that, the first cleavage cuts through the egg normally as through nothing had happened near the macromere pole.

Pari passu with the coming-down of the cleavage furrow, the ectoplasm thickens near the micromere pole as has been observed by ZIEGLER. And the "cleavage head" meets the ectoplasmic accumulation there (Pl. II, Fig. 15,). The walls of the cleavage furrow near the "head" become irregularly wrinkled and the spinning activity increases (*cf.* ANDREWS '98). The hole now assumes a triangular shape. As the entoplasmic alveoles quickly retreat, the ectoplasmic bridge[1] is left between two fartmeres (Pl. II, Figs. 17, 18, 21, 21). It is interesting to recall that LOEB observed cytoplasm flow away from the furrow towards the end of unilateral cleavage ('06 p. 66). No particular movement as seen by BUNTING in *Hydractinia* ('94 p. 216) takes place (*cf.* ZIEGLER's experiments). Finally the ectoplasm also goes into the blastomeres, leaving behind a fine thread of homogeneous layer. By the time one finds an ectoplasmic thickening with radially arranged entoplasmic alveoles near the micromere pole of each blastomere (Pl. II, Fig. 18) In studying this stage with a low power one soon notices that the greater part of the ectoplasm has come down towards the micromere pole (Pl. II, Figs. 31, 22)

Incidentally I might mention that the cell-wall between the two blastomeres of *Eucharis* and *Callianira* has a sieve-like appearance (Pl. II, Figs. 22, 24, 27). In the latter form I was able to see this peculiarity between two entoderm cells as late as the gastrula stage. The fenestrated appearance is due to the lenticular accumulation of a certain fluid as correctly observed by CHUN ('80 p. 102) (*cf.* FOL '73 Tf. 24, Fig. 5).

1 The surface of the bridge has radiating wrinkles.

b) *Subsequent Cleavages.*

Soon after the first cleavage is completed, the ectoplasmic thickening near the micromere pole disappears and at the same time an accumulation comes in view over the macromere pole (Pl. II, Fig. 23). The second cleavage takes place in exactly the same manner as the first (Pl. II, Fig. 24). In the beginning of the third cleavage an accumulation of the ectoplasm near the macromere pole is also seen (Pl. II, Figs. 25, 26, 27). At the fourth division the micromeres are formed, which are almost entirely made up of the greenish ectoplasm (Pl. II, Figs. 29, 30). Subsequent divisions of the micromere are carried on in unilateral fashion similar to the division of the entire egg. So also the divisions of the macromeres (Text fig. II).

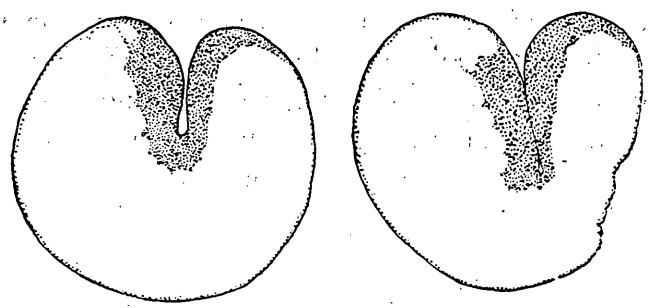

FIG. II.

Two dividing entoderm cells of *Beroë ovata*. ×60.

V. Experiments on Cell-division.[1]

Fifty eggs of *Beroë ovata* were operated on during the first cleavage in twelve different ways with the intention of testing, if possible, hypotheses hetherto put forth regarding the division mechanism of the ctenophore egg. On account of the large size and favorable consistency of the egg, the operations were performed with the greatest ease by means of a small knife. Sometimes, however, in case the jelly around the egg was unusually hard,

1 This part of the present paper was read as a preliminary note before the Seventh International Zoological Congress at Boston 1907.

cutting was accompanied with some difficulty. Very soon after the operation the cut surfaces close; so rapid is the closure that one cannot, as a matter of fact, see exposed entoplasm(*cf.* MAAS '03 p. 45). Each of the eggs operated on was placed in a compressorium[1], and the subsequent progress of cleavage was followed. From a single egg several sketches of successive stages were made. In the plates of the present paper in most cases only the first and the last stages have been reproduced, since the intervening ones would be of little value in illustrating the following experiments.

a) *Experiment I* (four cases).

A portion of the egg was cut below the "cleavage head" at various angles and along various levels, and the enucleated pieces were watched to see if they showed any sign of division activity.[2] Even in the case in which the cutting plane passed very near the "cleavage head," nothing happened in the enucleated piece—it simply rounded up and ceased to develop further (Pl. III, Fig. 31).

b) *Experiment II* (one case).

The above experiment was modified in the following way. An incision extending two thirds of the diameter of the egg was made below the "cleavage head" to see if the connection with the nucleated part of the egg would impart some division activity (Pl. III, Fig. 32). The cleavage went on normally, cutting the upper part in two, but the lower past remained undivided (Pl. III, Fig. 33).

The above two experiments (Exp. I and Exp. II) clearly show that portions devoid of the "cleavage head" do not manifest any division activity whatever.

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1 This was not used for compressing the eggs but as a sort of live-box for keeping them alive.

2 I have a case in which both pieces produced by a horizontal cut cleaved. It can hardly be doubted that it was a dispermic egg.

c) *Experiment III* (thirteen cases).

Sections were made on the egg above the "cleavage head" at various periods and along various planes, and the behavior of the enucleated fragments containing the "cleavage head" were studied.

In eight cases out of thirteen the cleavage went on normally. The rate of downward progress of the furrow was normal, or a little slower than the normal that is 15μ per minutes on an average. It was sometimes 10μ or even as slow as 8μ per minutes. In Fig. 34 (Pl. III) the section passed through the middle of the cleavage furrow and in Fig. 39 (Pl. III) the cut was made when the cleavage had just begun. In both the enucleated pieces cleavage went on as though they were a part of the whole egg. It is interesting to note that the movement of the ectoplasm is the same as in the entire egg, that is, a thick ectoplasmic accumulation is formed about the micromere pole towards the end of the division (Pl. III, Fig. 35), and after that thickenings in the neighborhood of the macromere pole (Pl. III, Figs. 36, 40). Here one notices that the thickness of the above ectoplamic accumulation depends upon the size of the enucleated pieces. It also may be remarked that similar up-and-down movement of the ectoplasm takes place in the nucleated pieces (Pl. III, Fig. 41).

In studying carefully the relation between the angle of the cuts and the direction of the cleavage furrow, the following results were obtained. If the section be made when the cleavage furrow is shallow, then the division goes on normally irrespective of the angle of the cut (Pl. III, Figs. 39. 40). If, on the other hand, the operation is performed in later stages, the cleavage is usually accomplished normally only when the section is horizontal or approximately so; if otherwise, the furrow is bent and the bending is always towards the side with more cytoplasm, so that the resulting blastomeres are of nearly the same size (Pl. III, Figs. 37, 38).

In three cases out of thirteen something unexpected happened. One of these cases is represented in Figs. 41 and 42 (Pl. III). The other two were very much like this. The cleavage furrow went down near the micromere pole and turned upward so

that in the end a bridge was formed between the two blastomeres. The bridge was not cut through. One case of this peculiar mode of cleavage was the result of a horizontal section. In the other two, the curving of the furrow faced the side of more cytoplasm (in Fig. 41, Pl III to the left). CONKLIN observed in the egg of *Linerges mercurius* the turning-up of the cleavage furrow (Pl. 3, fig. 13) and thought it probable that this might be due to the flowing of cytoplasm through the bridge from one cell to the other (p. 160). In the *Beroë* egg no streaming phenomenon accompanies this curling-up of the cleavage furrow.

In two cases for some unknown reason the division stopped at a certain stage and did not cut through. In one case at the beginning of the division the "cleavage head" was thick but as it proceeded the ectoplasm thinned out somewhat (Pl. III, Figs 43, 44).

From this experiment it may be concluded that after the "cleavage head" is established, the cell-division is accomplished without the instrumentality of either the uncleus or the centrosomes. Furthermore it should be noted in this experiment that there is no perceptible difference between the cut and the uncut eggs in the thickness of "cleavage head," though in some cases a little retardation of the progress of the cleavage is seen in the cut eggs. The up-and-lown flow of the ectoplasm takes place independently of the presence or absence of either the nucleus or the centrosome.

d) *Experiment IV* (Six cases).

In order to test whether either the nucleus or the centrosome exerts any influence on the deepening cleavage furrow, the nucleated portion of one side was cut off at various angles and periods, and the behavior of the cleavage furrow was studied.

In all cases division took place undisturbed by the operation (Pl. III, Figs. 45, 46). In one case, however, the cleavage furrow was bent near its end towards the nucleated side, but such a bending occurs so often in eggs not operated upon (Fig 11 Pl. I) that the cause of the bending in this particular case cannot be ascribed to the operation.

This experiment strengthens the results obtained from the preceding one (Exp. III); that is, the nucleus, centrosome, and the amount of cytoplasm above the cutting plane have little to do with the growth and direction of the cleavage furrow.

e) *Experiment V* (One case).

To slightly modify the above experiment, the cut was extended to the middle of the nucleated part as is shown in Fig. 47 (Pl. IV). The "cleavage head" came down uninterrupted by the operation. But the cleavage furrow stopped at a certain point. (Pl. IV, Fig. 48) owing probably to the fact that the nuclei had already enterd the phases of the second cleavage.

f) *Experiment VI* (Seven cases).

In this experiment the "cleavage head" were split in two at various stages by a vertical cut to see if the furrow proceeds from the end of the cut or from some other place.

In no cases were two "cleavage heads" formed. In one case out of seven the cleavage furrow made its appearance at the bottom of the incision, dividing the egg in two equal blastomeres.

In the six cases a remarkable phenomenon was met with. A new "cleavage head" emerged on one side of the incision below the original bottom of the furrow and in most cases a little above the end of the cut. It proceeded almost at right angles to the old cleavage plane. The portion of the cut below the new cleavage furrow dwindled and was either obliterated entirely or remained as a hole for a fairly long time. In Fig. 49 (Pl. IV) the operation was made when the cleavage furrow was very shallow,[1] and the result was Fig. 50 (Pl. IV). In passing, it may be remarked that in the egg of *Pennaria* HARGITT ('00) observed a similar figure (his Pl. II, Fig. 2).

It is interesting to note that in this case an ectoplasmic thickening was formed at the side of the egg (Pl. IV, Fig. 50). When the

1 The original bottom of the cleavage furrow is marked with ×× in this and the following figures.

operation was performed at a later stage (Pl. IV, Figs. 51, 52) a new accumulation of the ectoplasm appeared at the end of the incision which reinforced that of the old "cleavage head" to form the new one. The resulting cleavage furrow was exactly the same as that in the foregoing case.

In five cases the new "cleavage head" was formed on the side with more cytoplasm, while in one case it was just reverse. This may be interpreted in two different ways, *viz.*, (a) that a new cleavage furrow is formed on the side of more cytoplasm, or (b) on the side of more ectoplasm or "cleavage head"-substance due to oblique section. The above experiment fails to decide which of the alternatives is the correct view. The following experiments were especially directed to this point.

g) *Experiment VII* (One cass).

The "cleavage head" was split vertically and a nucleated portion was cut off as is shown in Fig. 52 (Pl IV). A new cleavage furrow was formed at an angle to the old and on the side of more cytoplasm.

h) *Experiment VIII* (Three cases).

For the same purpose, the "cleavage head" was split and a portion of the cytoplasm was cut off. In all cases as in Exp. VI a new "cleavage head" appeared at some angle to the old one. In one case a new division plane was directed towards the cut surface (Pl. IV, Figs. 55, 56) while in the other two the new furrow was formed on the side of the larger cytoplasmic mass and turned upwards as in some cases in Exp. III (Pl. IV, Figs. 58, 59).

This experiment clearly shows that the new "cleavage head" develops on the side of larger cytoplasmic portion.

i) *Experiment IX* (One case).

In one egg the "cleavage head" was split and another incision was made on one side of it (Pl. IV, Fig. 60). A new "cleavage head" was formed on the left hand side which turned

to the right (Pl. IV, Fig. 61). This cleavage furrows finally cut off an enucleated portion (Pl. IV, Fig. 62).

j) *Experiment X* (One case).

This is a modification of Exp. VIII. The "cleavage head" was cut in two by a vertical incision, a small nucleated portion was cut off (from the left side of the figure Fig. 63, Pl. V), and the micromere portion was removed. A new cleavage plane was established at the end of the vertical incision that cut through almost straight. This failed to yield anything of interest, being exactly the same as Figs. 55, 56 (Pl. IV).

k) *Experiment XI* (Eleven cases).

In this experiment an incision was made in the egg at the micromere pole to see if that would affect the course of cleavage. In nine cases out of eleven the cleavage furrow passed by the incision as though nothing had happened to the egg (Pl. V, Figs. 65, 67, 68). One notices in Fig. 66 an ectoplasmic accumulation at the left hand corner. In one case the cleavage plane stretched towards the incision and became continuous with it (Pl. V, Figs. 61. 70). In another case the cleavage furrow, which had attained a considerable length, dwindled owing to the operation, and two new furrows were formed giving rise to a three lobed egg (Pl. V, Figs. 71, 72, 73). As the original cleavage furrow shriveled up, a peculiar ray-like arrangement of entoplasm was seen. Whether it was due to the effect of the incision or to preparation for the formation of two new "heads" I could not determine. At any rate this double "headed" cleavage seemed to be an exceptional case, and should not be taken as of constant occurrence.

l) *Experiment XII* (One case).

Two cuts were made on one egg (Fig. 75 Pl. V) and the result was as in Fig. 76 (Pl. V). The cleavage furrow stretched towards one of the incisions and cut through the egg.

VI. Summary.

Observational part:

1. The ctenophore egg is composed of (a) the outer homogeneous layer, (b) ectoplasm and (c) entoplasm.

2. The outer homogeneous layer is homologous with ZIEGLER's "hyaline Aussenschicht" of the echinoderm egg.

3. The ectoplasm is an alveolar plasm and rays may be formed in it.

4. The ectoplasm is phosphorescent.

5. The sperm-rays and pole-rays of the first division enter the entoplasm, the alveolar walls of the latter taking a radial arrangement.

6. Polyspermic eggs may cleave normally.

7. Cleavage is not strictly unilateral, the furrow being formed in the micromere region.

8. Fine spinning of the homogeneous layer can be seen at entrance and at the bottom of the cleavage furrow.

9. In the "cleavage head" radiations are seen in the homogeneous outer layer and ectoplasm, but they do not extend into the entoplasm.

10. Beneath the "cleavage head" the entoplasmic alveoles are considerably compressed.

11. The micromeres consisting almost entirely of the ectoplasm cleave very similarly to the whole egg.

12. In the beginning of each cleavage, ectoplasmic accumulation is seen at the macromere pole. Towards the end of cleavage, an accumulation appears in the micromere region.

Experimental Part:

13. Enucleated fragments destitute of the "cleavage head" not manifest any division activity.

14. The cleavage plane is not predetermined in the egg.

15. An enucleated piece provided with the "cleavage head" divides by itself without the aid of either nucleus or centrosome. Nor is the ray system necessary for the cleavage of enucleated pieces.

16. The cytoplasm above the level of the cleavage head has little influence upon the accomplishment of the division.

17. The accumulation of ectoplasm over the micro-[1] and macromere poles is formed in enucleated fragments in the same way as in the whole egg.

18. If the removal of the nucleated portions is done at the beginning of the division, the cleavage furrow goes on normally irrespective of the angle of the section. If, however, the same operation is performed upon an egg in which the cleavage has further advanced, the division plane is in most cases turned towards the side of larger amount of cytoplasm, the enucleated fragment being divided into nearly equal parts.[2]

19. Sometimes in the egg operated on the cleavage furrow curls up towards the macromere pole.

20. If the "cleavage head" be split lengthwise, a new head" forms nearly at right angles and towards the portion with larger amount of cytoplasm.

21. If an incision is made in the egg in the micromere region the cleavage is not affected.

VII. General Discussion.

Three views have been put forth regarding the mechanism of the cytodieresis of the ctenophore egg. ZIEGLER maintains that the cleavage is accomplished by the constriction of a meridional ectoplasmic thickening,[3] which is in turn caused by the "action at a distance" of the centres and no rays are necessary for cleavage ('03 p. 162).

1 Strictly speaking, at the end of the cleavage furrow, since the accumulation takes place at the side of the egg in case a new cleavage furrow is formed at right angles to the old, *e.g.*, Pl. IV, Fig. 50.

2 This result was obtained when a large portion was cut off from the egg. No experiment was carried out, to my regret, to test whether or no the removal of a small amount of cytoplasm from an egg with an already far advanced cleavage furrow affects the remaining course of the cleavage plane.

3 It is interesting to recall how KOWALEVSKY was impressed when he observed cleaving ctenophore eggs: "wie sonderbar es auch klingen mag, so scheint mich doch diese, so zu sagen, todte unbewegliche centrale Masse ganz der mechanischen Pressung von Aussen : u und keine innen active Kraft zu besitzen" ('67 p. 3).

Based upon ZIEGLER's observations RHUMBLER tried to explain the cytodieresis of the ctenophore egg by adding a few subsidiary assumptions to his own theory of cell-division in general previously put forth ('99), viz., that (a) the nuclear fluid is present along the axis of the egg; (b) at the expense of the nuclear fluid the membrane grows rapidly; (c) the "cleavage head" is a structure comparable to the centrosome; and (d) the rays radiating from the "cleavage head" contract and pull down the cleavage furrow to the micromere pole.

FISCHEL expresses his view of the probable existence of the pole-rays, which function as in ordinary cases of cytodieresis ('98 p. 620 *et seq.*).

My experimental study on the ctenophore egg makes it impossible for me to accept any of the above three hypotheses for the following reasons. If, as ZIEGLER maintains, the cleavage is due to the contraction of an elastic ring around the egg, the curling-up of the cleavage furrow towards the macromere pole after the removal of the nucleated portion is a thing not easily accounted for. Still more difficult is it to apply his view to the case in which a new cleavage furrow is formed at right angles to the old. The above two facts are also against RHUMBLER's assumption. And the fact that there are no rays radiating from the "cleavage head" into the entoplasm makes his view untenable. It is certain that the cleavage is not accomplished by the contraction of pole rays, as FISCHEL incidentally states, as is seen in the cases in which the nucleated part is removed.

In his paper on the development of *Linerges mercurius*, CONKLIN puts forth the view that the unilateral cleavage of the cœlenterate egg in general is at least in part due to the structure of the oöplasm itself, that is, thin central entoplasm with a firmer peripheral layer ('08 p. 167). This we have no reason to deny, yet how such a structure is favorable to one-sided constriction is hard to understand. When we come to study the unilateral cleavage of the micromeres of the ctenophore egg, which are almost entirely made up of ectoplasm, it becomes doubtful how much influence the original structure of the oöplasm exerts on the performance of

such cell division. It is also interesting to note, as I have done elsewhere, that unilateral cleavage is seen in some parthenogenetically developing sea-urchin eggs and also in lamprey eggs, whose oöplasm is uniformely laden with yolk granules.

At present I am not in a position to construct any hypothesis to account for the cleavage mechanism of the ctenophore egg. Further detailed biophysical experimentation on the egg will undoubtedly shed a new light on the problem. As a working hypothesis this much can be said. Through the action of the centres (centrosomes) surface tension is increased along the cleavage plane first at the animal region and then towards the micromere pole[1] and thus the ectoplasm is gradually collected. The optical section of the bottom of the cleavage furrow is the "cleavage head, that is a passive structure. The entoplasm now tends to round up around two centres (geometrical) and the two blastomeres are formed. My experimental study seems to have furnished two important data regarding the above rather vague general interpretation of the cleavage phenomenon. Firstly, the cleavage furrow tends to divide the egg equally, as for instance in the cases where a portion of oöplasm is removed and thus the symmetry is disturbed, the new cleavage furrow being bent toward the larger mass of cytoplasm. Secondly, the ectoplasm flows up and down just as well without the nuclei and centres, as with them. This change may be caused by the unequal increase of surface tension due to the internal division phases. At any rate my results do not indicate that the ectoplasm alone is an active cleaving agent as ZIEGLER and RLUMBLER seem to believe.

Misaki Marine Biological Station
Aug. 11. 1910.

1 As has been pointed out by ZIEGLER unilateral cleavage is doubtless in some way connected with the eccentric position of the nuclei and centres. But it should be noted that their being in the ectoplasm is not an essential condition of one-sided cleavage. In the egg of a good many cœlenterates the nuclei are in the entoplasm and the cleavage is unilateral, *e. g.*, *Linerges* (CONKLIN), *Geryonia* (FOL), *Hydra* (BRAUER, TAUNREUTHER).

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PLATE I.

PLATE I.

Figs. 1–8, 10 and **11** *Beroë ovata.*
Fig. 9 *Eucharis multicornis.*

Figs. 1, 2 and **3.** Three successive stages of the division of the first polocyte. ×270.

Fig. 4. Surface view of three polocytes. Clear spot indicates the egg-nucleus. ×270.

Fig. 5. Sperm-head in the egg (surface view). Notice an aster around the centre and a refringent body situated a little apart from the sperm-head. ×270.

Fig. 6. Side view of an ectoplasmic accumulation caused by the spermatozoon. ×270.

Fig 7. The same drawn from a polyspermic egg. ×270.

Fig. 8. Trispermic egg (surface view). ×60.

Fig. 9. Dividing egg of *Eucharis* (optical section), a stage preceding Figs 23 and 24 (Pl. II), 3.23 P.M. ×140.

Fig. 10. Dividing egg, the cleavage having proceeded nearly one third the diameter. ×60.

Fig. 11. Dividing egg, showing outlines of four stages; a–a' 10 A.M., b 10.8 A.M., c–c' 10.25 A.M., and d–d' 10.35 A.M. ×60.

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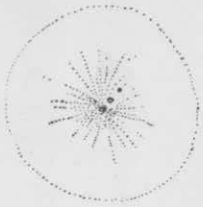

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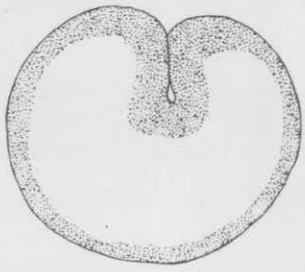

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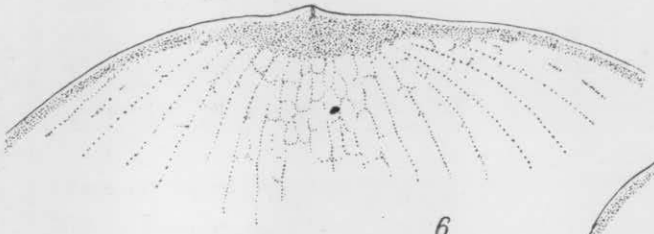

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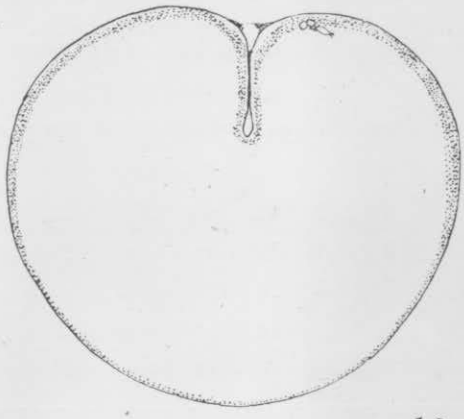

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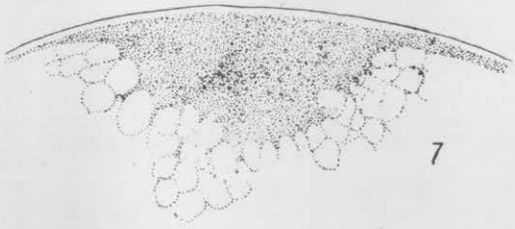

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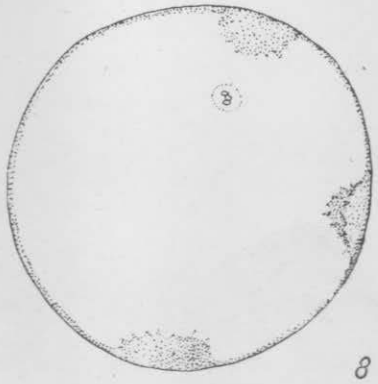

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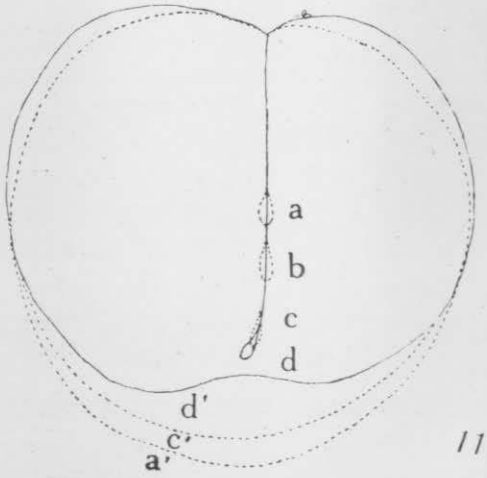

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PLATE II.

PLATE II.

Figs. 12–18, 21, 25 and 26 *Beroë ovata*

Figs. 19 and 20 *Beroë forskålii*

Figs. 22, 27–30 *Callianira bialata.*

Figs. 23 and 24 *Eucharis multicornis.*

Fig. 12. Very young cleavage-head. ×270.

Figs. 13 and 14. Two stages of the elongation of the cleavage furrow. ×270.

Fig. 15. Cleavage-head having fused with the ectoplasm in the micromere region. ×270.

Figs. 16–18. Last three stages of the first division. ×270.

Figs. 19 and 20. Two stages of the first division of the egg of *B. forskålii*, drawn respectively at 10.4 A.M. and 10.15 A.M. ×71.

Fig. 21. Early two-cell stage showing the ectoplasmic accumulation in the micromere region. ×60.

Fig. 22. Early two-cell stage of *Callianira*. Notice fenestrated cell-wall and thicker ectoplasm at the micromere region. ×390.

Fig. 23. Two-cell stage of *Eucharis*, in which the cleavage is about to begin. 3.55 P.M. ×140.

Fig. 24. The same; in one of the blastomeres the second cleavage has been taking place. ×140.

Fig. 25. Blastomere of the four-cell stage. ×71.

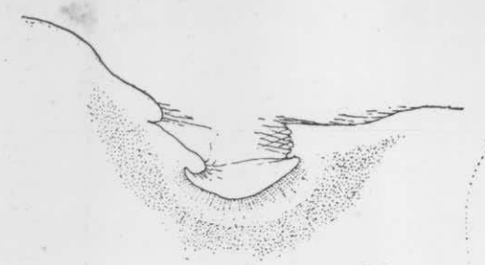
Fig. 26. Beginning of the third division. ×60.

Fig. 27. Beginning of the third division (*Callianira*); *cf.* Fig. 22. ×390.

Fig. 28. Blastomere of the four-cell stage, in which the third cleavage has more advanced (*Callianira*). ×200.

Fig. 29. Upper cell of the eight-cell stage, giving off a micromere towards the micromere pole (*Callianira*) ×390.

Fig. 30. Lower cell of the eight-cell stage giving off a micromere horizontally (*Callianira*). ×390.



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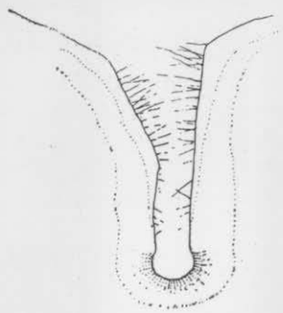

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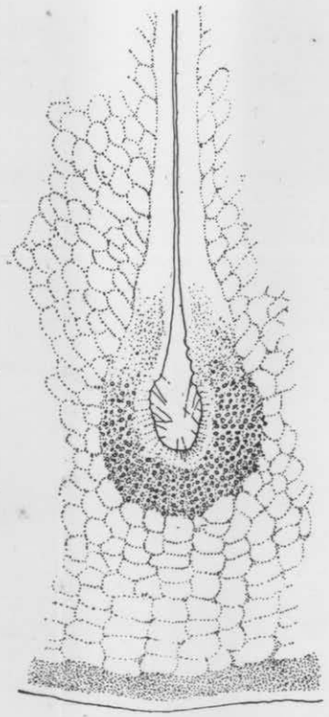

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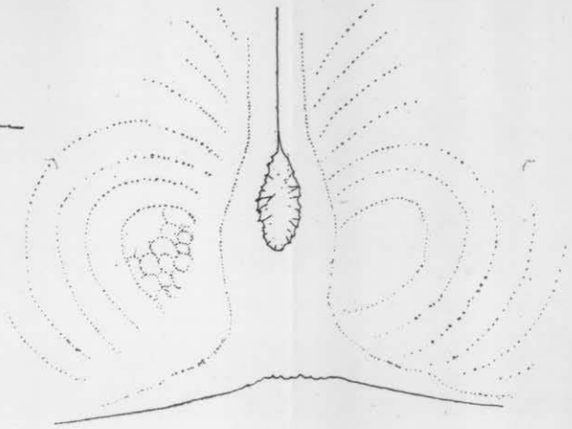

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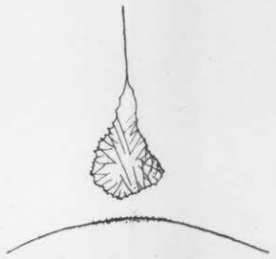

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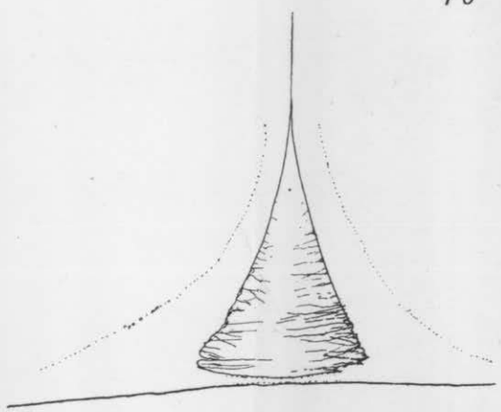

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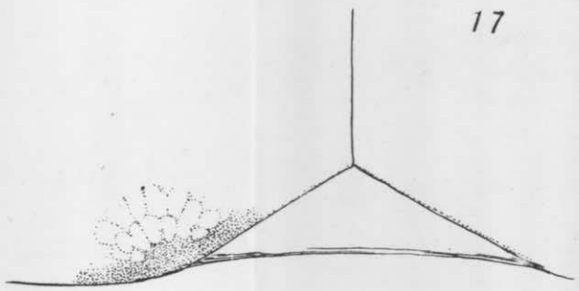

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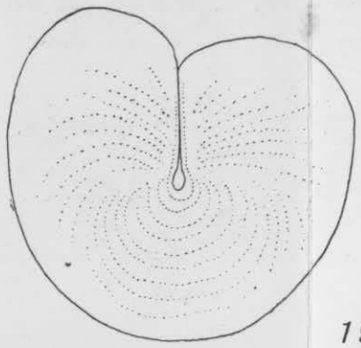

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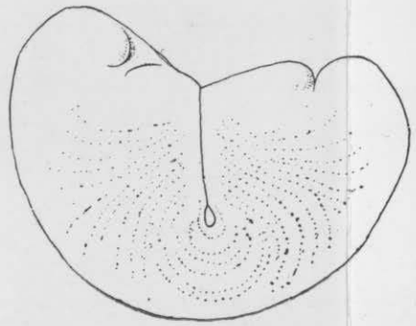

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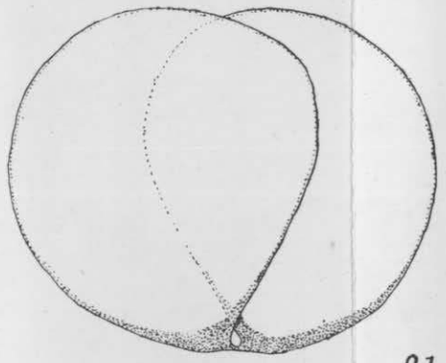

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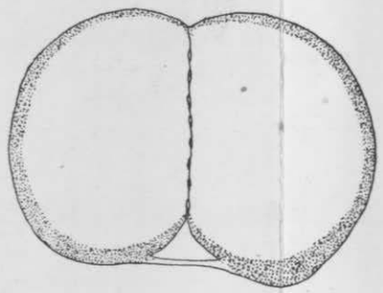

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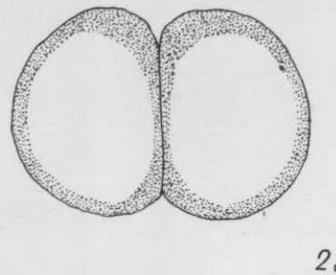

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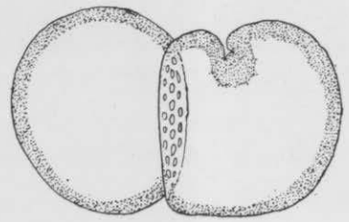

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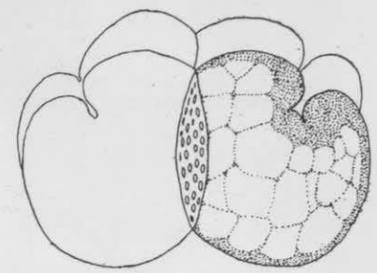

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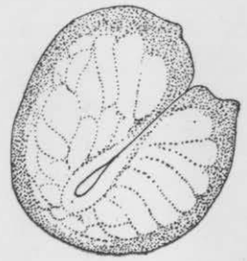

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PLATE III.

PLATE III.

Beroë ovata. ×60 (with the exception of Fig. 44 ×102).

Fig. 31. Egg cut horizontally below the "head." The enucleated cytoplasm cut off did not show any division activity.

Fig. 32. Egg cut below the "head" horizontally nearly two third of its diameter. 10.15 AM.

Fig. 33. The same. 10.38 AM.

Fig. 34. Egg cut in two horizontally at the stage when the "head" had come down nearly two-thirds of its course. 10.10 AM.

Fig. 35. Enucleated fragment of the same showing ectoplasmic thickening in the micromere region as in the normal case. 11;25 AM.

Fig. 36. The same showing a very thin ectoplasmic layer over the cut surface. 11.45 AM.

Fig. 37. Egg cut obliquely just above the "head." 11.22 AM.

Fig. 38. Enucleated fragment of the same divided by a slightly curved cleavage plane. 0.10 PM.

Fig. 39. Egg cut a little above the "head." 9.35 AM.

Fig. 40. Enucleated fragment of the same showing a thick ectoplasmic accumulation along the cut surface. The nucleated part was at the four-cell stage. 10,55 AM.

Fig. 41. Egg cut horizontally a little above the "head." Notice the thickening of the ectoplasm along the cut surface of the nucleated pieces. 2.40 Pm. The "head" at a, 2.50 PM., and at b, 3.25 PM.

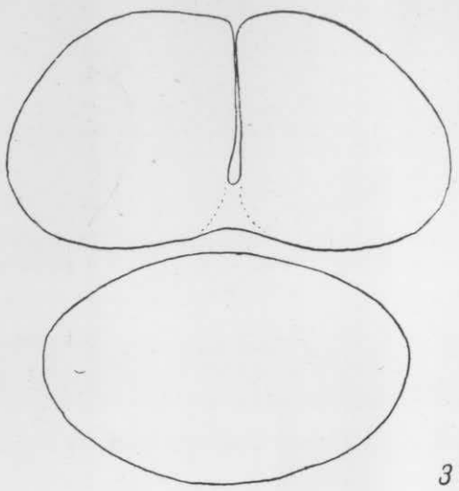
Fig. 42. Enucleated piece of the same. 4.40 PM. At 6 PM. the upper bridge became broader.

Fig. 43. Enucleated fragment obtained by a horizontal cut (outline in dotted line) 10.2 AM.; the same (in full line) 11.15 AM. In the beginning the "head" came down at the rate of 18 μ per minute, and stopped at the spot represented in the latter.

Fig. 44. Cleavage furrow and "head" of the same magnified ×102. 11.15 AM.

Fig. 45. Egg from which the right-hand nucleated portion has been cut off horizontally. Irregular mass attached to the nucleated fragment is the portion that flowed out. 10.45 AM.

Fig. 46. The same. 11.27 AM.



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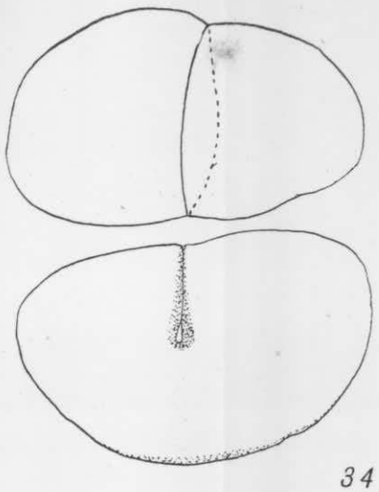

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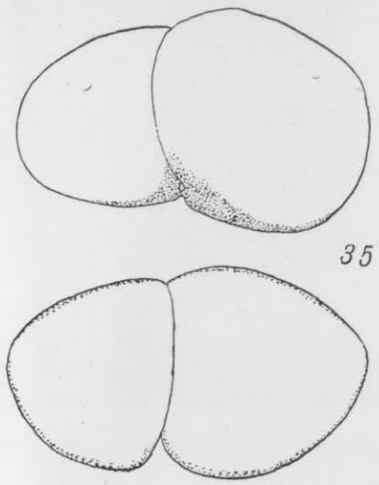

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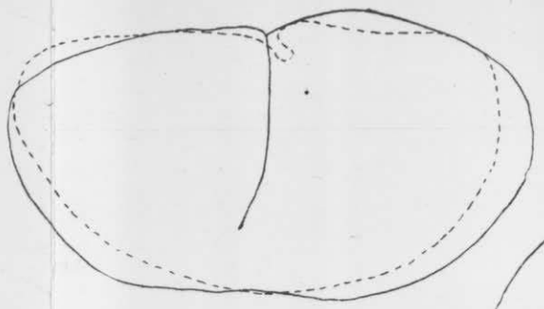

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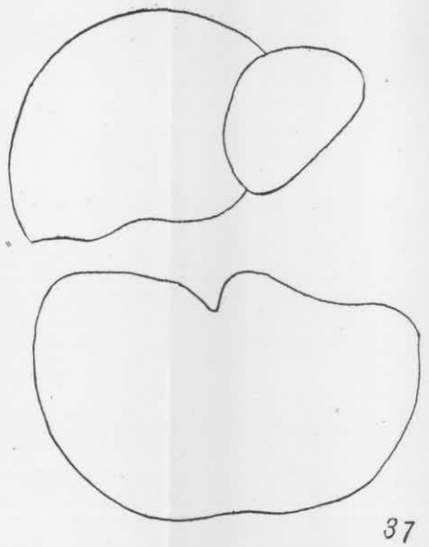

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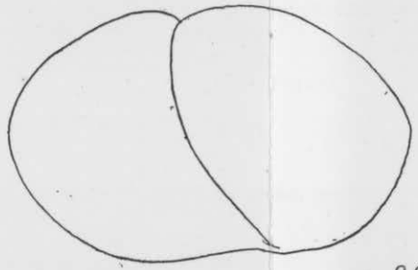

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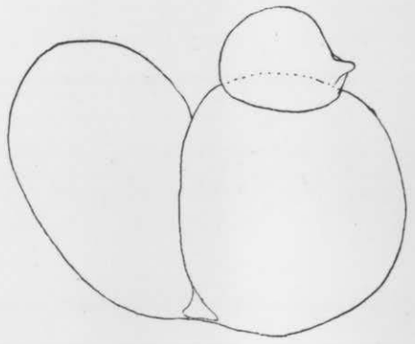

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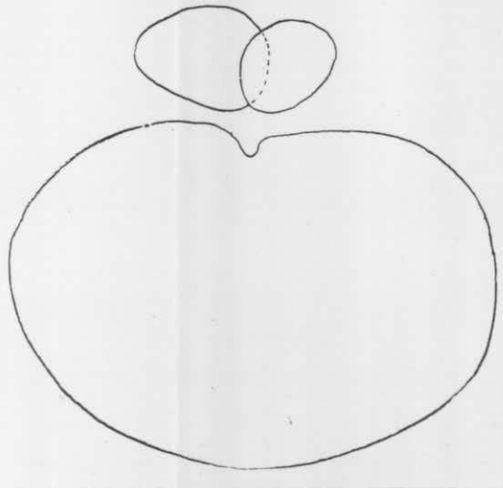

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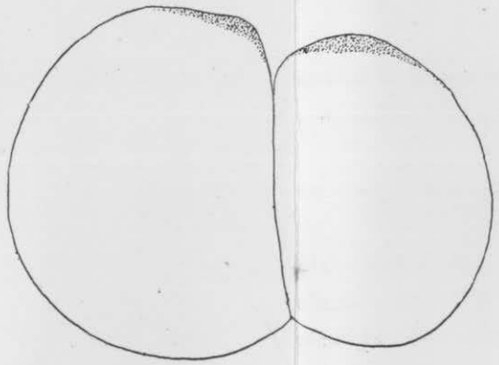

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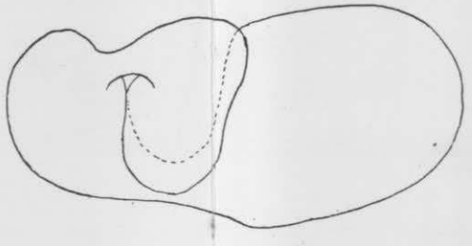

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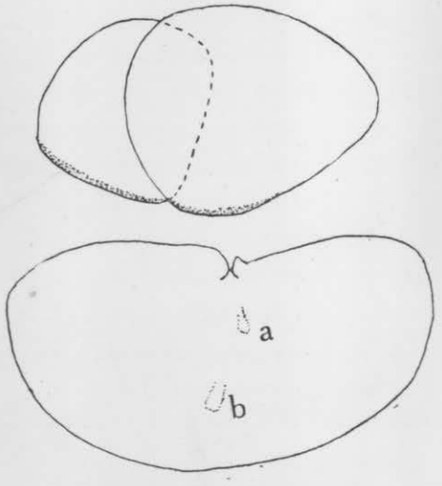

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PLATE IV.

PLATE IV.

Beroë ovata. ×60.

×× in some of the figures in this plate indicate the bottom of cleavage furrow at the time of operation.

Fig. 47. Egg partially cut in two horizontally, a little above the "head" 0.45 PM.

Fig. 48. The same. 2 PM.

Fig. 49. Egg an which young "head" has been split lengthwise by a vertical incision. 9.55 AM.

Fig. 50. The same 11.35 AM.

Fig. 51. Operation similar to Fig. 49. The "head" had got halfway when cut. 11.15 AM.

Fig. 52. The same. 11.35 AM. The cleavage furrow cut through the egg at 0.5 PM.

Fig. 53. Egg with two cuts, one longitudinal and the other horizontal, separating nucleated portion from right-hand prominence. 10.29 AM.

Fig. 54. The same. 11.24 AM.

Fig. 55. Egg from which a portion of cytoplasm has been cut off and the "head" split lengthwise. 11.15 AM.

Fig. 56. The same. 11.55 AM.

Fig. 57. Egg operated on similarly to Fig. 55.

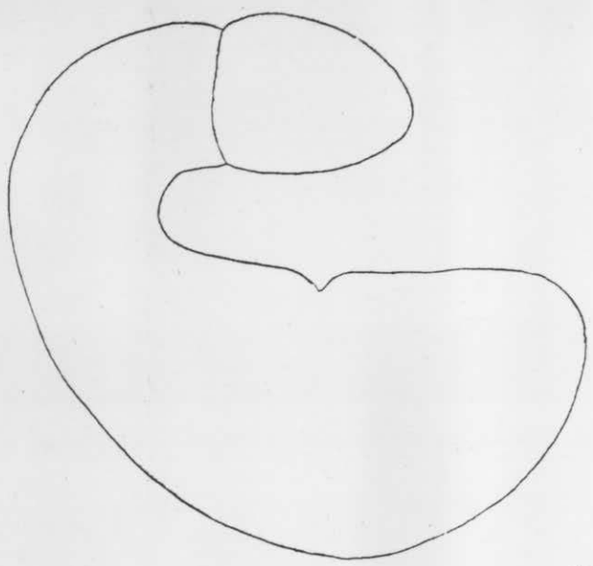
Fig. 58. Cleavage furrow has been formed from the bottom of the cut towards the left.

Fig. 59. Cleavage furrow has cut through the egg. Nucleated portions of both the blastomeres have divided.

Fig. 60. Egg in which the "head" has been split lengthwise and an incision made at the micromere pole. 9.42 AM.

Fig. 61. The same. 10.40 AM.

Fig. 62. The same. Cleavage furrow has cut through the egg, forming an enucleated mass on the right.



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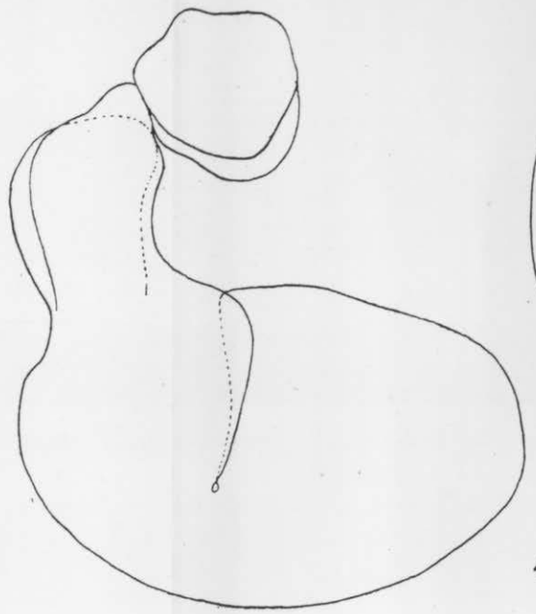

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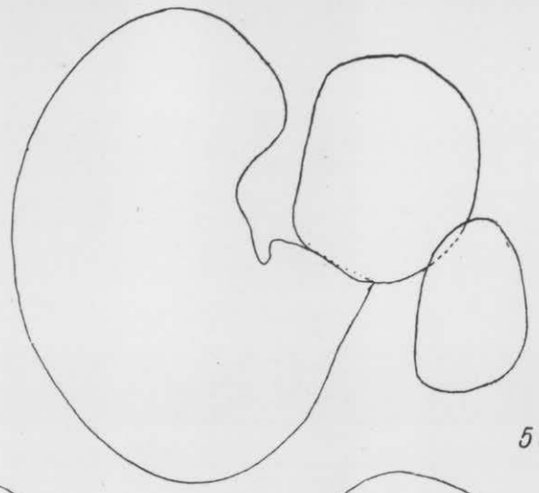

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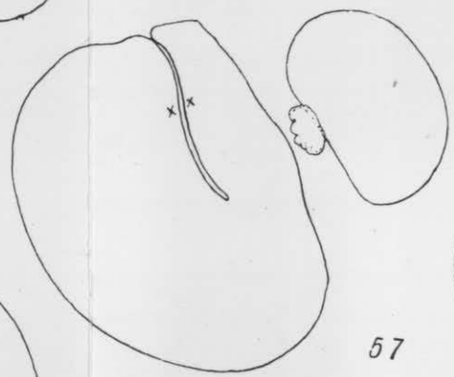

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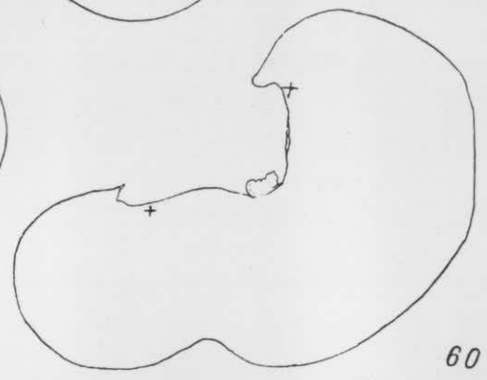

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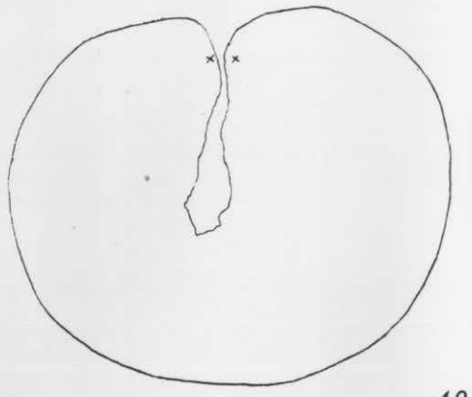

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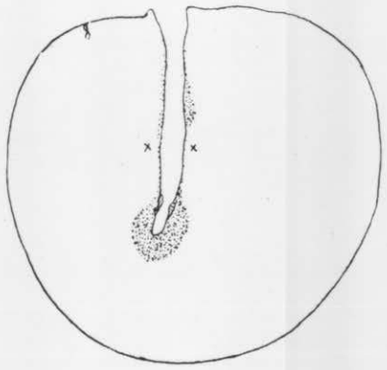

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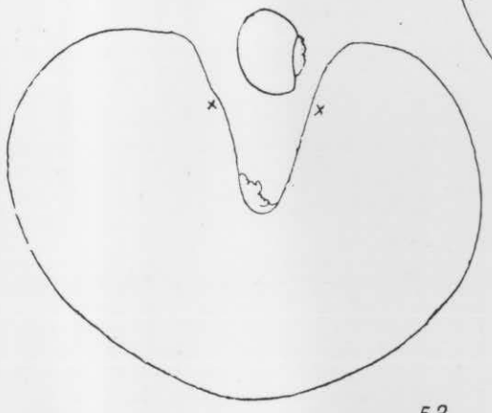

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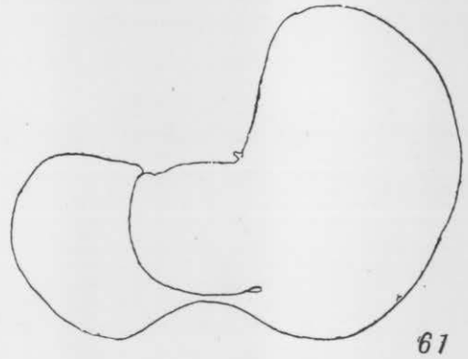

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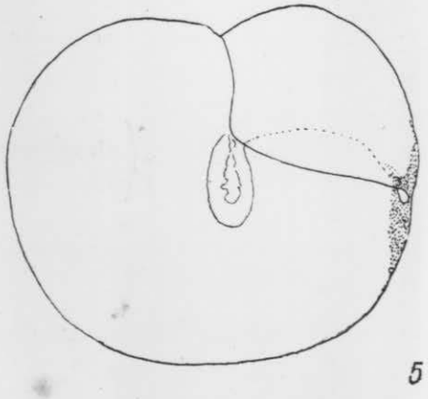

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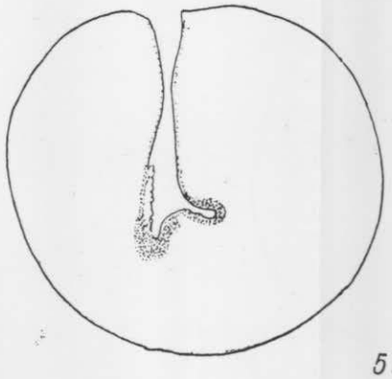

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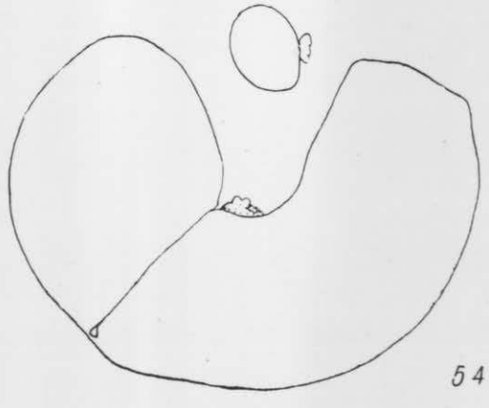

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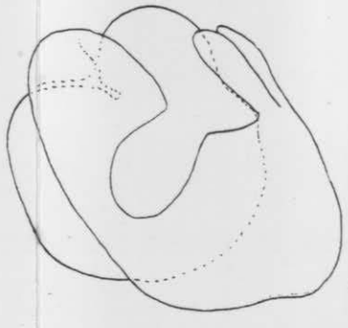

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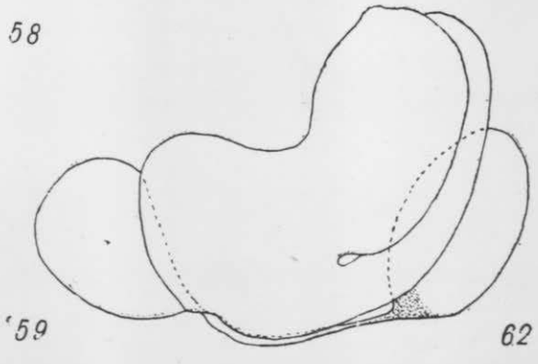

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PLATE V.

PLATE V.

Beroë ovata. ×60.

Fig. 63. Egg with three cuts, namely, the nucleated part was cut off from the left half, which is found attached to the right portion, the "head" was split lengthswise (it was at ×× when operated upon) and a portion of cytoplasm was cut off obliquely from the micromere region. 11.10 AM.

Fig. 64. The same. 0.35 PM.

Fig. 65. Egg with an incision at the micromere region. 9 AM.

Fig. 66. The same. Ectoplasmic thickening is very conspicuous over a prominence to the left of the incision. 11.45 AM.

Fig. 67. Egg with an incision on the right side. 0.5 PM.

Fig. 68. The same. 0.50 PM.

Fig. 69. Egg with an incision at the micromere region. 9.45 AM.

Fig. 70. The same. Cleavage furrow has become continuous with the cut. 10.18 AM.

Fig. 71. Egg with a vertical incision at the micromere region. 2.15 PM.

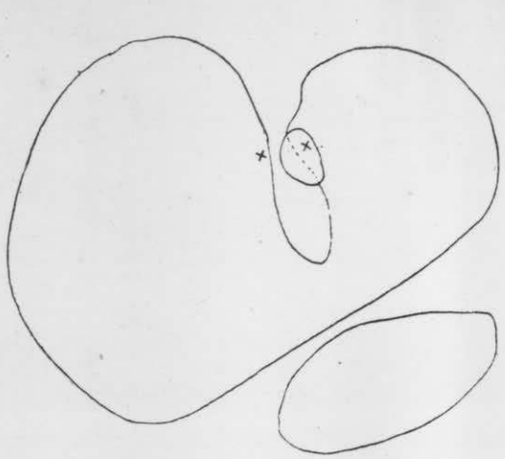
Fig. 72. The same. "Head" has become irregular and a flow figure is seen.

Fig. 73. The same. Cleavage furrow has fused and two new furrows have been formed. 3.11 PM. (at 2.55 PM. two slight indentations were formed).

Fig. 74. The same. 4.18 PM.

Fig. 75. Egg with two incisions on both sides. 10.53 AM.

Fig. 76. The same. 11.28 AM.



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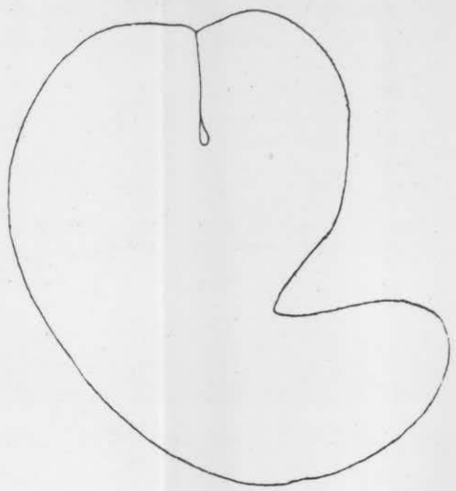

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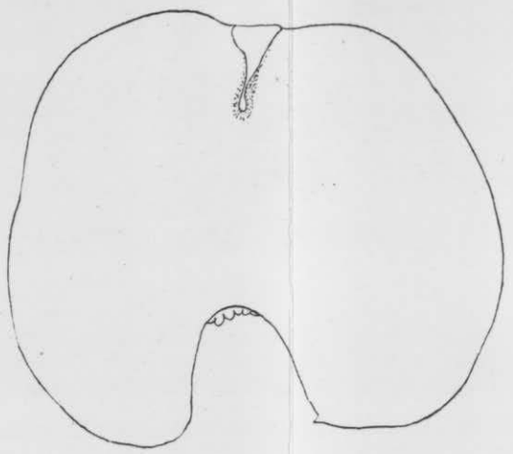

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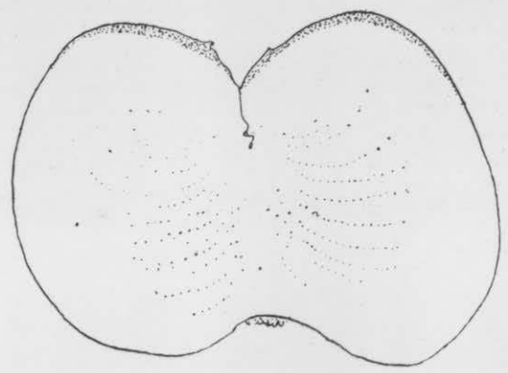

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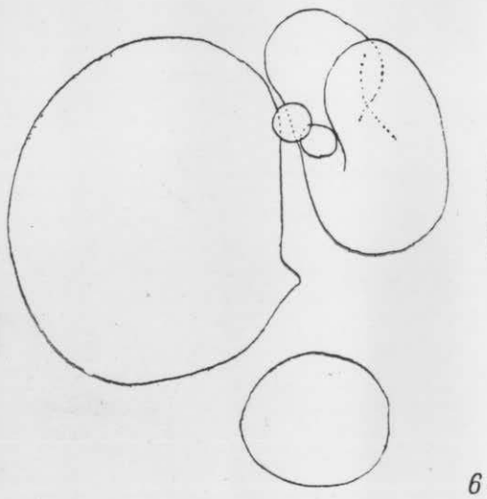

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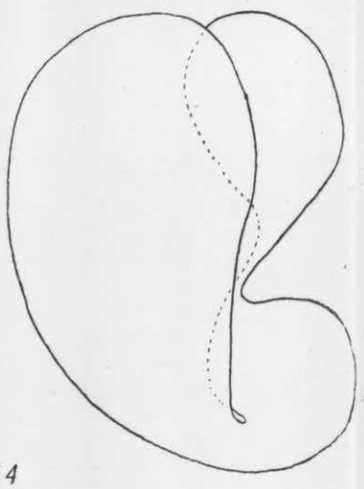

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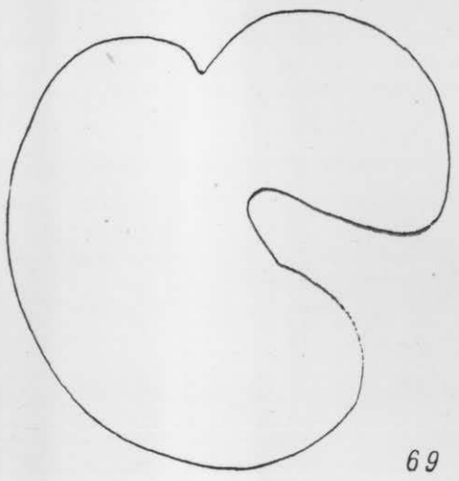

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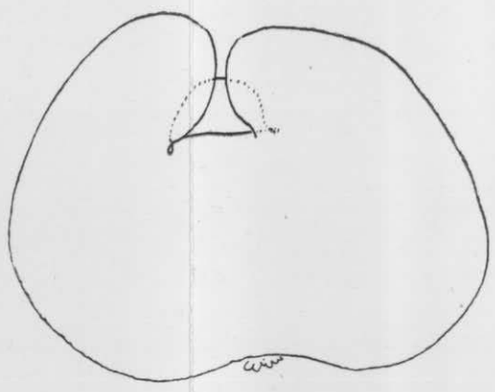

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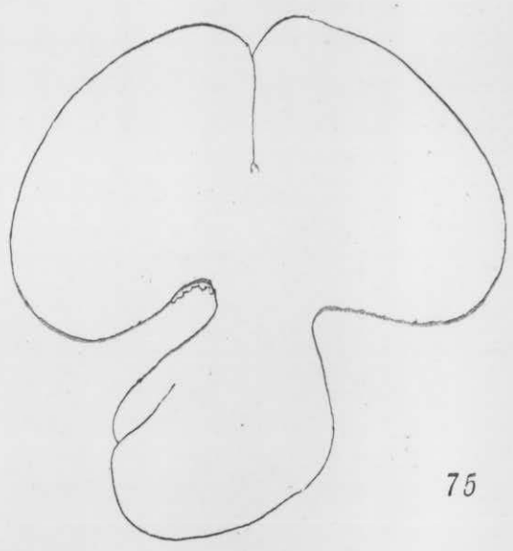

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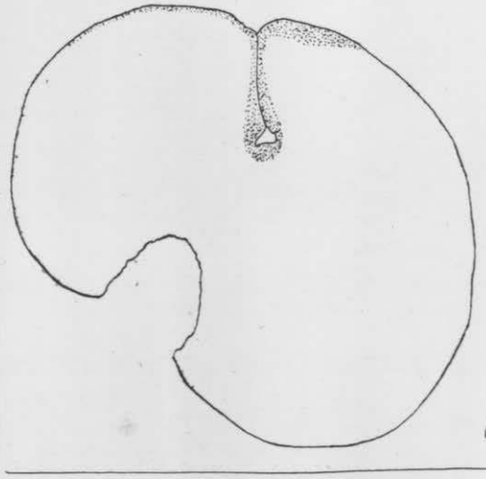

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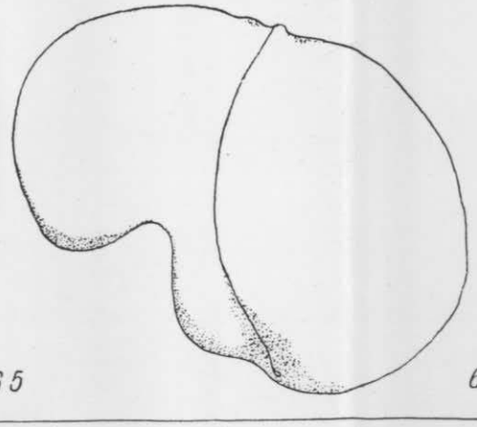

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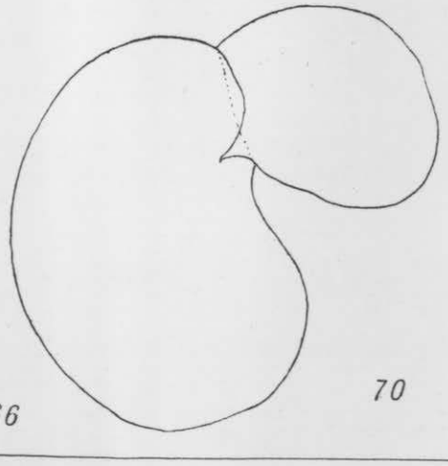

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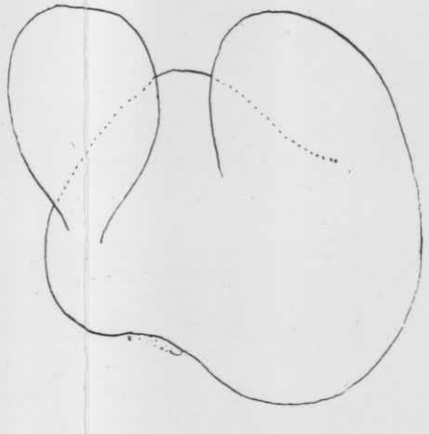

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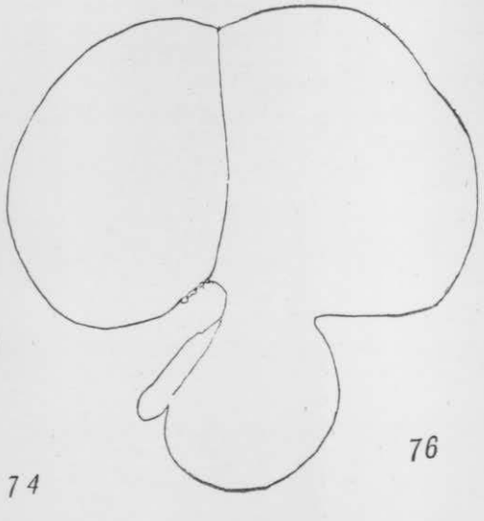

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