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The Fate of Polar Bodies in the Egg of *Urechis unicinctus*

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It was shown many years since by I. Ikeda ('02) that the polar bodies in the egg of *Urechis unicinctus* remained intact in its blastocoele of the blastula and the gastrula, or even in the young trochophore. This fact was later confirmed by H. Sato and M. Tanaka ('22) who reported that the slipping-in of the polar bodies into the blastocoele occurred at the 36-cell stage. As this behavior seems unusual with regard to the polar bodies in general, it is aimed in this paper to re-examine the fact, and to find out further, if possible, its probable causes. Here, we are glad to acknowledge our indebtness to Prof. M. Ohkawa for his help for securing the materials.

Observations

Formation of polar bodies. As is already described by many workers, the egg of *Urechis*, when shed, is irregular in shape, having indentations varying in number and size. A rounding off of the egg, however, results from insemination, and the membrane elevation then follows, at which, connections between the membrane and the egg surface are maintained by a number of fine protoplasmic strands that are radiating from the latter. These connections continue to exist even after the development proceeds, and can be found at least till the end of the blastula stage.

When polar bodies are formed, they are likewise connected with the overlying membrane by a few plasmic strands, which sometimes become thicker, probably due to their fusion*. Generally, two polar bodies are formed, the first and the second, and the first one rarely divides again, but remains somewhat larger than the second. Both of them are placed, when formed, in close contact with each other and are connected with the egg surface by a short stalk: hence their detaching or dislocation rarely occurs at least during cleavage stages.

Behavior of plasmic strands and polar bodies during cleavages.

* At the time of the first polar body formation, a slight indentation appears in the overlying membrane in 59 out of 94 (62.7%) cases observed. Here, a pulling action of plasmic strands caused by a form change of the egg body during maturation division, seems to be operating.

Several minutes before the onset of the first segmentation, there comes a stage where a few plasmic strands around the polar bodies condense at their roots, and thus tend to incline themselves slightly outwards

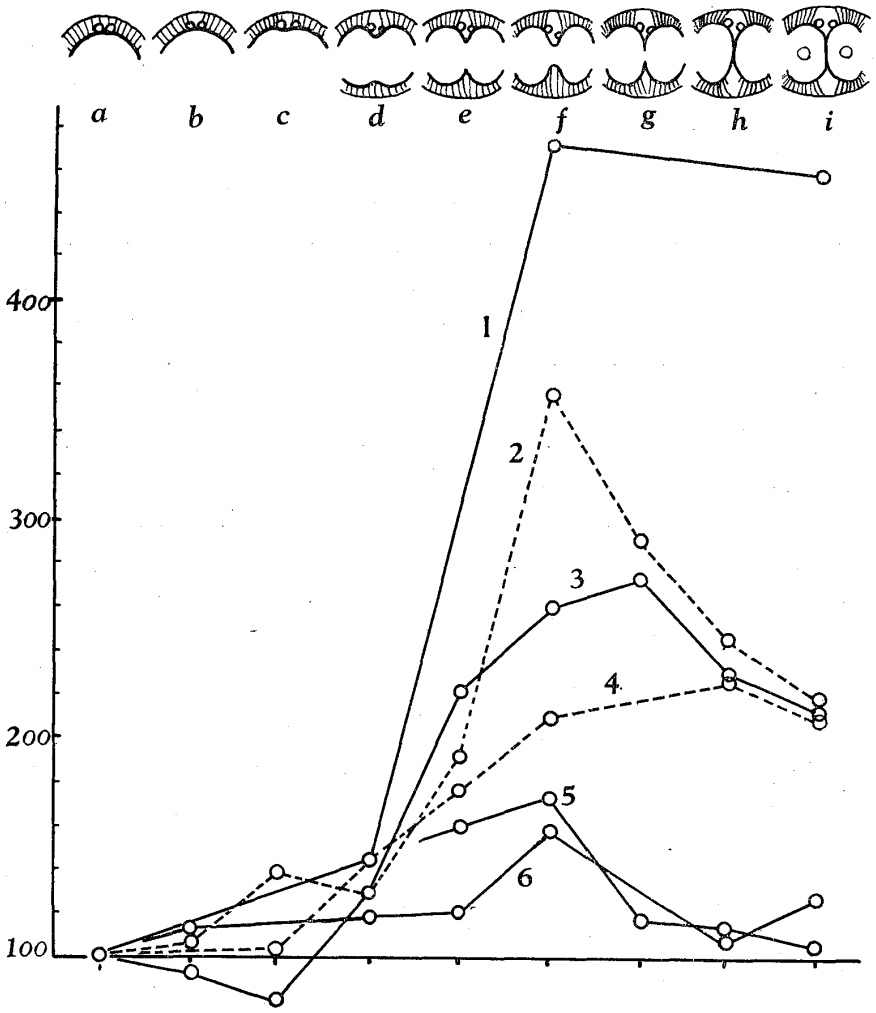


Fig. 1. Graphical representation of the results shown in table 1. Figures (a-i) at the top represent each successive stage where measurements were made. Stage a ; formation of the polar bodies completed, the plasmic strands being stretched between the membrane and the egg surface. Stage b ; plasmic strands around the polar bodies somewhat inclined outwards. Stage c ; first sign of the furrow at the animal pole. Stage d ; the same sign at the vegetal pole. Stages e-f ; the furrow gradually deepens, the plasmic strands at the animal side beginning to incline. Stage g ; condensation and inclination of plasmic strands at the outer angles of the dividing egg become conspicuous. Stage h ; segmentation nearly completed. Stage i ; the resultant blastomeres come together and flatten each other.

(stage b), But, when the furrow of the first segmentation begins to appear near the polar bodies (stage c), these plasmic strands around the polar bodies gradually come to disperse, and the more the furrow deepens, the wider becomes the area with plasmic strands thus changing

(stages d-f). While this is on, the plasmic strands at both outer angles of the dividing egg gradually become denser, this time far more markedly than those around the polar bodies, and begin equally to lie obliquely, now bending inwards instead of outwards (stage g).

These changes of plasmic strands in their density as well as direction during segmentation may, perhaps, be due to some regional shrinkage or expansion occurring on the surface of the dividing egg, the problem clearly verified by the efforts of K. Dan and others ('37–'47) in the eggs of sea-urchin, a marine gasteropod *Ilyanassa*, and of a medusa *Spirocodon*. However, the existence of such activities on the surface of the dividing *Urechis* egg seems to be more clearly manifested by the behavior of polor bodies during cleavage.

When the furrow appears at the animal pole, it passes either by the outer side of polar bodies, or between them. In both cases, the polar bodies which have been closely placed at first are pulled apart as the furrow deepens. In the former case, however, both polar bodies remain attached to either one of the resultant blastomeres, while in the latter case, the degree of pulling apart is far greater, and thus each one of the polar bodies comes to attach itself to different one of the resultant blastomeres. Since no dislocation of the polar bodies occurs because of their connection with the egg surface by a stalk, the changes of distance between them can reasonably be understood to be of the same nature with those of the adhered kaolin particles used by

Table 1

	a	b	c	d	e	f	g	h	i
1	100.0			144.0		468.0			456.0
2	100.0	107.1	138.0	128.8	190.4	375.1	290.7	245.2	219.0
3	100.0	93.3	80.0	130.0	220.6	260.0	273.3	230.0	213.3
4	100.0		103.0	145.5	175.7	209.0		227.0	209.0
5	100.0				160.0	173.3	116.6	114.0	106.6
6	100.0	113.3		116.6	120.0	156.6		106.6	126.6

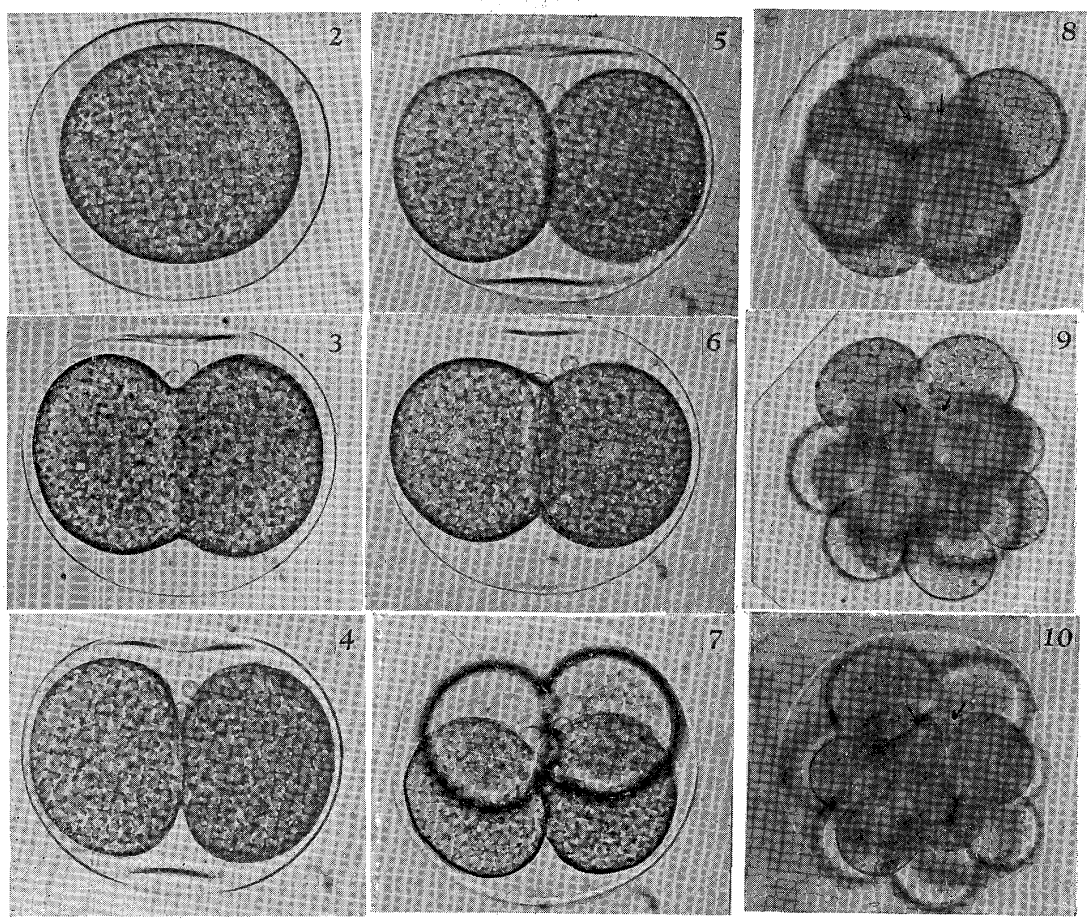
Table 1. Distance changes of polar bodies during the first segmentation expressed in terms of percentages, the initial readings being taken as 100.

1 and 2 are the cases in which the polar bodies were far separated, and each one of them came to belong to each of the blastomeres. In 3–6, both the polar bodies remained attached to either of the blastomeres. (a-i) are the successive stages during segmentation, for the explanations of which see under fig. 1.

Dan and others. Therefore, these distance changes were measured by means of camera lucida or photographic records, in which measurements, only those cases, where both polar bodies moved along almost on the same optical plane, were adopted for the sake of accuracy. Table 1 and figure 1 are the results of the measurements thus made. In these,

the results of measurements are expressed in terms of percentage to the initial readings.

As is plain from the above table and figure, the general trend of curves closely simulate those obtained by Dan and others in the egg of a sea-urchin, particularly those of the denuded egg*. Differences found on the side of the *Urechis* egg are, in the first place, the less dis-



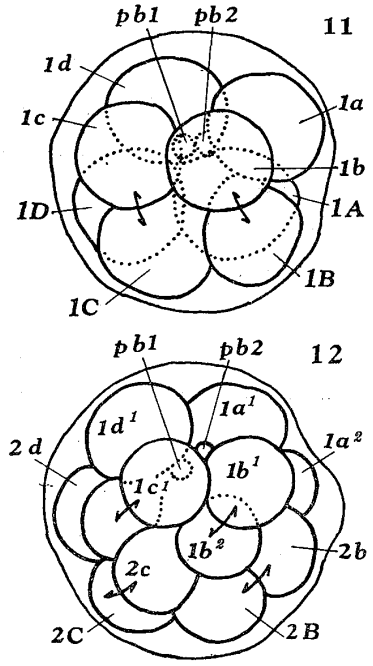
Figs. 2–7. Successive stages of segmentation of an *Urechis* egg. The measurements of distance changes of these polar bodies are presented as case 6 in table 1 and figure 1. Figs. 2, 3, 4 and 5 correspond stages b, d, f and i respectively. Fig. 6; a stage preparing for the following cleavage. Fig. 7; 4-cell stage of the same egg.

Fig. 8–10. Successive stages of later segmentation of another egg. The arrows indicate the position of poiar bodies. Fig. 8; 8-cell stage in which the two polar bodies, the upper and the lower, are interlocked between the upper blastomeres. Fig. 9; 16-cell stage in which the lower polar body is enclosed within the blastocoele. Fig. 10; View of the more animal surface of the same egg with fig. 9, showing the position of both the polar bodies.

* In this point, the presence of the plasmic strands seems to have some meaning. Since the plasmic strands are radiating out from the surface of the endoplasm through the outer membrane, which is comparable with the hyaline layer of the sea-urchin egg, a slipping of this membrane over the endoplasm does not so easily occur; hence, the curves simulate those of the denuded cases rather than those of the intact ones.

tinctiveness of the initial phases of shrinkage, which is of a constant occurrence in the sea-urchin egg; secondly, the much higher values of expansiveness shown both in maximal and final periods, though the former apparently comes earlier than that of the sea-urchin (the mean maximal and final values being 270.7% and 221.8% respectively in the *Urechis* egg as compared with 150.0% in that of the sea-urchin); and lastly, the gradual descent of the curves of the *Urechis* egg at the end of segmentation, instead of being horizontal.

As is indicated by the final values shown above, when the first segmentation is completed and the resultant blastomeres flatten each other, the two polar bodies remain far more separate than before: in most cases, one of them which has been carried deeper being left lying near the bottom of the furrow between the blastomeres, while the other lying nearer to its entrance. Quite rarely, there are cases in which both polar bodies travel together and both come to remain either near the bottom or the entrance of the furrow. Such cases result in either both the polar bodies being enclosed within the blastocoele, or excluded outside the embryo. For the sake of brevity, such cases were omitted from our observations.



Figs. 11–12. Camera lucida drawings of the same egg shown in fig. 8 and 9. Fig. 11; *pb1* is the lower polar body, while *pb2* the upper polar body. Fig. 12; One of the polar bodies (*pb1*) is already enclosed within the blastocoele.

That the similar expansive activities will be repeated in the furrow region at each of the following segmentations, may easily be expected. And so it was verified by Dan and others that this was actually the case at least at the second cleavage of the sea-urchin egg, and moreover, the accompanying photograph showing the early phase of the second cleavage also indicates the same thing (fig. 6). However, in spite of this sort of repetitions, the positions of polar bodies are not so markedly changed throughout the successive stages, their upper or lower positions being constantly maintained as a consequence. Thus, at the stage of four cells, when the blastomeres touch each other only laterally and the space between them opens at the poles, the opening at the animal side seems as if it were plugged by the two polar bodies, the upper and lower ones. The situation is also similar even at the 8-cell stage, where towards the animal pole are budded off the first quartet of micromeres, to which both the polar

bodies now come to belong.

At the time when 16 cells are formed and the first quartet of micromeres divides again into upper and lower halves ($1q^1$ and $1q^2$) the segmentation furrow crosses a little underside the lower polar body; therefore, both the polar bodies now come to belong to the upper half of the first quartet of micromeres ($1q^1$). But, at this time, these upper blastomeres come together, and close over the pole, which makes the lower or the deeply lying polar body enclosed entirely within the blastocoele, while the upper one remains outside the embryo.

Discussion

It is plain from the foregoing observations on the behaviors of plasmic strands as well as of polar bodies, that there exists on the surface of dividing *Urechis* eggs the regional expanding or shrinking activities which are comparable to those of sea-urchin eggs. The changes in direction as well as in density of a few plasmic strands around the polar bodies may, perhaps, be a manifestation of the initial shrinkage that commonly occurs at the animal pole of the sea-urchin egg before its segmentation. Similarly, the dispersion of the plasmic strands at the furrow region, and the following condensation and change in direction of them at the outer angles of the egg may both be understood as representing the expansion and shrinkage that occur at the corresponding regions of the sea-urchin egg during segmentation. However, more clear-cut examples, though limited within the furrow region, seem to be given by the behavior of polar bodies, that is, by their changes of mutual positions during segmentation.

Thus, the curves obtained by the measurements of these changes, when compared with those of sea-urchin eggs, show several interesting facts. In general, trends of both curves are quite similar with a few differences. Of these differences, the one which appears to have some bearing on the fate of polar bodies, is that both the maximal and final values are maintained much higher in the case of the *Urechis* egg.

The higher expansive capacity thus shown at the furrow region during the period of segmentation may, perhaps, be related with the corresponding lower capacity at some other regions in the egg. But, the reason why such a higher value is maintained even at the end of segmentation when the resultant blastomeres come together and flatten each other, seems not to be so simple to explain. It may be related either with lower capacity of other regions in the egg, or with the less extent of flattening done by both blastomeres, or it may be related to both. We have no adequate data at hand for comparing the degrees of flattening in the eggs of the two species, but, the flattening in the egg of *Urechis* seems to be in far less extent as can be seen in fig. 5.

If this be the case, the polar body that has been carried down deeper gets that condition for maintaining its deeper position, and therefore, a condition for its being enclosed within the blastocoele.

According to Ikeda ('02), the slipping-in of the polar body occurs at 74-cell stage, and is due to some peculiar movement of some apical micromeres, which the authors could not confirm. As described in the foregoing section, the final enclosure of the polar body occurs at the stage of 16 cells, and is simply done by the closing-over of the pole when the apical quartet of micromeres come together. It is interesting to notice here that the fact of the enclosure of the polar body within the blastocoele without detaching or dislocation from its original position on the egg surface, indicates that the original egg surface does not necessary form the outer surface of the embryo of later stages, but, at least some part of it is involved in the formation of the inner surface of the wall of the blastocoele.

Summary

1. It is aimed in this paper to re-examine the fact about the polar bodies in the egg of *Urechis* that they are said to be enclosed within the blastocoele during early stages of development, and to find out futher, if possible, its probable causes.

2. The enclosure of the polar bodies within the blastocoele occurs at the stage of 16 cells. In most cases, only one polar body is enclosed, but, in quite rare cases, two are enclosed. Polar body or bodies thus enclosed can remain as such even until the stage of young trochophore. Since their detaching or dislocation occurs because of their connection with the egg surface by a stalk, the enclosure of polar bodies within the blastocoele indicates that the original egg surface does not necessary form the outer surface of the embryo of later stages, but, some part of it is involved in the formation of the inner surface of the wall of the blastocoele.

3. Occurrences of the regional shrinkage or the expansion on the surface of the dividing *Urechis* egg are conceivable from the observations on the behaviors of plasmic strands as well as polar bodies. Curves obtained by the measurements of distance changes between two polar bodies show that the furrow region of the dividing *Urechis* egg has far higher expansive capacity when compared with that of the sea-urchin.

4. The higher capacity of expansion in the furrow region and the less extent of flattening on the side of the blastomeres of the *Urechis* egg are suggested as probable causes for the enclosure of polar bodies within the blastocoele.

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