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The effect of removing the polar lobe in centrifuged eggs of *Dentalium*

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Classical evidence for the existence of morphogenetic substances was provided by experiments with spiralian eggs possessing a polar lobe: *Ilyanassa* (Crampton, 1896; Clement, 1952, 1956, 1962); *Dentalium* (Wilson, 1904); *Chaetopterus* (Tyler, 1930); *Sabellaria* (Hatt, 1932; Novikoff, 1938); and *Mytilus* (Rattenbury & Berg, 1954). Eggs from which the polar lobe had been removed developed into embryos with specific abnormalities. In *Dentalium*, after removal of the polar lobe at the trefoil stage, a trochophore larva is formed without post-trochal region and apical tuft. Removal of the polar lobe at second cleavage causes a larva without post-trochal region, but with an apical tuft. Wilson concluded that specific cytoplasmic materials essential to the formation of the apical tuft are contained in the first but no longer in the second polar lobe.

Centrifuging the uncleaved egg just before first cleavage will disturb the normal distribution of substances. The morphogenetic substance for the apical tuft may be also displaced, so that at the formation of the first polar lobe this substance is not only present in the lobe, but also in the blastomeres. The object of the present investigation was to study the effect of removing the first polar lobe in eggs centrifuged immediately before the beginning of first cleavage.

MATERIAL AND METHODS

All experiments were carried out at the Caribbean Marine Biological Institute at Curaçao (Neth. Ant.) with the local species *Dentalium antillarum*, which is abundant in inner Piscadera Bay. The animals could be obtained easily by collecting the superficial bottom material at a depth of 3–4 m. The material was sieved on the spot and the animals were picked out in the laboratory. They could be kept alive for weeks in small glass aquaria with a bottom of sand and in running sea-water.

Mature animals are $\frac{3}{4}$–1 in. in length with a translucent shell, through which the gonads can be observed under a dissecting microscope. In the ovary the

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brownish grey oocytes are packed as coins. The testes are milky-white tubules. As a result, it is possible to determine the sex of the living animals with a rather high degree of certainty.

Oocytes and sperm may be obtained by breaking the shell with two pairs of forceps and opening the ovary or testes with a sharp needle. Another method is to push up the animal in its shell with a blunt needle. It then releases oocytes or sperm through the small opening at the rear side. Both methods have their disadvantages as the oocytes are easily destroyed.

Ripe oocytes are biscuit-shaped with a diameter of 175–200 μ. They are olive-coloured with a large bright centre nearly devoid of pigment. In histological sections this appears to be the germinal vesicle. The oocytes are surrounded by a membrane with a wrinkled appearance (Text-fig. 1, no. 1). About 20 min after release from the ovary, the germinal vesicle suddenly breaks down in some of the oocytes, which become spherical. The original membrane ruptures and the remains become attached to a new membrane, which is elevated from the egg surface. In this manner a clear capsule is formed around the egg, which then becomes 160 μ in diameter (Text-fig. 1, no. 2).

The eggs appeared to be sensitive to polyspermy, which causes abnormal cleavage. In order to avoid polyspermy as much as possible the following method was used. The sperm, which just after opening of the testes are immobile and start moving only after about 20 minutes, were collected in a dish with sea-water. With a braking pipette a very small quantity of motile sperm was sucked up and disseminated over the eggs. In this way nearly all eggs were fertilized at the same moment and polyspermy was practically avoided.

Eggs of *Dentalium antillarum* appeared to be rather sensitive to centrifugation. When exposed to a centrifugal force of 400 ***g*** for 10 min, the eggs did not cleave or cleaved abnormally. In the present experiments a centrifugal force of 300 ***g*** for 10 min was applied, which causes a good stratification of the egg substance. This treatment is certainly not harmless to the eggs, but as for our purpose the effect of centrifugation on the egg substances was to be as great as possible, the consequences had to be accepted. Furthermore, all eggs showing an abnormal first or second cleavage were eliminated.

By using a sharp tungsten needle, the polar lobe can be removed easily at first or second cleavage.

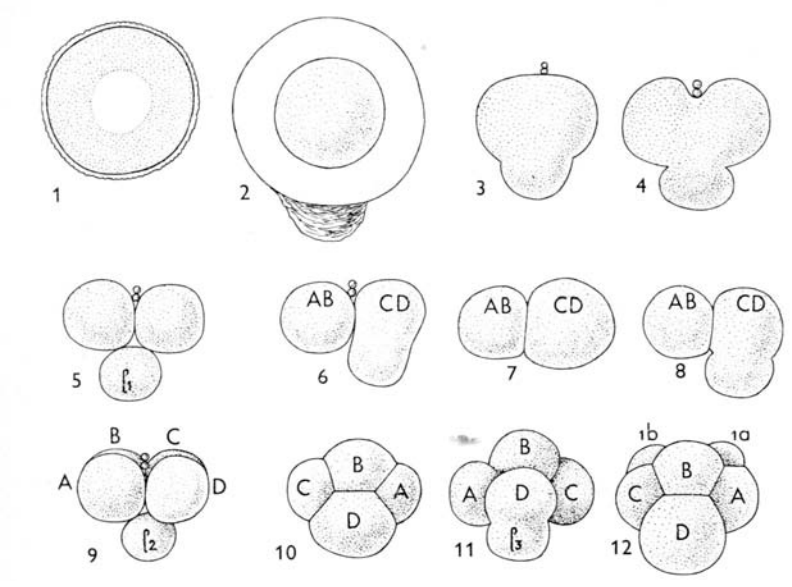
The eggs were cultured in filtered and boiled sea-water in dishes with a cover-glass in an air-conditioned room at about 25 °C.

Eggs fixed in Bouin's fluid were sectioned at 5 μ and stained with Heidenhain's haematoxylin or Herlant's tetrachrome staining. Embryos fixed with Kleinenberg's fixative were stained with Delafield's haematoxylin. They were mounted between slide and coverglass, supported by a piece of paper.

RESULTS

(*a*) *Normal development*

During the formation of the first and second polar bodies (15 and 35 min after fertilization) no polar lobe forms in *Dentalium*. About 55 min after fertilization a polar lobe appears at the vegetative pole of the egg, marking the beginning of first cleavage (Text-fig. 1, no. 3). While the lobe rounds off, a cleavage furrow is formed at the animal side of the egg and a little later also at the vegetative side.



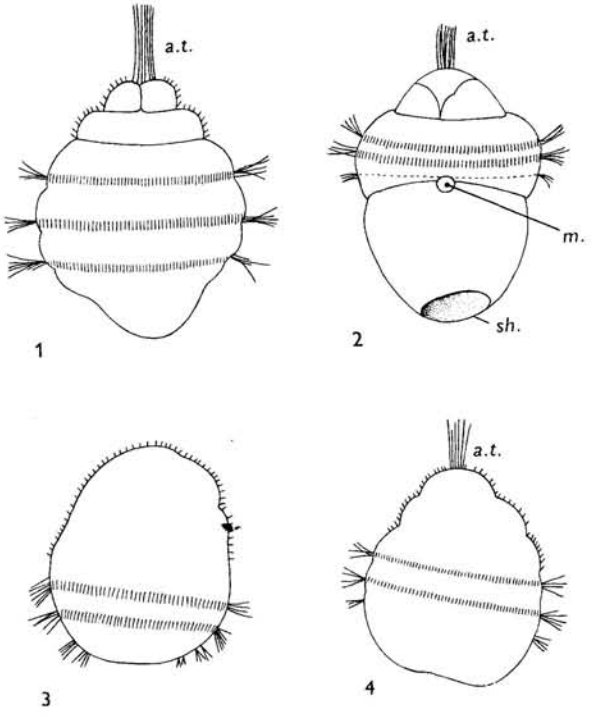
Text-fig. 1. 1, Ripe oocyte after release; 2, egg, after throwing off the original membrane and the formation of the capsule; 3 and 4, formation of the 1st polar lobe; 5, trefoil stage with polar lobe (l.1); 6, fusion of the polar lobe with the CD cell; 7, 2-cell stage; 8 and 9, formation of the 2nd polar lobe (l.2); 10, 4-cell stage seen from the vegetative pole; 11, formation of the 3rd polar lobe (l.3); 12, 8-cell stage seen from the vegetative pole.

When the egg reaches the trefoil stage, no connexion between the lobe and the blastomeres can be observed (Text-fig. 1, no. 5). After 4–5 min. a connexion between the polar lobe and one of the blastomeres appears, and subsequently the lobe fuses with the CD blastomere (Text-fig. 1, nos. 6 and 7).

About 30 min after the beginning of first cleavage, a second polar lobe is formed, which is well rounded with respect to the four blastomeres (Text-fig. 1, nos. 8 and 9).

Finally, at third cleavage, a polar lobe is present, which, however, does not separate completely from the D-blastomere. A first quartette of micromeres is split off at the animal side of the egg (Text-fig. 1, nos. 11 and 12). In the subsequent cleavages, which follow each other rapidly, a polar lobe is not formed.

Gastrulation starts about 4 h after first cleavage; 3 h later trochophores with a well-developed apical tuft are swimming in the dish (Text-fig. 2, no. 1). These larvae are very delicate and difficult to rear. As for our purpose a study of the trochophore stage was most important, the embryos were studied immediately after they had left the capsules.



Text-fig. 2. Normal trochopore of 7 h; 2, Normal trochophore of 24 h.; 3, larva of 7 h., after removal of the first polar lobe; 4, larva of 7 h., after removal of the second polar lobe (*a.t.*, apical tuft; *m.*, mouth; *sh.*, shell gland).

The surviving larvae start metamorphosing at the end of the first day (Text-fig. 2, no. 2). They can be found at the bottom of the dish, moving only sluggishly. After 48 h metamorphosis is complete and a young *Dentalium* is present, complete with foot and shell.

(*b*) *The structure of the egg*

In the ripe oocyte a conspicuously large germinal vesicle is present, surrounded by an endoplasm which is nearly free of yolk granules (Plate 1, fig. A).

These granules are localized in a wide zone beneath the surface. Only a few small granules are situated at the two flat sides of the biscuit-shaped oocyte. The surface of the oocyte shows a fine striation, most probably due to the microvilli, which are also present in the eggs of some other molluscs (*Barnea*, Pasteels & de Harven, 1962; *Mytilus*, Humphreys, 1962; *Spisula*, Rebhun, 1962).

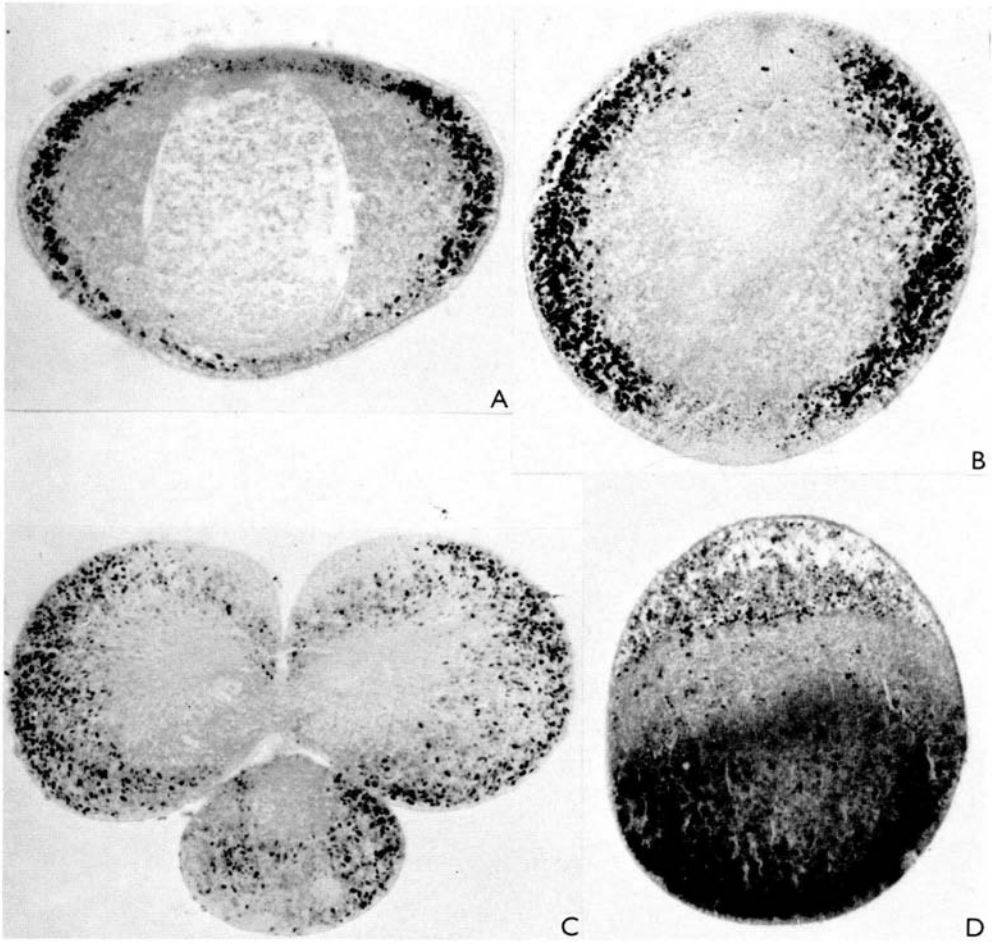


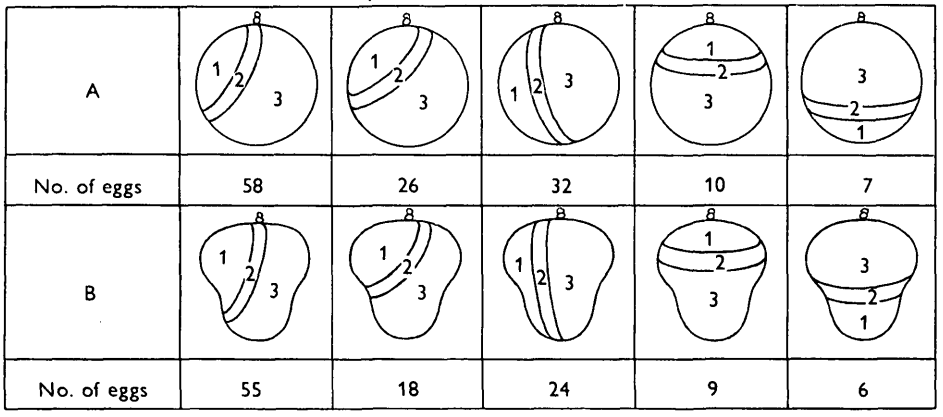
Fig. A. Section of a ripe oocyte with large germinal vesicle.

Fig. B. Section of an unfertilized egg with first maturation spindle near the animal pole; animal and vegetative pole plasm.

Fig. C. Section of a trefoil stage.

Fig. D. Section of an egg cell centrifuged 40–50 min. after fertilization.

When the germinal vesicle disappears and the egg becomes rounded, a clear endoplasm occupies the entire egg centre. The yolk granules are arranged in a wide zone beneath the egg surface, which still shows a clear striation. At the animal and vegetative poles, pole plasm is present nearly free of yolk granules (Plate 1, fig. B). The polar lobe at first cleavage receives part of the endoplasm and of the yolk granules, and the vegetative pole plasm is enclosed in it (Plate 1, fig. C).



Text-fig. 3. Stratification in eggs centrifuged 40–50 min. after fertilization. A, Immediately after centrifugation; B, 5 min later during the formation of the first polar lobe. 1, Fat zone; 2, zone of hyaloplasm; 3, yolk zone.

(*c*) *The influence of centrifugation on the structure of the egg*

In a number of eggs, centrifuged from 40 to 50 min after fertilization, the stratification was determined immediately after removal from the centrifuge. The results are summarized in Text-fig. 3. Three zones may be distinguished: 1st, the fat zone; 2nd, the zone of hyaloplasm; and 3rd, the yolk zone. Studying the relation between the direction of stratification and the egg axis, it appears that in most eggs the polar bodies are situated in the hyalin zone or very near to it. In a few cases only they are situated near the centre of the fat or the yolk zone. At the formation of the polar lobe, in the majority of cases a part of all three zones becomes incorporated into the lobe (groups 1 and 3 in Text-fig. 3). In groups 2 and 4 the lobe consists nearly entirely of yolk material and in group 5 of the fat zone, and the hyaloplasm.

The plane of cleavage in all centrifuged eggs, without any exception, is at right angles to the direction of stratification. Consequently, at first cleavage each blastomere receives a part of all zones. The polar lobe is always formed just opposite the polar bodies at the vegetative pole of the egg, even when first cleavage is abnormal, for instance equatorial instead of meridional.

Sections of eggs fixed immediately after centrifugation (Plate 1, fig. D) also show the three zones which are observed in the living egg. The yolk granules

are generally displaced to the centrifugal side of the egg. In the centre of the yolk zone the endoplasm is situated, displaced more or less as a whole, as may be seen in sections stained with Herlant's tetrachrome, in which the endoplasm becomes deeply blue. A small part of the yolk granules is situated at the opposite side in the fat zone, which contains many vacuoles. The hyaloplasm is situated beneath this zone; in most eggs it contains the asters and the spindle. At the boundary of the hyaloplasm and the yolk zone very small granules are present, which may be mitochondria, as these collect at this place in centrifuged eggs of *Physa* (Clement, 1938) and *Limnaea* (Raven, 1946).

(*d*) *Effect of removing the polar lobe in untreated eggs*

Removal of the first polar lobe from more than 70 eggs always resulted in a larva without an apical tuft. The post-trochal region was in all cases absent. The prototroch was well developed (Text-fig. 2, no. 3). The larvae moved in a normal way through the water during the first day, but they died on the second day without showing any sign of metamorphosis.

After removing the second polar lobe in 26 eggs, 24 embryos with an apical tuft were obtained. The post-trochal region was present but reduced in size (Text-fig. 2, no. 4). These larvae also died without metamorphosing.

(*e*) *Effect of removing the first polar lobe in centrifuged eggs*

The results of a first series of experiments, in which the eggs were centrifuged at from 40–50 min. after fertilization, are shown in Table 1. A certain percentage of the untreated eggs develops abnormally, a fact mentioned also by Wilson (1904) for *Dentalium entalis*. In these embryos the post-trochal region is generally abnormal, but in many cases the prototroch bands and the pre-trochal region are also affected. The apical tuft is missing in most of the abnormal embryos.

In typical 'lobeless embryos' the apical tuft and the post-trochal region is in all cases absent.

After centrifugation the number of abnormal embryos increases; an apical tuft is present in more than 40 % of the embryos. After removal of the polar lobe an apical tuft was never found, neither in the controls nor in the centrifuged embryos.

In a second series of experiments, eggs were centrifuged at from 50 to 60 min after fertilization. As first cleavage starts 55 min after fertilization with the appearance of the first polar lobe, the eggs were at the trefoil stage when removed from the centrifuge. The polar lobe was then immediately cut off in some of the eggs, while others were kept as a control. The results, summarized in Table 2, are not different from the first series. Centrifuged eggs, from which the polar lobe had been removed, never showed an apical tuft.

Table 1.

	Controls		Centrifuged eggs	
	With polar lobe	Without polar lobe	With polar lobe	Without polar lobe
No. of eggs	63	51	52	47
Normal embryos	43	—	18	—
Typical lobeless embryos	—	46	—	28
Atypical embryos with apical tuft	2	—	5	—
Atypical embryos without apical tuft	18	5	29	19

Table 2.

	Controls		Centrifuged eggs	
	With polar lobe	Without polar lobe	With polar lobe	Without polar lobe
No. of eggs	24	24	27	33
Normal embryos	14	—	14	—
Typical lobeless embryos	—	17	—	30
Atypical embryos with apical tuft	6	—	3	—
Atypical embryos without apical tuft	4	7	10	3

DISCUSSION

Since the experiments of Wilson (1904) the morphogenetic role of the polar lobe for larval development has become well established, and Wilson's conclusion that the lobe contains specific cytoplasmic materials essential to the formation of certain larval structures is generally accepted. Attempts have therefore been made to identify the special constituents of the polar lobe which may be related to its morphogenetic importance.

Reverberi (1958) studied the mitochondrial distribution in the egg of *Dentalium* and found that the bulk of the material contained in the polar lobe consists of mitochondria. During cleavage these are distributed among the future mesoderm cells. Another supply of mitochondria is situated near the animal pole during the uncleaved stage and the early cleavage stages. These are transmitted to the ciliary cells of the larva. Reverberi (1958, p. 85) concluded: 'The fact that the polar lobe in the egg of *Dentalium* is particularly well supplied with mitochondria explains the consequence of its removal (Wilson, 1904).' From the

data of Reverberi it is, however, not evident that the mitochondria which during development are passed to the ciliary cells originate from the polar lobe, as some are already present at the animal pole of the uncleaved egg. The fact that they are also present in the AB cell at the two-cell stage, and also that larvae from AB halves show ciliary cells, proves the contrary. Furthermore, the bulk of mitochondria is present not only in the first but also in the second polar lobe. Nevertheless, the effect of removing the first or second lobe is quite different. Finally, our experiments have shown that centrifugation of the uncleaved egg does not influence the effect of removing the polar lobe, although the mitochondria are likely to be displaced so that they are not removed or only partly removed with the polar lobe.

The chemical constitution of the polar lobe has been studied in *Ilyanassa* by Collier (1960*a*, *b*, 1961, 1965). The polar lobe has a higher concentration of phospholipids but a lower concentration of RNA. The major part of the pool of nucleic acid precursors present in the egg is probably localized in the lobe. Removal of the lobe results in a decrease of protein and DNA synthesis, whereas RNA synthesis is comparable to that of the normal embryo. Collier (1965) suggested that there may be two reasons why the lobeless embryo fails to differentiate: informational RNA synthesis is selectively repressed by the deficiency of nucleic acid precursors, and the polar lobe contains an informational RNA essential to determination and/or differentiation.

The experiments described in this paper show, however, that the effect of removing the polar lobe in centrifuged and uncentrifuged eggs is similar, although the cytoplasmic constitution of the lobe has been altered by centrifugation. It seems therefore more obvious to assume that the factors determining the lobe-dependent structures are restricted rather to the cortex than to the cytoplasm of the lobe, a suggestion made already in 1950 by Raven *et al.* on the basis of their studies on the development of the annelid *Sabellaria*.

From the data of Wilson (1904) it is known that after removal of a part of the first lobe larvae are produced which in some cases possess and in other cases do not possess, an apical tuft. This may be dependent on the particular part of the cortex being removed in such experiments. Further investigations may supply additional information on the localization of the determining factors.

SUMMARY

1. The effect of removing the first lobe in centrifuged eggs of *Dentalium antillarum* has been studied.

2. Eggs were centrifuged at 300 ***g*** for 10 min just before the beginning of first cleavage.

3. Removing the polar lobe at first cleavage causes larvae to develop without the apical tuft and the post-trochal region. After removal of the lobe at second cleavage the apical tuft is present and the post-trochal region is reduced.

4. Centrifugation does not influence the effect of removing the first polar lobe.

5. These results are consistent with the view that the morphogenetic factors of the first polar lobe are restricted to its cortex.

RÉSUMÉ

L'effet de l'excision du lobe polaire chez les œufs centrifugés de Dentalium

1. L'effet de l'excision du lobe polaire chez les œufs de *Dentalium antillarum* a été étudié.

2. Des œufs ont été centrifugés à 300 *g* pendant 10 min avant le commencement de la première segmentation.

3. L'excision du lobe polaire à la première segmentation aboutit à la formation de larves sans organe apical et sans région posttrochale. Après l'excision du lobe polaire à la deuxième segmentation l'organe apical est présent et la région posttrochale est reduite.

4. La centrifugation n'influence pas le résultat de l'excision du lobe polaire.

5. Le résultat des expériments suggère que les facteurs morphogénétiques du lobe polaire sont localisés dans le cortex plutôt que dans le cytoplasme.

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