Incorporation of Phenylalanine-H^3 in the Fragments of the Fertilized Ascidian Egg

Investigation of the Ascidian egg with the optical and electron microscope has shown that the cytoplasmic components of the unfertilized egg are uniformly distributed. Cytochemical treatments have also not demonstrated any evidence of segregation[1-4]. If the virgin egg is cut equatorially, these halves after fertilization can develop into normal larvae[5-7].

Following fertilization a few cytoplasmic components are 'segregated' and several presumptive territories are formed[8]: the distribution during development of these components has been extensively studied[9-15]. If the egg is cut equatorially after fertilization, the vegetal fragment divides with the same pattern as the normal egg and gives rise to a normal embryo; the animal fragment however divides radially giving rise to blastular form with a testal membrane[6,16]. These results suggest that certain cytoplasmic components segregated in the vegetal hemisphere are necessary for normal segmentation and development[17].

Since neither cytological or histochemical investigations have yet demonstrated what kind the components are, we have tried to undertake the problem of investigating the protein metabolism in the two halves following the incorporation of phenylalanine-H^3. The protein metabolism of the two halves of the unfertilized egg has already been taken in consideration[17,18].

Materials and methods. Fertilized eggs of *Ascidia malaca*, freed from their membranes, were cut equatorially with glass needles into 2 halves of about equal size. The orientation of the unsegmented egg is possible because the polar bodies formed after fertilization are visible at the animal pole. The cutting operation was performed after the elimination of either the first or second polar body.

It is known[8] that after fertilization there is a movement of cytoplasmic material from the animal pole toward the vegetal pole: the spermatozoon enters at the vegetal pole.

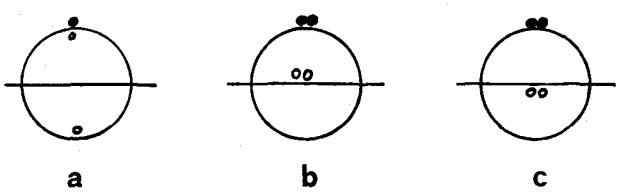

Fig. 1. Scheme illustrating the equatorial cut of the egg: a) after the extrusion of the 1st polar body; b) and c) after the extrusion of the 2nd polar body.

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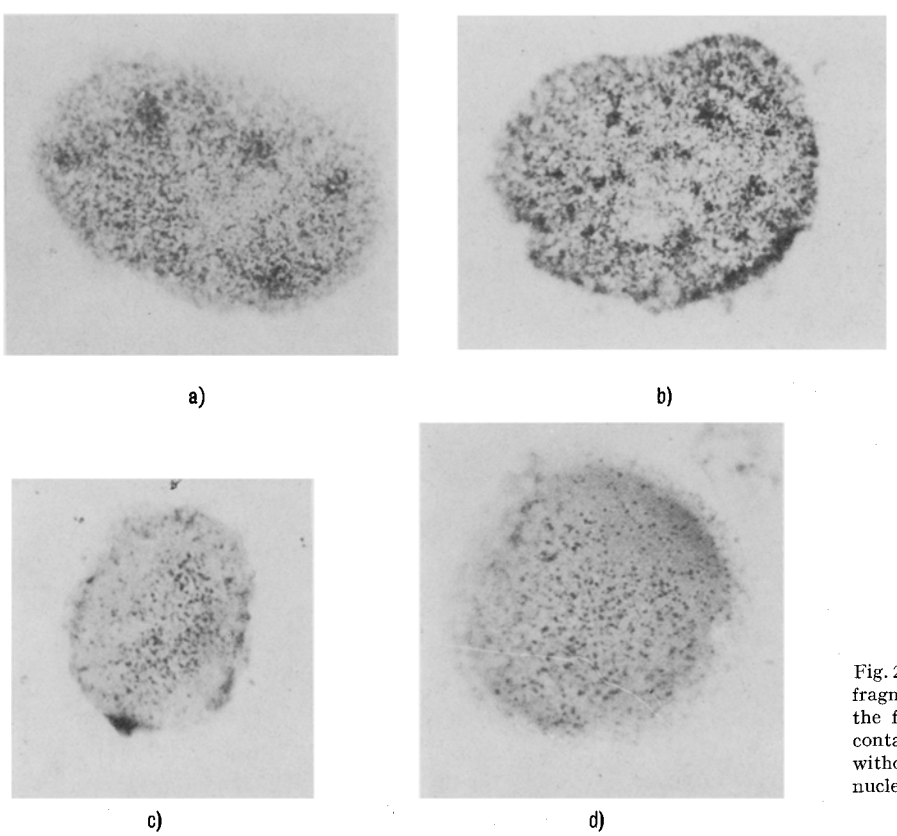

Fig. 2. Autoradiography of animal and vegetal fragments of the egg: a) fragment containing the female pronucleus; b) vegetal fragment containing the male pronucleus; c) fragment without nucleus; d) vegetal half without nucleus.

An equatorial cut, therefore, if performed at the first polar body stage, isolates an animal half with a female pronucleus. On the other hand, if the cut is made after the elimination of the second polar body, the male and female pronuclei are at the center of the egg; in this stage it is possible to cut the egg so that one half contains both pronuclei and the other lacks them.

The animal and vegetal fragments were incubated in sea water to which phenylalanine-H^3 (att. 12.5 μc; conc. 0,25 m*M*) had been added; afterwards they were fixed with Carnoy, embedded in paraffin and sectioned at 7 μm. The sections were washed with a solution of unmarked phenylalanine. The slides were covered with Kodak AR-10 film and exposed for 10 days. The sections were stained with pyronine.

Results. Equatorial cuts made after the emission of the first polar body. After cutting, the 2 halves (Figure 1a) were immediately transferred into sea water with phenylalanine-H^3 added. After an incubation time of $1^1/_4$ h they were washed with unlabelled phenylalanine and fixed (controls at early blastula stage). On examination of the sections, they showed silver grains at the same rate (Fig. 2a, b).

Equatorial cuts made after the emission of the second polar body. As remarked above, at this stage the 2 pronuclei are together at the center of the egg: it is thus possible to obtain, by cut, animal or vegetal fragments with both pronuclei (Figure 1b,c). The 2 sorts of fragments were incubated in phenylalanine-H^3 for $1^1/_2$ h, and after the usual treatment, their sections were studied: the anucleate fragments, of course, did not segment: however, they incorporate the amino acid (Figures 2c, d). The nucleated fragments which were at blastula stage also showed radioactivity in their sections.

Conclusion. The results show that the animal and vegetal halves of fertilized ascidian eggs incorporate phenylalanine-H^3 at the same rate. The conclusions were unexpected as the respiratory metabolism of the vegetal quartet is higher than that of the animal quartet[19,20].

In conclusion, our problem whether the wider developmental potentialities of the vegetal region of the egg are linked with a more intense protein metabolism remains unsolved. The protein metabolism of the quartets of the egg with others radioactive amino acids will be checked.

Riassunto. E'stata studiata l'incorporazione di fenilalanina-H^3 nelle metà animali e vegetative delle uova fecondate di Ascidie tagliate subito dopo l'emissione del 1° e del 2° globulo polare, allo scopo di vedere se le potenzialità di sviluppo delle metà vegetative fossero legate con un diverso metabolismo proteico. I risultati hanno mostrato che entrambe le metà incorporano fenilalanina-H^3.

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Fibrinolytic Activity in the Closed Ductus Arteriosus

The recent note[1] on the fibrinolytic activity of the patent ductus arteriosus of the human foetus prompts us to report some observations on the localization of fibrinolytic activity in the obliterated ductus arteriosus (DA) of young animals.

The obliteration of the DA represents a natural tissue repair process caused by involution of an organ. We have studied the localization of areas of fibrinolytic activity in frozen sections of the DA from young, adult animals by the histochemical fibrin slide technique of TODD, as modified[2]. The study was undertaken in an effort to substantiate by the findings in a physiological repair process, the proposed role of fibrinolysis in the regulation of reparative connective tissue formation following tissue injury[3,4].

Figure 1A shows a cross section of the wall of the pulmonary artery of the pig, cut at the level of the junction with the now obliterated DA. For comparison, Figure 1B shows a cross section of the adjacent regular wall of the pulmonary trunk. In both figures the luminal side of the vessel is at the bottom of the figure and the adventitia at the top. Both sections were collected on the slide, briefly dried in the air, covered with fibrin, and incubated for 30 min at 37 °C. As usual in the pig, high activity was present in the adventitial layers. Endothelial activity was absent in both sections. In the regular section (Figure 1B), scattered foci of fibrinolytic activity were observed in the outer half of the media. The cross section in Figure 1A cuts obliquely into the atrophied DA close to its origin at the pulmonary trunk. An area of repair tissue is seen forming part of an enlargement of the vessel wall protruding in-

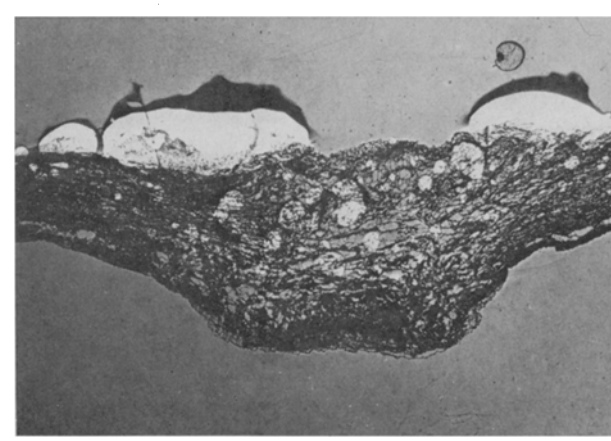

Fig. 1. A) Transverse section of the wall of the pulmonary trunk of an adult pig at the junction of the now atrophied ductus arteriosus (DA) and oblique to the latter. Frozen section. Fibrin slide technique. Incubated for 30 min. Fixed in formalin and stained with Harris haematoxylin. Adventitial layer at top, luminal side at bottom. × 7.

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