Normal Larvae Obtained from Dark Fragments of Centrifuged *Ciona* Eggs

The unfertilized Ascidian eggs, if deprived of their surrounding membranes, can be broken, by appropriate centrifugal forces, into two or more fragments[1–3], which are quite different in their morphological and chemical structure. When two fragments are obtained from the same egg, one is clear and the other pigmented; the clear fragment is smaller than the dark one. Both types of fragments can be fertilized; in previous experiments with *Ciona intestinalis* eggs, only the pigmented fragments were able to develop[1]. In *Ascidia malaca*, however, both kinds of fragments developed[2], although larvae were never obtained from either type of fragments. These facts were interpreted as resulting from the absence (in *Ciona*) or presence (in *A. malaca*) of certain substances in the clear or in both fragments respectively[2]; no suggestions however were made about the nature of such substances.

In new centrifugation experiments on the virgin eggs of *Ciona*, results were obtained which can be summarized as follows:

(1) The clear fragments, if fertilized, do not develop at all; they are not even able to segment, but show ameboid modifications. This ameboid activity lasts for some hours, then the fragments reassume their original spherical shape and degenerate. They never extrude polar bodies, consequently they do not possess the egg nucleus and must be haploid. As they contain some lipid globules, they correspond to the centripetal part of the egg. As the eggs assume different positions in the centrifuge tube, they may be considered as unoriented fragments.

(2) The dark fragments, on the contrary, if fertilized, develop in a normal way. After the penetration of the sperm, they change their spherical shape, as normal eggs do, extrude two polocytes, segment typically, gastrulate, and finally give rise to small but completely normal larvae. The larvae have straight tails, which are morphologically normal; they also possess vesicular brains with sensory spots, and palps; they swim rapidly and normally. This result was never reached in the past, and it gives another indication that the unfertilized egg of Ascidian must be considered as a totipotent or regulative system.

(3) By means of some histochemical reactions, the nature of the content of the clear and the dark fragments was investigated. The Nadi reaction and the vital staining with Janus green demonstrate that the mitochondria are absent in the clear fragments and present in the dark ones. The fact that the mitochondria are present only in the pigmented fragments which develop and give rise to normal tadpoles, and are absent in the clear fragments which do not develop at all, suggests the hypothesis that the mitochondria have an important role in Ascidian development and morphogenesis. Such a suggestion fits in with some earlier observations[4–7]. The morphogenetic role of the mitochondria might be related to the enzymes which are present in their structure, and which are implicated mainly in the liberation and utilization of the chemical energy.

(4) If the morphogenetic role of the mitochondria is admitted, one can explain why both fragments in *A. malaca* can develop[2]. In fact URBANI and URBANI-MISTRUZZI[3] showed that in *A. malaca* both fragments resulting from the centrifugation of the egg show a positive Nadi reaction, that is both fragments contain mitochondria.

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Riassunto

Dei due frammenti, jalino e pigmentato, ottenuti per centrifugazione dall'uovo vergine di *Ciona intestinalis*, solo il frammento scuro è capace di svilupparsi. Da esso può aversi una larva completamente normale. Questo risultato è spiegato attribuendo un valore morfogenetico ai mitocondri che sono presenti esclusivamente nel frammento scuro.

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Über die Wirkung von Elastase auf die Blutgerinnung

Die von BALÓ und BANGA entdeckte Elastase[1] kann unseren heutigen Kenntnissen nach als ein eiweißspaltendes Ferment betrachtet werden, dessen spezifisches Substrat das Elastin, ein Eiweiss der elastischen Fasern, ist. Die Elastase ist kein spezifisches Enzym und baut ausser Elastin auch andere Eiweisskörper ab: denaturiertes Hämoglobin, Serum-Albumin, Kasein und gewisse synthetische Substrate[2]. Da diese Substrate auch durch Trypsin abgebaut werden, das die Blutgerinnung durch Aktivierung des Prothrombins fördert[3], lag es nahe zu prüfen, ob auch die Elastase die Blutgerinnung beeinflusst.

Wir haben frisches Zitrat- oder Oxalatblut, menschliches, Rinder-, Schweine- und Kaninchenblutplasma untersucht. Fibrinogen wurde nach unseren Verfahren[4], rohes Prothrombin nach MELLANBY[5] hergestellt, Thrombokinase nach CHARGAFF[6,7]. Die Elastase wurde mit unseren Adsorptionsverfahren gewonnen[8,9]. Das in unseren Versuchen gebrauchte hellgelbe, lyophile Endprodukt enthielt – gemessen an BANGA's Elastin[10] mit einer kombinierten Biuret-Folin-Reaktion[11,12,13] – 100 Elastase-Einheiten (E. E.) pro mg. In Kontrollversuchen haben

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