

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  $\mu$ c; conc. 0.25 mM) had been added; afterwards they were fixed with Carnoy, embedded in paraffin and sectioned at 7  $\mu$ m. 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 $\frac{1}{4}$  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 $\frac{1}{2}$  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<sup>19,20</sup>.

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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<sup>19</sup> M. DE VINCENTIS and G. ORTOLANI, *Rc. Accad. naz. Lincei* 32, 8 (1962).

<sup>20</sup> M. DE VINCENTIS and G. ORTOLANI, *Rc. Accad. naz. Lincei* 35, s. 8, 604 (1964).

## Fibrinolytic Activity in the Closed Ductus Arteriosus

The recent note<sup>1</sup> 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<sup>2</sup>. 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<sup>3,4</sup>.

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-

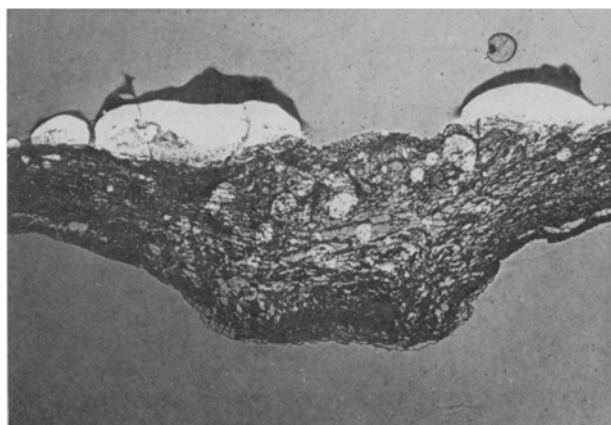


Fig. 1. A) Transverse section of the wall of the pulmonary trunk of an adult pig at the junction of the now atrophied ductus arteriosus (DA) and oblique to the latter. Frozen section. Fibrin slide technique. Incubated for 30 min. Fixed in formalin and stained with Harris haematoxylin. Adventitial layer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

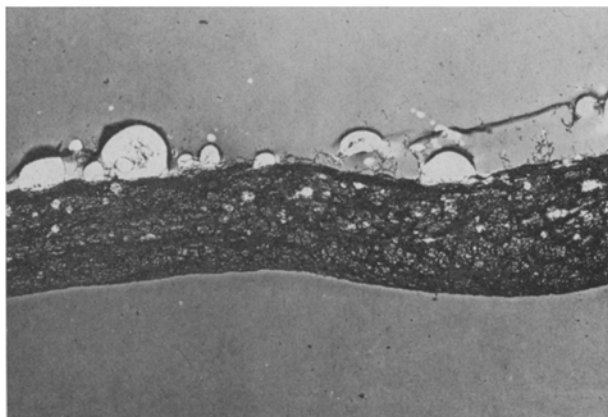
<sup>1</sup> G. GENNSER and B. ÅSTEDT, *Experientia* 27, 679 (1971).

<sup>2</sup> H. C. KWAAN and T. ASTRUP, *Lab Invest.* 17, 140 (1967).

<sup>3</sup> T. ASTRUP, *Lancet* 11, 565 (1956).

<sup>4</sup> T. ASTRUP, *Biochem. Pharmac. Suppl.*, 241 (1968).

to the lumen of the pulmonary trunk. It shows numerous focal areas of high fibrinolytic activity randomly distributed in the thickened vessel wall. This pattern is characteristic for an area of tissue repair undergoing organization such as observed following experimentally induced tissue injury<sup>5</sup>. The same pattern was observed in samples from another pig heart.



B) Transverse section of the wall of the pulmonary trunk adjacent to the area in Figure A.  $\times 8.5$ . Other details as in Figure A.

In the rabbit, cross sections of the DA showed well demarcated zones of lysis in the peripheral parts of the obliterated areas after 60 min of incubation. An abundance of cells indicated that organization was still progressing. Lysis was absent in other vascular structures of the rabbit, including the adventitia of the aorta and pulmonary artery, even after 3 h of incubation. Similarly, in 2 guinea-pigs scattered focal lysis appeared in relation to the obliterated DA after 30 min of incubation, while lytic zones became visible in the aorta and pulmonary artery only after 60 to 120 min and were restricted to the adventitia<sup>6</sup>.

*Zusammenfassung.* Im obliterierenden Ductus arteriosus vom Schwein, Kaninchen und Meerschweinchen erscheinen Zonen von intensiver fokaler fibrinolytischer Aktivität, die charakteristisch für neugebildetes vaskularisiertes Bindegewebe im Wundheilungsgebiet sind.

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## The Histochemically Demonstrable Monoamines of Human Fetal Carotid Body

Several biochemical and histochemical reports have revealed that the mammalian carotid body contains catecholamines and 5-hydroxytryptamine (for ref. see HAMBERGER et al.<sup>1</sup>, CHIOCCIO et al.<sup>2</sup>). The localization of different amines in the cells of the carotid body has been discussed (MORITA et al.<sup>3</sup>, ZAPATA et al.<sup>4</sup>) together with their functional importance for the generation of the chemosensory response. However, the cytochemical basis of the chemosensory function of the carotid body is still a matter for discussion. The presence of the monoamines has been demonstrated in all mature mammalian carotid bodies studied, and also in man. These compounds, or at least some of them, could be considered necessary for the normal chemosensory response of the organ.

In the embryological papers on the development of carotid body (BOYD<sup>5</sup>, ROGERS<sup>6</sup>), conventional light microscopic methods alone have been used. No investigations dealing with the catecholamine histochemistry of fetal carotid bodies were available. The present paper is a preliminary report of studies concerning the fetal function and histochemical differentiation of the human carotid body.

*Material and methods.* The carotid bodies of 6 human fetuses (Cr 10.5–14.5 cm) were prepared immediately after the disconnection of the fetoplacental circulation. The specimens were frozen quickly in isopentane cooled with liquid nitrogen. For the study of the distribution of histochemically demonstrable catecholamines and 5-HT, the formaldehyde induced fluorescence method was essentially the same as described by ERÄNKÖ<sup>7</sup>. An efficient chemical water trap in the form of large amounts of phosphorous pentoxide was used (OLSON and UNGERSTEDT<sup>8</sup>). The exposure to formaldehyde was performed at 60°C for 30 min and then at 80°C for 60 min. The freeze-dried specimens were embedded in a mixture of Epon and Araldite (ERÄNKÖ and ERÄNKÖ<sup>9</sup>). The blocks were cut at

2–5  $\mu$ m using glass knife and LKB Pyramitome. For fluorescence microscopy, a Leitz Ortholux microscope was used with a HBO 200 mercury lamp (Osram) and with the following filters: A 3 mm thick BG 38 heat absorbing filter, one 3 mm thick BG filter, a TAL 408 interference filter (all filters by Schott & Gen., Mainz), an epi-illuminator by Ploem and Leitz ultraviolet absorbing filter K 470.

*Observations.* The carotid bodies were easily recognizable between the base of internal and external carotid arteries (Figure 1). The organ was clearly separated from the adventitia of the vessels. A smaller artery, the ascending pharyngeal artery was seen between body and the external carotid artery.

The organ was composed of small tight groups of brightly yellow fluorescent cells, probably comparable to the chemoreceptor or glomus cells of adult carotid bodies. The carotid bodies were located in loose perivascular connective tissue and there was not any continuous capsule surrounding the fluorescent cells. The fluorescent cells or groups of them were organized around capillaries

<sup>1</sup> B. HAMBERGER, M. RITZEN and J. WERSÄLL, J. Pharm. exp. Ther. 152, 197 (1966).

<sup>2</sup> S. R. CHIOCCIO, M. P. KING and E. T. ANGELAKOS, Histochemie 25, 52 (1971).

<sup>3</sup> E. MORITA, J. R. CHIOCCIO and J. H. TRAMEZZANI, J. Ultrastruct. Res. 28, 399 (1969).

<sup>4</sup> P. ZAPATA, A. HESS, E. L. BLISS and C. EYZAGUIRRE, Brain Res. 14, 473 (1969).

<sup>5</sup> J. D. BOYD, *Embryology* (Carnegie Institute, Publication 26, Washington 1937), No. 152, p. 1.

<sup>6</sup> D. C. ROGERS, J. Anat. 99, 89 (1965).

<sup>7</sup> O. ERÄNKÖ, J. R. microsc. Soc. 87, 259 (1967).

<sup>8</sup> L. OLSON and U. UNGERSTEDT, Histochemie 22, 8 (1970).

<sup>9</sup> O. ERÄNKÖ and L. ERÄNKÖ, Progr. Brain Res. 1970, 34.