excess, and Araldite embedding with acetone as dehydrator and thinner[2]. These methods preserve the bodies, which are eroded by alcohol and epoxypropane.

In normal lungs of the non-simians, about 75% of LOPBs with recognizable structure consist of lamellae, 20 to 100 nm thick, which form, in section, straight or arcuate cross-bars, with lines at 4 nm spacing within them; these meet the periphery at right or acute angles[3]. There is generally a single bounding membrane, separeted from the 4 nm lines by a space of nm or more (Figure, left). Similar LOPBs exist in the hedgehog (*Erinaceus europaeus*)[4], and in the dog[5]. We have taken multiple serial sections which show that the lamellae composing this type of body are flat or dome-shaped throughout, and that the sections showing straight cross-bars are not produced by axial sectioning of a scroll or of a body composed of cylindrical sheets. The few (10%) LOPBs showing concentric bands in section, in non-simian lung, may be explained as sections cut through a body with domed lamellae, at right angles to the axis of the dome. About 1 LOPB in 10 shows signs of origin from a multivesicular body[6]. Extracellular osmiophilic bodies are found in the alveolar spaces of mature foetal lung (human and non-simian), and sometimes in adult lung; they are, if of recognizable shape, always concentric, and appear to be formed by curling up of strips of osmiophilic material extruded through openings in the cell membrane, not by release of intact LOPBs, from the cells. We have found no sign of the species differences which have been reported to exist[7] between the LOPBs of some of the non-simians.

Bodies containing osmiophilic whorls associated with mitochondrial cristae, and possibly transitional between mitochondria and LOPBs, are rare in untreated animals. They are found under conditions of stress[8], and we have found them in a premature (28 day) rabbit after $4^1/_2$ h breathing; a double bounding membrane is present.

The LOPBs of man and of the monkeys examined are quite different from those of the non-simians. They are mainly concentric (Figure, right) with thinner lamellae, which often contain only two 4 nm layers. It is difficult to trace a continuous bounding membrane; this suggests that they are not of mitochondrial origin. Their real organellar origin is not clear, but their structure would be compatible with formation by fusion of the walls of flattened vesicles of rough endoplasmic reticulum, followed by winding into a scroll. This has been illustrated from the human fetus[9], and we have found traces of it in the fetal and newborn mouse. In the adult simians the cross-barred or arcuate bodies appear to be absent; the literature[10], where it shows the details of LOPBs of human origin, agrees. Rather similar concentric bodies appear in the chick[11]. In RDS, concentric LOPBs are found, though in smaller numbers than in adult lung, in line with the deficiency of surfactant in this condition[12]; one arcuate body of non-simian type has been seen. Multivesicular bodies are rare in simians. The morphological differences described here may be connected with the biochemical differences reported by GLUCK et al.[13], who consider that humans have an additional pathway for surfactant synthesis which assists survival in the event of premature birth.

Résumé. Les inclusions lamellaires des cellules du second type du poumon sont la source de la substance tensioactive. Chez l'homme et les singes de l'ancien et du nouveau monde, ces inclusions sont de forme concentrique et peuvent provenir du réticulum endoplasmique. Chez d'autres mammifères, y compris un lémurien, elles ont, pour la plupart, des barres rectilignes ou arguées et proviennent des corps multivesiculaires.

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24 September 1973.

[2] C. SCHOCK, R. E. PATTLE and J. M. CREASEY, J. Microsc. *97*, 321 (1973).
[3] R. E. PATTLE, C. SCHOCK and J. M. CREASEY, Experientia *28*, 286 (1972).
[4] W. BARGMANN and A. KNOOP, Zt. Zellforsch. *44*, 263 (1956).
[5] B. SIEGWART, P. GEHR, J. GIL and E. R. WEIBEL, Resp. Physiol. *13*, 141 (1971).
[6] S. P. SOROKIN, J. Histochem. Cytochem. *14*, 884 (1967). – C. MEBAN, J. Anat., *112*, 195 (1972).
[7] Y. KIKKAWA and R. SPITZER, Anat. Rec. *163*, 525 (1969).
[8] R. E. PATTLE, C. SCHOCK, P. DIRHUNBER and J. M. CREASEY,
[9] Nature, Lond. *240*, 468 (1972); Br. J. exp. Path., in press (1974). J. U. BALIS and P. E. CONEN, Lab. Invest. *13*, 1215 (1964).
[10] R. Y. HATT and CH. ROUILLIER, Path. Biol. *34*, 1371 (1958). – J. U. BALIS, M. DELIVORIA and P. E. CONEN, Lab. Invest. *15*, 530 (1966). – J. M. LAUWERYNS, Human Path. *7*, 175 (1970).
[11] P. PETRIK, Folia morph., Praha *15*, 176 (1967).
[12] M. E. AVERY and J. MEAD, J. Dis. Child. *97*, 517 (1959). – G. GANDY, W. JACOBSON and D. GAIRDNER, Archs Dis. Childh. *45*, 289 (1970).
[13] L. GLUCK, M. V. KULOVICH, A. I. EIDELMAN, L. CORDERO and A. F. KHAZIN, Pediat. Res. *6*, 81 (1972).
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Catecholamines in Human Fetal Heart

The heart of adult mammals has been observed to have an extensive adrenergic nerve supply, and catecholamine-containing cells have been found in the atrial myo- and epicardium[1–4]. Organs composed of small catecholamine-containing cells, which receive their vascular supply directly from the coronary arteries, have been described[5]. These organs, called aortic and pulmonary bodies, are situated in the wall of the main arterial trunks and they are considered homologous with the carotid bodies[6].

[1] E. T. ANGELAKOS, K. FUXE and M. L. TORCHIANA, Acta physiol. scand. *59*, 184 (1963).
[2] D. JACOBOWITZ, J. Pharmac. exp. Ther. *158*, 227 (1967).
[3] W. F. FRIEDMAN, P. E. POOL, D. JACOBOWITZ, S. C. SEAGREN and E. BRAUNWALD, Circulation Res. *23*, 25 (1968).
[4] T. H. SCHIEBLER and J. WINCKLER, Progr. Brain Res. *34*, 405 (1971).
[5] H. KNOCHE, G. SCHMITT and E.-W. KIENECKER, Z. Zellforsch. *118*, 532 (1971).
[6] C. P. ABBOTT and A. HOWE, J. Physiol,. Lond. *209*, 18P (1970).

The functional adrenergic sympathetic innervation of the heart has been observed to develop relatively late during the ontogenesis of the laboratory mammals[3,7]. No fluorescence histochemical studies have been carried out on these adrenergic compartments in human fetal heart, and thus no knowledge is available on the sympathetic innervation or the monoamine-containing cells in it. The present study was carried out to fill this gap.

Material and methods. 4 human fetuses, aged 10, 13, 15 and 16 weeks, were obtained from legal interruptions of pregnancy. The hearts were processed for the histochemical demonstration of catecholamines according to the formaldehyde-induced fluorescence (FIF) method[8]. The hearts were thereafter embedded in Epon-Araldit and serially cut at 10 µm. Every 20th section was stained with Toluidine Blue for general orientation. For the study of the FIF, a Leitz Orthomat fluorescence microscope, with an epi-illuminator and appropriate filter combinations for catecholamine studies, was used[8,9].

Results and discussion. The fundamental observations on the adrenergic structures were the same in all the specimens studied. In the atrial epi- and myocardium and in the adventitia of the main arterial trunks, both non-fluorescent nerve trunks and trunks with a weak green fluorescence were seen. In both types of nerve trunks, small intensely fluorescent cells with long processes were a constant feature (Figure 2). These cells showed the characteristics of the small intensely fluorescent (SIF) cells first described in the sympathetic ganglia[10,11]. Similar cells were also detected in the atrial myocardium, scattered between muscle bundles and blood vessels, without contact to nerve trunks (Figure 1). No fluorescent adrenergic terminal nerve fibres could be observed in the atrial or ventricular wall, or in the nodal tissues.

At the base of the aortic and pulmonary trunks, several clusters of small fluorescent cells were seen. The fluorescence intensity varied between individual cells from weak to intense (Figure 3). The fluorescent cells had, again, long processes, which were often in contact with perikarya of other fluorescent cells. Non-fluorescent nerve trunks, probably branches of the vagus nerve, were observed in the intimate vicinity of the fluorescent cell groups. In general, numerous clusters of fluorescent cells were seen in the connective tissue upwards from the level of the semilunar valves around the aortic and pulmonary trunks. These cell clusters closely resembled the human fetal carotid bodies[12,13] fluorescence histochemically, and they are obviously identical with the aortic or pulmonary bodies described in other mammals[5].

[7] T. H. Schiebler and R. Heene, Histochemie *14*, 328 (1968).
[8] O. Eränkö, J. R. microsc. Soc. *87*, 259 (1967).
[9] O. Eränkö and L. Eränkö, Progr. Brain Res. *34*, 39 (1971).
[10] O. Eränkö and M. Härkönen, Acta physiol. scand. *58*, 285 (1963).
[11] O. Eränkö and M. Härkönen, Acta physiol. scand. *63*, 511 (1965).
[12] A. Hervonen and O. Korkala, Experientia *27*, 449 (1971).
[13] O. Korkala and A. Hervonen, Histochemie *37*, 287 (1973).

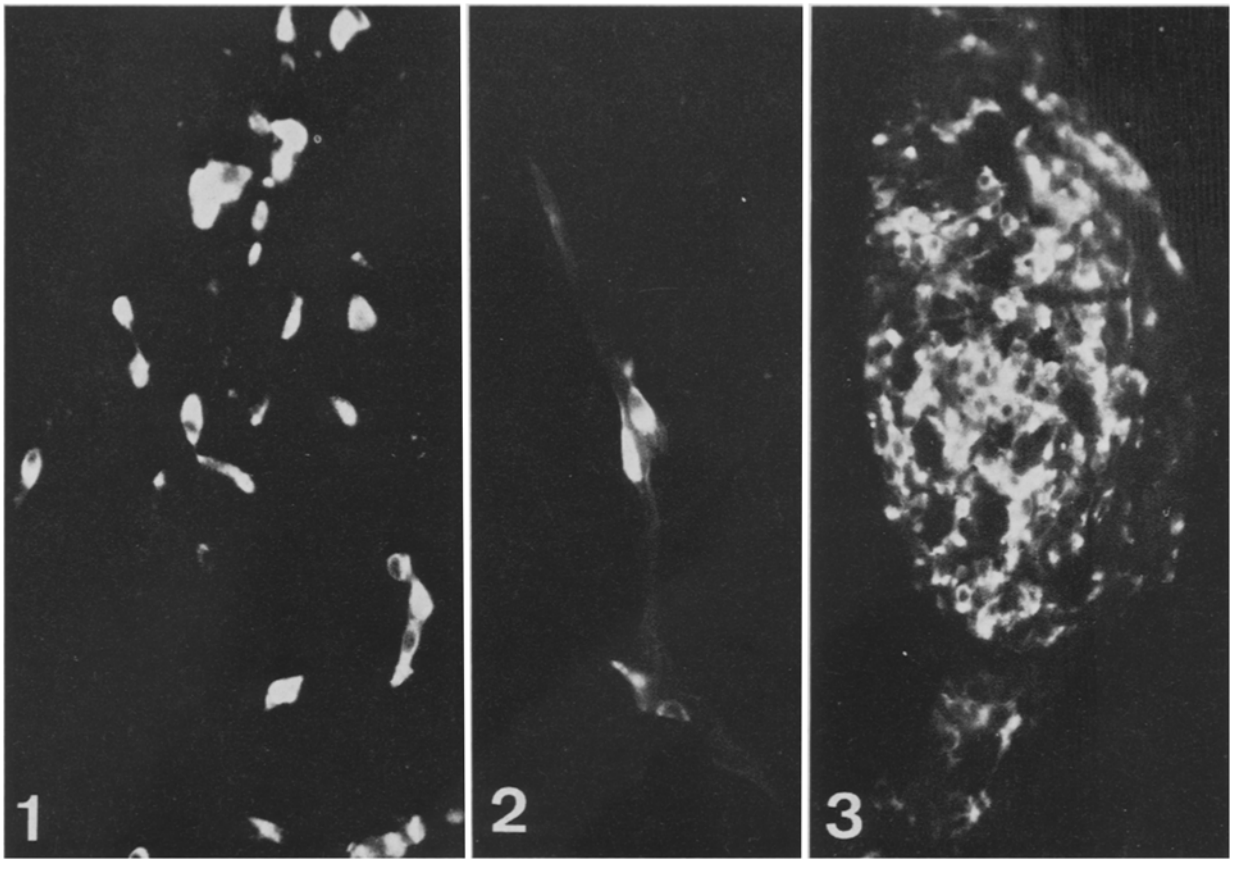

Fig. 1. Single and small clusters of fluorescent cells in the atrium of a human fetus aged 15 weeks. ×250.

Fig. 2. Small intensely fluorescent cells in a weakly fluorescent nerve in the adventitia of the aortic trunk of a human fetus aged 15 weeks. ×250.

Fig. 3. The aortic main body of a human fetus aged 10 weeks. The fluorescence intensity of the cells varies from weak to intense. ×250.

The lack of terminal adrenergic nerve fibres in the cardiac wall of human fetuses suggests humoral, rather than neural, adrenergic control of the human fetal heart, at least during the first half of pregnancy. The paraganglionic tissue and the scattered single catecholamine-containing cells in the atrial wall might represent a pool for catecholamine liberation to the coronary circulation, or the catecholamines from these cells might directly affect the atrial muscle. This view is well compatible with observations on the release of catecholamines from the pre-aortic paraganglia during asphyxia[14,15]. The fetal aortic and pulmonary bodies, together with the carotid bodies, might exert chemoreceptor nature possibly combined with catecholamine liberation.

Addendum. After the present manuscript was completed, the paper by DALE and PALMER[16] came to our attention. Their results are in accordance with the concept of humoral adrenergic control of the human fetal heart, presented in this paper.

Zusammenfassung. In 4 fötalen menschlichen Herzen im Alter von 10–16 Wochen fanden sich wohl intrazelluläre Katecholamine, hingegen keine adrenergischen Nervenendigungen. Das fötale Herz dürfte somit zumindest im Beginn seines Lebens adrenergisch gesteuert sein.

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14 January 1974.

[14] T. BRUNDIN, Acta physiol. scand.; suppl. *290*, 70 (1966).
[15] A. HERVONEN and O. KORKALA, Acta obstet. gynec. scand. *51*, 17 (1972).
[16] W. C. DAIL and G. C. PALMER, Anat. Rec. *177*, 265 (1973).

Cytotoxic Effect of Di(2-ethylhexyl) Phthalate on Cultured Chick Embryonic Cells

Plasticized polymeric materials are widely used in delivery of medical services. Blood can extract plasticizers from devices (e.g., polyvinyl chloride tubings) and such plasticizers have been identified in human tissues[1,2]. A number of investigations have been made of the possible toxic and teratogenic properties of commonly used plasticizers, phthalate esters. The results so far obtained from adult humans, dogs, guinea-pigs, mice, rats, and rabbits, and chick embryos are inconsistent[3–9]. Furthermore, there is no information available on the toxicity of phthalate esters on embryonic cells. In view of this, the present study was undertaken to investigate the potential cytotoxic effect of di (2-ethylhexyl) phthalate, the most extensively used plasticizer in manufacturing of various plastic devices, on cultured chick embryonic cells.

Materials and methods. Di(2-ethylhexyl) phthalate (Matheson, Coleman and Bell, Cincinnati, Ohio), like many other phthalate esters, is sparingly soluble in nutrient medium. For preparing a saturated solution of di(2-ethylhexyl) phthalate in medium 199, the following procedure was used: 50 ml of phthalate ester and 150 ml of medium 199 were added to a separatory funnel. The funnel was shaken vigorously for 10 min and the layers were allowed to separate. The aqueous layer was centrifuged to remove all droplets of phthalate ester. The amount of phthalate ester, dissolved in medium 199, was estimated by a conventional procedure using diethyl ether as an extraction solvent. The solubility of di(2-ethylhexyl) phthalate in medium 199 was found to be ca. 0.1 mg/ml.

Nine-day-old White Leghorn embryos were used to obtain the desired structures, e.g. heart, aortic arches, and

[1] R. J. JAEGER and R. J. RUBIN, Science *170*, 460 (1970).
[2] Y. L. MARCEL and S. P. NOEL, Lancet *1*, 35 (1970).
[3] C. B. SHAFFER, C. P. CARPENTER and H. F. SMYTH JR., J. ind. Hyg. Toxicol. *27*, 130 (1945).
[4] C. P. CARPENTER, C. S. WEIL and H. F. SMYTH JR., Arch. ind. Hyg. occup. Med. *8*, 219 (1953).
[5] R. S. HARRIS, H. C. HODGE, E. A. MAYNARD and H. L. BLANCHET, JR., Am. med. Ass. Archs ind. Health *13*, 259 (1956).
[6] T. MCLAUGHLIN, JR., J. P. MARLIAC, M. J. VERRETT, M. K. MUTCHLER and O. G. FITZHUGH, Toxic. appl. Pharmac. *5*, 760 (1963).
[7] D. CALLEY, J. AUSTIN and W. L. GUESS, J. Pharm. Sci. *55*, 158 (1966).
[8] W. L. GUESS and S. HABERMAN, J. biomed. Material Res. *2*, 313 (1968).
[9] R. K. BOWER, S. HABERMAN and P. D. MINTON, J. Pharm. exp. Ther. *171*, 134 (1970).

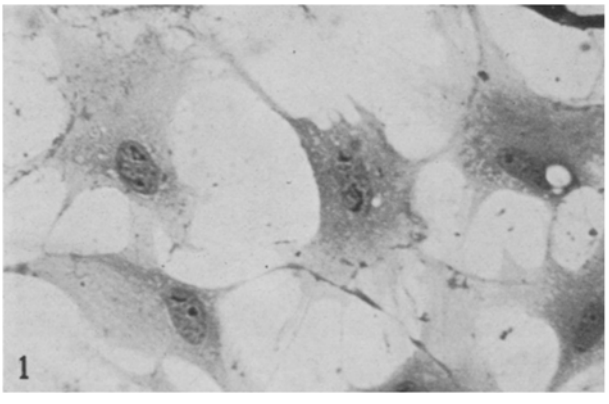

Fig. 1. Normal aortic cells in monolayer culture. Stained May Grünwald-Giemsa. ×450.

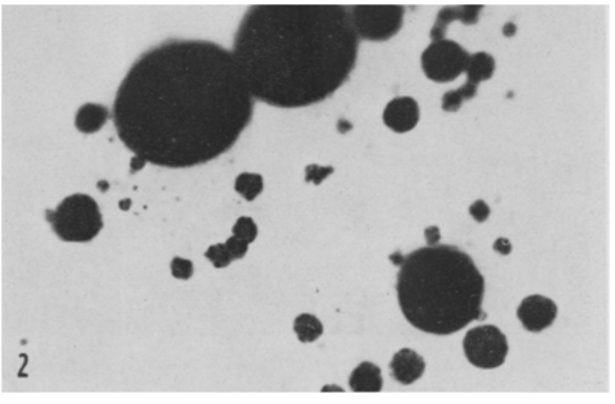

Fig. 2. Aortic cells grown for 19 h in medium with ca. 0.05 mg/ml di(2-ethylhexyl) phthalate. Stained Sudan black B. ×1100.