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.

S. PARTANEN and O. KORKALA

*Department of Anatomy, University of Helsinki, Siltavuorenpenger 20, Helsinki 17 (Finland),*
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## 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

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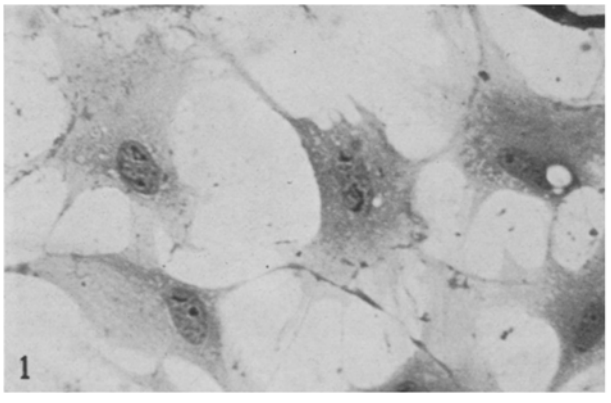


Fig. 1. Normal aortic cells in monolayer culture. Stained May Grünwald-Giemsa. ×450.

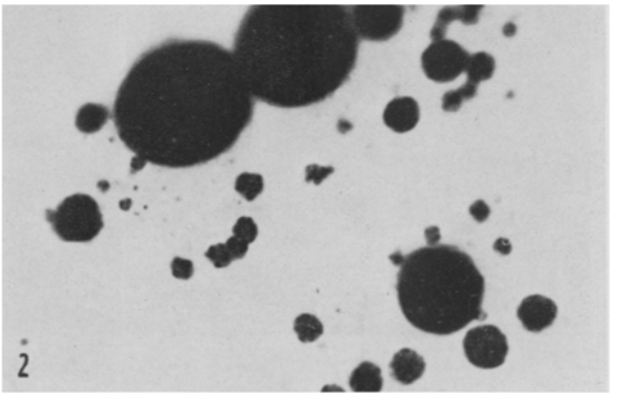


Fig. 2. Aortic cells grown for 19 h in medium with ca. 0.05 mg/ml di(2-ethylhexyl) phthalate. Stained Sudan black B. ×1100.

liver. The latter were washed several times in Earl's BSS and cut into small pieces (0.3 × 0.3 mm). The pieces (explants) were rinsed twice in nutrient medium and 2 explants were placed in chicken plasma on a flying coverslip. After plasma clotting, the coverslip was inserted into a roller tube. 2 ml of nutrient medium was added to each tube which was then gassed with a $CO_2$–$O_2$ 5%–95%) mixture. The tubes were placed in a roller drum and incubated at 37.5 °C. Once the growth of cells was firmly established, i.e., by the 3rd day, the explants were carefully removed from the coverslips. The monolayer cultures thus obtained were subcultured for 8–21 h in nutrient medium with or without di(2-ethylhexyl) phthalate. After the incubation period cultures were frozen or fixed in absolute methanol and stained with Sudan black B or May Grünwald-Giemsa.

The nutrient medium consisted of medium 199 (25 parts), horse serum (15 parts), 20% chick embryo extract (10 parts), L-glutamine (1.46 µg/ml), penicillin G (80 units/ml), and streptomycin sulfate (40 µg/ml). The pH of the medium was adjusted to 7.2–7.4 with 10% $NaHCO_3$. All materials were obtained from Difco Laboratories, Detroit, Michigan.

*Results.* Cells grown from the explants of various embryonic tissues, such as heart, aortic arch, and liver, were equally susceptible to treatment with ca. 0.05 mg/ml of di(2-ethylhexyl) phthalate. This is briefly illustrated in the following paragraph using aortic cells as an example.

The appearance of normal aortic cells in monolayer culture is shown in Figure 1: polygonal- or spindle-shaped cells with two or more protoplasmic processes, coarse and vacuolated cytoplasm, and a round or oval nucleus containing usually 2 nucleoli. Treatment of aortic cells for 8–10 h with ca. 0.05 mg/ml of di(2-ethylhexyl) phthalate often resulted in a retraction of protoplasmic processes and the formation of various cytoplasmic vacuoles. After 17–21 h of treatment, many cells became rounded and sudanophilic granules increased in number and size (Figure 2). At ca. 0.01 mg/ml or lower, no visible morphological effect was noted.

*Discussion.* This study showed that di(2-ethylhexyl) phthalate, at a concentration of ca. 0.05 mg/ml, was toxic to cultured chick embryonic cells. The mechanism of its toxic action is presently not known. JAEGER and RUBIN[1] reported that 1. a rather high concentration (27 mg/100 mg of dry weight) of di(2-ethylhexyl) phthalate was present in tissues of patients who were known to have received blood transfusions; 2. the perfused rat liver could hydrolyze butyl glycolybutyl phthalate, but not di(2-ethylhexyl) phthalate, the latter being accumulated in the liver. MARCEL and NOEL[2] found that the blood, which had been stored in plastic bags for 4–21 days, contained 4–11.5 mg of dihexyl phthalate per 100 ml of plasma. These concentrations were higher than those of di(2-ethylhexyl) phthalate used in our experiments. It is highly unlikely that such high concentrations will often be found in human blood, but the possible cumulative nature of phthalate esters in the tissues should not be overlooked.

*Zusammenfassung.* Nachweis, dass Di(2-ethylhexyl) Phthalat in einer Konzentration von ca. 0,05 mg/ml eine toxische Wirkung auf kultivierte Hühnerembryonalzellen hat, während Konzentrationen von 0,01 mg/ml oder darunter keine deutliche Wirkung mehr haben.

H. LEE[10] and G. W. KALMUS

*Department of Biology, Rutgers University, Camden (New Jersey 08102, USA), 19 February 1974.*

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## Secondary Teratogenic Factors

During the process of organogenesis, certain anomalies in embryonic development may bring into contact tissues which are normally either separated or are in contact only in some distant regions of the embryo. The interaction of these tissues abnormally brought into contact may well be a secondary teratogenic factor.

We arrived at this hypothesis after studying in detail the following interactions between embryonic tissues: a) interaction between the notochord and the digestive tract; b) interaction between the neural tube and the digestive tract; c) interaction between the digestive tract and the ectoderm.

*Material and methods.* We succeeded in provoking abnormal interactions between chick embryonic tissues by applying diathermic coagulation either to the caudal end of the neural tube, or the region immediately below it, the primitive node or the remnants of the primitive streak. The operations were carried out in embryos at 48 h of incubation. 52 embryos in all were operated. The 37 which survived long enough to be included in our study were fixed in 10% formalin or Bouin solution at ages varying from 4 to 13 days. All the fixed embryos were then embedded in paraffin, cut serially into transverse and sagittal sections, the former being more numerous. The stain used in most cases was hematoxilin-eosin.

*Results. Deviation of the axial structures.* As a result of the diathermic coagulation applied to the caudal portion of the axial structures, the growth of the neural tube and the notochord deviated ventrally; and consequently, both the neural tube and the notochord came into contact or even fused with the digestive tract. In some cases, this deviation also affected the gut, resulting in contact with the abdominal ectoderm at regions craneal to the anal plate.

*Interactions between embryonic tissues.* a) Interaction notochord/intestinal tract. The contact between the notochord and the intestinal tract gave rise, in all cases, to the following phenomena: Firstly, when the notochord approached the intestinal tract, (this normally took place near the hindgut or the cloaca) the cells lining the intestinal wall became flattened and the mitosis in these cells decreased notably in number. Secondly, once the notochordal tissue was in contact with the intestinal cells, the latter appeared to be necrotic and were later discarded into the intestinal space (Figure 1). On its approach to the intestinal tract, the notochord was not sheathed, as is usual, but rather presented cells which seemed to intermingle freely with the tissues that happened to lie in their path. In embryos sacrificed several days after the commence of the notochord/intestinal tract