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.

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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

contact, a firm adherence was observed between these two tissues. 1 embryo in particular, fixed on the 13th day of incubation, presented a clear adherence between the hindgut and the notochord at the ventral surface of one of the last lumbar vertebrae. This adherence, or pinching, because of further growth in length of the gut, caused a cul-de-sac which, in transverse sections, gave the impression of a duplicated tail gut.

Another fact that must be stressed in connection with the interaction between the notochord and the digestive tract, is the tropism that the former seems to exercise over the latter. This tropism appeared very clearly in cases where the free portion of the notochord remained close to the cloaca. The cloaca, separating itself from the anal plate, migrated dorsally towards the notochord (Figure 2).

b) Interaction neural tube digestive tract. This interaction is very different in nature from that described for the notochord. When the neural and intestinal tissues approached each other, their outlines became less clearly defined and, eventually, became fused. The tissues were mutually tolerant, although their cells did not intermingle. It could easily be seen where one type of tissue began and where the other ended; and it was not infrequent to find a long stretch of digestive epithelium bordering the neural tube, or vice versa.

c) Interaction intestinal tract/ectoderm. In 3 of our embryos, as the notochord and the neural tube progressed ventrally, the intestinal tract became displaced in a ventral-lateral direction, thus establishing contact with the embryonic ectoderm craneal to the anal plate. The ectoderm next to the intestinal epithelium showed a considerable thickening. At the area of contact, an aperture was formed – ectopic cloaca – by necrosis of the ectodermic and endodermic cells. The first 2 of the 3 embryos used presented only this ectopic cloaca, whereas the 3rd presented, at the same time, another opening, which, judging from its location, corresponded to the normal cloaca.

Discussion. The abnormal interaction between tissues, such as the type that we have described above, is not a rare phenomenon. Developmental anomalies of the axial structures are not infrequent, and the conditions that we have obtained experimentally are in fact very often seen clinically. The existence of diverticula, gut duplication, rectal atresia, etc. has often been related to abnormal axial structures formed as a consequence of any anomalies of the notochord and the vertebrae that might be present (ELLIOT et al.[1], FEDELE and SIMONETTI[2]). Likewise, it is also not rare to find in perinatology anorectal anomalies that are present together with malformations of the posterior third of the neural axis (WILLIAMS and NIXON[3], EGINETE and VERRENCIA[4]). On the other hand, these anomalies of the posterior third may be due to non-specific causes, such as: Anaemia (GONZALO[5,6]), hypoxia (HICKLIN[7], GALLERA[8]), hypoglycemia (LANDAUER[9], SMITHBERG and RUNNER[10]).

Our experimental results suggest that frequently in the congenital malformations there is a primary teratogenic factor (hypoxia, anaemia, etc.) producing axial anomalies, which in turn acts as a secondary teratogenic factor responsable for the intestinal malformations.

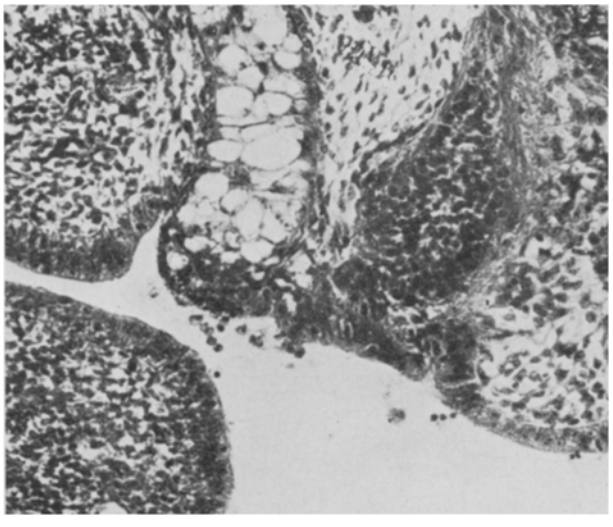

Fig. 1. Transverse section through the cloacal region of a 5-day chick embryo. The notochord has destroyed the cloacal wall.

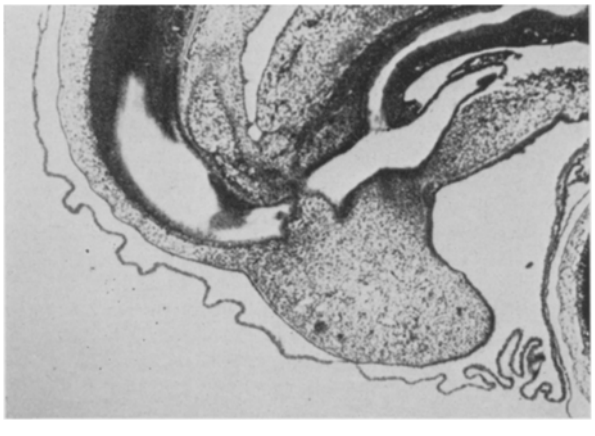

Fig. 2. Sagittal section through the caudal third of a 5-day chick embryo. The deviation of the cloaca is apparently approached to the notochord and its separation from the anal plate.

Resumen. La desviación del tubo nervioso y de la notocorda en dirección ventral, provocada por electrocoagulación en la proximidad del del nódulo primitivo en embriones de pollo de 48 horas, da lugar a la fusión de esas estructuras con el tracto intestinal. La interacción entre estos tejidos, que normalmente no están en contacto, origina malformaciones intestinales. La interacción anormal entre tejidos se puede considerar, pues, como un factor teratógeno secundario.

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