liver (4fold less than controls). The lysosomal membrane permeability to acid phosphatase was 4 times higher than controls in the tumor and 3 times higher in tumor-invaded liver however, approximately $^{9}/_{10}$ of the total tissue acid phosphatase activity remained membrane-sequestered. Lysosomal membrane permeability to substrate in tumor and tumor-invaded liver was not significantly different from control liver, suggesting that loss of membrane integrity in intact lysosomes, is not associated with either the cancerous state or with host response to invasive activity.

Histochemically, fewer lysosomes were observed in Novikoff hepatoma cells (figure 4) than in normal liver cells (figure 1). In many tumor sections the only acid phosphatase activity to be detected was that associated with the white blood cells. In the tumor-invaded liver (figure 3) the lysosomes were more plentiful than in the tumor, but less abundant than in normal liver, and cellular atrophy and evidence of hemmorhage was pronounced. An intermediate condition was seen in the livers of tumor bearing rats that had not been actively invaded by hepatoma (figure 2).

Discussion. Our data allows us to derive several conclusions concerning the nature of lysosomal enzyme release, if such a phenomenon indeed occurs, in Novikoff hepatoma and tumor-invaded liver. The low intracellular levels of acid phosphatase present in these tissues indicates that, if any loss of lysosomal enzyme to the extracellular space occurs, it is not totally compensated for within the cell, e.g. by increased protein synthesis. Since the lysosomes of these tissues show nearly normal membrane integrity, leakage from intact but structurally damaged lysosomes (a common mechanism in various pathological processes other than cancer[10–12]) cannot be evoked as an explanation for primary enzyme loss. An hypothesis, based on our observation of decreased numbers of morphologically normal lysosomes in both the cancerous tissues analysed, would support a mechanism of selective lysosomal rupture. Indeed, such a mechanism might explain the extensive necrosis that we observed in the host liver tissue, since selective lysosomal rupture is known to cause the destruction of cellular and extracellular materials[8].

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Direct intercellular contacts at the ectomesodermal interface during the duck embryologic preen gland development[1]

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Summary. The epidermis, which lines the uropygial invaginations and which then forms the primary and secondary buds, is separated from the mesenchyma by an uninterrupted basal lamina. Between the end of the internal morphogenesis and the beginning of the secretory activity, direct intertissular contacts are established through the gaps in the basal lamina. They appear to be related to the induction of glandular differentiation.

Numerous studies have shown that the morphogenetic development of different organs depends on the interaction between epithelial and mesenchymal components of organ rudiments. The available data concerning this interaction have been the subject of several reviews[2,3], but the transmission mechanism of the inductive signals have not been clearly defined. The suggestion that diffusion of high-molecular-weight material might mediate the inductive effects without direct contacts between the mesenchymal and epithelial cells was originally based on transfilter experiments believed to exclude cell contacts[4]. However, a positive correlation was observed between successful transfilter mouse kidney tubules induction and the establishment of a direct apposition of cytoplasmic processes from the interacting cells in the filter channels[5,6]. Direct cell contacts occurring during epithelio-mesenchymal interaction in the normal kidney tubules induction in vivo support the hypothesis that direct intercellular contact is the induction mechanism in this system[7]. Such contacts are observed in several developing tissues: foetal mouse liver[8], tooth buds in the cat[9], in the rat[10], human regenerating skin[11], rat duodenal mucosa[12] and rat submandibular gland[13].

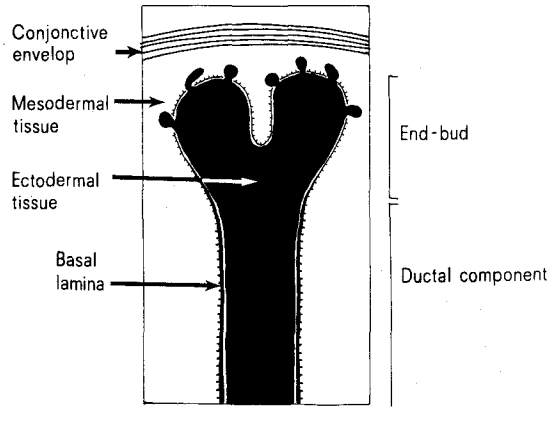

Fig. 1. Schematic representation of the uropygial tubule terminal part at the end of the morphogenesis, showing the zone of direct ecto-mesodermal contacts.

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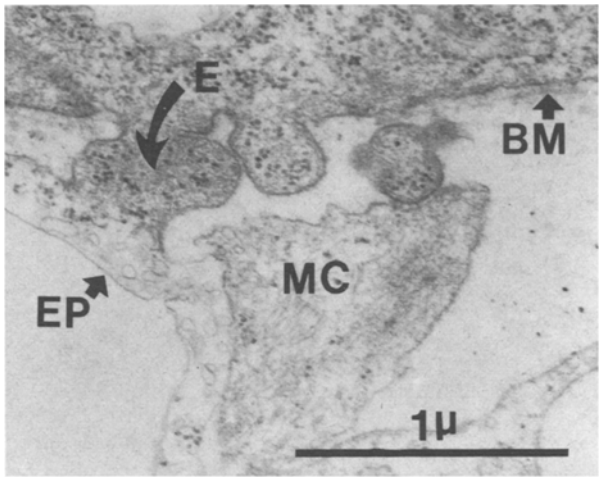

Fig. 2. An ectodermal pseudopode-like process (EP) containing smooth vesicles projects from an end-bud cell (E) through a gap in the basal membrane (BM) and makes a close contact with a mesenchymal cell (MC) (in 20 day duck embryo).

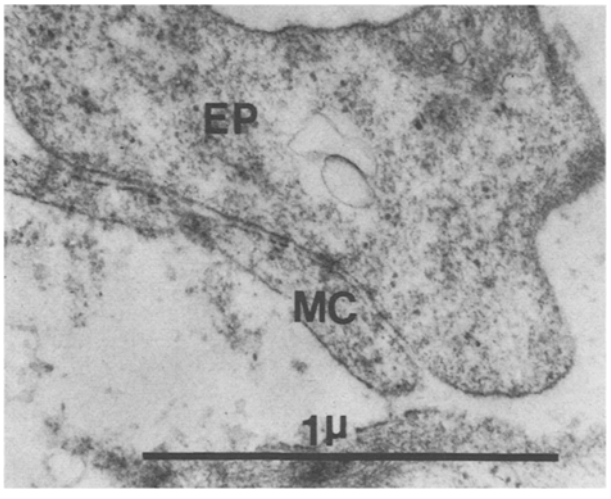

Fig. 3. In another area of contact between an ectodermal cell process (EP) and a mesenchymal cell (MC), the plasma membranes are separated by a space (in 20 day duck embryo).

During the Anas platyrhynchos duck preen gland morphogenetic development which depends on ecto-mesodermal interaction[14], ectoderm appeared to be completely separated from the mesoderm by a continuous basal lamina without direct contact between the tissues[15]. However, modifications occurred at the ecto-mesodermal interface after the branched tubular structure of the gland had been established by branchings of buds from the primordial invagination epithelium. At the end-buds of the branched tubules (figure 1), cytoplasmic processes of the ectodermal cells projected into the surrounding mesenchyme through the gaps in the basal lamina. These pseudopode-like projections were never surrounded by a basal lamina, and they made contact with the plasma membrane of neighboring mesodermal cells (figure 2 and 3). A type of direct contact was charaterized by an apparent membrane fusion between the apposed epithelial and mesenchymal cell membranes (figure 2). In other contact zones, the membranes were separated by a gap of approximately 100, 150 Å (figure 3), that was often filled with a material of moderate electron density. No specialized junction was identified at the contact zones. The cytoplasm of the ectodermal cell processes contained abundant ribosomes, varying numbers of smooth-walled vesicles (figure 2) and occasional pinocytic vesicles (figure 4). The number of ectodermal projections decreased from the 21st day of incubation, when the first glandular differentiation signals appeared. On the 24th day of incubation, the end-buds were actively secreting and the extruding ectodermal projections had disappeared. Along the ductal component, whose epithelium had a similar keratinization pathway to avian embryonic epidermis, such direct contacts were not observed.

The ultrastructural studies showed that direct contacts characterize the ecto-mesodermal interface at the end-bud during a rapid proliferation of the ectodermal cells before the onset of glandular secretory production. It is evident that the mesodermal tissue, which initiates the uropygial invagination morphogenesis[14], may be able to induce glandular cytodifferentiation. The morphological specialization which appears at the ecto-mesodermal interface suggests that a new interaction mechanism would

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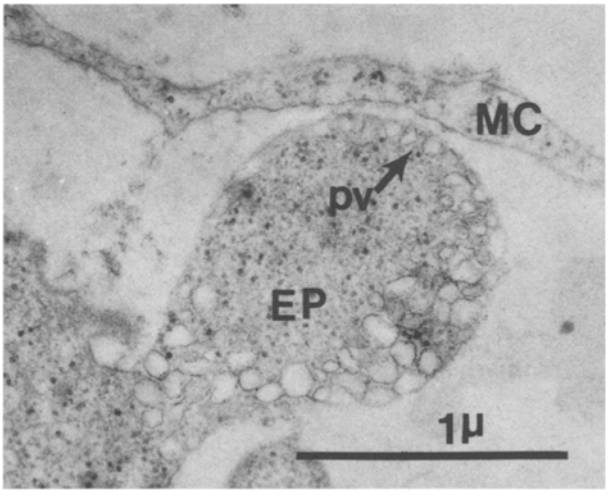

Fig. 4. At the contact zone between an ectodermal process (EP) and a mesenchymal cell (MC), pinocytic vesicles (pv) can be seen (in 20 day duck embryo).

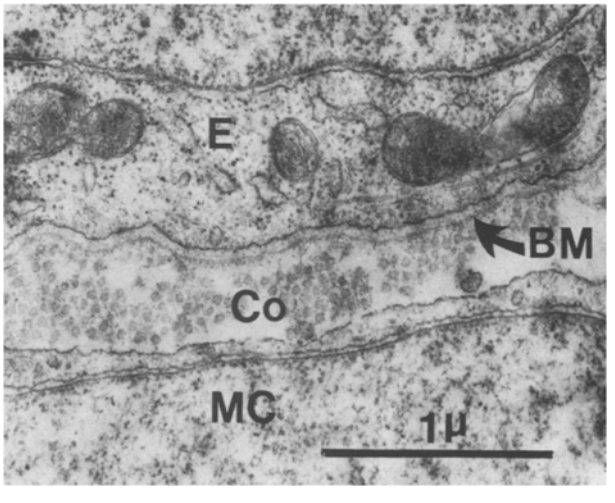

Fig. 5. Along the future collecting duct, the ectodermal ductal cells (E) and the mesenchymal cells (MC) are separated by an uninterrupted basal membrane (BM) and collagen fibres (Co) (in 20 day duck embryo).

be required to induce the glandular differentiation and the inhibition of the keratinization in the ectodermal end-buds. The basal membrane disruption and the presence of pinocytic vesicles could facilitate the transfer of large molecules. However, the presence of direct intercellular contacts between the interactive tissues would suggest a direct inductive signal transmission from one cell to another, rather than a matrix interaction, which has been demonstrated during the morphogenetic period[16,17].

Thus, during the preen gland development, several interaction mechanisms exist at different stages, in different anatomical sites, and they initiate a local differentiation of the uropygial tubules.

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Placental permeability of arsenate ion during early embryogenesis in the hamster[1]

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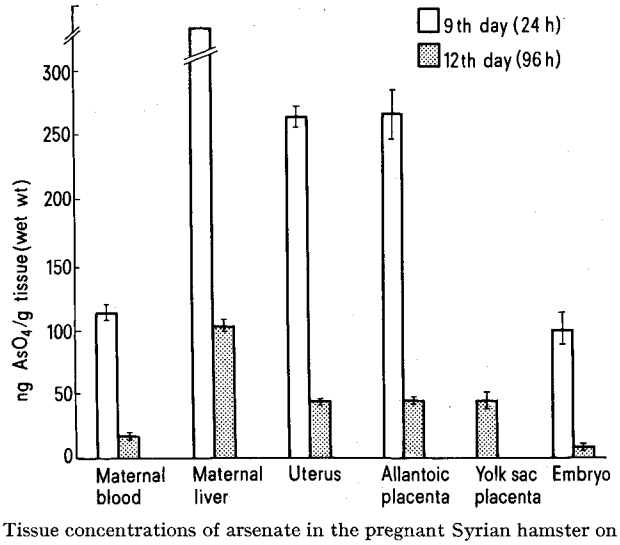
Summary. Sodium arsenate crosses the placental barrier in pregnant Syrian hamsters following injection in teratogenic or tracer doses on the 8th day of gestation.

Arsenate ion produces specific malformations in surviving offspring of hamsters if injected on day 8 of gestation[2]. Teratogenic lesions could result from an effect of arsenate on the maternal system, on placental permeability, or directly on the embryo. Obviously, a direct effect on the developing embryo requires placental transport of the teratogen. We have examined this point with the aid of radioisotopic arsenic (^{74}As). Our data show that ^{74}arsenate crosses the placental barrier when injected in a teratogenic dose, or trace amounts, during the critical phase of organogenesis.

Materials and methods. Procedures generally followed those described by Hanlon and Ferm[3] with regard to timed matings of female hamsters, injection of teratogen, and collection and radioassay of tissue samples. However, a Beckman Biogamma D counter replaced the previously used radiation analyzer system. The whole energy spectrum channel was employed. Counting efficiency was 55%. Sufficient $Na_2H^{74}AsO_4$ (Amersham-Searle) was added to an aqueous solution containing 4.0 mg $Na_2HAsO_4 \times 7 H_2O$/ml to yield an initial calibration of 10 μCi/ml. On the 8th day of gestation, 12 hamsters were injected (sublingual vein) with 0.5 ml of solution/100 g maternal b.wt to give dose levels of 8.37 mg AsO_4/kg. 24 h, and 96 h after injection, 6 animals were killed by chloroform anesthesia and tissue samples taken. Due to the difficulty of separation, 9th day placental tissue includes yolk sac and chorioallantoic placenta together with a small amount (about 15%) of maternal decidua. 12-day-old chorioallantoic placentae could be separated from yolk sac placentae and their radioactivity measured individually. Corrections for the loss of radioactivity in the samples (^{74}As has a half-life of 18 d) were made by comparing sample counts with that of a standard labelled arsenate solution on the day of assay. A limited study of the distribution of radiolabelled arsenate following injection of trace doses was also carried out. 4 hamsters were injected on day 8 of pregnancy with a 0.85% sodium chloride solution containing 12.8 μCi and 75 ng arsenate (AsO_4) per ml. Doses were administered at the level of 0.5 ml solution/100 g of animal, i.e., 375 ng/kg. 2 animals were sacrificed on day 9 and 2 on day 12. The remainder of the procedure followed methods described above.



Tissue concentrations of arsenate in the pregnant Syrian hamster on day 9 and day 12 following injection of radiolabelled arsenate on the 8th day of gestation. Vertical bars represent standard errors of mean values. Experimental conditions are described in the text.

Results and discussion. The distribution of ^{74}arsenate in maternal and fetal tissues of the Syrian hamster following injection of a teratogenic dose is shown in the figure. 24 h post injection significant amounts of ^{74}As appeared in embryonic tissues, indicating that the placentae do not function as completely effective barriers to the passage of arsenate (or some metabolite of arsenate) into the embryo. Arsenate concentrations undergo identical decreases (84%) for maternal blood, uterus, and placentae between 24 h and 96 h. The figures for maternal liver and embryos are 77% and 91%. These data are best explained by a steady state condition in which arsenate rapidly equilibrates between compartments. The overall decrease in concentration is probably due primarily to excretion via the kidneys[4].

1 This work was supported by USPHS grant ES-00697.
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