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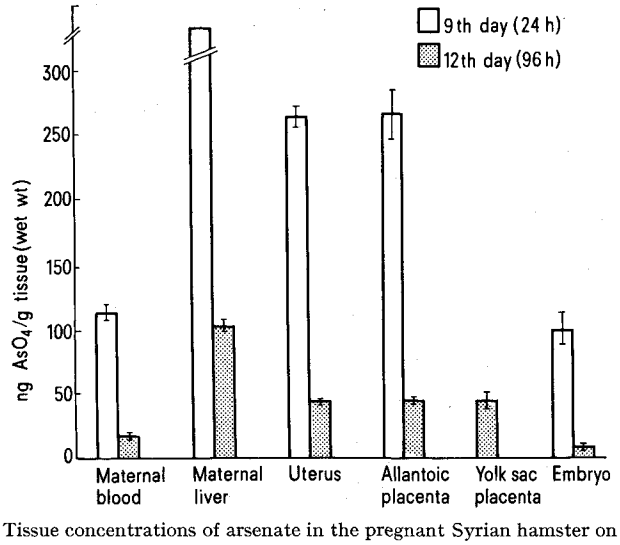
Summary. Sodium arsenate crosses the placental barrier in pregnant Syrian hamsters following injection in teratogenic or tracer doses on the 8th day of gestation.

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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Our limited study of the distribution of a trace dose (375 ng/kg) of arsenate revealed the following: a) day 9 embryos contained detectable amounts of arsenate – approximately 3 pg/g tissue; b) the pattern of arsenate distribution and the fractional decrease in tissue arsenate between day 9 and day 12 were the same as for the teratogenic dose. Thus, arsenate is partitioned in identical fashion for doses of teratogen differing by more than 2100 times. Constancy in the pattern of arsenate distribution over a wide concentration range implies that very little teratogen is bound to macromolecules or other tissue components, contrary to expectations for heavy metal ion teratogens.

The biochemical basis of arsenate-induced teratogenesis may depend on the chemical similarity of arsenate and phosphate. For example, arsenate competes with inorganic phosphate in a variety of metabolic reactions[5] and could interfere with one or more biochemical steps at a critical stage of organogenesis. Alternatively, arsenate might be reduced to arsenite, as Ginsburg has demonstrated in renal clearance studies[4], and further metabolized to an organo-arsenical with teratogenic properties. Regardless of the biochemical mechanism of arsenate-induced teratogenesis, our data show that the teratogen does enter embryonic tissues during organogenesis. These findings, therefore, offer support to Ferm's suggestion that arsenate exercises a specific effect on embryonic cephalic mesenchyme[6].

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Histochemical demonstration of adrenergic nerve fibres in the renal capsule of rats

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Summary. Adrenergic innervation in rat renal capsule was demonstrated using the histochemical fluorescence method with glyoxylic acid.

The fluorescence histochemical method of Falck and Hillarp[2] has revealed adrenergic innervation in the renal blood vessels[3–5] and tubules[6]; however, localization of monoamines in the renal capsule has not been reported. A new histochemical technique using glyoxylic acid instead of formaldehyde has been developed[7,8] and good results are seen with the method using aqueous solution and heating in the stretch preparation of peripheral thin tissues[9]. We attempted to demonstrate adrenergic innervation in the renal capsule using this glyoxylic acid method.

Male Wistar rats weighing 150–200 g were sacrificed under pentobarbital anesthesia. The kidneys with the capsules were rapidly excised and immersed in ice-cold 2% glyoxylic acid solution. Glyoxylic acid monohydrate was dissolved in 0.1 M phosphate buffer adjusted to pH 7.0 with NaOH. 10 min later, the capsules were removed and stretched on clean glass slides. The specimens were dried with a hair-dryer for 20 min, heated at 100 °C for 5–10 min and then mounted with the entellan-xylene mixture. For microscopic analyses, a Zeiss epifluorescence microscope was used with a high pressure mercury lamp as a light source. The excitation filter used was a BG12 and the barrier filter was Zeiss '47' or '50'.

The outer surface of the kidney is covered with a delicate fibrous capsule composed of collagenous and elastic fibres and a few smooth muscle cells. Outside the tunica fibrosa is a fatty layer called the tunica adiposa. Fluorescent nerve fibres with varicosities were found to innervate the hilus arteries of the kidney and their ramifications to enter the renal capsule. The renal capsular arteries were accompanied by 2 main bundles in which ran 2 or more intensely fluorescent fibres and both bundles were connected by a few fine varicose terminals (figure 1). There were 2 types

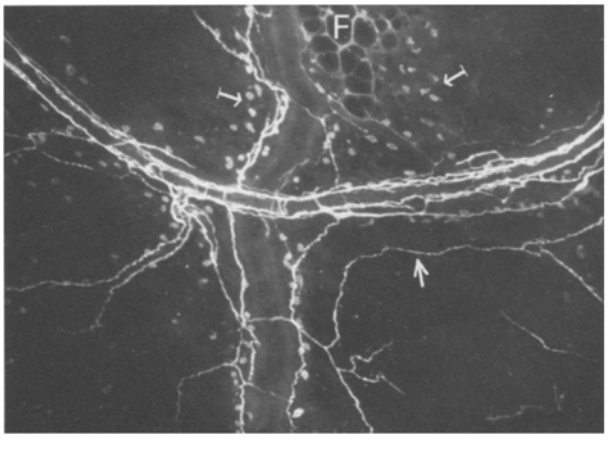

Fig. 1. The renal capsule of the rat. The adrenergic nerve fibres run along the blood vessels, on to the fat cells (F) and freely on the renal capsule (↑). Yellow fluorescent mast cells (↑) were seen around the blood vessels and the fat cells. ×30.

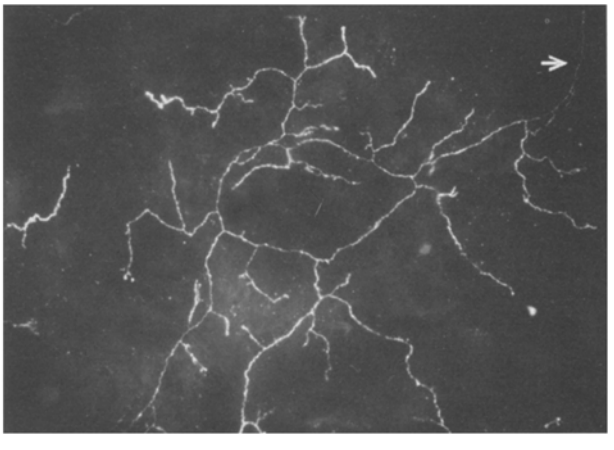

Fig. 2. The tunica fibrosa of the rat kidney. A system of branching terminals arising from one preterminal axon (↑) is visible in the tunica fibrosa. ×30.