

INTESTINAL TRANSPORT OF SUGARS AND AROMATIC AMINO ACIDS IN MARINE FISH AND SELECTED FRESH WATER FISH

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Our studies in the Summer of 1964 have shown that certain aromatic amino acid derivatives were transported across the mucosal membrane of the flounder's intestine against a concentration gradient, while glucose was not transported. It was the aim of the present investigation to pursue this problem in other marine fishes and study the structural specificity of this transport mechanism. The technique of everted intestinal sac or isolated mucosal membrane as described in our previous publications was used. D-galactose and L-tryptophan in different concentrations were placed on both the mucosal and serosal sides. The sacs or mucosal membranes were then incubated at 25°C for an hour. Inulin was used to estimate the flux movement of water. Totally eight species of marine fishes as well as two species of fresh water lungfishes were studied. They can be grouped into 3 classes according to their intestinal transport function.

- I. Both galactose and L-tryptophan were transported in Fundulus heteroclitus, Myoxcephalus octodecimspinosus, Squalus acanthias,* and Lepidosiren paradoxa*
- II. L-tryptophan was transported, but galactose was not transported in Pseudopleuronectes americanus, Hemitripterus americanus, Pollachius virens, and Macrozoares americanus
- III. Neither galactose nor L-tryptophan was transported in Cyclopterus lumpus or Propterus aethiopicus

The structural specificity of sugars and amino acids was further studied on the intestine of the Fundulus heteroclitus. It was found that D-glucose, D-galactose and 3-O-methylglucose were transported across the intestinal mucosa against a concentration gradient, but levulose, mannose, D-xylose, sorbose and alpha-methyl-glucoside were not transported. The amino acids, L-tryptophan and L-tryosine, were transported, but D-tryptophan was not transported against a concentration gradient. The transport of L-tryptophan was temperature dependent and was inhibited by L-phenylalanine and 2,4-DNP.

FINE STRUCTURAL ACCOMMODATIONS FOR MATERNOFETAL EXCHANGE IN Squalus acanthias†

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The purpose of the summer's work has been to examine electron microscopically the membranes of the dogfish across which pseudoplacental/placental exchanges take place. In the spiny dogfish, fetal and maternal contributions to the exchange system are not closely apposed; and it has been generally assumed that the system functions solely in respiratory exchange. A better knowledge of the changes which allow for such functional adaptation, both in the maternal mem-

*The technique of mucosal membrane was used in these two fishes.

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brane (viz., the pregnant endometrium) and in the fetal pseudo-placenta (viz., the vascularized yolk sac) will help to elucidate problems in placental transport in general. Such a study fits into a comparative survey, which is being made by the investigators, on materno-fetal exchange systems in various ovoviviparous and viviparous vertebrates (1-5).

The specific aims of the current investigation are two: First, the ultramorphology of endometria of nonpregnant specimens will be compared with those of early pregnancy, late pregnancy and post-partum uteri. Fine structural alterations which convert the uterine mucosa into a "pseudoimplantation" substrate and maternal pseudoplacenta will be compared with recent studies on ultrastructural changes in the endometrium of mammals during decidualogenesis (6;7). Second, the fine structure of the yolk sac at two different stages, viz., 1) a stage at which the embryo is about one inch long (ca. 3 months of gestational age); and 2) a stage near term, (ca. 20 months) will be compared to determine fine structural changes in this portion of the barrier with increasing gestational age. Age changes which have been observed in the placentas of higher forms (1;4), are believed to reflect changes in transport function.

Materials and Methods: To date 21 mature female dogfish have been autopsied. These were categorized as follows: 3 non-pregnant, 8 pregnant in the egg candle stage; 9 pregnant near term (i.e., with fully-formed, free-living pups); and 1 post-partum. A final group of specimens will be autopsied late in August to procure additional post-partum specimens.

For electron microscopy, small pieces of uterus and yolk sac (the latter only in late egg candle and in term pup stages) were fixed cold for 2 hours in 5% gluteraldehyde, buffered to pH 7.5 with 0.1M phosphate and rendered isosmotic with dogfish plasma with sucrose. They were post-fixed for 2 hours in buffered, sucrose, 1% osmium tetroxide and embedded in British araldite. Thin sections were cut on a Porter-Blum ultramicrotome and mounted on copper grids.

For optical microscopy, similar pieces of tissue were fixed in formol-sublimate-dichromate, followed by post-chromation, or in Bouin's picric acid-formol and were embedded in paraffin.

Electron microscopic grids and light microscopic slides will be examined in the fall when the investigators have returned to Tulane, the former with an RCA EMU-3G.

Discussion: Examination of the tissues which contribute to the formation of placental-like membranes in the dogfish raises the question of the degree of viviparity attained by this species. The degree of viviparity achieved by different elasmobranchs varies; some are strictly ovoviviparous, others, e.g., Mustela, are partly truly viviparous. The status of Squalus acanthias in this regard is not known. From a gross comparison of the total mass of term pups (neonate plus appended yolk) to the mass of the fertilized egg in the candle stage, it is evident, however, that there is an increase in mass during gestation. Such increase implies that, at least at certain stages of pregnancy, in addition to respiratory needs, embryonic nutritive requirements are transported across the placenta as well.

It is hoped that in a continuing program for studying accommodations for materno-fetal exchange in this species, determinations both of the degree of viviparity and of the mode of qualitative selection of transplacental exchange can be made. Such determinations could feasibly be undertaken by the injection of tracer materials at varying gestational ages into 1) the maternal circulation, 2) the uterine lumen, and 3) the yolk sac cavity. In our laboratory at Tulane, placental passage of tracers has effectively been visualized in the rat, both by using electron opaquely tagged colloidal particles, the passage of which can be observed directly under the electron microscope (8-10), and by injecting radioisotopically labeled metabolites, followed by

radioautography (11;12). In addition, by applying standard cytochemical techniques at the e/m level to the placental barrier of the dogfish during such visualized transport, it will also be possible to correlate localization of specific enzymatic activities with mechanisms of transplacental exchange.

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MICROSPECTROPHOTOMETRIC STUDY OF CHLORPHENOL RED TRANSPORT IN ISOLATED RENAL TUBULES OF THE FLOUNDER*

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In two recent studies employing *in vitro* techniques the efflux (run-out) of pre-accumulated organic anions (Diodrast and p-aminohippurate) from renal tissue (goldfish and dog) was accelerated by the presence of low concentrations of competitors in the efflux medium and decelerated by higher concentrations of the same competitors (Kinter and Cline, *Amer. J. Physiol.*, 201: 309, 1961 and Farah, *et al.*, *J. Pharm. and Exptl. Therap.*, 139: 120, 1964). If it were certain that the pre-accumulated anions were leaving from tubular fluid, i.e., not being displaced from intracellular binding sites, then these biphasic effects would constitute strong evidence that the efflux process was more complex than simple diffusion and involved a "carrier" of the sort generally proposed for the uphill transport producing accumulation. To clarify this point, direct photometric measurement of anionic dye efflux from luminal fluid was undertaken with flounder tubules.

The original isolated flounder tubule preparation was developed by Forster (*Science*, 108: 65 1948). Luminal concentration of anionic dye, initially phenol red and later chlorphenol red, was estimated by eye (zero to 4+) using a standard microscope. With this method Hong and Forster

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