

water and urea data from Table 1); telencephalon = 0.1; and spinal cord = 0.04. The reported value for the rabbit choroid plexus (Am. J. Physiol. 205: 617-624, 1963) is comparable to the above estimate for the dogfish choroid plexus whereas the mammalian values for similar CNS regions are five to 10 times those for the dogfish (J. Appl. Physiol. 27: 296-300, 1969).

The data presented in this study suggest that a special transport system for urea exists in the choroid plexus of the dogfish but not in the capillary endothelium of the telencephalon and spinal cord. Furthermore estimates of blood flow to these tissues indicate that the choroid plexus is perfused at a surprisingly high rate and that the neural tissue is perfused at a much lower rate.

#### ACKNOWLEDGEMENTS

The authors thank Ms. Leslie Hogben for her careful calculations of the data.

1973 #15

#### GILL PERMEABILITY TO LIPID-INSOLUBLE MACROMOLECULES IN THE DOGFISH

*Squalus Acanthias*

J.A. Fishman and G.G. Pietra, Departments of Pathology and Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

Previous observations (Pietra, G.G. et al. Science 166:1643, 1969; Schneeberger, E.E. and Karnovsky, M.J., J. Cell Biol. 49:319, 1971) using lipid-insoluble macromolecular tracers in a variety of mammalian species have shown that the permeability of inter-endothelial junctions in the pulmonary microcirculation may be strongly modified by hemodynamic influences. Thus the minute pulmonary vessels that are normally impermeable to hemo-proteins of varying molecular weights (myoglobin, m.w. 18,000; horseradish peroxidase, m.w. 40,000; hemoglobin, m.w. 68,000; catalase, m.w. 240,000) become exceedingly permeable at high pulmonary capillary pressures. In contrast to the abrupt and striking change in permeability at high pulmonary capillary pressures is the gradual change that occurs in muscle capillaries: at normal capillary pressures myoglobin and hemoglobin traverse the inter-endothelial junctions slowly and their rate of passage increases gradually as capillary pressures are increased (Pietra, G.G. unpublished observations). Despite these differences the results on both the pulmonary and systemic microcirculations contradict the conventional concepts of rigid endothelial pores (Landis, E.M. and Pappenheimer, J.P., Handbook of Physiol., Circulation 2:961, 1963) and favor the hypothesis that capillary walls are permeated by distensible pores that can be stretched by physical (hemodynamic) or other influences (Shirley, H.A. Jr., et al. Amer. J. Physiol. 190:189, 1957).

In the present study we investigated the permeability of the microvasculature of the gills at different pressures using horseradish peroxidase and ferritin as tracers. Two major considerations prompted this investigation: the anatomical and hemodynamic similarities between the microcirculations of the gill and of the lung: the dependence of the dogfish for survival on impermeability of

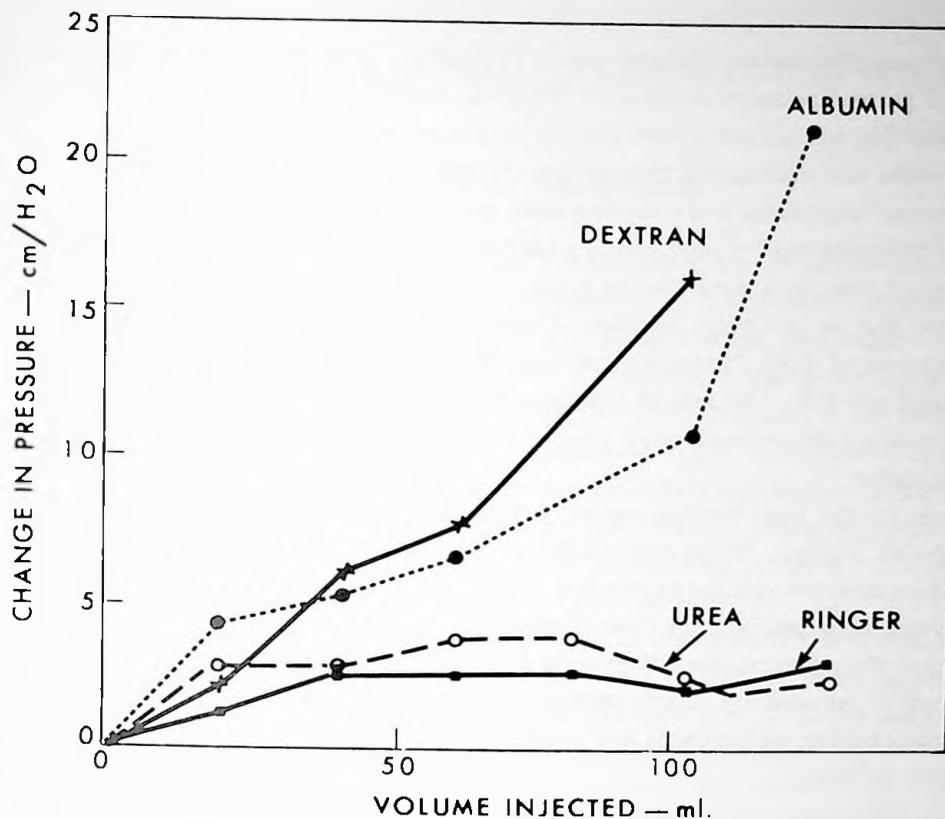


Figure 1: Effects of various injectates on blood pressure in the ventral aorta. In contrast to urea and Ringers solution, dextran and albumin elicited considerable increments in blood pressure.

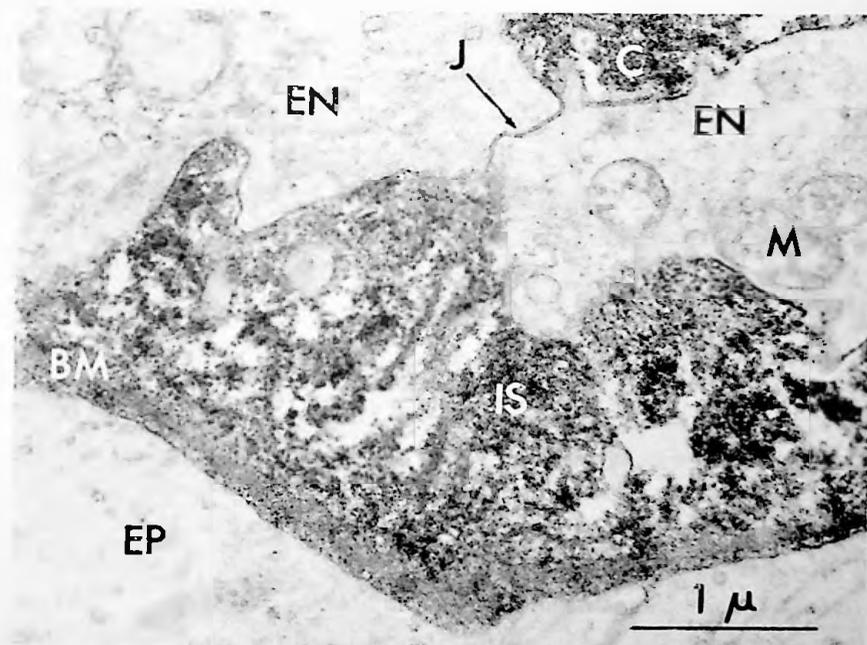


Figure 2: Electron micrograph of gill capillary showing horseradish peroxidase in the lumen of the capillary (C) and in the pericapillary interstitial space (IS). Horseradish peroxidase permeates the junction (J) between two adjacent endothelial cells (EN). The plasmalemmal vesicles appear free of the tracer. Thus horseradish peroxidase traverses the capillary wall solely via the interendothelial junctions.

Only a small segment of epithelium (EP) is shown in this figure. In other electron micrographs, the horseradish peroxidase was seen penetrating part of the way between epithelial cells but arresting at the level of the tight junctions.

Section reacted with 3,3' diamino-benzidine and stained with uranyl acetate and lead citrate.

its gills to urea.

Successful experiments were performed in 25 dogfish. Anesthesia was accomplished by immersing the fish in a 0.5 percent solution of MS-222 (Ethyl-m-Aminobenzoate Methanesulfonate, Eastman). The anesthetized fish was placed ventral side up on a narrow shallow tank and a continuous flow of cool fresh sea water was maintained throughout the experiments by cannulae introduced through the opercular openings. Injections and sampling were done via the caudal vein. Blood pressures were measured after thoracotomy by connecting a cannula in the ventral aorta to a water manometer.

Five different injectates were used as volume expanders: urea (20 percent solution); elasmobranch Ringers (Forster, R.P. et al, *Comp. Biochem. Physiol.* 42A:3, 1972); isotonic elasmobranch saline; dextran (4.5 percent of T-70, Pharmacia); and egg albumin (two percent in dogfish Ringers). One injectate was used per fish. The rate of injection was controlled to deliver 120 ml of injectate within five minutes; repeated determinations of pressure were made after each injection of 20 ml and for 30 minutes thereafter.

Two tracers are the basis for this report: T-1824 (Evans blue, Fisher) and horseradish peroxidase (20 mg/kg, type VI, Sigma). In the control fish the tracer was injected into the caudal vein taking special precaution to avoid volume expansion. During the test period the same procedure was followed after volume expansion has been completed.

At the close of each experiment segments of gill arches were removed between ligatures for optical and electron microscopy. Each segment was promptly immersed in gluteraldehyde (2.5 percent in cacodylate buffer, 456 mOsm) and was further trimmed to smaller size within a few hours in order to improve fixation.

Preliminary observations by optical microscopy after injection of T-1824 (Gersh, I. and Catchpole, H.R., *Amer. J. Anat.* 85:457, 1949) both unbound and saturated with albumin indicated that volume expansion increased the permeability of the gills. Subsequent observations involved hemodynamic measurements and electron microscopy. The effects of injectates on pressures in individual fish are illustrated in Figure 1. A diverse range of increments in pressure were elicited ranging from only a few cm H<sub>2</sub>O after urea or Ringers solution or elasmobranch saline up to 23 cm H<sub>2</sub>O after albumin.

The ultrastructural studies using horseradish peroxidase indicated that at normal pressures the permeability of the microvasculature of the gill resembles that of mammalian muscle capillaries rather than mammalian pulmonary capillaries. Thus tracer was seen traversing inter-endothelial junctions to enter the interstitial space (Figure 2). However passage was slow and inconsistent so that not all capillaries were affected. However as pressures were increased the rate of passage and the numbers of capillaries involved appeared to increase. Even at the higher pressures the leakage remained focal. In contrast to the permeability of the microvasculature the epithelium of the gill did not become permeable to the tracer.

These preliminary observations using horseradish peroxidase indicate that even under normal conditions the microvasculature of the gills is permeable to lipid-insoluble macromolecules and that the inter-endothelial junctions traversed by the macromolecules are distensible. Our unfinished observations using ferritin as a tracer may provide additional insight into the macromolecular size that can traverse the gill capillaries under the different conditions of the present experiments. It should be noted that these observations provide no information concerning the mechanism by which the macromolecules entering the interstitial space are restored to the circulation.

This work was supported by a grant from the National Heart and Lung Institute, National Institutes of Health (HE-08805).