

coloration and turned silver, resembling the silver hue of eels spontaneously migrating to the sea. Freshwater eels prepared by 10 days of hydrocortisone injections (400  $\mu$ g/100 gm/day) withstood sudden transfer to full strength seawater without preliminary residence in 50% seawater, and their plasma chloride did not rise as high as that of control eels transferred abruptly to full strength seawater (W. MacKay, report #17 this issue).

The data indicate that hydrocortisone induces a series of changes in freshwater eels, including a rise in Na-K-ATPase of gill and intestine, that successfully prepares these euryhaline teleosts to combat the osmotic stress of migration to seawater.

1969 #7

RELATION OF SALT AND WATER TRANSPORT TO FINE STRUCTURE OF THE INTESTINE AND GILLS OF THE EEL, Anguilla rostrata AND THE DOGFISH, Squalus acanthias

W. L. Doyle, The University of Chicago, Chicago, Ill.

(1) The spiral valve of the dogfish was isolated and the lumen and artery perfused with saline to establish conditions of maximal and minimal rates of water transport across the mucosa. Under such conditions the morphological configuration of the intercellular space in the epithelia should show correlative change. An additional factor is the intraluminal hydrostatic pressure and the experimental procedure was designed to determine the effects of changes in this parameter. Specimens of these preparations were fixed and embedded for electron microscopy. They are currently being studied.

(2) The transfer of eels from fresh to salt water is accompanied by increased salt and water transport in the gills and gut. In collaboration with Franklin Epstein, Ralph Janicki, and William MacKay samples were obtained for electron microscopy of the gills and the proximal gut to provide morphological correlates of the functional states observed by them. The existence of elevated Na-KATPase levels in gills of fresh water eels treated with hydrocortisone (see Epstein this bulletin) should provide interesting comparisons with corresponding levels of enzyme activity and the morphological changes usually associated with animals adapted to sea water.

(3) Preliminary observations on the fine structure of mesothelia found on dogfish mesentery indicate the presence of junctional complexes of the kind found in gill epithelia (close junction, zonula adherens and maculae densa). Other aspects of the fine structure may provide clues to the low rates of absorption of drugs and metabolites from the peritoneal cavity in this animal.

This work was supported by a grant from the National Science Foundation, GB 8312.

1969 #8

THE TRANSPORT OF VARIOUS ORGANIC MATERIALS FROM BLOOD TO TISSUE IN Squalus acanthias

Joseph Fenstermacher, Andres Roomet, Jeffrey Ratner, and David P. Rall, National Cancer Institute, Bethesda, Md. 20014

Studies of the blood to tissue transport of eight organic compounds have been undertaken in

Squalus acanthias. The molecules chosen covered rather a broad spectrum of molecular sizes and lipid-water solubilities. By selecting such a group of molecules, information could be obtained concerning capillary permeability, size of the extracellular space, and cellular permeability of the various tissue. The compounds used, with their molecular weights (MW) and approximate olive oil: water partition coefficient (PC) noted in parentheses, were:  $^{14}\text{C}$ -inulin (MW = 5,000, PC < .00003);  $^{14}\text{C}$ -sucrose (MW = 342, PC = .00003);  $^3\text{H}$ -mannitol (MW = 182, PC = .00003);  $^{14}\text{C}$ -antipyrine (MW = 188, PC = .05);  $^{14}\text{C}$ -thiourea (MW = 76, PC = .002);  $^3\text{H}$ -ethylene glycol (MW = 62, PC = .00049);  $^{14}\text{C}$ -urea (MW = 60, PC = .00015); and  $^3\text{H}$ -water (MW = 18, .0007). Cardiac muscle, skeletal muscle, cerebrum, medulla, and cerebrospinal fluid (CSF) were the tissues and fluid studied.

A constant or nearly constant blood level of the radioactive compound was achieved by a combination of an initial priming injection and subsequent intramuscular sustaining injections. Plasma samples were taken at regular intervals to monitor this. Animals were sacrificed at various times, ranging from 10 minutes to 24 hours. Tissues and fluid samples plus a final plasma sample were obtained at the time of sacrifice. All samples were analyzed for radioactivity by liquid scintillation spectrometry. All spaces reported in this note are calculated by dividing the sample radioactivity per milligram by the final plasma sample radioactivity per microliter. No corrections for residual blood volume or tissue water content were applied to the numbers reported herein.

Antipyrine and water would be expected to equilibrate with most tissues and CSF rapidly. Indeed this was observed for cardiac muscle, brain, and CSF. Equilibration with plasma water was essentially complete within ten minutes. Water distribution spaces of 75-80% were obtained for the tissues indicating equilibration with both extracellular and intracellular water. With antipyrine the time to achieve apparent equilibrium was longer (between 10 and 30 minutes) and the final level attained was higher (90 to 110%) for these same tissues and the CSF. Of these tissues equilibration of  $^3\text{H}$ -OH and antipyrine seemed to be most rapid for cardiac muscle. As suggested above, there was an appreciably slower rate of uptake of antipyrine and water by skeletal muscle. After 30 minutes the skeletal muscle levels for these two compounds were about 75% of their anticipated equilibrium levels and still rising. It is likely that this is a result of relatively slow cellular uptake. No later results were obtained.

Inulin, sucrose, and mannitol are generally considered to cross the capillary endothelium readily, equilibrate with the tissue extracellular fluid rapidly, and not enter cells to any appreciable extent. For these reasons they are often used as extracellular space markers. The results with mannitol indicate that this is certainly the case for cardiac and skeletal muscle of Squalus. Twenty minutes after injection the mannitol spaces for both type of muscle reached a maximum level and remained constant for as long as 22 hours. The cardiac muscle mannitol space was about 28%; the skeletal muscle mannitol space was 8-9%. Nearly identical results were obtained with sucrose in cardiac and skeletal muscle, although in the former tissue the sucrose space may be several percent lower than the mannitol space. The inulin data for these muscle tissues is incomplete at present; but they appear to be similar to those of mannitol and sucrose. Uptake of these three compounds by cerebrum, medulla, and CSF, is quite slow. The tissue levels after 18 to 24 hours appear to still be rising. At this time the respective levels of cerebrum, medulla, and CSF (expressed as a percent of the plasma level) are: for mannitol - 12%, 10%, and 37%; for sucrose - 13%, 9%, and 27%, and for inulin - 8%, 5%, and 9%. These are

all well below what we have calculated by other methods as the potential equilibrium levels for these compounds in brain and CSF.

Urea, ethylene glycol, and thiourea are molecules of similar molecular weight but of differing lipid solubility, urea being the least lipid soluble and thiourea the most of these three. Equilibration of ethylene glycol between blood and tissue or CSF was essentially complete within four hours with the exception of skeletal muscle which again appeared to be more "impermeable" than the other tissues. For urea equilibration between blood and cardiac muscle was very rapid, being essentially complete in 30 minutes. Cerebrospinal fluid urea equilibration was complete in about one hour. Blood urea appeared to equilibrate with skeletal muscle in about 4 hours (distribution space = 90%) while cerebrum and medulla urea levels were still rising after 22 hours. The results with thiourea are very similar to those with urea except that CSF seemed to equilibrate more slowly with the plasma thiourea and skeletal muscle appeared to equilibrate more rapidly.

1969 #9

#### RENAL HEMODYNAMIC VALUES IN THE SKATE (Raja erinacea)

Roy P. Forster and Leon Goldstein, Dartmouth College, Hanover, N. H. and Brown University, Providence, R. I.

Except for the dogfish, Squalus acanthias, little or nothing is known about standard renal hemodynamic values in elasmobranchs that could be used for comparisons with the more extensively studied teleosts and other aquatic vertebrates. In the course of a series of environmental dilution experiments on the common small skate, Raja erinacea, several individuals turned up with plasma levels of p-aminohippurate (PAH) in a range appropriate for measuring minimal renal plasma flow (RPF) under control conditions and after adaption to 50% sea water. The essential findings on two of these are presented in Table 1.

Table 1

#### URINE VOLUMES, GLOMERULAR FILTRATION RATES, RENAL PLASMA FLOWS AND FILTRATION FRACTIONS IN SKATES MAINTAINED IN 100% AND IN 50% SEA WATER

Hours	ml per kg x hr			FF
	$\dot{V}$	GFR	RPF	
1.13 kg skate in 100% sea water				
6.75	.26	.45	30.8	.015
6.15	.22	.40	34.3	.012
10.50	.19	.50	40.4	.012
1.5 kg skate in 50% sea water				
3.25	2.06	5.80	67.0	.087
2.75	2.00	5.70	85.7	.067