

The rather surprising finding that none of the very lipid soluble drug is excreted by the gill is explicable on the basis of its extremely low water solubility (1 ng/ml), with free drug being reduced even more by plasma binding, now under investigation. Thus, virtually no free drug is available for either gill or renal excretion.

The liver of this species, which contains approximately 50% fat, is an obvious depot for drug, and is found to sequester it quantitatively.

Preliminary studies showed that there is marked toxicity at 10 mg/kg i.v., of a delayed type. Fish became rigid and paralyzed. Additional studies are planned to investigate the toxicity in more depth.

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1970 #10

#### THE DISTRIBUTION OF VARIOUS POLAR MATERIALS BETWEEN BLOOD AND TISSUE IN Squalus acanthias

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The tissue distribution of nine intravenously administered polar materials were studied in Squalus acanthias. By choosing materials of various sizes and lipid solubilities, information can be obtained concerning the size of the vascular and extracellular space (ECS) and the permeability of the capillaries and cells for the various tissues. The materials used, with their molecular weights (MW) and approximate olive oil:water partition coefficients (PC) noted in parentheses, were:  $^{14}\text{C}$ -dextran (MW  $\cong$  60,000; PC  $<$  .00003),  $^{14}\text{C}$ - and  $^3\text{H}$ -inulin (MW  $\cong$  5,000; PC  $<$  .00003),  $^3\text{H}$ - and  $^{14}\text{C}$ -sucrose (MW = 342; PC = .00003),  $^3\text{H}$ -mannitol (MW = 182; PC = .00003),  $^{14}\text{C}$ -thiourea (MW = 76; PC = .002),  $^3\text{H}$ -ethylene glycol (MW = 62; PC = .00049),  $^{14}\text{C}$ -urea (MW = 60; PC = .00015),  $^{36}\text{Cl}^-$  (MW = 36; PC = ?), and  $^3\text{H}$ -water (MW = 18; PC = .0007). Skeletal muscle, heart, intestine, rectal gland, pancreas, and liver were the tissues examined. In addition, pericardial fluid (PCFO) and bile were sampled.

A constant or nearly constant blood level of the radioactive material was achieved in un-anesthetized, freely swimming dogfish by a combination of intravenous and intramuscular injections. Plasma samples were taken at regular intervals to monitor this. Animals were sacrificed from 10 minutes to 24 hours after the initial intravenous injection, and samples of the various tissues and fluids were rapidly obtained. Tissue samples (20-40 mg) were digested in Nuclear Chicago solubilizer to which liquid scintillation fluid was then added. All samples were analyzed for radioactivity by liquid scintillation spectrometry with appropriate corrections for quenching. All spaces are calculated by dividing the sample's radioactivity per milligram by the final plasma sample's radioactivity per microliter x 100. Determinations of total tissue or fluid water were made by comparison of wet and dry weights. Binding of the labeled materials to plasma proteins was checked by ultrafiltration and was found to be negligible.

Skeletal muscle. The ratio of tissue water to plasma water was 0.77 for this tissue. The dextran space (plasma space) equaled 1%. The distribution spaces at 20 hours were 8% for inulin, 11% for sucrose, 10% for mannitol, and 12% for chloride. The chloride space reached this level at 30 minutes and remained constant thereafter. Longer periods of time (4-20 hours) were re-

quired for mannitol, sucrose, and inulin to reach steady tissue levels. It appears that the size of the skeletal muscle ECS is 8-11%. The 20 hour distribution spaces for ethylene glycol and urea are virtually equal (75-80%) and match the skeletal muscle water space; however, the uptake of ethylene glycol is somewhat more rapid than that of urea. The thiourea results are more equivocal, but suggest a similarity in uptake rates between thiourea, urea and ethylene glycol; however, a larger distribution space (95%) was found for thiourea. The distribution of  $^3\text{HOH}$  was only followed for 30 minutes. At this time equilibration of labeled water with tissue water is not complete.

Heart. The tissue water to plasma water ratio is 0.87 for the heart. The dextran space is 11%. The inulin space at four hours is 22% and remains constant thereafter. Sucrose (26%), mannitol (28%), and chloride (28%) all give somewhat larger spaces than inulin. The apparent rates of uptake for the latter three materials are more rapid than for inulin; an apparent tissue-plasma steady state is obtained for them in one hour or less. The distribution of urea and ethylene glycol in the heart is very similar. A tissue steady state space of 85-90% is reached at one hour. The thiourea space appears to be around 100% at both one hour and four hours. Tritiated water appears to equilibrate with tissue water in about 30 minutes.

Pericardial fluid. The fluid:plasma water ratio is 1.03 for PCF. The fluid:plasma concentration ratios for inulin, sucrose, and mannitol after twenty hours are less than 5%. At this same time the chloride, urea, and ethylene glycol ratios are 30%, 40%, and 95%, respectively. Tritiated water appears to equilibrate with this fluid within ten minutes.

Intestine. The tissue:plasma water ratio is 0.84, and the dextran space is 6% for the intestine. Constant tissue distribution spaces for inulin (24%), sucrose (31%), mannitol (32%), and chloride (37%) are reached within 30 minutes. Urea and ethylene glycol reach a tissue steady state space of 80-83% at one hour. Thiourea appears to enter the intestine more rapidly than the latter two compounds and gives a higher distribution space (94%). Labeled water equilibrates with the water of the intestine in about 30 minutes.

Rectal gland. The tissue:plasma water ratio for the rectal gland is 0.83. The dextran space equals 13%. The inulin distribution space reaches 24% at four hours and remains constant thereafter. Likewise, the sucrose and mannitol spaces become steady at four hours, but the spaces are higher (28-30%). Within 30 minutes the chloride space of the rectal gland becomes constant; the size of the steady state chloride space is 37%. The uptake of  $^3\text{HOH}$ , urea, and ethylene glycol is very rapid. Spaces of 80-83% were measured at 10 minutes for these three materials and the spaces remained constant thereafter. The thiourea spaces at 10, 30, and 60 minutes were all around 100%.

Pancreas. The ratio of tissue water to plasma water is 0.83 for the pancreas. The inulin and sucrose spaces from one hour to twenty hours were 15% and 19%, respectively. The mannitol and chloride spaces at twenty hours were around 30%. This tissue level was reached within one hour for chloride; however, longer periods of time (4-20 hours) were required before the mannitol space attained this level. The urea and ethylene glycol spaces were approximately 80% at one hour and remained constant thereafter. Tritiated water seemed to equilibrate with tissue water in about 10 minutes.

Liver. The tissue:plasma water ratio is 0.29 for liver. The distribution spaces for inulin, sucrose, and mannitol at 20 hours were 6%, 8%, and 22%, respectively. The chloride space was 12% from 10 minutes through 20 hours. The urea and ethylene glycol spaces were virtually equal

with the water space at 60 minutes (about 30%) and remained constant thereafter. Equilibration of  $^3\text{HOH}$  between plasma and tissue water was complete within 10 minutes.

**Bile.** The ratio of bile water to plasma water is 0.78. Virtually no  $^{14}\text{C}$ -inulin was present in the bile after 20 hours. The space at 20 hours for sucrose, mannitol, and chloride ranged from 5 to 22%; ethylene glycol and urea had 35% and 78% spaces, respectively, at this time. At 30 minutes the  $^3\text{HOH}$  space was considerably less than the bile water space.

1970 #11

#### TRANSPORT OF SODIUM AND Na-K-ATPase IN GILLS OF Anguilla rostrata DURING ADAPTATION TO SALTWATER: THE EFFECT OF PRETREATMENT WITH CORTISOL

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A feature of saltwater adaptation in the European eel, Anguilla anguilla, is reported to be a prompt increase in outflux of isotopic sodium across the gill when freshwater eels are immersed in seawater, so that within 24-48 hours the rate of efflux characteristic of fully adapted saltwater fish is attained. The present studies indicate that the course of adaptation to seawater by the American eel, Anguilla rostrata, is considerably more prolonged, permitting a close analysis of the relationship between Na-K-ATPase activity and gill transport of  $\text{Na}^+$ , at a time when both are changing.

Hypophysectomy of saltwater eels results in a lowered sodium outflux across the gills and an elevated serum sodium which may be restored to normal by either ACTH or cortisol. In freshwater Anguilla rostrata, treatment with cortisol induces a rise in gill Na-K-ATPase similar to that elicited by transfer to saltwater (Bull. MDIBL, 9:9-10, 1969). The present experiments were designed to measure both the specific activity of Na-K-ATPase and the gill transport

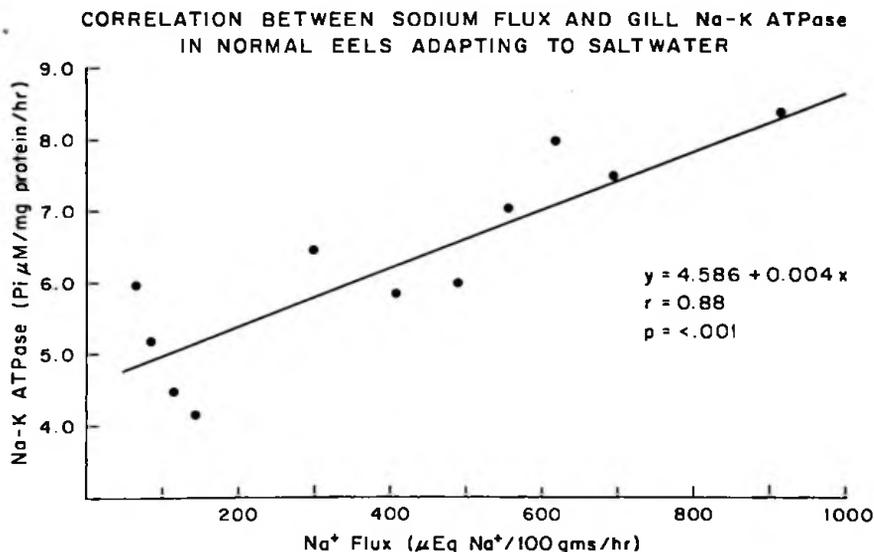


Figure 1