rate of secretion into the lumen. Concentrations in the tissue of inulin and PAH originating from the lumen were also measured. The PAH concentration in the tissue was .57 times that in the perfusion fluid, whereas inulin in the tissue was .04 times that in the perfusion fluid. Thus, both the luminal and pertibular membranes provide significant barriers to PAH loss from the lumen.

Glycine transport was studied in 8 experiments using glycine-H^3 and glycine-C^{14}. Equal concentrations (5×10^{-5} M) of glycine were placed in the perfusion fluid and external bath with a different radioactive label in each solution. Glycine concentration (estimated as the sum of C^{14} and H^3 activities) was .31 times as high in the collected as in the perfused fluid, consistent with net glycine absorption from the lumen. Movement of glycine from bath to lumen was small (concentration ratio .11, tubule fluid/bath). Tissue glycine concentration was 25 times greater than in the bath (or original perfusion fluid) suggesting that active transport into the cells from the lumen is a step in amino acid absorption. However, a large fraction of the glycine in the tissue (76%) originated from the bath suggesting that there is also transport into the cells across the peritubular border.

Glucose transport was studied in a similar manner. With 1.4 to 5.5 mM glucose in both the bath and perfusion fluid, glucose concentration in the fluid collected from the tubule lumen was .50 times that perfused, indicating net absorption (8 experiments). Movement of glucose from bath to lumen was small (concentration ratio .11, tubule fluid/bath). In contrast to the findings with glycine, however, concentrations of radioactivity in the tissue were relatively low and differed according to the position of the radioactive label in the glucose perfused. When uniformly labeled glucose was initially present in the perfusion fluid, total concentration of radioactivity in the tissue was 4.9 times that in the surrounding fluids (3 experiments), but with C-1 or C-6 labeled glucose this ratio was only 1.8 (5 experiments). The difference is due to the retention of products of glucose metabolism in the tissue from the uniformly labeled glucose, as was confirmed by paper chromatography.

When an isotonic Na-free perfusion fluid ($MgSO_4$ plus $CaSO_4$) was used, there was no inhibition of either glycine or glucose transport. This finding, however, does not rule out the possibility that the transport processes are Na-dependent since Na entered the tubule lumen from the bathing medium in large quantities during perfusion. This was indicated by measurements with Na^{22} and also by the occurrence of net fluid movement into the lumen under these conditions (inulin ratio .67 collected/perfused in 4 experiments). This high Na permeability is comparable to that found in mammalian proximal tubules.

1967 #5

SOME PARAMETERS FOR THE DOGFISH, Squalus acanthias

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Some general parameters for the spiny dogfish as determined here at the Laboratory are presented in three tables. Table 1 is self-explanatory, although one is cautioned that values may vary widely. Table 2 is an effort to organize the dogfish in terms of organ weights, tissue water, and organ water pools. In Column 1 are the organ weights for a single fish. No effort was made to idealize the fish or select an archtype. Weights from two other fish are given for comparison

Table 1

Blood flow	1.60 ± 1.00 1/kg-hr	(M & R)
Blood volume	4.5 ± 6.5% body weight	(M & R, self)
Total body water	66 ± 7% body weight	(M & R)
Extracellular space	20 ± 4% body weight	(M & R)
Intracellular water	45 ± 6% body weight	(M & R)
Skin area	891 - 738 cm^2/kg (2.5 & 5.0 kg fish)	
Gill area	3700 cm^2/kg	(Bo)
Gill water flow	25 1/kg-hr	(M & R)
Oxygen consumption	1.7 mmol/kg-hr	(R & M)
Carbon dioxide production	1.5 mmol/kg-hr	(R & M)
Respiratory exchange ration	0.98 ± 0.1	(R & M)
Metabolic water	0.025 ml/kg-hr	(R)
Water influx via gills	1 ml/kg/hr	(Bo)
Urine flow	0.5 ml/kg-hr	
Glomerular filtration	2-5 ml/kg-hr	
Rectal gland flow	0.5 ml/kg-hr	
Bile flow	0.08 ml/kg-hr	
Cerebral ventricular fluid production	0.05 ml/kg-hr	(O)

(Bo, Boylan; M, Murdaugh; O, Oppelt; R, Robin)

in Columns 2 and 3. The percentages of body weight agree quite well between the individual fish except for the liver; the female tract (due to number and size of ovarian eggs); gut contents; and uterine contents, especially in the pup stage when large volumes of uterine fluid exist which readily leak on handling (this latter is not evident from the table).

Certain organs are very mixed tissues. The gills as here recorded are not only lamellae but the septa and septal cartilages. Gill bars and opercula are not included. The skeleton includes cartilage, notochord, heavy connective tissue, cranial "jelly," teeth and dorsal spines. One notes the dogfish is almost two-thirds skin, skeleton, and muscle. Since pregnant females are widely used experimentally, one must remember that the female apparatus and embryos equal about 10 percent of the total body weight. One can ask parenthetically whether or not blood volume and gas exchange hypertrophies to handle the embryos.

Column 4 gives the percentage of water in various tissues. This water is a complex of cellular water, extracellular water, and blood water. The tissues analyzed were from fish bled by caudal vessel puncture. The fish in Column 1 yielded 86 ml whole blood. Assuming that the various tissues have a proportionate amount of blood, one can correct the Column-values for water by calculating the animal with all its blood or minus its entire blood volume. However, not all blood is in the tissues since some blood is in the larger vessels. Using another 2.7 kg fish, we gently extracted 54 ml blood from the aorta and cardinal veins under visual control through the opened abdomen. If one assumes arbitrarily that this blood was in the larger vessels, and subtracts it from a calculated blood volume of 180 ml, one has 126 ml which can be used as tissue blood for proportional calculations. For example, taking muscle at 42.7% body weight, muscle

Table 2

	% body weight			% water in tissues	ml tissue H_2O/Kg body weight
Weight of fish (female)	2.72	3.82	2.5		2.72
Skin with denticles	4.16				30
Skeleton	14.32	13.9			126
Cartilage				88.4 (7)	
Striated muscle	42.67	43.59	42.8	74.5 (1)	320
Digestive tube	6.34	4.47	6.7		52
Pyloric stomach				82.6 (6)	
Oesophageal stomach				82.5 (1)	
Spiral valve intestine				78.6 (2)	
Pancreas	0.36	0.30	0.36	75.8 (7)	3
Liver	9.36	14.11	8.5	19.6 (9)	18
Female tract minus embryos	3.61	1.86	4.6		26 est.
4 Pup embryos	6.33		6.0		
2 Candles (embryos)		6.56			
Uterus				87.2 (5)	
Brain & spinal cord	0.23	0.18	0.24	79.1 (4)	2
2 Eyes	0.66	0.44	0.64	87.8 (1)	
Rectal gland	0.07	0.05		78.9 (6)	
Kidneys	0.46	0.35	0.48	78.4 (4)	4
Gills	1.09			69.6 (1)	7
Spleen	0.32	0.35	0.32	79.9 (2)	3
Heart	0.12	0.11		75.6 (1)	1
Whole blood (drawn)	3.63			89.27 (3)	28
Plasma				92.98 (2)	
Gut contents	3.42				
Fluid & solid residue collected	2.53				12
TOTAL	99.68				638

( ) = number of fish studied.

blood as a static volume would be 124 x 0.427 = 63 ml. Water content of this blood would be 63 x 0.893 = 56 ml. It is desirable that the various fluid spaces of the various tissues be defined by more refined methods. The liver has surprisingly little water (range: 12 - 30 percent). These values are not errors.

Column 5 gives the calculated pools of water in the various organs. It is crudely possible to separate these pools into their compartments, but the figures here are raw figures.

Our data indicate that on a long term basis, unfed dogfish lose about 24 ml water/k -day through the urine and the rectal gland. Boylan has found an uptake of water by the gill of the same value. This loss is about 3.6% of total body water. It takes therefore about 28 days for a

Table 3

	Na^{22} cpm/gm tissue (half-time = 5.5 hrs)	Na^{22} cpm/ml tissue H_2O
Skin	19,700	27,800
Cartilage	20,300	23,400
Striated muscle	3,100	4,300
Pyloric stomach	14,100	16,400
Oesophageal stomach	12,100	14,600
Spiral valve	8,600*	10,900
Pancreas	8,500	11,100
Liver	2,000	15,500
Uterus	21,600	24,000
Brain and spinal cord	11,300	13,900
Rectal gland	12,000	14,000
Kidney	13,200*	16,700
Spleen	8,800*	11,000
Heart	10,000	11,600
Whole blood	31,700	35,400
Plasma	43,000	46,600
Urine	25,800	
Bile	20,800	
CSF	31,900	
Extradural fluid	21,800	
Stomach fluid	280	
Peritoneal fluid	722	
Pericardial fluid	4,400	
Vitreous humor	18,700	

*Another fish, half-time (1.75 hrs) 40,000 counts per min.

dogfish to turn over its body water. Another comparison is that the total daily circulation is about 38,400 ml blood or 34,300 ml water. The fluid loss against this volume is 0.0007 percent. The ratio of body water to circulated water is 0.019.

Yet, there is an active exchange between blood and the tissues. Our data on arterial-venous differenced for Na^{22} give an average of 25 percent. Using this as a working figure, and assuming a crude relation between water and sodium transcapillary movement, one can say that 25 percent of the plasma water is filtered and reabsorbed by the "capillaries in each circuit of blood." It is interesting to note that this system is driven by a heart which is 0.0012 of the body weight, and regulated by kidneys and a rectal gland which are 0.0053 of body weight. There is no evidence for a net efflux of water through gills or skin.

Column 1 of Table 3 gives the Na^{22} content for some tissues and fluids 11 hours after injection of the Na^{22}. The sodium content of the various tissues is meagerly defined. Intracellular sodium has been reported at 11.5 mEq/L (Boylan) and as 30 mEq/L (Murdaugh and Robin). These later investigators found sodium concentrations of brain at 120 mEq/L H_2O and red cell at 33 mEq/L H_2O. The Column-values for Na^{22} are different for various tissues, and one may assume that these counts are more or less equilibrium values for the whole tissues. Column 2 of Table 3 presents counts corrected to gm tissue water.

It turns out that of the counts injected, 12 percent wind up in the muscle which is 43 percent of the total body weight, while 8 percent are in the skin which is only 4 percent of total body weight. Apparently the largest sodium pool is the extracellular fluid, including blood, and then, strangely, followed by the skeleton.

Aided by PHS Grant 1 RO1 GM14296-01.

1967 #6

CONTINUED STUDIES ON SODIUM FLUXES AND RATES OF TISSUE UPTAKE OF SODIUM IN THE DOGFISH, *Squalus acanthias*

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Whether unidirectional or not, the exchange of water and electrolyte through the external surfaces of aquatic organisms is a basic problem. Data of Burger and Tosteson, Comp. Biochem. Physiol. 19:649-53, 1966, and Burger and Horowicz, Bull. M.D.I.B.L. 1966: 8-9, indicate a net sodium influx through the head end of the spiny dogfish. One of us (PH) developed a unique mathematical treatment which slightly revised the values published here in 1966. Mean efflux was 0.16 mEq Na/kg hr; mean influx was 0.68. Using the same methodology as previously, but calculating by conventional methods, the following new data were secured.

1. Sodium influx is not due to or augmented by the drinking of sea water during the experimental periods. All fish that were in an external bath containing Na^{22} had their stomach contents analyzed. There was no evidence of even minute drinking.

2. Sodium efflux into a choline chloride branchial perfusate averaged 0.064 mEq/kg hr (Farber, Gerstein, and Boylan, Bull. M.D.I.B.L. 1965:14). Immersing a dogfish head in a pure sucrose bath, isotonic to sea water gave values like those of Farber *et al*. The average efflux from four fish was 0.069 mEq/kg hr (0.053 - 0.086). It is noted that the efflux is reduced over what it was in sea water, possibly because of the absence of external ions for exchange. Since the dogfish has a positive arterial pressure, it seems reasonable to regard the efflux as hydrostatically induced leakage. As seen below, the skin is rich in sodium.

3. Influx from a sucrose bath isotonic to sea water was studied. In order to have some basis for calculation, Na^{22} was mixed in 5 ml sea water which was added to a 2 liter sucrose bath. For ten periods in three fish, influx averaged 0.0093 mEq Na/kg hr. If the bath were sea water (440 mEq/1), this rate would give an influx of 0.52 mEq/kg hr, which is in the range of previously determined normal influx. It appears that whatever the mechanism effecting influx, it operates at the same rate whether the external medium is low or rich in sodium.

4. Dogfish without a rectal gland maintain normal plasma electrolyte and osmolarity for a 21 day test period (Burger, Physiol. Zool. 38:191-96, 1965). It was reasoned that these glandless fish must have a decreased sodium uptake. To test this directly, four dogfish had their rectal glands removed and four controls had a dummy operation. After fourteen days, only two