Table 2

Group	N	$\sqrt{\text{Percent}}$ growth and standard error	Significant differences
Upper uncrowded	146	32.6 ± 1.1	$p < .01$
Upper crowded	164	21.8 ± 0.9	
Middle uncrowded	148	47.9 ± 1.4	$p < .01$
Middle crowded	125	31.9 ± 1.5	
All upper	310	26.9 ± 0.8	$p < .01$
All middle	273	40.6 ± 1.1	$p < .01$
All lower	112	34.9 ± 1.8	

higher at the middle level. Upper barnacles did not differ significantly in percent growth from lower barnacles.

Other t-test comparisons were made to determine the effect of the number of contacts with other barnacles on growth. Upper and middle barnacles with zero or one contacts significantly differed in percent growth from those contacting 4, 5 or 6 barnacles.

This work was supported by the General Research Fund of the Pennsylvania State University.

1966 #2

RENAL TUBULAR REABSORPTION OF GLUCOSE IN *Squalus acanthias*

John W. Boylan and Dorothy Antkowiak, State University of New York, Buffalo, N. Y.

The plasma glucose concentration (glucose oxidase method) of 16 female dogfish, taken 1 - 2 days following capture, averages 0.90 ± 0.49 mg/ml. This value agrees closely with that found by Oppelt, Bunin and Rall (Life Sciences No. 7, p. 497, 1963), and is somewhat higher than the earlier published figures of Kisch (Biochem. Zt. 211:276, 1929).

At these plasma glucose concentrations little glucose is excreted in the urine. The urine of 2 of the 16 fish was glucose-free (less than 0.1 mg/ml). Twenty urine samples taken from the remaining 14 fish gave an average glucose concentration of 0.38 mg/ml (range 0.1 to 0.83 mg/ml).

The rate of renal glucose reabsorption was measured in 25 female dogfish before and after elevation of the plasma glucose concentration by infusion. Inulin, 200 mg IV, was given on the day prior to the experiment; this procedure was found to yield appropriate plasma inulin values of good constancy over the period of the next day's experiment. On the morning of the experiment a polyethylene catheter was tied into the fish's ureter and the stored urine expressed by gentle pressure over the pelvic girdle. A balloon was affixed to the catheter and a one-hour urine collection period begun. Arterial blood samples for glucose and inulin bracketed the urine collection period. Inulin was determined after yeasting the plasma filtrate or urine by Harrison's diphenylamine method (Proc. Soc. Exptl. Biol. and Med. 49:111, 1942). Filtered glucose was taken as inulin clearance x plasma glucose. Reabsorbed glucose (T_G) was calculated as filtered glucose (mg/hr) minus urinary glucose (mg/hr).

Following one or two control periods, plasma glucose was elevated by the IV infusion of 2-6 gm glucose dissolved in water. An hour was allowed for equilibration following which urine collection periods were repeated as described. Between collection periods the fish swam free in a live car.

Table I lists the measured parameters of renal glucose reabsorption in 10 representative control experiments. Note that, with one exception, at these normal values for plasma glucose concentration (less than 1.0 mg/ml) 94-98% of filtered glucose is reabsorbed.

Table 1

RENAL GLUCOSE REABSORPTION IN *Squalus acanthias*

Fish # wt. kg.	$\dot{V}$ ml/hr	P_G mg/ml	U_G mg/ml	GFR ml/hr	Load mg/hr	$U_G \cdot \dot{V}$ mg/hr	T_G mg/hr	% R_G
4	1.5	0.83	0.16	11.3	9.4	0.24	9.1	97
	1.3	0.89	0.19	10.5	9.4	0.25	9.1	97
3.7	1.6	0.92	0.24	9.7	8.9	0.38	8.5	95.6
8 3.2	1.5	0.82	0.35	16.8	13.8	0.4	13.3	96.4
9 2.6	1.5	0.46	0.23	28	12.9	0.35	12.6	97.6
12	3.0	0.37	0.175	67	24.8	0.52	24.3	98
3.0	2.2	0.35	0.18	46	16.1	0.39	15.7	97
13 2.8	1.0	0.50	0.33	21.8	11.0	0.33	10.7	97.4
14 3.2	1.6	0.86	0.36	12.2	10.5	0.58	9.9	94
19 4.4	2.0	0.80	0.81	13.6	10.7	1.60	9.10	85
23 4.1	3.8	0.48	0.16	23.6	11.3	0.599	10.7	94.6
24 4.1	1.8	0.71	0.17	9.76	6.93	0.30	6.63	95.6
28 4.35	1.3	0.80	0.24	9.57	7.65	0.31	7.34	96

Table 2 records the data for glucose reabsorption as filtered load was increased by elevation of plasma glucose and by spontaneous variation in GFR. Several points are notable in Table 2:

1. With increases in filtered load there were corresponding increases in the amount of both excreted and reabsorbed glucose. Reabsorbed glucose showed no tendency to plateau at high plasma concentrations.

Table 2

GLUCOSE REABSORPTION AT HIGH PLASMA GLUCOSE LEVELS

Fish # wt. kg.	$\dot{V}$ ml/hr	P_G mg/ml	U_G mg/ml	GFR ml/hr	Load mg/hr	$U_G \dot{V}$ mg/hr	T_G mg/hr	T_G/GFR
2 2	2.25	2.1	2.6	7.8	16.6	5.8	11.8	1.4
	1.5	2.4	3.9	7.3	17.3	5.8	11.4	1.6
	1.8	3.4	9.6	11.4	38.8	17.5	21.3	1.9
3 3.4	3.6	2.9	8.3	19.0	55.9	30.0	25.9	1.4
	0.63	3.2	9.6	4.2	13.4	6.0	7.3	1.7
	2.6	3.8	12.5	13.2	50.2	32.5	17.7	1.3
5 4.6	4.1	1.6	2.4	58.5	95.0	9.9	85.6	1.5
	4.6	1.7	2.1	50.0	85.0	9.6	75.4	1.5
	4.4	2.1	2.5	36.2	77.0	10.8	66.2	1.8
7 4.6	1.7	2.2	3.1	27.0	58.6	5.2	53.4	2.0
8 3.2	1.9	1.5	2.1	18.3	27.5	3.8	23.7	1.3
9 2.6	2.0	1.9	2.1	22.0	41.0	4.2	37.2	1.7
14 3.2	1.2	2.3	6.5	7.0	16.0	7.8	8.4	1.2
15 2.8	1.2	3.9	12.3	11.7	45.7	14.8	31.0	2.6
16 4.1	2.6	3.6	8.4	9.5	34.0	22.0	12.0	1.3
23 4.1	3.6	3.4	11.0	19.0	64.0	40.0	24.0	1.3
28 4.4	1.7	2.7	9.8	11.8	32.0	17.0	15.0	1.3
29 3.95	2.2	3.6	15.0	14.4	52.0	33.0	19.0	1.3

2. There is a positive correlation between the magnitude of the glomerular filtration rate (GFR) and the amount of glucose reabsorbed.
3. From (2) it follows that the ratio T_G/GFR should yield a constant value and within reasonable limits this is the case; the mean of the 18 ratios tabulated in Table 2 is 1.6 ± 0.35.

It appears, therefore, that the dogfish and the mammalian renal tubule reabsorb normally filtered glucose with like efficiency.

Unlike published data for dog and man, however, a tubular maximum for glucose could not be demonstrated in the spiny dogfish.

Instead, the tubules of the dogfish seem to reabsorb a fixed amount of sugar for each ml of glomerular filtrate presented to them.

Whether this is a manifestation of glomerular intermittence or of a flow-dependent mechanism for glucose reabsorption cannot be stated at this time. Supported by National Science Foundation Grant GB-2580.

1966 #3

ON THE COUPLING BETWEEN METABOLISM AND ANAEROBIC SODIUM TRANSPORT IN THE RED BLOOD CELLS OF THE DOGFISH SHARK (Squalus acanthias)

Neal S. Bricker, Levi Guerra,* William Beauman, and Carlos Marchena, Mt. Desert Island Biological Laboratories, Salisbury Cove, Me. and the Renal Division, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Mo.

The nucleated red blood cells of the dogfish shark transport sodium in vitro from cell water to extracellular fluid at a faster rate than mammalian red blood cells and at temperatures of 20 to 22° C. This transport occurs anaerobically as well as aerobically. During anaerobic transport, glucose is utilized as substrate and anaerobic glycolysis has been found to be approximately twice as great as aerobic glycolysis. Oligomycin, an inhibitor of oxidative phosphorylation in mitochondrial-linked energy producing systems, inhibits roughly 50% of anaerobic sodium transport via the erythrocytes. In oxidative systems, oligomycin depresses oxygen consumption; but in the anaerobic studies on the dogfish erythrocytes, glycolysis was markedly stimulated. The depression of sodium transport, all other things being equal, should result in an accumulation of cellular ATP stores in view of the fact utilization of energy for sodium transport is decreased. The increased rate of glycolysis, similarly should result in an increase in cellular ATP stores assuming that glycolysis occurs normally and that ATP is produced normally via the Embden-Meyerhof pathway. Yet the measured levels of ATP in the presence of oligomycin were moderately diminished rather than increased. Utilizing a cell membrane ATPase preparation from dogfish erythrocytes, it was found that oligomycin inhibited sodium, potassium stimulated ATPase activity, rather than stimulating it. Were stimulation to have occurred, the decrease in ATP concentrations despite the presence of inhibition of sodium transport and increased anaerobic metabolism might have been explicable. However hydrolysis of ATP by plasma membrane ATPase activity was decreased and this too should contribute to an accumulation of ATP in the cell water. The combination of effects suggests that glycolysis was "uncoupled" from phosphorylation. Moreover, the fact that sodium transport was inhibited in the presence of appreciable levels of ATP would provide evidence, albeit indirect, that ATP per se may not be the immediate source of energy for sodium transport. Electron micrographs of dogfish erythrocytes performed by Dr. W. L. Doyle of the University of Chicago have shown no discrete mitochondria in the cells. Thus, it seems unlikely that oligomycin acted conventionally on a high energy intermediate arising from glycolytic ATP recycled in a reversed order through mitochondria. We believe that these data provide phenomenologic evidence for a high energy intermediate of glycolysis; if such an intermediate exists, it presumably serves as the energy source for anaerobic erythrocyte sodium transport.

Supported by U. S. Public Health Service Research Grant AM-09976, Department of the In-

---

*Supported by NATO Fellowship #E5/65.