to plasma. Rates of bile secretion were measured over a three-day period. Bile secretion in 20 studies ranged from 0.54 - 4.5 ml/kg body weight per day but averaged 1.99 ± 1.1 ml on day 1; 2.66 ml ± 0.89 on day 2; and 1.8 ± 1.02 ml by day 3.

The composition of hepatic bile obtained from the cannula was identical to plasma with respect to Na^+ and Cl^- concentrations. Osmolarity and HCO_3^- concentration slightly exceeded plasma values whereas K^+ and Mg^{++} were significantly greater. In contrast gallbladder bile composition was identical to plasma except for slightly smaller osmolarity and Cl^- concentrations. Gallbladder bile salt concentration was four to five times the concentration in cannula (hepatic) bile suggesting that the gallbladder reabsorbed water. However these studies differed from previous findings in the dogfish shark where gallbladder reabsorption of water, HCO_3^-, and Cl^- were more striking (Boyer, Bull. MDIBL 11:4-5, 1971). The increase in hepatic-plasma bile ratios of K^+, HCO_3^-, Mg^{++}, and Ca^{++} together with an increase in hepatic bile osmolarity were not observed in the dogfish suggesting that the biliary cannula may stimulate ductular secretion of these ions in the skate. The large variation in range of bile flow and the possibility that cannulation alters ion transport suggests that *in vivo* studies of bile secretory function in the little skate may be difficult. An isolated perfused organ system may be preferable.

TABLE 1

COMPOSITION OF PLASMA, HEPATIC AND GALLBLADDER BILE IN THE SMALL SKATE, *Raja erinacea*

		OSMOLARITY (mosmoles/L)	BILE SALTS (mmoles/L)	Na^+ (mmoles/L)	K^+ (mmoles/L)	Cl^- (mmoles/L)	HCO_3^- (mmoles/L)	Mg^{++} (mg%)	Ca^{++} (mg%)
PLASMA	Time 0	958 ± 14 (4)	------	285 ± 13 (10)	4.3 ± .6 (10)	281 ± 13 (10)	4.4 ± .5 (4)	2.5 ± .2 (10)	16.4 ± .22 (10)
	24 hrs.	946 + 15 (4)							
CANNULA (Hepatic) BILE	0-24 hrs.	1010 ± 28* (4)	7.96 ± 5.21 (5)	295 ± 12 (5)	13.0 ± 2.1* (5)	280 ± 25 (5)	6.2 ± 2.0* (5)	6.2 ± 1.7* (4)	32.8 ± 4.0* (5)
GALLBLADDER BILE	Time 0	920 ± 9** (5)	35.16 ± 10.09 (10)	267 ± 31 (10)	4.6 ± .8 (10)	221.5 ± 25** (10)	5.1 ± 1.7 (5)	2.7 ± .3 (10)	20.7 ± 3.1 (10)

*Significantly greater than plasma ($p < .01$)
**Significantly less than plasma ($p < .01$)
numbers in parentheses = # of studies

1973 #7

MAXIMUM RATES OF BICARBONATE REABSORPTION BY THE DOGFISH KIDNEY

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The dogfish is credited with an extraordinary capacity for the renal reabsorption of filtered bicarbonate. Smith (J. Cell. Comp. Physiol. 14:95, 1939) first reported that urinary pH in this species is fixed at 5.7 and unaffected by the infusion of sodium bicarbonate, alkaline phosphate or phlorizin. Hodler et al. (Am. J. Physiol. 183: 155, 1955) later demonstrated that a carbonic anhydrase—dependent system is not involved in the acidification of urine in the dogfish. Maren (Physiol. Rev. 47:595, 1967) has calculated the theoretical maxima for bicarbonate and H^+ production by the renal tubular cells of Squalus at the uncatalyzed rate of CO_2 hydration and compared the calculated values to those observed by Smith and by Hodler et al. (see above). Observed values were two and a half to four times the theoretical maxima, a finding supporting the ionic reabsorption of bicarbonate. More recently Maren and Deetjen (Bull. MDIBL 12: 22, 1972) observed the titration of bromcresol purple with and without added bicarbonate, in droplets injected into surface nephrons of the skate kidney. Added bicarbonate did not increase the time for color change of the indicator. The investigators concluded that some mechanism other than protonation is responsible for most of the bicarbonate reabsorption in this elasmobranch. Because neither Smith nor Hodler et al. seriously challenged the reabsorptive mechanism for bicarbonate in Squalus we have undertaken to provide data in an extended range of filtered load for this anion.

Data are reported from 17 female dogfish weighing from 2.8 to 5.6 kg. One day before the experiment the fish were given 200 mg inulin i.v., a procedure now known to provide steady plasma values for inulin on the following day. On the day of the experiment a polyethelene catheter was tied into the urinary sinus and a second catheter threaded into the dorsal aorta via percutaneous #18 needle. The needle was removed, the aortic catheter secured in place, and the fish placed in a well-aerated tank maintained at 13-14°C. Each fish received a priming dose of 2 g $NaHCO_3$ IV followed by a sustaining infusion (Howard pump) of eight percent $NaHCO_3$ at a constant rate for each fish but varying for different fish from 0.42 to 1.1 ml per minute. Approximately 30 minutes after the priming dose of bicarbonate the urinary sinus was emptied by suprapelvic pressure and a balloon attached for urine collection. From three to five urine collection periods of about one hour each were taken. Blood was sampled at the beginning and end of each urine collection. Blood and urine pH were read within a few minutes of sampling using a Corning pH-meter. Inulin in urine and plasma was determined by the resorcinolthiourea method, bicarbonate by manometric Van Slyke.

At its normal plasma bicarbonate concentration of 4 - 7 mM/l a 3 kg dogfish has a filtered bicarbonate load of 1 - 1.5 μM/min (μmoles/min). Priming and infusion of bicarbonate yielded plasma values in the experimental fish of between 40 and 200 mM/l and filtered loads of 5 - 25 μM per minute. Figure 1 demonstrates that reabsorption of filtered bicarbonate remains at nearly 100 percent until plasma values exceed 140 mM/l. Above this plasma level there appears to be a distinct reduction in percentage reabsorption although plasma values are scattered at reabsorption below 80 percent. Increasing plasma bicarbonate concentration was without systematic effect on GFR and a plot of reabsorbed bicarbonate against plasma bicarbonate showed no correlation. However when reabsorbed bicarbonate is expressed per unit of GFR—the device traditionally used to demonstrate reabsorptive tubular maxima—it is directly proportional to plasma bicarbonate concentration. The proportionality holds to plasma bicarbonate concentrations of approximately 140 mM/l. Above this value the splay is scattered downward and no steady state TM can be demonstrated.

Figure 2 displays the absolute reabsorptive rate in uM/min at increasing filtered loads of bicarbonate. The highest values are more than 15 x the theoretical maximum (1.5 μM/min) calculated by Maren for the uncatalyzed rate of CO_2 hydration. The urine of all fish infused with eight

Figure 1

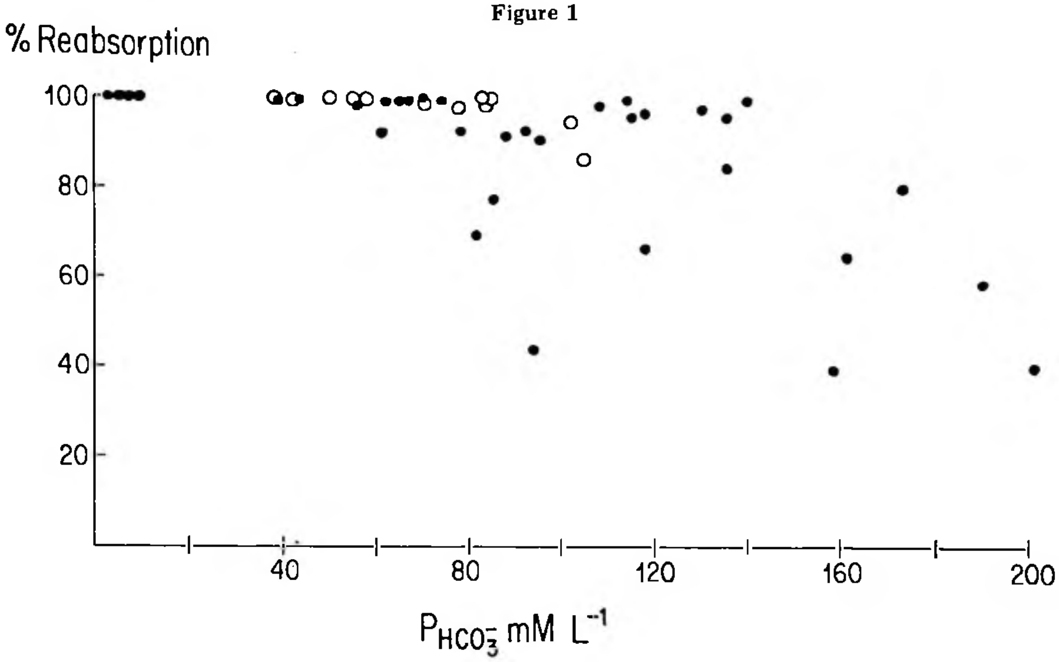
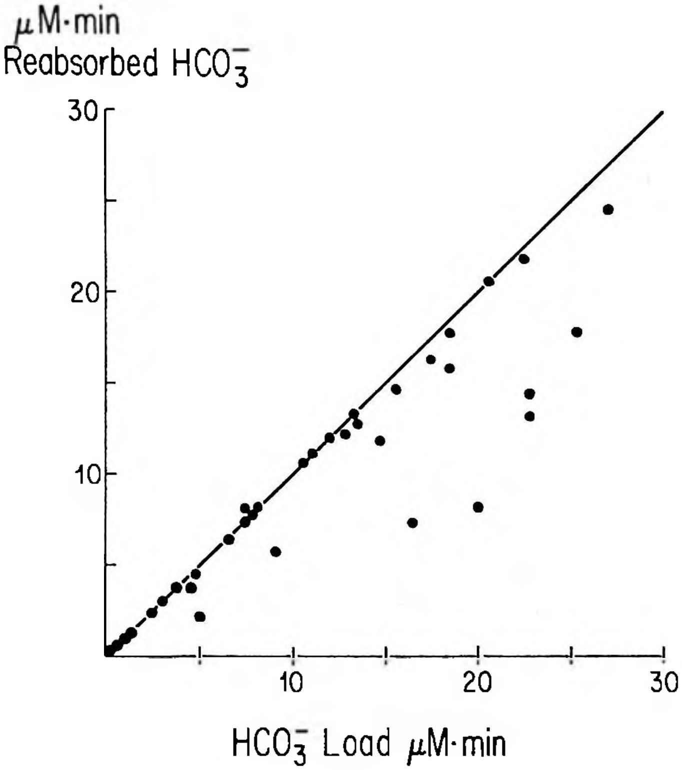


Figure 2



percent $NaHCO_3$ became alkaline usually within the first hour and at plasma bicarbonate levels above 60 mM/l. The threshold for bicarbonate is set therefore at about ten times the normal plasma value. Urinary pH ranged from 5.8 to 8.3 and urinary bicarbonate from trace to 162 mM/l. Even the most marked degree of alkalosis was compatible with return of blood and urine pH to normal values in several fish examined 24 hours after the experiment.

The data are inconsistent with a bicarbonate reabsorption system that depends on protonation through intracellular hydration of CO_2 and luminal dehydration of carbonic acid. Bicarbonate reabsorption demonstrated in Figure 2 reaches values more than 15 times the theoretical maximum expected at the uncatalyzed rates of these reactions.

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1973 #8

VOLUME REGULATION OF WINTER FLOUNDER *Pseudopleuronectes americanus* RED BLOOD CELLS

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This study was designed to determine the volume regulatory capabilities of the red cells of the flounder *Pseudopleuronectes americanus.* The role of inorganic ions and the passive and active pathways by which these ions move during volume regulation were studied. The broader interest of the study was to establish the nucleated red blood cells of the flounder as a model for other nucleated, less accessible cells.

The cells were obtained by withdrawal in a heparinized syringe. The blood was centrifuged and the plasma and buffy coat removed by aspiration. The cells were then washed four times in solution A: 170 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, .75 mM $CaCl_2$, 5 mM Dextrose, 3 mM imidazole, 10^6 units penicillin, .25 gm/1 streptomycin sulphate, pH 7.95. These cells were then pre-incubated overnight in solution A to insure that a true steady-state was reached prior to experimental treatment. Cells used for tracer efflux studies were loaded with either ^{86}Rb or ^{22}Na at 10-15 μCi/ml for the last two hours of pre-incubation. At the end of the pre-incubation period cells were split into equal groups to be used as experimentals (volume-perturbed) and controls (volume-static). These cells were suspended at hematocrit of eight percent if chemical analysis was to be performed and three percent if tracer flux was monitored. All cells upon which determinations of Na, K, Cl, and water were to be performed were suspended in media containing ^{14}C polyethylene glycol (m.w.=4000) as an extracellular space marker. If tracer influx was to be determined the cells were suspended in the appropriate medium containing 10-15 μCi/ml of ^{22}Na or ^{86}Rb. The other solution used, solution B, was the same as solution A with the exception that it contained only 100 mM NaCl and was therefore hypotonic to A. There were two sampling procedures employed: one for ouabain-free and one for ouabain-poisoned cells. Prior to sampling the cells designated as experimentals were placed in solution B which was hypotonic and induced osmotic swelling while cells used as controls were resuspended in solution A which was isosmotic and caused no volume change. Both experimental and control cell