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

Peter M. Cala, Case Western Reserve University, Cleveland, Ohio; and The Mount Desert Island Biological Laboratory, Salsbury Cove, Maine

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

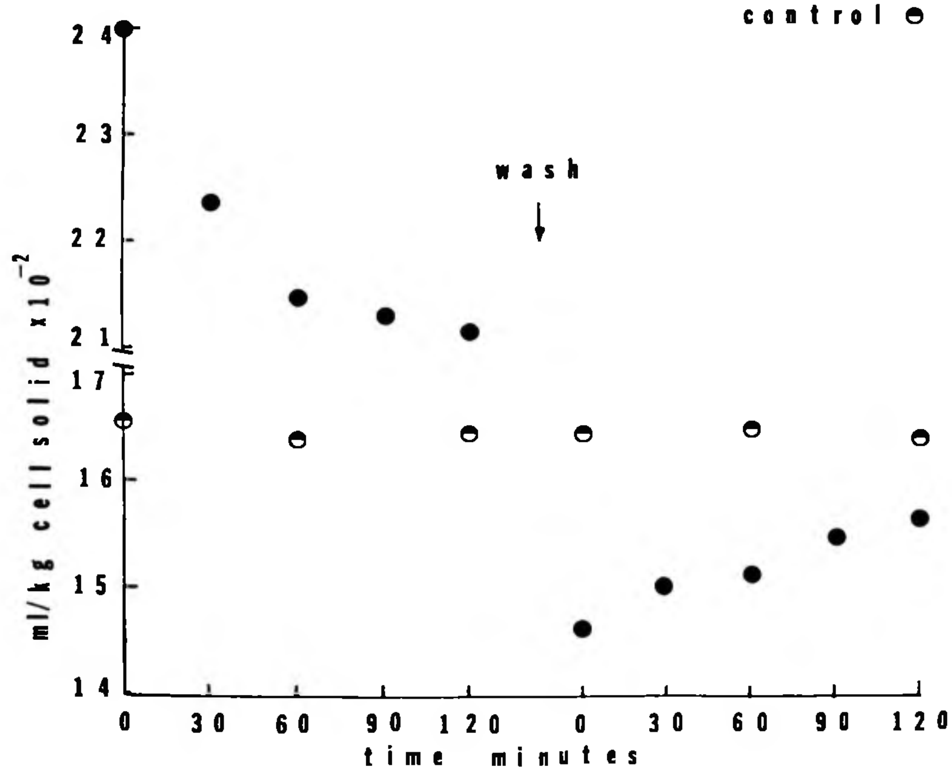

Figure 1. First 120 minutes is representative of experimentally-treated cells (closed circles) which have been transferred from solution A (346 mOs) to solution B (200 mOs) and become osmotically swollen as seen by T = 0 water content relative to controls (half circles). The experimental cells immediately proceed to lose water, approaching the steady-state volume indicated by control cells.

The second 120 minutes depict cell volume increasing subsequent to transferring experimental cells from solution B to solution A. There is an initial rapid osmotic shrinkage, followed by volume increases back toward steady-state values.

TABLE 1

Flux Expressed as Per Cent of Ouabain Free Volume Static Cells

CONDITIONS	OUABAIN	Na EFFLUX	Na INFLUX	K EFFLUX	K INFLUX
Volume decreasing	-	138.69 ± 13.0 (8)	138.67 ± 13.0 (8)	217.37 ± 7.0 (8)	173 ± 11.0 (8)
	+	143.0 ± 15.0 (7)	309.0 ± 21.0 (7)	269.3 ± 27.0 (7)	173 ± 16.0 (7)
Volume increasing	-	141.0 ± 12.0 (7)	221.0 ± 18.0 (7)	106.0 ± 5.0 (8)	109 ± 5.0 (8)
	+	210.5 ± 23.0 (7)	415.0 ± 20.0 (7)	135.2 ± 14.0 (8)	120 ± 11.0 (8)
Controls	-	100.0 ± 8.9 (8)	100.0 ± 8.9 (8)	100.0 ± 8.4 (8)	100 ± 8.4 (8)
	+	124.1 ± 15.0 (7)	136.0 ± 13.0 (7)	128.3 ± 9.0 (8)	123 ± 8.0 (8)

All fluxes are expressed as t = 0 flux. Volume decreasing refers to cells osmotically swollen and undergoing volume loss at time of flux measurements. Volume increasing refers to cells osmotically shrunken and in the process of regaining volume at the time flux data were obtained.

suspensions were sampled immediately and then at 30-minute intervals for two hours. After two hours of sampling the suspensions were centrifuged and the supernatant removed. The cell pellets were washed once in solution A and finally suspended in solution A. Since the experimental cells had equilibrated with solution B solution A was hyperosmotic and induced osmotic shrinkage. Samples were again taken at time zero and at 30-minute intervals thereafter for two hours. If ouabain was used sampling differed from that described above only in that samples were taken at 15-minute intervals for two 1-hour periods.

Figure 1 shows changes in the water content of cells expressed as ml/kg dry cell solid versus time in minutes. The experimental or osmotically-perturbed cells are indicated by closed circles while control or volume-static cells are represented by half circles. The first 120 minutes represents experimental cells which had been osmotically swollen at time zero by suspension in solution B following pre-incubation in solution A. The graph clearly illustrates that subsequent to osmotic swelling, the cells regulate their volume back toward steady-state or control levels.

Associated with the water loss during volume decrease is a decrease in intracellular K and anion. The tracer flux data for volume decreases (Table 1, rows preceded by –) show that the net K loss is the result of large increases in unidirectional K efflux. Since the increased K flux is in the direction of the gradient for K it is considered a passive flux resulting from an increase in the membrane's permeability to K (P_K). The flux data for Na are interesting in that they show a bidirectional increase in Na flux. This suggests a lack of absolute specificity for K by the permeability pathway which is countered by active extrusion of Na at a rate equal to its entry. The second 120 minutes of Figure 1 depict cells which have been washed once in solution A at the point indicated by the wash arrow, and then suspended in A at T = 0. Since solution A is hypertonic to experimental cells they undergo osmotic shrinkage. The water movements shown following shrinkage are associated with net increases of intracellular Na, K, and Cl, with Na comprising three-quarters of the cation gained. The flux data obtained during volume increases (Table 1, rows preceded by –) show that unidirectional Na influx increased by 121 percent over volume-static control cells. This suggests an increase in the membrane's permeability to Na (P_{Na}) with the result that sodium moves down its electrochemical gradient into the cells. The data concerning K flux in this case show increases in both the influx and efflux of the ion. It appears that volume is regulated as a result of permeability changes to Na which, as in the case of volume decreases, are not absolutely specific since K efflux is stimulated. The increase in K efflux is however compensated for by an increase in K influx which is presumed to be active since it leads to a net accumulation of intracellular K.

Experiments using ouabain (10^{-4}M) were performed in order to determine the degree of and necessity for active Na-K pumping during the volume regulatory process. Figures 2a and 2b depict cell volume changes subsequent to osmotic perturbation in the presence of ouabain. The time course and extent of the water movements as seen in these two figures is indistinguishable from the response of identically-treated ouabain-free cells. Figure 2a is an illustration of the water movement of osmotically-swollen and control cells taken from the same animal with those points shown as closed circles representing cells exposed to ouabain. The points shown in Figure 2b are all representative of ouabain-poisoned cells. The first 60 minutes show water loss from experimental cells (closed circles) subsequent to osmotic swelling as a result of suspension in solution B. At that point marked by the wash arrow the cells were washed once and then suspended in solution A which caused osmotic shrinkage. The second 60 minutes in this figure show volume increase in response to osmotic shrinkage.

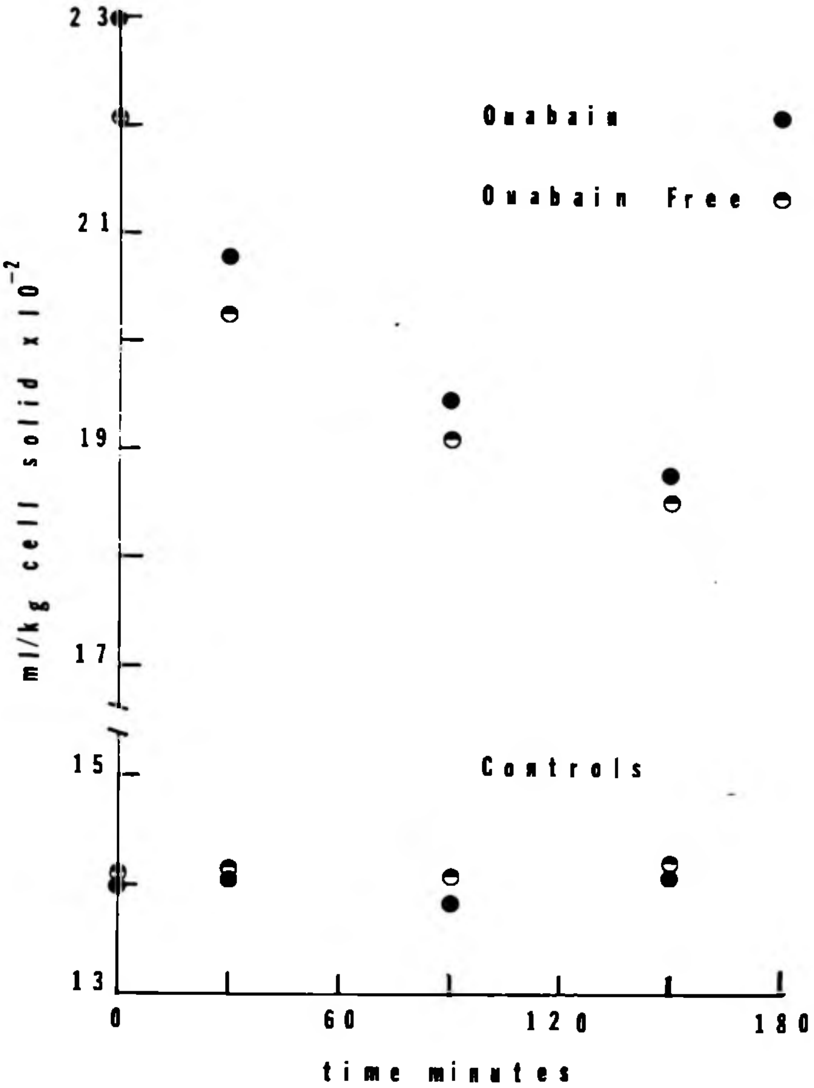

Figure 2a. Water movements in osmotically-swollen cells and volume-static controls in the absence (half circles) and presence (closed circles) of ouabain. It can be seen that the volume status of both osmotically-perturbed and volume-static cells is unaffected by the presence of ouabain.

Although ouabain had no effect upon water movements associated with volume regulation it did cause alterations in ion flux.

Following osmotic swelling the water loss is due to net K efflux but in the presence of ouabain there is also a net Na influx with the result that nearly twice as much K must move out of the cells during volume decreases in order to offset the net inward movement of Na. The end result is a net decrease in the absolute amount of intracellular cation and water equal to that seen in identically treated ouabain-free cells. During volume increases subsequent to osmotic shrinkage an increase in intracellular Na and anion was responsible for water uptake. Under conditions of ouabain-poisoning

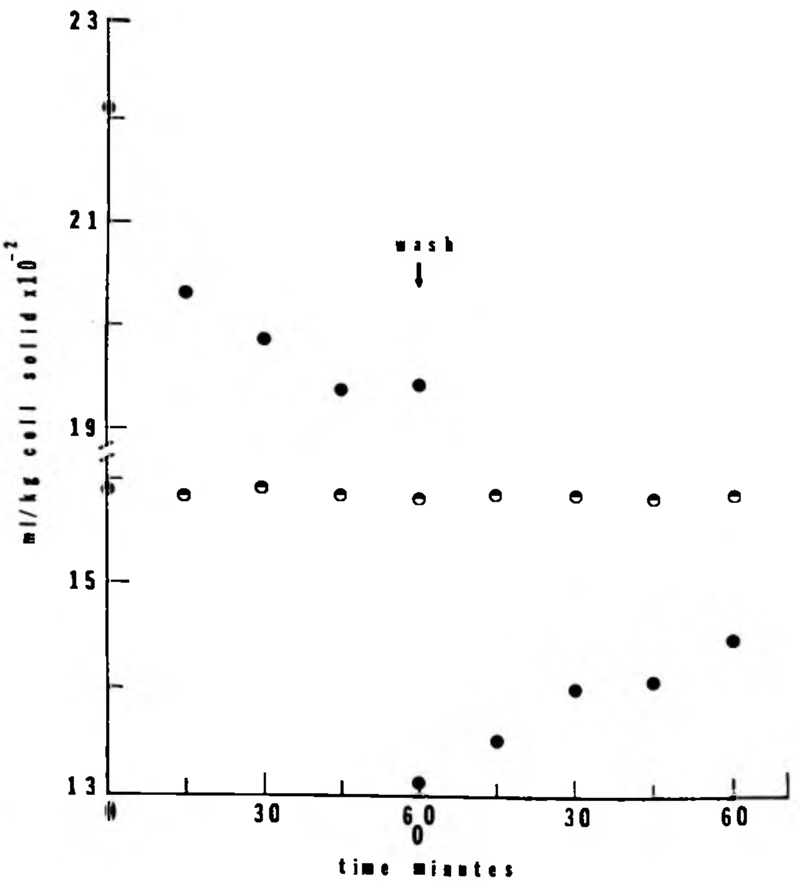

Figure 2b. This graph shows water status of cells exposed to ouabain. The osmotically-perturbed cells (closed circles) show volume regulation after osmotic swelling (first 60 minutes) and osmotic shrinkage (second 60 minutes). See Legend of Figure 1 for treatment resulting in volume perturbation.

the net gain in Na was nearly doubled while net K loss was increased yet when K loss was subtracted from Na gain it was apparent that the net gain in intracellular cation equalled values for volume-increasing ouabain-free cells.

The flux data for ouabain (10^{-4}M)-treated cells (Table 1, rows preceded by +) show that Na and K influx and efflux are stimulated. The passive flux of these two species is however increased to the greatest extent. This finding at first appears to be contradictory to the expected effect of ouabain yet when these data are viewed in light of the behavior noted for ouabain-free volume-perturbed cells the apparent contradiction is explained. The ouabain-free volume-perturbed cells show large increases in P_K or P_{Na} depending on the direction of the induced volume change. The data also show that the permeability increases are not absolutely specific. The lack of absolute Na or K specificity is actively compensated for as seen in the bidirectional flux increases of Na and K during volume decreases and increases respectively. Inhibition of the Na-K volume, ionic level maintenance pump (Tosteson and Hoffman, J. Gen. Physiol., 44: 169, 1960) should lead to disruption of cell volume as a consequence of the Gibbs-Donnan effect. Pump inhibition would also negate the supportive aspect of limiting net flux to only that ion whose net passive movement would favor volume restoration. Since cessation of ion pumping would further insult cell volume it would be expected that membrane permeability might undergo large increases in the presence of inhibitors of active transport. This is in fact the case as seen in the data for Na influx and K efflux associated with ouabain-treated cells. In light of the magnitude of the permeability changes it is not at all unlikely that contra-gradient flux should be increased to values equal to or in excess of values seen during ion pumping. In other words ouabain has affected the ion pumping of the cells but superimposed upon its effect is the volume regulating response of increased membrane permeability.

In summary the data show that flounder cells respond to volume perturbation by regulating volume back toward steady-state values. The water movements during the regulatory process are associated with movements of the inorganic cations Na and K down their respective electrochemical gradients. These passive ion movements are a consequence of changes in membrane permeability which are only partly selective to Na and K. Ion pumping plays a supportive role during the process of volume regulation but is not essential as shown by the data on ouabain-treated volume-perturbed cells. Yet this is not to say that these cells have no need of active ion pumping since it is the energy stored in the Na and K electrochemical gradients established and maintained by the Na-K pump that produces the driving force for ion and therefore water flux during volume regulation.

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1973 #9

THE ACTIVITIES OF HEPATIC MICROSOMAL ENZYME SYSTEMS IN FISH

Theresa S.T. Chen, University of Louisville, Department of Pharmacology, Louisville, Kentucky

The activities of three hepatic microsomal enzymes were determined in winter flounder *Pseudopleuronectes americanus*, killifish *Fundulus heteroclitus*, the small skate *Raja erinacea*, and mackeral *Scomber scombrus*. Enzyme assays were performed on whole liver homogenates. Phosphorothioate detoxification was measured by the method of Neal and DuBois (J. Pharmacol. Exp. Therap. 148:185, 1965) using O-ethyl-O-(4-nitrophenyl)-phenyl phosphonothioate (EPN) as the substrate and O-demethylase activity was measured by the procedure of Netter and Seidel (J. Pharmacol. Exp. Therap. 146:61, 1964) using p-nitroanisol as the substrate. The N-demethylation of aminopyrine was measured by the method of La Du et al. (J. Biol. Chem. 214: 741, 1955) and the activity expressed as micrograms of 4-aminoantipyrine formed per 100 mg. of liver in 30 minutes. The activities of the EPN detoxification system and O-demethylase are expressed as micrograms of p-nitrophenol formed by 50 mg. of liver in 60 minutes from EPN and p-nitroanisol respectively.

Table 1 summarizes our studies on three enzyme activities in the four species of fish examined. These activities are quite low, approximately one-third those observed in mammals. Since pheno-