$t_{1/2}$ indicates that it depends on both blood flow and blood-brain barrier permeability. For those compounds with small $t_{1/2}$'s (water and ethanol), the exchange across the capillaries is so rapid that the washout is essentially limited by the flow of blood to and from the medulla. For those compounds with large $t_{1/2}$'s (propanediol to thiourea), the rate of exchange is dominated by the permeability of the brain capillary complex and the $t_{1/2}$'s are inversely related to the blood-brain barrier permeability coefficients of each molecule.

The size of the brain extracellular space, the reduction in the tissue diffusibility of extracellular markers, and the rates of capillary exchange agree quite well with published mammalian values when considerations of the differences in body temperature, tissue composition, and cerebral blood flow are included.

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INTRACELLULAR OSMOREGULATION FOLLOWING ENVIRONMENTAL DILUTION IN THE LITTLE SKATE, *Raja erinacea*

ROY P. FORSTER, LEON GOLDSTEIN, JOHN W. DANFORTH, CHRISTINE C. ROGERS, MARTHA WINSOR AND LINDA D. BAKER. DARTMOUTH COLLEGE, HANOVER, NEW HAMPSHIRE; AND BROWN UNIVERSITY, PROVIDENCE, RHODE ISLAND

The osmoregulatory functions of marine cartilaginous jawed fishes are of special interest because of the important part that nitrogenous compounds, especially urea and trimethylamine oxide, play in the characteristic osmotic "superiority" of their body fluids relative to the strongly saline external environment. Plasma osmolarity of some of the Chondrichthyes such as the little skate *Raja erinacea* is considerably reduced during transfer from full

strength to diluted seawater (Goldstein and Forster, Am. J. Physiol. 220: 742, 1971). Our current experiments evaluate the redistribution of water and solutes that occurs between intra- and extracellular compartments of muscle and erythrocytes when skates are gradually transferred from their fully marine environment to 50 percent seawater.

Relatively few studies have been made on changes that occur in the intra- and extracellular pools of free nitrogenous compounds in euryhaline vertebrates as they adapt to different environmental salinities (Colley, Fox and Huggins, Comp. Biochem. Physiol. 48A: 757, 1974), and none has previously been reported on a chondrichthyan. The most surprising finding in our current study on the skate is the very important part played by amino acids (ninhydrin-positive substances) in the accommodation of their intracellular milieu to an osmotically diluted plasma.

Little skates of either sex weighing slightly less than a kilogram were collected off Mount Desert Island by dragnet. They were maintained in a large pool supplied with separate seawater and freshwater circulations. For the seven experimental animals dilution of the pool from full strength to 50 percent seawater was accomplished over a period of four to five days by regulating the inflow of freshwater until at steady state it equalled the flow rate of seawater. Chloride concentrations were determined using coulombmetric titration with silver electrodes to monitor salinities. Stingrays, *Dasyatis americana*, were similarly maintained in the somewhat more saline waters at the Lerner Marine Laboratory, Bimini Bahamas. There the final tank concentration of chloride was 298 mEq/L and the initial seawater concentration was 561 mEq/L, considerably higher than that of the waters off Mount Desert Island. Samples of wing muscle, plasma, and separated erythrocytes taken from four stingrays at the Lerner Laboratory were stored

frozen and then analyzed four months later along with similar samples taken from the skates at MDIBL.

Blood samples were drawn from caudal vessels into a heparinized syringe. The animals were killed quickly by transection of the spinal cord and muscle samples were then taken from the base of the "wings". Water content of erythrocytes, plasma, and muscle was determined as wet weight minus dry weight, and percentage water as water content divided by wet weight x 100. Osmometry was done by cryoscopy (Fiske Osmometer) on undiluted plasma samples, and on the supernatant of diluted boiled muscle.

Chloride "space" in muscle was used to estimate extracellular volume (Manery, Physiol. Res. 34: 334, 1954). Tissue chloride concentration in nmoles/kg wet wt. tissue factored by the Gibbs-Donnan ratio for chloride (0.977) and by plasma water content in ml/kg wet wt. plasma was divided by plasma chloride concentration in nmoles/L plasma.

Muscle extract was prepared by pulverizing frozen muscle, precipitation with trichloracetic acid, and extraction of TCA with hydrated ether. Chloride was determined with an Aminco-Cotlove Antomatic Chloride Titrator. An Instrumentation Laboratory 343 Flame Photometer was used for sodium and potassium analyses. Moore and Stein's method was used to determine total amino acid concentration expressed colorimetrically as total ninhydrin-positive substance (J. Biol. Chem. 176: 367, 1948). Trimethylamine oxide was assayed by microdiffusion with both the reduction step to free amine and back titration carried out in the Conway dish (Forster et al. J. Gen. Physiol. 42: 319, 1958). Urea analyses were made by a modification of Archibald's colorimetric procedure (J. Biol. Chem. 157: 507, 1945).

Mean extracellular volume (Chloride space) in muscle of six skates maintained in full strength seawater was 9.02%±SE2.5%; and in seven skates

TABLE 1

SOLUTE CONCENTRATIONS IN PLASMA, ERYTHROCYTES AND MUSCLE OF SKATES MAINTAINED IN SEAWATER AND IN HALF-STRENGTH SEAWATER

SAMPLE	SEAWATER	K^+ mEq/L	Na^+ mEq/L	AA mmoles/L	UREA mmoles/L	TMAO mmoles/L	OSMOLYTES mosmoles/L
PLASMA	100%	4.96±0.38 (6)	299±0.49 (6)	10.9±0.49 (6)	361±17.8 (6)	39.2±2.7 (6)	965±18 (6)
	50%	4.25±0.24 (7) N.S.	217±5.05 (7) <.001	12.2±0.79 (7) N.S.	264±11.9 (7) <.01	30.2±2.7 (7) < .05	719±14 (7) < .001
ERYTHROCYTES	100%	120.8±4.44 (6)	50.7±7.38 (6)	280±6.18 (6)	413±19.9 (6)	35.8±1.5 (6)	
	50%	135.7±4.11 (7) < .05	32.7±2.12 (7) < .05	150±13.9 (7) < .001	283±15.4 (7) < .001	29.3±2.2 (7) < .05	
MUSCLE	100%	161.8±6.20 (6)	9.6±2.58 (6)	214±21.8 (7)	398±18.2 (6)	63.9±14.2 (6)	1080±47 (6)
	50%	134.2±1.90 (7) <.01	4.1±1.84 (7) N.S.	144±19.8 (7) < .05	264±15.3 (7) <.001	35.8± 9.4 (7) N.S.	769±20 (7) < .001

Value for solute concentrations are means ± S.E. Number of fish per group indicated in parentheses.

N.S. = not significant, i.e. probability >.05 indicates no significant difference between the means of solute concentrations for skates in 50% and those in full strength seawater.

Solute concentrations in muscle are intracellular concentrations.

in 50% seawater this increased to 15.4%±SE1.15 (P value<.01). In the same fish the osmolarity of plasma decreased from 965 mosmoles/L±SE18.1 to 719±13.6 (P value<.001); osmolytes in muscle dropped from 1080±47 to 769±20.9 (<.001). Hematocrits were 16.8±.99 and 16.03±1.23% respectively.

The percentage water in plasma, erythrocytes, and muscle corresponded to dilution of environmental salinity: percent water of plasma rose from 92.7±.35 to 94.9±.56 (P<.05); erythrocytes from 68.1±.25 to 74.3±.70 (P<.001) and that of muscle increased from 77.8±.58 to 82.4±.27 (<.001).

Table 1 summarizes the shifts of solute concentrations that occur. Cell volumes presumably remain relatively constant while extracellular fluid is being diluted because intracellular solutes are unloaded. Hematocrits were essentially unchanged and intracellular water in muscle increased less than 5 percent following dilution while plasma and muscle osmolarities dropped 25 percent and 29 percent respectively, and extracellular fluid volume in muscle increased more than 40 percent. Most of the reduction in intracellular solute concentrations can be accounted for by the efflux of nitrogenous organic solute, with free amino acids playing a surprisingly large role. Intracellular concentrations of urea are slightly higher than in plasma, probably due to some binding to cell protein, but their efflux from red cells and muscle following dilution rather closely parallels the drop in osmolarity. Considerable variability was encountered in muscle trimethylamine oxide determinations which accounts for the relatively high S.E. values and the probability well beyond the 5 percent level of significance. Sodium appears to be extruded from erythrocytes and muscle cells during dilution, although the very low levels in muscle make accurate determinations difficult as is indicated by the high S.E. values. Intracellular potassium levels are closely regulated. The increase in erythrocyte K^+ that follows dilution appears to

be significant and may reflect maintenance of cationic strength to compensate for Na^+ loss, and thereby to assure intracellular electrical neutrality. In muscle however regulation of $[K^+]$ is mirrored in the fixed electrical membrane potential as calculated using the Nernst equation for K^+ which was -85.54 mV before environmental dilution and -84.75 mV after.

Findings on the stingray, *Dasyatic americana*, closely paralleled those on the skate. Intracellular and extracellular urea levels in this species were very high, probably the highest recorded: 630 and 574 mmoles/L in plasma, and 604 and 666 respectively in muscle intracellular water of two control fish. TMAO levels in stingray muscle were much higher than in skate muscle, and total amino acid levels were lower. In stingray erythrocytes however amino acid levels were very high. Following environmental dilution Cl^- space in muscle almost doubled. Redistribution of inorganic electrolytes was generally similar to the skate and dilution of the pool of nitrogenous compounds also corresponded in degree to that noted in the skates.

Probably the most significant findings here are the large contributions made by free amino acids to total intracellular osmolarity of skate erythrocytes and muscle and the important regulatory role which they play in accommodating cells to an extracellular osmotic challenge. We plan now to identify and characterize membrane processes that maintain these high free amino acid levels intracellularly and control the dramatic shifts that occur when skates are exposed to environmental dilution.

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