

whereas the permeability characteristics of atrial preparations appear to remain intact. They also continue to beat spontaneously throughout 4 hr incubation periods.

As in the flounder ventricular muscle (Vislie and Fugelli. *Comp. Biochem. Physiol.* 52A:415-418, 1971) there is a positive correlation between the decrease in intracellular K^+ and total osmolality decrease of the incubation medium. Our atrial observation appears to be reliable, but, again, the in vitro ventricular strip preparation is suspect because of the spontaneous drop in $[K^+]$ during the 2 hr control incubation period.

Total atrial osmolality of 1091 ± 97 (see Table 1) is fairly closely accounted for by the identified constituents shown in Table 4 and the covering anions for Na^+ and K^+ . To these should be added approximately 75 mmoles for trimethylamine oxide. Our intracellular Na^+ values are considerably lower than the very high concentrations reported by Segama and Irisawa for *Dasyatis akajei* taken from the Sea of Japan. Intracellular K^+ and Na^+ concentrations agree closely with Vislie and Fugelli's findings in the flounder heart ventricle which has much lower plasma osmolalities, even in sea water adapted fish. Our NPS findings on the atrium also are in agreement with their conclusion that amino acids (mainly taurine) take part in preventing volume changes caused by alterations in plasma osmolality. Our control in vivo ventricle intracellular concentrations of Na^+ and K^+ are identical with Lee and Fozzard's determinations made with cation selective glass microelectrodes in rabbit ventricular papillary muscles (*J. Gen. Physiol.* 65:695-708, 1971).

Conclusions. The hemi-atrial in vitro preparation of the skate shares the advantage with the hemi-diaphragm of young rats and other small mammals in that it is thin enough so that cells can receive oxygen and nutrients from the external surface without slicing. Furthermore the spontaneous beat that persists for many hours in vitro serves as an indicator of functional viability. Slices or strips of ventricle are well suited because they spontaneously allow Na^+ to leak into cells and K^+ to leave.

In situ the atrium and ventricle regulate cell volume by adjusting intracellular osmolality to lower plasma osmolality by extruding solute during environmental dilution.

The ECF of skate heart muscle is much higher than that of skeletal muscle, a characteristic shared with vertebrate hearts generally. This is reflected in total wet wt. concentrations of Na^+ in ventricular and atrial tissue approximately twice that of K^+ .

Some as yet undetermined component of ninhydrin-positive substances, probably taurine, contributes to cell volume regulation in vitro in the skate atrium. In agreement with earlier findings on the flounder ventricle, K^+ also takes part in preventing volume changes challenged by alterations in osmolality of the incubation medium.

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OXIDATION OF β -ALANINE AND TAURINE BY TISSUES FROM SKATES (*R. erinacea*) ACCLIMATED TO NORMAL AND DILUTE SEAWATER

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Recent studies have shown the importance of free amino acids for intracellular osmoregulation in various osmotically tolerant elasmobranchs including the little skate, *Raja erinacea* (Forster and Goldstein, *Am. Physiol.* 230:925-931, 1976). High concentrations of β -alanine and taurine, among other amino acids identified in skate tissues, have been found to decrease significantly in skates acclimated to 50% seawater. In this investigation, we studied the oxidation of these amino acids by skate tissues to determine mechanisms by which cellular amino acid pools are regulated.

Little skates, *Raja erinacea*, of mixed sex and weighing 0.5-1.0 Kg were used. Blood was withdrawn from the red blood cells suspended in Forster's elasmobranch saline solution. The skate was then killed by

transection of the spinal cord and the other tissues to be studied were removed quickly. In addition to erythrocytes, the tissue preparations included strips of pelvic depressor muscle and slices of telencephalon, liver, and kidney. These tissues (50 mg each) were incubated in 25 ml Erlenmeyer flasks containing 3 ml of Forster's elasmobranch saline (970 mOsm) that was 0.1 mM in β -alanine or taurine and contained 0.1 μ Ci of 14 C- β -alanine or 14 C- β -taurine. In some experiments a diluted incubation medium (790 mOsm) was used. The flasks were incubated in an oscillating water bath at 15°C; the gas phase was 99% O₂ and 1% CO₂. After one hour of incubation, 0.2 ml of a phenylethylamine-ethoxyethanol mixture (1:2) was injected into the center well of the vessel for CO₂ absorption and 0.3 ml of 6 N sulfuric acid was added to the incubating medium. The vessels were incubated for an additional hour and the trapped 14 CO₂ was then measured by liquid scintillation counting.

In a second set of experiments, β -alanine and alanine oxidations were measured in liver and kidney tissues from skates acclimated to either 100% seawater or 50% seawater. Acclimation took place over seven days (Goldstein and Forster, Am. J. Physiol. 220:742-746, 1971) during which time the skates were not fed. In these experiments, concentrations of protein and ninhydrin positive substances (NPS), i.e., mainly free amino acids, in the kidney and liver were also measured (Layne, E. Methods in Enzymology, Vol. 111, 450-451 and 468-471, 1963). Profiles of individual amino acid concentrations in the livers of the skates were determined on salicylic acid extracts of freeze-clamped tissue. Amino acid analysis was performed using a Durrum D-500 automatic amino acid analyzer.

Table 1 shows the values for β -alanine oxidation by normal skate tissues incubated in dilute and full strength salines. Oxidation was highest in the skate kidney followed by liver and brain and no activity was

Table 1

Oxidation of β -alanine by skate tissues incubated in full strength (970 mOsm) and diluted (790 mOsm) elasmobranch salines.

Tissue	Full strength saline	Diluted saline
	μ mol CO ₂ /g·hr	
Kidney	0.94 \pm 0.08 (6)	0.87 \pm 0.05 (4)
Liver	0.44 \pm 0.07 (6)	0.46 \pm 0.11 (4)
Brain	0.16 \pm 0.03 (4)	0.17 \pm 0.02 (4)
Erythrocytes	ND (4)	ND (4)
Muscle	ND (4)	ND (4)

Values are means \pm S.E. Number of fish for each group is shown in parentheses. ND = not detectable.

detected in erythrocytes and muscle preparations. None of the tissues studied displayed taurine oxidation. Incubation in diluted saline did not significantly change β -alanine or taurine oxidation for any of the tissues.

Acclimation of the skates to a diluted environment increased oxidation of β -alanine by 68% in skate liver and 46% in kidney (Table 2). Alanine oxidation was increased by 57% in the liver but environmental dilution caused no significant change in alanine oxidation by the kidney.

Concentrations of ninhydrin positive substances in liver and kidney of skates kept in 100% seawater were 64.0 \pm 8.5 and 48.5 \pm 6.6 mmol/Kg, respectively. In fish acclimated to 50% seawater, liver NPS decreased

Table 2

Oxidation of β -alanine and alanine by kidney and liver of skates acclimated to 100% or 50% seawater.

	Seawater	β -alanine	Alanine
$\mu\text{mol CO}_2/\text{g}\cdot\text{hr}$			
Kidney	100%	0.94 \pm 0.08 (6)	0.97 \pm 0.04 (6)
Kidney	50%	1.37 \pm 0.13 (8)	0.90 \pm 0.08 (8)
		p < .05	NS
Liver	100%	0.44 \pm 0.07 (6)	0.19 \pm 0.04 (6)
Liver	50%	0.74 \pm 0.11 (8)	0.29 \pm 0.02 (6)
		p < .05	p < .05

Values are means \pm S.E. Number of fish for each group is shown in parentheses. P values indicate significance between fish acclimated to 100% seawater vs. 50% seawater. NS = not significantly different.

significantly (37.8 ± 5.7 mmol/Kg, $p < .05$) but there was no significant change in kidney value (37.9 ± 3.3 mmol/Kg). Analysis of concentration profiles of individual amino acids revealed that taurine was the major amino acid in the liver (Table 3). Acclimation of the skates to 50% seawater decreased the

Table 3

Amino compounds in the livers of skates acclimated to 100% and 50% seawater.

Compound	100% seawater ($\mu\text{mol/g}$)		50% seawater ($\mu\text{mol/g}$)
Taurine	25.72 \pm 0.87	p < .001	10.03 \pm 1.40
Aspartate	0.36 \pm 0.03		0.30 \pm 0.07
Threonine	0.72 \pm 0.12		0.54 \pm 0.11
Serine	0.46 \pm 0.10		0.41 \pm 0.05
Glutamic acid	3.69 \pm 0.60	p < .05	1.45 \pm 0.37
Glutamine	ND		ND
Proline	0.07 \pm 0.01		0.09 \pm 0.04
Glycine	0.28 \pm 0.05		0.40 \pm 0.05
Alanine	0.22 \pm 0.03		0.24 \pm 0.11
Citrulline	* 0.01		* 0.03
Valine	0.20 \pm 0.01	p < .01	0.09 \pm 0.02
Cystine	0.05 \pm 0.01		0.05 \pm 0.01
Methionine	0.06 \pm 0.01		0.06 \pm 0.02
Isoleucine	0.16 \pm 0.01	p < .05	0.07 \pm 0.02
Leucine	0.26 \pm 0.02	p < .05	0.16 \pm 0.03
Tyrosine	ND		0.03 \pm 0.01
Phenylalanine	ND		ND
β -alanine	0.97 \pm 0.21		0.94 \pm 0.21
Ammonia	1.59 \pm 0.08		1.68 \pm 0.04
Ornithine	0.21 \pm 0.03	p < .05	0.12 \pm 0.01
Lysine	0.21 \pm 0.03		0.12 \pm 0.03
Arginine	* 0.03		* 0.04

Values are means \pm S.E. 100% seawater values are averages from 4 fish and 50% seawater values are averages from 3 fish. ND = not detectable. *Analysis was done on pooled sample from 3 or 4 fish. P values indicate significance between fish acclimated to 100% seawater vs. 50% seawater.

taurine concentration by 61%. Liver concentrations of glutamic acid, valine, isoleucine, leucine, and ornithine also decreased significantly when skates were acclimated to a diluted environment. Protein concentrations were 87.5 ± 3.3 mg/g liver and 31.8 ± 5.7 mg/g kidney for skates maintained in 100% seawater. Acclimation of the fish to 50% seawater significantly decreased the protein concentrations of liver to 74.8 ± 3.2 mg/g ($p < .01$) and kidney to 18.9 ± 1.4 mg/g ($p < .05$). Environmental dilution also resulted in a 10% decrease in skate liver dry weight percentage. The decrease in liver protein concentration in skates acclimated to 50% seawater can be accounted for by this change in dry weight.

Investigation of the mechanisms by which amino acid pools are altered in response to environmental salinity change can provide important information for understanding cell volume regulation. The present study revealed that oxidation of β -alanine occurs primarily in the liver and kidney of the little skate. This suggests that the dilution-induced decreases in β -alanine concentration in tissues that cannot oxidize the amino acid (e.g. muscle) may be achieved by transport of the amino acids, via the circulatory system, to the liver where deamination takes place. We found that acclimation of skates to 50% seawater caused a rise in capacity of the liver to oxidize β -alanine and alanine. This increased oxidation may have been brought about by the increased load of amino acids transported to this organ from other tissues and would facilitate a faster removal of amino acids during the acclimation period.

The inability to demonstrate taurine oxidation in any of the skate tissues suggests that this amino acid may be excreted intact. Taurine has been shown to be poorly reabsorbed by the mammalian renal tubule (Danzler and Sibernagl, Pflugers Archiv. 367:123-128, 1976). If it is handled comparably by the elasmobranch kidney, taurine released by tissues during environmental dilution would be readily excreted.

In the present study, we observed a decrease in liver NPS and an increase in liver amino acid oxidation when skates were acclimated to 50% seawater. A previous study by Goldstein and Forster (Am. J. Physiol. 220:742-746, 1971) demonstrated that acclimation to a diluted environment caused a decrease in total nitrogen excretion. Thus despite increased oxidative capacity of the liver, lowered amino acid concentrations may cause a decrease in total nitrogen excretion for skates acclimated to 50% seawater.

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HYBRIDIZATION EXPERIMENTS AND SPECIATION IN *Fundulus heteroclitus* AND *Fundulus diaphanus*

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The discovery by one of us (M.J.R.) of what is apparently a hybrid between *Fundulus heteroclitus* (Linn.) and *Fundulus diaphanus* (Lesueur) in the estuarine waters of Narragansett Bay, Rhode Island has led to the experiments listed below. This report concerns itself with those experiments conducted at M.D.I.B.L. while a later paper will describe the natural hybrids and hybridization experiments conducted in Rhode Island. Previous reports of hybridization are few. Hubbes et al (Contrib. Lab. Vert. Biol. Univ. Mich. 23, 1943) Breder and Rosen (Modes of reproduction in fishes. Natur. Hist. Press, New York, 1966) and Newman (J. Exp. Zool. 16:447-499, 1914) have reported the event in nature and experimentally in the laboratory.

One of us (C.E.W. Jr.) has for many years been concerned with the apparent differences in developmental rate of zygotes fertilized in the laboratory at M.D.I.B.L. and those reported from experimental fertilizations at Woods Hole and other places on the south shore of Cape Cod and in Narragansett Bay. In spite of water temperature differences there remained, perhaps, the possibility that *Fundulus heteroclitus* was not the same to the north of Cape Cod as that to the south of this barrier.

A series of fertilizations, following meticulously the methods established and reported by Wilde and Crawford (Exp. Cell. Res. 44:471-488, 1966) were carried out as follows: