the second media change there is a significant decrease in the basal QO_2 but not in the Db C-AMP stimulated tissue. Following the addition of ouabain during the third incubation time period (40-60 min), there was no change in the basal QO_2; however, in the stimulated slice there is significant decrease from the 20-40 min time period.

The data from these QO_2 measurements illustrate that there is a basal non-transporting QO_2 in isolated dogfish rectal gland which can be increased following stimulation. These data also compare favorably with the studies of Rosa et al. (Bulletin, MDIBL 16:1976) who measured QO_2 by AV difference in isolated perfused rectal gland. The basal QO_2 from tissue slices of 0.3 to 0.5 μM O_2/min/gWW is similar to their data on whole glands. Following TDb C-AMP stimulation, the tissue slice values of 0.5 to 0.65 μM O_2/min/gWW are at the low end of the whole gland QP_2's but comparable. Ouabain inhibition lowered the tissue slice value to 0.38 μM O_2/min/gWW, a value similar to whole gland inhibition with ouabain.

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CELL VOLUME REGULATION BY SKATE HEART

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The little or hedgehog skate *Raja erinacea* is an imcomplete osmoconformer and, as such, is a useful model for studying mechanisms that regulate cell volume when interstitial fluids are osmotically perturned. This is a problem of interest both from an environmental and a clinical point of view. This elasmobranch is common in the outer estuaries of the American Atlantic coast from South Caroline to Nova Scotia and it has been collected at survey stations where salinities were as low as $20°/_{oo}$ (Fitz, E. S. Jr. and F. C. Daiber. Bull. Bing. Oceanogr. Coll. 18(3):69-97, 1963). In the laboratory they thrive well in sea water gradually diluted to half strength or lower causing plasma osmolality to drop 26-35%. In the current study on cardiac muscle we sought to differentiate between the osmoregulatory activities of atrial and ventricular fibers and to develop an in vitro method that would perhaps be useful later to examine transport kinetics, metabolic events and electrophysiology of the myocardium.

Methods. In vivo environmental dilution experiments were generally similar to those used in our earlier studies on the intracellular osmoregulatory role of amino acids and urea in skates and stingrays (Forster, R. P. and L. Goldstein. Am. J. Physiol. 230:925-931, 1976; Boyd, T. A., Chung-Ja Cha, R. P. Forster and L. Goldstein. J. Exp. Zool. 199:435-442, 1977). *Raja erinacea* of mixed sex with weights ranging from 1-2 Kg were collected by drag net from waters off Mount Desert Island. Gradual dilution of circulating sea water in the pool was accomplished by reducing the salinity by 10% per day down to 50% sea water, and the skates were then allowed to equilibrate for two days before samples were collected. Blood was taken from the caudal vessels and the skates then killed by swift transection of the spinal cord. The beating excised heart was left in a beaker of balanced isotonic elasmobranch medium gassed with 99% O_2 - 1% CO_2 (Forster et al. Comp. Biochem. Physiol. 42A:3-12, 1972) for 1-2 min until cleared of blood. The atrium and ventricle were surgically separated, the conus arteriosus removed, and the pericardium and adjoining supporting structures trimmed off. Samples of atrium and ventricle were quickly blotted lightly, weighed, placed in aluminum foil and frozen between blocks of dry ice. Tissue was ground to a powder by cold mortar and pestle on dry ice, and extracted. Extracts of tissue for analysis of ninhydrin-positive substances (NPS) were prepared by adding one volume of cold 50% trichloracetic acid (TCA) and 8 volumes of cold distilled water; then mixed, allowed to stand on ice for 30 min, and centrifuged. TCA was extracted 3 times with 3 volumes of hydrated ether. Plasma extracts were similarly prepared using equal volumes of plasma and 10% TCA. NPS determinations were made by the photometric method of Moore & Stein (J. Biol. Chem.

176:367-388, 1945). Osmometry and analyses of Na^+ and K^+ were done on frozen tissue added to 4 volumes distilled water and mixed. The slurry was transferred to a centrifuge tube and placed in a boiling water bath for 2 min. It was then allowed to stand overnight at 6°C, centrifuged, and a 0.2 ml aliquot of the supernatant taken for cryoscopic osmometry (Fiske). Na^+ and K^+ were determined on the remaining supernatant by flame photometry. For determining water content, the atrium and ventricle were separated, rinsed, blotted lightly, weighed in tared vials and dried for 24 hours at 100°C.

For the *in vitro* experiments, the excised heart was placed in gassed (99% O_2 - 1% CO_2) elasmobranch bicarbonate medium until clear of blood and then transferred to fresh medium. The separated thin walled single atrium, which is a large chamber lying above the ventricle in elasmobranch fish, was divided in the and flattened to provide maximal surface area for diffusion. Strips of the thicker-walled ventricle were cut free hand with a razor blade. It was generally possible to obtain 3 strips weighing 30-70 mg each fr a ventricle. The slices and the hemi-atria were blotted, weighed and separate samples placed in Erlenmeyer flasks containing 1 ml of medium for each 10 mg of tissue sample. Following preincubation for 15 min in the 99%-1% CO_2 atmosphere they were transferred to fresh medium and incubated for 2 hours at ro temperature. Medium was prepared for the "dilute" experiments by reducing urea and NaCl concentrations diluted one-third. Following incubation the tissue fragments were blotted lightly, reweighed and quickly frozen between blocks of dry ice. Extraction was identical to the method used in the *in vivo* procedures.

The volumes of distribution of ^{14}C mannitol and ^{14}C inulin were measured to find a suitable method to estimate extracellular fluid volume (ECF). Hemi-atria and ventricular strips were prepared as for the *in vitro* analyses and preincubated for 15 min. The tissue fragments were then transferred to flasks containing elasmobranch medium with the isotopically labeled compound and then incubated in the O_2-CO_2 atmosphere for 2 hours in the case of mannitol, and for 1, 2 and 4 hours with labeled inulin (Table 2). The atrial and ventricular tissues were removed, rinsed in normal nonradioactive medium, blotted lightly and placed in tared scintillation vials. The vials were weighed, 1 ml Protosol added to each, and the vials incubated overnight in a shaking water bath at 40°C. Upon removal from the bath 15 ml of a scintillation cocktail (4g PPO and .05g POPOP added to 1 liter toluene) was added to each; the vials vortexed and assayed for ^{14} by liquid scintillation counting.

TABLE 1

Osmolality and Water Content of Atrium, Venticle and Plasma Following Gradual Environmental Dilution

Seawater	Osmolality (mosm/l)			Water Content (% wet wt)		
	Atrium	Ventricle	Plasma	Atrium	Ventricle	Plasma
100%	1091±97 (6)	1052±50 (9)	1041±23 (7)	86±1 (5)	83±1 (7)	91±1 (3)
50%	715±81 (3) p<.05	685±58 (3) p<.001	701±13 (4) p<.001	90±2 (5) p<.01	86±1 (9) p<.01	95±1 (5) p<.001

Values are means ± standard error. Number of fish per group shown in parentheses.

Results and Discussion. In a gradual environmental dilution procedure that reduced plasma osmolality by one-third (Table 1) both the atrium and ventricle underwent similar reductions in terms of mosmol/Kg wet weight of whole organ while the percentage water content simultaneously increased slightly in plasma, atrium and ventricle. This compliance of heart tissue to a lowered plasma osmolality is in agreement with our earlier observations on skeletal muscle in the skate.

The distribution of inulin in vitro after 1, 2 and 4 hours is shown in Table 2. The 4 hour values for inulin were used to calculate ECF volume and to determine the intracellular concentrations of NPS, Na^+ and K^+ shown in Table 4. Seyama and Irisawa also used the 4 hour inulin distribution period for their study of

TABLE 2

In Vitro Uptake of ^{14}C Inulin in Atrium and Ventricle (Inulin Space, % wet wt) in Full Strength and Diluted Elasmobranch Medium

	One hour	Two hours	Four hours
Control atrium	32±2 (4)	39±1 (3)	42±1 (3)
Dilute atrium	30±1 (4) ns	37±1 (3) ns	41±3 (3) ns
Control ventricle	24±1 (5)	27±2 (5)	37±3 (5)
Dilute ventricle	25±3 (4) ns	22±3 (4) ns	32±1 (4) ns

Values are means ± standard error. Number of fish per group shown in parentheses.

the effect of high sodium concentration on the action potential of the skate heart (J. Gen. Physiol. 50:505-517, 1967). Mannitol uptake is generally higher than inulin and unlike the latter does not equilibrate after 2-3 hours as was shown by Armstrong et al. in the isolated frog ventricle (Am. J. Physiol. 217:1230-1235, 1969). Ventricular strips became more permeable to inulin after 2 hours and there is a significant increase in its distribution presumably due to the trauma resulting from cutting the strips, and perhaps also to inner fibers becoming increasingly permeable to inulin because of hypoxic conditions at the core of these relatively thick sections. The latter explanation is supported by the evidence that Na^+ leakage into ventricular fibers and K^+ loss occurs as early as 2 hours after incubation. Armstrong et al concluded that virtually all the Na^+ in fresh tissue appears to be extracellular, and that during incubation these ions freely enter the fiber water. Note in Table 4 that ventricular strips under control conditions after a 2 hour incubation period have 94 mmole/l of intracellular Na^+ compared with 31 in the original intact ventricle, when simultaneously the intracellular K^+ content of 137 mmole/l drops to 96.

ECF was calculated as percentage wet wt. of tissue = $\frac{\text{counts per min/g tissue}}{\text{counts per min/g medium}}$. ECF volumes are generally higher in heart than in skeletal muscle and most other tissues, and atrial ECF is higher than ventricular. For example Poole-Wilson and Cameron (Am. J. Physiol. 229:1299-1304, 1975) consider ^{51}Cr EDTA a satisfactory ECF marker in intact rabbits, and they report 34% in atria, 27 and 21 of right and left ventricle, respectively; whereas skeletal muscle (quadriceps) was 8% which is typical for striated muscle throughout the vertebrates. Our current results on the skate show also that cardiac muscle ECF is approximately 4X that of the 9% we found earlier in "wing" muscle of *Raja erinacea*.

Table 3 shows that some ninhydrin-positive material is released by atrial tissue during osmodilution under both in vivo and in vitro conditions, and also by ventricular tissue, at least in vivo. Note that these values are for total wet wt. of tissue. However, most of this is intracellular; NPS concentrations

TABLE 3

Ninhydrin Positive Substances (NPS), Na^+, and K^+ Concentrations (mmoles/kg wet weight) in Vivo and in Vitro, Before and After Dilution, in Atrium and Ventricle

	NPS	Na^+	K^+
		in vivo	
Control atrium	103±9 (10)	135±6 (9)	59±4 (10)
Dilute atrium	56±8 (7) p<.01	94±8 (8) p<.001	49±3 (9) ns
Control ventricle	118±10 (14)	116±4 (11)	66±5 (13)
Dilute ventricle	79±5 (14) p<.01	73±4 (12) p<.01	54±2 (12) p<.05
		in vitro	
Control atrium	112±10 (12)	125±8 (7)	64±4 (7)
Dilute atrium	59±4 (7) p<.001	101±4 (10) p<.05	49±1 (11) p<.001
Control ventricle	91±10 (11)	133±3 (8)	47±2 (8)
Dilute ventricle	77±7 (9) ns	133±12 (9) ns	42±1 (10) p<.05

Values are means ± standard error. Number of fish per group shown in parentheses.

plasma are only 10-12 mmole/l. Shown also are the corresponding values for Na^+ and K^+. The most striki feature is the very high Na^+ concentration in the skate. Similar values in the literature expressed in

mmole/kg wet wt. Na^+ are, atrium: rabbit, 64.2; ox(rt.), 96; ox(l.), 72. For ventricle [Na^+] are: rabbit (rt.), 53; rabbit (l.), 45; ox(rt.), 47; ox(l.), 55; frog 31; and cat, 62. The high skate heart values for [Na^+] reflect its relatively higher ECF than that in other vertebrates and also higher absolute concentrations in intracellular water.

Table 4 summarizes intracellular concentrations calculated as in our earlier paper on striated muscle in the skate. Atrial cells in vitro, in contrast to the ventricle, regulate cell volume in a diluted environment by releasing significant quantities of amino acids (NPS). In an earlier study analyzing the concentrations of individual amino acids in vivo we showed that free amino acids (mainly taurine) did not play an osmoregulatory role when skates were gradually acclimated to a dilute environment. Ventricular tissue only was used (Boyd et al. J. Exper. Zool. 199:435-442, 1977), so now it would be interesting to find whether taurine behaves differently in atrium and ventricle in this regard, or whether some other

TABLE 4

Intracellular Concentrations of Ninhydrin Positive Substances (NPS), Na^+, and K^+ in Atrium and Ventricle Following In Vitro Incubation

	NPS (mmoles/l)	Na^+ (mmoles/l)	K^+ (mmoles/l)
Control atrium (in vivo)	218±21 (10)	42±10 (9)	127±9 (10)
Control atrium (in vitro)	225±19 (11)	14±6 (7)	138±9 (7)
Dilute atrium (in vitro)	116±8 (7) p<.001	24±8 (9) ns	105±3 (11) p<.01
Control ventricle (in vivo)	244±19 (14)	31±9 (11)	137±10 (13)
Control ventricle (in vitro)	185±22 (11)	94±24 (8)	96±5 (8)
Dilute ventricle (in vitro)	141±13 (9) ns	85±8 (7) ns	79±3 (10) p<.05

Values are means ± standard error. Number of fish per group shown in parentheses.

unidentified NPS is unloaded by atrial fibers when they are challenged by a diluted interstitium. Again, the hemi-atrium in vitro preparation appears to be the more viable. No NPS is lost from hemi-atria following the 2 hour in vitro incubation period under control conditions, whereas the ventricular strips do release some. Furthermore the latter gain Na^+ and lose intracellular K^+ in the control incubation period

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, 1975) 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 finding 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, 1975).

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 ill-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 lowered 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. J. 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 and the red blood cells suspended in Forster's elasmobranch saline solution. The skate was then killed by