

the fraction of high molecular weight proteins (#1) is $22 \pm 5\%$ (n3) in urine and $59 \pm 2.8\%$ (n5) in plasma.

Although the disappearance curve of PVP from *Lophius*' plasma (Fig. 3) follows a time course similar to that in mammalian species the renal clearance of this molecule is incomparably less. In the rat, for example, clearance of PVP (MW 40,000) is 10,000 x the value calculated here for *Lophius*. The extrarenal disposal of this macromolecule is therefore of considerable interest.

When relative clearances of PVP and protein are compared within species another striking difference is found. In *Lophius* (Table 1) the clearance of PVP is 3 or 4 x tht of total plasma protein, in the rat it is about 1000 x. This discrepancy may be explained by the relatively small molecular weight of fish plasma proteins and the large fraction of LMW proteins in the plasma of *Lophius*.

These preliminary data allow no more than inference regarding the mode of access of plasma proteins to the urine in this aglomerular species. The fact that all plasma proteins find urinary representation speaks against secretion since it seems unlikely that secretory mechanisms for every protein would be evolved. Also LMW proteins predominate in urine whereas known secretory proteins (IgA, uromucoid) are of high molecular weight. It is possible that the very small rate of urinary protein excretion (12 g/hr/kidney) represents leakage of plasma proteins through intercellular spaces. The bulk flow of urine across these epithelial structures may assist the movement of proteins. The predominance of LMW proteins in the urine of *Lophius* is consistent with this process of proteinuria.

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42

19 • 1975

Metabolism of the Standing Dead Plant Community in Several Maine Salt Marshes

John L. Gallagher and William J. Pfeiffer. The University of Georgia Marine Institute, Sapelo Island, Georgia

The salt marsh, like many natural grasslands, is a detritus based ecosystem. Relatively few grazers are present and most of the photosynthate enters the food web via microbial degradation. Microbes are active in southeastern United States marshes throughout the year, but in Maine a strong seasonality in plant decay would be expected.

Aerial and aquatic respiration of the dead plant communities and dissolved organic carbon (DOC) release were measured in stands of *Spartina alterniflora* Loisel., *Spartina patens* (Ait.) Muhl. and *Juncus gerardi* Loisel. Samples were collected from marshes at Northeast Creek and Hog Bay in Hancock County. Aquatic respiration of the community associated with the standing dead plants was measured as oxygen consumption by the Winkler method and aerial respiration was quantified as CO₂ release with an infrared gas analyzer. DOC discharge was measured by the method described by Gallagher *et al* (*Estuar. Coast Mar. Sci.*, *in press*).

Aquatic respiration rates of the three dead plant communities were similar in April when the temperature

was 1°C. They were 14.9(\pm 0.6), 12.6(\pm 1.0), 15.4(\pm 0.5) g C hr⁻¹ gram dry weight⁻¹ (\pm SE) for the *alterniflora*, *S. patens* and *J. gerardi* respectively. Over the wide temperature range used to compare the Maine (ambient 1°C) and Georgia (ambient 25°C) communities the Q₁₀ was 2.75. Aerial respiration rates in April were slightly lower than those measured under aquatic conditions. In May the aerial Q₁₀ for the *S. alterniflora* community averaged 3.89 over the temperature range 2-18°C, while that for *S. patens* was 2.77. These values are higher than similar values for Georgia and indicate the saprophages in Maine communities are adapted to take advantage of short periods of warm weather. Carbon dispersion from the dead plant communities by DOC leaching was more than 4 times that of respiration rate. These compounds released from the dead plant communities are readily absorbed by planktonic heterotrophs. Chemical analyses of the dead plant communities for sugars, starch and crude protein are not complete, but similar studies in Georgia have shown a strong correlation between respiration rate and protein content of the dead plants.

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Free Amino Acids In Tissues Of The Skate, *Raja erinacea*: Regulation Of Concentrations And Transport During Adaptation To A Dilute Sea Water Environment.

Leon Goldstein, Thomas A. Boyd, Anne E. McElroy, Chung-Ja Cha and Roy P. Forster Brown University, at Dartmouth College

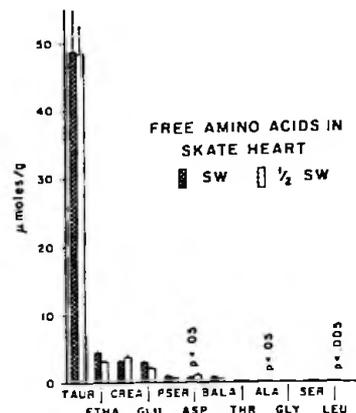
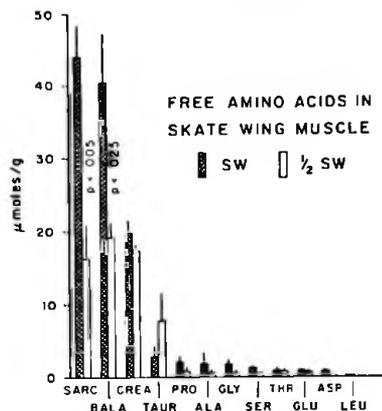
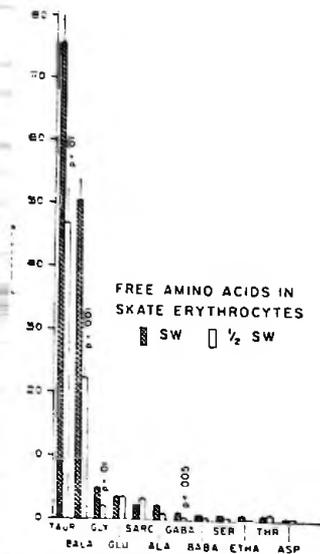
Study of intracellular osmoregulatory processes is difficult in higher vertebrates because these animals do not survive large changes in osmolarity of body fluids. However, certain fish (euryhaline elasmobranchs) are suited ideally for this type of study, for they tolerate relatively large alterations in body fluid osmolarity. Previous studies (*Forster and Goldstein, Am. J. Physiol. in press*) have shown that amino acids play a major role in intracellular osmoregulation in the skate *Raja erinacea*. Total free amino acids in skeletal muscle and RBC of skates kept in seawater (SW) comprise more than 1 percent of the osmotically active solutes, and this concentration is reduced significantly when the fish are adapted to half-strength sea-water (1/2 SW). In the present study, we measured the levels of individual amino acids in RBC, wing muscle and heart of skates maintained in SW and 1/2 SW.

Blood was drawn from a caudal vessel. The skate was pithed and a piece of wing muscle and heart quickly removed and immediately freeze-clamped with aluminum blocks precooled in liquid nitrogen. Blood was separated into packed RBC and plasma and the latter deproteinized with 10 percent trichloroacetic acid (TCA). The TCA was removed with ether. Frozen, packed RBC, wing muscle and heart were powdered separately in mortar and surrounded with dry ice and extracted with 90 percent sulfosalicylic acid. The tissue and plasma extracts were dried under vacuum, over conc. H₂SO₄, at 40°C. The dried residues were dissolved in appropriate volume

Figure 1 (left) Twelve most common free amino acids in skate erythrocytes. 1/2 SW values significantly different from SW values are indicated by p values.

Figure 2 (middle) Twelve most common free amino acids in skate wing muscle.

Figure 3 (right) Twelve most common free amino acids in skate heart.



buffer and analyzed for free amino acids with an automatic amino acid analyzer (Durrum D500).

The major amino acids of RBC were taurine and β-alanine (BALA) (Figure 1); their concentrations were 47 ± 4 and 51 ± 4 moles/g RBC, respectively. In skates adapted to 1/2 SW the levels of these same amino acids dropped to 47 ± 5 and 22 ± 2 moles/g, the decreases being significant in both cases ($P < .01$). Sarcosine and creatine were the major amino acids in wing muscle (Figure 2) and their concentrations fell from 44 ± 4 and 41 ± 7 moles/g muscle to 16 ± 5 and 19 ± 2 moles/g during adaptation to 1/2 SW. In contrast the major amino acid of skeletal muscle, taurine, did not show any change in concentration during environmental dilution (Figure 3); the concentration was 49 ± 7 moles/g in SW and 48 ± 4 moles/g in 1/2 SW. In agreement with previous reports we found relatively low concentrations of individual amino acids (1.0mM) in skate plasma and environmental dilution had no significant effect on these concentrations. Thus, it appears that a relatively small number of amino acids — BALA, taurine, and sarcosine — account for most of the free amino acid concentration in the three tissues examined and that in RBC and skeletal muscle, but not heart, the levels of these amino acids are regulated according to osmotic demands of the environment.

In an attempt to determine the mechanism of regulation of tissue amino acid levels in skates adapted to 1/2 SW, we examined the effects of extracellular Na concentration on the rate of uptake of BALA by RBC in vitro. A 0.2 ml aliquot of a suspension of washed RBC was placed in 2.5 ml incubation medium (300mM NaCl, 10mM KCl, 2.7mM MgSO₄, 15mM Tris, HCl pH7.5, 370mM urea; osmolarity 960mOsm) containing 0.25 Ci [1-¹⁴C]-β-alanine and 2.5 moles ¹²C-β-alanine. The mixture was incubated, with shaking, in 25 ml Erlenmeyer flasks for 1 hour at 15°C. After incubation, 2.0ml of the RBC mixture was suspended and washed twice with Na⁺-free (choline) medium. The washed, packed RBC were extracted with 1.0ml 5 percent TCA and 0.3ml of the extract was mixed with 15ml POPOP-PPO-toluene scintillation containing 1.0ml Protosol. The mixture was

assayed in a Nuclear Chicago Mark I liquid scintillation counter with external standard. Under these conditions BALA uptake was linear for at least 2 hours. A separate experiment showed that BALA was not metabolized by RBC after uptake: RBC were incubated for 3 hours with ¹⁴C-BALA and the washed cells extracted with TCA. The extract was chromatographed on thin-layer cellulose sheets. The sheets were subdivided and analyzed for radioactivity by liquid scintillation counting. Approximately 90-95 percent of the radioactivity on the sheets was associated with the BALA spot.

The rate of BALA uptake was directly dependent on medium Na concentration in the range of 50-300 mEq Na/L (Figure 4). BALA uptake from medium containing 200 mEq Na/L (Na concentration in plasma of skates in 1/2 SW) was 45 percent lower than uptake at 300 mEq Na/L (plasma Na concentration in SW). The effect of Na on BALA uptake was specific for that ion. There was no uptake of the amino acid in the absence of Na in the medium, and partial replacement of medium Na by choline, lithium or sucrose produced the same decrease in BALA uptake. These results suggest that extracellular Na concentration regulates the uptake of certain amino acids in skate RBC and that this effect may be important in controlling the concentration of free amino acids, and thus volume regulation, in the RBC and skeletal muscle of this fish. (Supported by NSF Grant PCM75-14322; NIH Grant HL-04457; The Holden Fund of the Rhode Island Hospital).

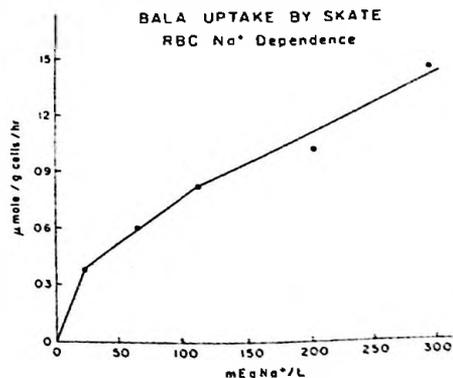


Figure 4 Sodium dependency of β-alanine (BALA) uptake by skate RBC.