experiments with a purified, functioning carrier would be of value in studies concerned with transport energetics and molecular pharmacology. Now that we have a functional assay for this carrier, we will attempt to purify it further, using, for example, affinity chromatography. Supported by USPHS Grant ES 01678.

SYNTHESIS OF β-ALANINE, TAURINE AND SARCOSINE BY TISSUES OF THE LITTLE SKATE (*Raja erinacea*)

Patricia King, Leon Goldstein and Teresa Massagli, Division of Biology and Medicine, Brown University, Providence, Rhode Island

Free amino acids have been shown to play a role in intracellular volume regulation of euryhaline and osmotically tolerant elasmobranchs. In particular, tissues of the little skate, *Raja erinacea*, and the stingray, *Dasyatis americana*, contain high concentrations of β-alanine, taurine and sarcosine that decrease significantly upon acclimation of these fishes to 50% seawater (Boyd et al., J. Exp. Zool., 199(3):435-442, 1977). Regulation of these amino acid levels most likely involves an integration of degradation, synthesis, and cellular transport mechanisms. Previously we investigated the capacity of the skate tissues to oxidize β-alanine and taurine (Bull. MDIBL, 17:16-19, 1977). In the present study, possible pathways of amino acid synthesis, their tissue distribution and their regulation were examined.

Capacity for amino acid synthesis was assayed in the appropriate skate tissues depending on the amino acid being studied. Tissues were chosen in which a particular amino acid was highly concentrated and its level changed significantly upon acclimation to a diluted environment. Liver and kidney were assayed as well. 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 Blood was withdrawn and the red blood cells suspended in Forster's elasmobranch saline-urea solution.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 muscles and slices of telencephalon, liver and kidney.

β-alanine (BALA) synthesis was assayed by following the evolution of $^{14}CO_2$ from ^{14}C-uracil: uracil $\longrightarrow$ dihydrouracil $\longrightarrow$ β-ureidopropionic acid $\longrightarrow$ β-alanine + NH_3 + CO_2. Fifty mg of each tissue and 0.1 ml of red blood cell suspension were incubated in a 25 ml Erlenmeyer flask containing 3 ml of Forster's elasmobranch saline-urea solution (980 mOsm) that contained 0.1 mM uracil and 0.1 µCi of ^{14}C-uracil. In some experiments a diluted incubation medium (680 mOsm) was used. The flasks were closed with stoppers fitted with cups that extended into the vessel and incubated in an oscillating water bath at 15°C; the gas phase was 99% O_2 and 1% CO_2. After one hour of incubation, 0.2 ml of phenethylamine:ethoxyethanol mixture (1:2) was injected into the cup within the flask for CO_2 absorption and 0.3 ml of 6 N sulfuric acid was injected into the incubation medium to release $^{14}CO_2$. The vessels were incubated for an additional hour and the trapped $^{14}CO_2$ was then measured by liquid scintillation counting.

β-alanine synthesis from ^{14}C-uracil was also assayed in slices prepared from skates acclimated to 50% seawater. Acclimation took place over 7 days during which time salinity was followed by chloride determinations. Skates were fed on the third day of the acclimation period. The incubations were as described above; a diluted medium was used.

For the remainder of the synthesis experiments, tissues were incubated with putative radioactive precursors and the amino acid end products isolated by thin layer chromatography on cellulose sheets. The precursors used included ^{3}H-uracil for BALA formation; ^{14}C-choline chloride for sarcosine and glycine formation; and ^{35}S-sodium sulfate and ^{14}C-cystine for taurine synthesis. Each precursor was appropriately labeled so that the amino acid product could be detected by liquid scintillation. A 30 mg slice of each tissue to be tested or 0.06 ml of red blood suspension was placed in a vial

containing 0.5 ml of Forster's elasmobranch saline-urea solution and 0.1 mM and 5 to 10 µCi of the appropriate radioactively labeled precursor. In some of the experiments 5 µmoles of the final amino acid was added to the incubation medium to decrease degradation of the desired labeled end product. The vial was placed in a 10 ml beaker which was inside a 20 ml beaker (with each beaker containing a small amount of H_2O for temperature equilibration) and incubated in a shaking water bath at 15°C for 5 hours. At the end of the incubation approximately 0.45 µmoles of unlabeled amino acid product was added to insure detection during color development of the chromatography. Four volumes (2 ml) of pure ethanol were then added, the tissue ground with a glass rod, and centrifuged. The supernatant was removed and 5 µl were spotted on thin layer cellulose sheets and run in a solvent containing 120 ml n-butanol, 50 ml H_2O, and 30 ml acetic acid. The plates were sprayed with ninhydrin and color developed by heating at 100°C for 5 minutes. After recording R_f values, the spot for the amino acid product was cut out and incubated overnight at 37°C in 3% Protosol toluene cocktail. The radioactivity was then measured by liquid scintillation counting.

Table 1. Formation of ß-alanine from ^{14}C-uracil in isolated tissues of skates acclimated to 100% and 50% seawater.

		nmol CO_2/g-hr	
Seawater	Tissue	Full strength saline*	Diluted saline**
100%	liver	362.0 ± 54.0 (6)	440.0 ± 93.0 (4)
	kidney	33.1 ± 9.4 (6)	
	brain	0.3 ± 0.2 (6)	
	rbc	2.8 ± 1.7 (6)	
	muscle	1.9 ± 0.9 (6)	
50%	liver		325.0 ± 60.0 (4)

Values are means ± S.E. Number of fish for each group is shown in parentheses.

*Incubation of tissues in full strength elasmobranch saline (980 mOsm).

**Incubation of tissues in diluted elasmobranch saline (680 mOsm).

Table 1 shows the values for BALA synthesis by tissues of skates acclimated to either 100% or 50% seawater. Synthesis of BALA from ^{14}C-uracil was highest in the liver followed by the kidney. Incubation of liver slices in dilute saline or acclimation of the skates to 50% seawater did not significantly affect the rate of BALA synthesis in this tissue.

The remainder of the amino acid synthesis results are shown in Table 2. Using ^{14}C-cystine or ^{35}S-sodium sulfate as precursors, skate tissues displayed only trace amounts of taurine synthesis (experimental cpm < 2 x background cpm) when the incubation medium included 10 mM taurine. Synthesis of sarcosine from ^{14}C-choline chloride was demonstrated in the kidney and liver at significant levels; these incubations included 10 mM sarcosine in the saline medium. Glycine synthesis from ^{14}C-choline chloride was also demonstrated in the liver.

Elucidation of the mechanisms regulating the intracellular amino acid levels is critical for understanding the dynamics of cell volume control. Although BALA is accumulated in high concentrations in red blood cells and muscle, the present study reveals that the synthesis of this amino acid occurs predominantly in the liver. Likewise, sarcosine which accumulates in the muscle appears to be synthesized mostly by liver and kidney. These results suggest that synthesis is localized to the liver with the amino acid being transported via the circulatory system to the rest of the organs and accumulated in the various tissues by selective uptake. Incubation of the tissues in diluted saline and acclimation of the skate to 50% seawater did not significantly change the capacity of the liver

TABLE 2

Appearance of precursor label in amino acids synthesized by isolated skate tissues

Precursor	Amino acid	Tissue	dpm/g Incubation 1*	dpm/g Incubation 2**
^{35}S-sulfur	Taurine	Liver	ND	Trace
		Brain	ND	Trace
		RBC	ND	Trace
		Heart	ND	Trace
^{14}C-cystine	Taurine	Liver	ND	-
^{14}C-choline chloride	Sarcosine	Liver	Trace	2010
		Kidney	Trace	6103
		Muscle	-	Trace
	Glycine	Liver	-	1317

Values are the means of duplicate chromatographic runs from one fish.
Trace activity indicates experimental cpm < 2 x background cpm.
ND = not detectable.

*Incubation medium was elasmobranch saline that contained 0.1 mM labeled precursor (20 µCi/ml).

**Incubation medium was elasmobranch saline that contained 0.1 mM labeled precursor (20 µCi/ml) and 10.0 mM amino acid end product.

slices to synthesize BALA. Thus, unlike the degradation and membrane transport of BALA, synthesis of this amino acid does not appear to be regulated during acclimation to a dilute environment.

Five metabolic pathways have been identified for taurine synthesis in biological systems (Jacobson and Smith, Physiol. Rev. 48(2):424-511, 1968). Our study tested four of the five pathways and significant taurine synthesis could not be demonstrated in any of the tissues. Considering the high levels of taurine in the skate and the lack of evidence supporting *de novo* synthesis of this amino acid, a high dietary source of taurine or its synthesis in the gut by bacteria may be important.

The present study also provides information concerning sarcosine degradation. We have previously been unable to test sarcosine oxidation due to low sensitivity of nonradioactive assays and the unavailability of an appropriately labeled preparation of the amino acid. The results of the present work indicate that the skate liver has the capacity to synthesize glycine from choline chloride thus providing evidence for sarcosine metabolism: choline ⟶ sarcosine ⟶ glycine. This work was supported by NSF grant PLM 75-14322.

ELECTROPHYSIOLOGICAL STUDY OF THE APICAL BARRIER OF THE INTESTINAL EPITHELIUM OF WINTER FLOUNDER (*Pseudopleuronectes americanus*)

Sandy I. Helman and Klaus W. Beyenbach, Dept. of Physiology and Biophysics, University of Illinois, Urbana, Illinois

The intestinal epithelium of the winter flounder (*Pseudonectes americanus*) absorbs NaCl by a process that is thought to involve a neutral uptake of NaCl at the apical (mucosal) barrier of the cells (Field et al., J. Memb. Biol. 41:245, 1978, and Frizzell et al., J. Memb. Biol., in press). To characterize the apical barrier electrophysiologically the voltage at the apical barrier (V_a) was determined with intracellular microelectrodes and the changes of voltage observed in response to alteration of the ionic composition of the mucosal solution. Of particular interest was the finding that changes of mucosal potassium concentration caused large depolarizations of the V_a while changes of Na and Cl concentrations had either little or paradoxical effects on the V_a.