the nonspecific binding characteristics for cAMP, the minimal stimulation by cAMP, and the absence of an effect of protein kinase inhibitor. This investigation was supported by U.S. Public Health Service Grant AM-03858 (to Dr. Richard M. Hays).

INCORPORATION OF AN ORGANIC ANION CARRIER FROM WINTER FLOUNDER (*Pseudopleuronectes americanus*) KIDNEY PLASMA MEMBRANES INTO LIPOSOMES.

David S. Miller, Linda A. Butler and John B. Pritchard, Mount Desert Island Biological Laboratory, Salsbury Cove, Maine, and Laboratory of Pharmacology, NIEHS, Research Triangle Park, North Carolina

Experiments with isolated membrane fractions have provided mechanistic information on transport processes in several specialized transporting epithelia. Recent developments in membrane biology now indicate that membrane proteins can be inserted into defined artificial lipid membrane systems, e.g., liposomes, and that specific transport activity is retained in the reconstituted system (see, e.g., Biochem. J. 168:311-314, 1977). We present here results of preliminary experiments which show reconstitution of p-aminohippuric acid (PAH) transport in a flounder kidney plasma membrane (PM) protein-liposome system.

Kidney membranes were isolated by the procedures of Eveloff et al. (in preparation), membrane proteins were solubilized and incorporated into liposomes using a modification of the procedure of Kinne and Faust (Biochem. J. 168:311-316, 1977). Briefly, membranes were solubilized in KHT buffer (150 mM KCl, 10 mM $MgSO_4$ and 5 mM Tris-HEPES, pH 7.5) with 0.5% Triton X-100 and membrane fragments were removed by ultracentrifugation. The supernatant was passed through six Bio-Bead SM 2 columns to remove the Triton and the Triton-free extract (4 ml) was mixed with an equal volume of a 20 mg/ml suspension of phosphatidyl choline (Sigma type III=E, with hexane removed under vacuum) in KHT. This mixture was sonicated for 2 min in an ice bath and the proteoliposomes were collected by ultracentrifugation. Proteoliposomes were suspended in KHT and used immediately for transport experiments employing the Millipore filtration procedure of Hopfer et al. (J. Biol. Chem. 248:25-32, 1973).

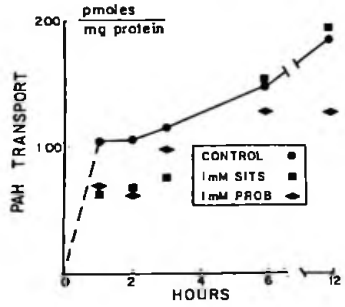

Figure 1. Representative experiment showing time course of PAH uptake by proteoliposomes prepared from flounder kidney PM and phosphatidyl choline. Proteoliposomes, suspended in KHT, were added to an equal volume of KHT containing 20 μM ^{3}H-PAH with or without inhibitor. Fifty μl aliquots were removed at timed intervals, diluted with 1 ml KHT containing 1 mM SITS and filtered through 0.22 Millepore filters. Proteoliposomes on the filters were washed and then counted using standard liquid scintillation procedures. Data given as mean of duplicate determinations from a single proteoliposome preparation (PM from 8 fish kidneys).

Initial experiments with proteoliposomes from flounder kidney brush border membranes showed Na-dependent and phloridzin sensitive transport of D-glucose, but no specific uptake of PAH. With proteoliposomes from PM, PAH transport was inhibited by both SITS and probencid (Figure 1); these chemicals are strong inhibitors of transport in both intact tubules and isolated PM. In addition, PAH uptake was reduced when mannitol was added to the incubation medium (not shown) indicating that substrate was moving into an osmotically active space rather than binding to the proteoliposomes.

The renal organic anion system mediates active excretory transport of xenobiotics and natural metabolites. At present, the driving forces for transport have not been determined and the effects of drugs and chemical pollutants are just beginning to be explored at the subcellular level. Clearly,

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