gans of teleost and elasmobranch under differing circumstances of osmotic stress. The enzyme is likely, therefore, to play an important role in the active transport of sodium across epithelial membranes and the adjustment of marine animals to their environment.

Supported by PHS grants HE-00834 and AM-5015.

1968 #19

UREA: EXPERIMENTS DEMONSTRATING ITS INERT CHARACTER IN TWO PHYSIOLOGICAL SYSTEMS OF THE SPINY DOGFISH, Squalus acanthias

Ralph Janicki and Leon Goldstein, Harvard University, Boston, Mass., and Brown University, Providence, R. I.

The high concentration of urea (approximately 350 mM) in the tissues of elasmobranchs has been considered to be of physiological importance only in relation to its osmotic effect. Two test systems, the accumulation of p-aminohippurate (PAH) in kidney slices and oxygen (O_2) consumption in isolated liver mitochondria were used to examine this view. The uptake of PAH by kidney slices of Squalus was measured according to the procedure of Taggart and Forster (Meth. Med. Res. 5:228, 1952) at 25°C. Urea failed to effect the PAH S/M (slice/medium) ratio in eight assays. When the Ringers solution contained 360 mM urea the S/M ratio was 8.6 ± 1.1, whereas in Ringers solution without urea the S.M. ratio was 8.8 ± 1.2. Similarly urea failed to affect the uptake of PAH by slices prepared from the kidney of a teleost (Pseudopleuronectes americanus).

For studies on O_2 consumption, intact mitochondria were prepared from Squalus liver in 0.88 M sucrose according to the procedure of Massey and Smith (Comp. Biochem. Physiol. 25: 241, 1968) with the addition of enough mannitol, or mannitol and urea, to bring the final osmolarity to 1.0 molar. Succinate (9 mM) served as substrate and was added from the sidearm to initiate the reactions which were run in duplicate, at 25°C, in a Warburg apparatus. Oxygen consumption (μl O_2 consumed by mitochondria from one gram liver per hour) in the presence of 360 mM urea was 84 ± 3.7 and without urea 82.0 ± 6.4 (five assays each).

These observations support the thesis that marine elasmobranchs are not dependent upon urea for reasons other than maintaining an osmolarity slightly above that of their external environment.

It was noted, incidentally, that sucrose inhibits O_2 consumption in isolated liver mitochondria of Squalus. Sucrose concentrations as low as 0.12 M completely inhibited O_2 consumption. The "sucrose-effect" was reversible since mitochondria isolated in sucrose and resuspended in 1.0 M mannitol consumed oxygen at the normal rate. Liver mitochondria prepared from the short horned sculpin (Myosocephalus scorpius) readily consumed O_2 in the presence of sucrose indicating the inhibitory effect of sucrose may be peculiar to the elasmobranchs.

Research supported by NSF grant GB-5613.