aminohippurate), ^{125}I-Diodrast (-iodopyracet) and inulin in intact fish.

Active, well-fed flounder (Pseudopleuronectes americanus) weighing between 150 and 600 g were used. A catheter was inserted through the external urinary papilla and tied in place for urine collection. Inulin (300 mg/Kg body weight) was injected intraperitoneally at least 12 hours prior to an experiment. Organic acids at appropriate doses were injected either intravenously or intraperitoneally in 0.5 - 1.0 ml of saline. Urine was collected continuously in fractions of 0.2 - 2.0 ml (usually 0.3 ml) and when required 0.3 ml of blood was drawn by needle from the tail vein. Urine/plasma concentration ratios (U/P) were calculated using the concentration of unbound organic acid in the plasma at the midpoint of the period during which a urine sample was formed. The latter period was obtained by subtracting the lag time of the catheter from the start and finish of sample collection. Ultrafiltration of plasma from fish injected with organic acid indicated that 30% of CPR (8 fish), 95% of PAH (2 fish) and 94% of Diodrast (1 fish) were not bound to plasma proteins.

Experiments were carried out on 22 fish. The urine flow varied from 0.2 to 2.4 ml/Kg/hr with a mean of 1.1 ml/Kg/hr. Values for U/P inulin usually ranged from 1 to 4, indicated limited water reabsorption. When the plasma concentration of unbound CPR was greater than 1×10^{-5}M the U/P CPR ratios were less than 50. As the concentration of unbound CPR in the plasma fell, the U/P CPR ratios rose to almost 500 at 5×10^{-7} and approached 2,500 between 1×10^{-7} and 1×10^{-8}M. Results for PAH and Diodrast show similar high U/P ratios when the plasma concentrations were in the range 5×10^{-7} to 5×10^{-8}M. The maximal ratios were 1,200 and 4,500 for PAH and Diodrast respectively.

Since all segments of the flounder nephron except the neck region and the large collection ducts probably secrete organic acids (Trump and Bulger, Lab. Invest. 16:453, 1967), water reabsorption from the nephron, unless it occurs in the large collecting ducts, will not increase the U/P ratio for organic acid above the luminal fluid/plasma ratio produced by tubular cell secretion. Thus, U/P ratios in vivo may be compared to the luminal fluid/medium ratios in vitro and the present results demonstrate that the very efficient secretion of organic acids by flounder tubules in vitro also occurs under in vivo conditions. The fact that tubular cells are capable of maintaining equally high concentration ratios in both conditions, suggests that the brush border pump (Mackenzie et al, report #21, this volume) functions in vivo as well as in vitro. Currently we are preparing autoradiographs from kidney tissue exposed to organic acids in vivo in hope of answering this question definitively.

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COMPARISON OF IN VIVO AND IN VITRO ACCUMULATION OF ORGANIC ACIDS BY RENAL TISSUE OF FLOUNDER (Pseudopleuronectes americanus)

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The active transport of the organic acid dyes, phenol red and chlorophenol red (CPR), into the lumen of the flounder kidney tubule has been well established in in vitro experiments. It is

generally considered that the major uphill step in this transport is produced by a pump at the luminal (brush border) membrane of the tubular cells and that the concentration of dye in the cells remains relatively low (Cf. also Mackenzie et al, Bull. MDIBL, this volume). Much less is known about the secretion of the organic acids in vivo by flounder kidney tubules. Thus an attempt has been made to determine whether the concentration of ^{3}H-CPR and ^{3}H-p-aminohippurate (^{3}H-PAH) in flounder kidney tubules differs under in vitro and in vivo conditions.

Samples of renal tissue exposed to organic acids in vivo were obtained, as follows, at the end of experiments with ^{3}H-CPR and ^{3}H-PAH in intact flounder (Maack et al, Bull. MDIBL, this volume): a terminal blood sample was taken; the fish killed by decapitation; the kidneys removed and a portion (10-20 mg) blotted, weighed and transferred to vials for digestion and scintillation counting. The remainder of the kidney was teased in cold Forster's medium and 10-20 mg masses were incubated in the plasma of the fish from which the tissue was taken and in Forster's medium containing appropriate concentrations of either ^{3}H-CPR or ^{3}H-PAH. The tissue was incubated for 30 to 60 minutes at 20-25 C in shallow depression slides and midway through the incubations the plasma or medium was renewed. At the end of the incubation the tissue was blotted, weighed and transferred to vials for digestion and scintillation counting. Ratios of the concentration of organic acid in the tissue to that in the medium or plasma (T/M) were calculated from the radioactivity measurements. Correction was made for the amount of CPR (70%) bound to plasma proteins (Maack et al, report #20, this volume).

The T/M ratios obtained for tissue exposed to organic acid in vivo (11 fish) fell within the same range of values as the T/M ratios for tissue incubated in vitro (44 samples). If the results of the in vivo and in vitro experiments are combined, the extreme values for T/M ratio were 4 and 100 for CPR and 1 and 30 for PAH when the concentration in the medium or plasma ranged from 1×10^{-5} to 1×10^{-8}M. The ratios increased as the concentration of organic acid in the medium or plasma was decreased, i.e., highest ratios obtained with 1×10^{-8}M.

The results indicate that the total uptake of organic acid by the renal tubules is similar whether they are exposed to the acid in vivo or in vitro. Furthermore, if it is assumed that the organic acid is concentrated in the tubular lumens (approximately 6% of the tissue mass, Cf. Maack and Kinter, Am. J. Physiol. 216:1034, 1969) then the lumen/medium ratios would vary from 68 to 1,700 for CPR and 17 to 510 for PAH. Such values are similar to the urine to plasma ratios from intact fish (Maack et al, Bull. MDIBL, this volume) which varied from 50 to 2,500 for CPR and 50 to 1,200 for PAH over a similar range of concentrations in the plasma. Therefore, since most of the organic acid in the tissue under in vivo conditions can be accounted for in the tubular lumens, these data suggest the concentration of organic acid is low in the tubular cells and that an uphill, brush border pump is required to produce the high luminal concentrations.