



Figure 1. Photomicrograph of a cross section of a flounder tubule with overlying  $^{125}\text{I}$ -Diodrast autoradiograph.

which suggest that an uphill pump is not present at the brush border. However, we do have additional in vivo data (Maack et al and Mackenzie, reports #20 and #21, this volume) which suggest that the brush border pump is present and does function in the kidney of intact fish.

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RENAL EXCRETION OF CHLOROPHENOL RED, PAH, AND DIODRAST BY INTACT FLOUNDER (*Pseudopleuronectes americanus*)

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A considerable amount of data has been accumulated on the active transport of organic acids such as chlorophenol red into the lumen of the flounder kidney tubule in vitro. The present experiments were carried out to extend these data to the in vivo situation by measuring urine/plasma ratios for the concentration of chlorophenol red (CPR),  $^3\text{H}$ -CPR,  $^3\text{H}$ -PAH 9-p-

aminohippurate),  $^{125}\text{I}$ -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  $1 \times 10^{-5}\text{M}$  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  $5 \times 10^{-7}$  and approached 2,500 between  $1 \times 10^{-7}$  and  $1 \times 10^{-8}\text{M}$ . Results for PAH and Diodrast show similar high U/P ratios when the plasma concentrations were in the range  $5 \times 10^{-7}$  to  $5 \times 10^{-8}\text{M}$ . 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