1969 #19

HIGH-RESOLUTION AUTORADIOGRAPHY OF ^{125}I-DIODRAST, ^{3}H-PAH AND ^{3}H-CHLOROPHENOL RED IN ISOLATED FLOUNDER KIDNEY TUBULES

Duncan D. S. Mackenzie, Ming-Der Wong and William B. Kinter, State University of New York Upstate Medical Center, Syracuse, N. Y.

During in vitro incubation of flounder kidney tubules the anionic dyes phenol red and chlorophenal red (CPR) may be concentrated several hundred fold in the tubular lumen with respect to the medium and yet the dye is not usually detectable in the cells of the tubular wall. An estimate of the cellular concentration relative to that in the medium and the lumen is necessary in order to understand the overall process of transepithelial transport of these organic acids. With newly developed autoradiographic methods for water-soluble compounds, it is now possible to estimate the relative concentration of organic acid in the medium, cell and lumen when tubules from flounder kidney are incubated in vitro with either ^{3}H-CPR, ^{3}H-PAH (-p-aminohippurate) or ^{125}I-Diodrast (-iodopyracet).

Teased kidney fragments from flounder (Pseudoplueronectes americanus) were incubated in shallow depression slides for periods of up to 60 minutes in Forster's medium containing appropriate concentrations of the organic acid (Kinter, Am. J. Physiol. 211:1152, 1966). Incubations were terminated by freezing the tissue in liquid propane at approximately -160°C. The tissue, kept at liquid nitrogen temperature, was air-freighted to Syracuse where autoradiographs were prepared by the method of Stirling and Kinter (J. Cell Biol. 35:585, 1967). After the freeze drying and plastic embedding steps, certain modifications were required since it was found that the organic acids, particularly Diodrast and CPR, were leached from the plastic sections on contact with water. Thus to prevent the sections from coming in contact with water, they were cut with a dry knife and covered with a waterproof film of Saran F 310 before a semi-dry film of emulsion was applied.

An example of an autoradiograph of a tubule which was incubated in 2.5×10^{-5}M ^{125}I-Diodrast is shown in Figure 1. The microscope has been focused so that some of the silver grains appear as black and some as white dots overlying the medium (M), tubular cells (C) and lumen (L). In this particular autoradiograph the lumen/medium ratio of the number of grains per unit area was 53 and the cell/medium ratio was 9. Similar ratios were obtained for other tubules incubated in 2.5×10^{-5}M ^{125}I-Diodrast or ^{3}H-PAH. Ratios were lowered if the concentration of organic acid in the medium was increased to 1×10^{-3}M and were close to unity if the tissue was incubated in the absence of oxygen. Samples of tissue incubated in medium containing 1×10^{-6}M and lower (down to 1×10^{-9}M) concentrations of ^{3}H-CPR or ^{3}H-PAH are at present being processed. Preliminary results for tubules incubated in 1×10^{-6}M ^{3}H-CPR indicate a considerably higher lumen/medium ratio for CPR at this concentration than for Diodrast or PAH at 2.5×10^{-5}M.

Overall, the results of these autoradiographic studies and previous visual observations indicate that the movement of organic acid from the tubular cells into the lumen can proceed against a concentration gradient, thus indicating the presence of an uphill pump for organic acids at the brush border. In contrast Burg and Weller (Am. J. Physiol. 217:1053, 1969) found with microperfused, flounder kidney tubules that the concentration of Diodrast in the cells was always considerably higher than that in the medium or the lumen. We cannot explain their data

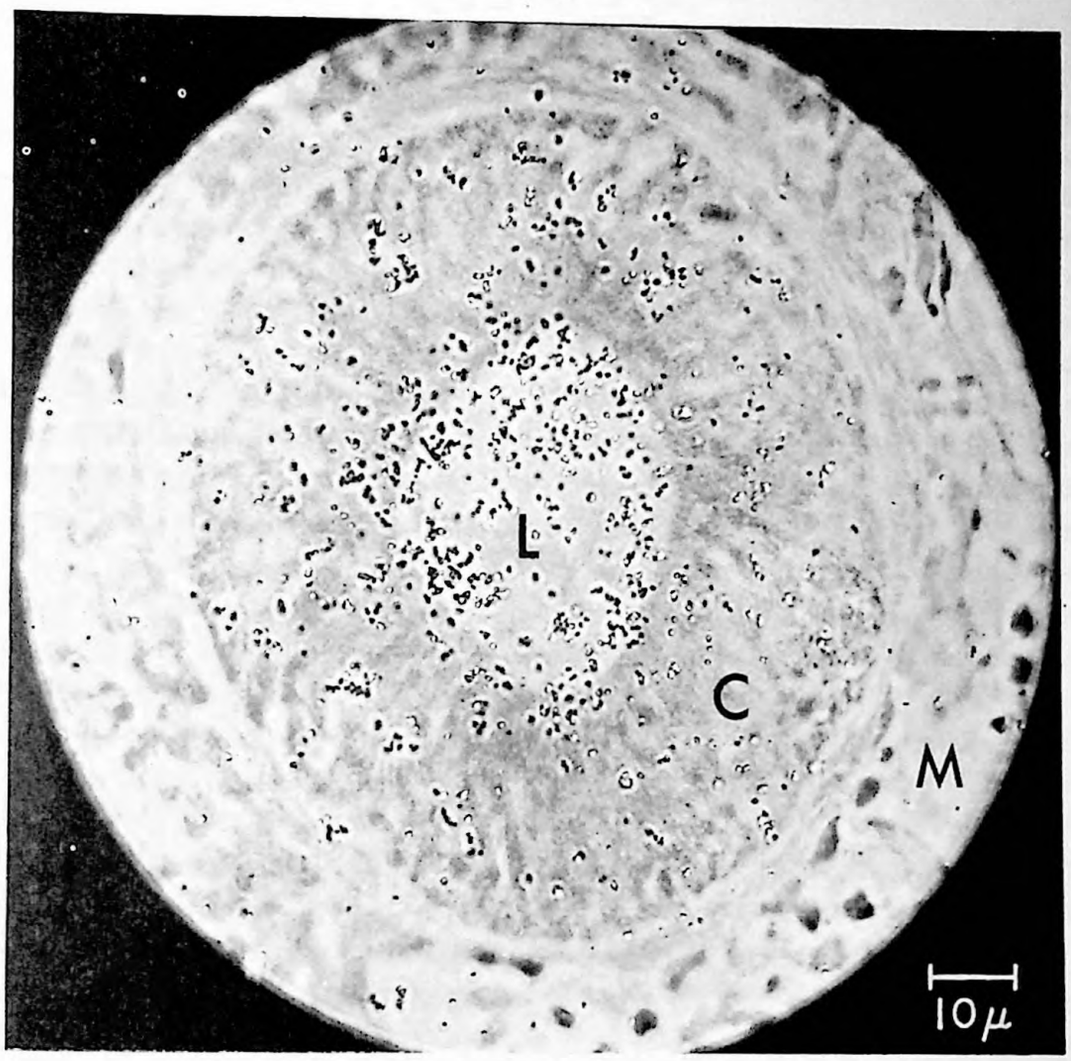

Figure 1. Photomicrograph of a cross section of a flounder tubule with overlying ^{125}I-Diodrast autoradiograph.

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.

This work was supported by USPHS Grant AM 06479.

1969 #20

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

Thomas Maack, Duncan D. S. Mackenzie and William B. Kinter, State University of New York Upstate Medical Center, Syracuse, N. Y.

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}H-CPR, ^{3}H-PAH 9-p-