In Vitro Transport of Dyes by Isolated Renal Tubules of The Flounder As Disclosed by Direct Visualization. Intracellular Accumulation And Transcellular Movement.

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Certain representative acidic and basic dyes have been observed directly while undergoing cellular transport in an isolated renal tubule preparation *in vitro*. For compounds capable of being actively transported across renal cells, movement into the cells from ambient medium is the rate-limiting process, and subsequent concentration in the lumen than proceeds without detectable intracellular accumulation. However, those compounds not transported actively themselves tend to accumulate within the cell, and they act most effectively as competitive inhibitors of the more actively transported members of an homologous series such as the phenolsulfonphthaleins. The behavior of various dyes during uptake and run-out under experimentally imposed conditions supports the view that transport across renal cells involves at least three phases, each of which for the different dyes may or may not be dependent upon energy-yielding metabolic events. These include the two steps which move substances across the peritubular and the luminal cell membranes, and an intracellular "trapping mechanism" which usually is exhibited most prominently with those basic and acidic compounds which are least actively transported.

The basic dye Neutral red was concentrated intraluminally by some energy-independent process which operated in the presence of 2, 4-dinitrophenol and cold; and general metabolic inhibitors did not facilitate run-out of the dye after tubules were transferred to dye-free medium subsequent to accumulation. Cyanine #863 was also taken up in the presence of DNP and in the cold but, in contrast to Neutral red, it exhibited very strong intracellular accumulation. Indigo carmine (Ponceau R), a typical sulfonic acid dye, was taken up intraluminally and to some extent intracellularly by an energy-demanding process. After accumulation, it appeared to be "trapped" and little run-out of dye occurred when tubules were transferred subsequently to cold dye-free medium.

Run-Out of Chlorphenol Red Following Luminal Concentration By Isolated Renal Tubules of The Flounder.

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In this series of studies observations were made after isolated tubules had been exposed for 60 minutes to 3 x 10^{-5}M concentrations of chlor-

phenol red under the standard conditions generally used for the isolated tubule preparation. After accumulation the tubules were transferred to oxygenated dye-free medium containing various agents to be tested for their possible effectiveness in altering run-out rates of chlorphenol red. In no instance was accumulation of chlorphenol red noted within cells, whether during uptake or during run-out in the presence of metabolic or competitive inhibitors. After dye accumulation had been achieved the cell membrane on the luminal side was the effective barrier; when an inhibitor was effective, run-out of dye back to medium was not impeded by the cell membrane on the peritubular side, nor was any evidence of an intracellular "trapping mechanism" noted. The energy requirements of step II (transfer from cell to lumen) are similar to those previously shown for step I (penetration of cell from medium). The metabolic inhibitors, NaCN, 2, 4-dinitrophenol and $HgCl_2$, effected essentially complete run-out with 30 minutes exposure, while cold (2-4°C) for 60 minutes resulted in about 50% loss of dye from lumena.

Equimolar concentrations of compounds which compete with chlorphenol red for uptake facilitate its run-out subsequent to luminal concentration, though the order of relative effectiveness is not identical in both instances. Compounds most effective in reversing luminal concentrations of chlorphenol red with p-aminohippurate, bromcresol green, xylenole blue, Benemid, Diodrast and bromphenol blue. These, after 60 minutes exposure, were at least as effective as cold in facilitating run-out. Indigo carmine, bromchlorphenol blue and p-aminobenzoic acid were ineffective.

These observations serve to characterize step II transfer as an energy-dependent process and a site of competitive transport inhibition.

Excretion of Sodium Bicarbonate By The Freshwater Catfish, (Ameiurus nebulosus)

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It has been previously demonstrated that in the marine dogfish (Squalus acanthias) a carbonic anhydrase sensitive to inhibition by Diamox* was involved in the branchial excretion of sodium bicarbonate. The present study was undertaken to explore if a similar mechanism exists in the freshwater catfish (Ameiurus nebulosus).

In the catfish, intraperitoneal injection of 10 - 20 mg of the sodium salt of Diamox elicited an increase in blood PCO_2, (HCO_3^-), (total CO_2), and a decrease in pH. These changes in blood were associated with an increase in urine volume, pH and total CO_2 excretion. The excretion of CO_2 by the gills and the loss of sodium into the surrounding fluid was unaffected.

The intraperitoneal injection of 1.1 - 1.5 mM of sodium bicarbonate elicited an increase in blood pH and (HCO_3^-) and a decrease in the plasma