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

(Cl^-). The urine volume, pH, total CO_2 and sodium excretion increased. Elimination of the sodium bicarbonate load was slow and blood levels of (HCO_3^-) did not return to normal within 24 hours. CO_2 excretion via the gills and sodium loss into the surrounding fluid was slightly increased.

The intraperitoneal injection of a combination of 1.5 mM of sodium bicarbonate and 20 mg of the sodium salt of Diamox elicited a less consistent rise in blood (total CO_2) and (HCO_3^-) and virtually no change in pH. The urine volume, pH, total CO_2 and sodium excretion increased. The rate of excretion of CO_2 via the gills did not differ significantly from that observed in control animals and in fish receiving Diamox alone. The rate of sodium loss into the surrounding fluid was similar to that observed following sodium bicarbonate alone.

Conclusion: in contrast to the marine dogfish, the freshwater catfish retains a sodium bicarbonate load for as long as 24 hours. Part of the bicarbonate is excreted by the kidneys thereby increasing the urinary pH. However, it could not be demonstrated in the catfish, that branchial excretion of sodium bicarbonate is significantly increased after a (sodium bicarbonate) load, nor was it possible to demonstrate inhibition of branchial bicarbonate exchange by Diamox. It was again shown that Diamox inhibits the formation of acid urine.

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The Transport of Indigo Carmine and Neutral Red by the Isolated Flounder Tubules

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Observations were made on the rate of transport of indigo carmine and neutral red by isolated flounder tubules suspended in modifications of the medium described by Forster.

The transport of neutral red across a cellular barrier is generally related to its dissociation constant and the difference in pH on both sides of the membrane. In the isolated flounder tubule, a low intraluminal pH and an alkaline suspending medium would theoretically facilitate the transport of neutral red from the medium to the lumen. Accumulation of the dye in the lumen may then be ascribed to trapping. This phenomenon was repeatedly observed in the present studies and the degree of concentration agreed with the prediction formula of Jacobs (Cold Spring Harbor Symposium 8: 1940). However, a similar but less marked intra-luminal accumulation occurred in the absence of a proper pH gradient (acid suspending medium). The following hypothesis seems to account for the observations: 1) neutral red enters the cell of the flounder tubule by virtue of an appropriate concentration gradient, 2) the dye is concentrated in plates along the luminal surface of the cell, thereby maintaining appropriate diffusion gradients, 3) enters the lumen by virtue of the difference in pH