Uptake and Run-out of Chlorphenol Red By Isolated Renal Tubules of Flounder *In Vitro*

Suk Ki Hong and Roy P. Forster
University of Buffalo and Dartmouth College

Attempts were made to elucidate further by direct observation the mechanism involved in the active transport of acidic dyes by the isolated renal tubules of flounder.

Neither alterations of magnesium concentration nor of the potassium/calcium concentration ratio in the medium modified the active process transporting dye from cell to lumen (Step II). However, the replacement of calcium by strontium blocked Step II as occurs in calcium-free medium. Chlorphenol red which had previously accumulated in the lumen ran out of the lumen at a faster rate in a dye-and calcium-free medium than in the control dye-free medium. During this facilitated run-out of chlorphenol red in dye-and calcium-free medium, there was no visible accumulation of dye within cells. These observations seem to indicate that the Step II process is influenced specifically by the calcium ion.

Various competitive inhibitors of chlorphenol red such as PAH, Diodrast, probenecid (Benemid), and Carinamide were also investigated for their effects on both the uptake and run-out processes at concentrations of 3×10^{-4} M of each competitor. Although all the competitors inhibited uptake of chlorphenol red and facilitated run-out of the dye, PAH showed the least inhibitory effect on dye uptake and it was the most effective in facilitating dye run-out, whereas Diodrast's relative effectiveness on the two processes was just the opposite. These observations are consistent with our earlier view that both Step I (intracellular uptake from medium) and Step II are subject to competitive inhibition, but the nature of competition at these respective sites seems to be quite different.

Phlorizin in the control medium at concentrations of 3×10^{-3} to 3×10^{-5} M inhibited chlorphenol red uptake and also facilitated dye run-out from lumen. These effects were graded as a function of the concentration of phlorizin in the medium. However, to what extent this phlorizin effect is competitive in nature or due to an inhibitory effect on oxidative phosphorylation is not yet established.

Distribution of Carbonic Anhydrase in Several Non-Mammalian Species, with a Few Notes on Function

T. H. Maren
University of Florida College of Medicine

Using a simplified and scaled-down (1/10) version of a method previously described (Bull. Johns Hopk. Hosp. **95**, p. 244, 1954), carbonic

anhydrase was determined in organs of several species, and in some cases exploratory functional studies carried out.

Fish

Following are figures for *S. acanthias,* with comparable mammalian data in parenthesis. In units/g. of tissue: blood 8 (800), gill 37, stomach epithelium 40 (240), ciliary process 21 (30), choroid plexus 23 (300). brain 24 (50), lens 0 (500), kidney 0 (300), pancreas 0 (100). Functional studies are reported separately in this Bulletin and by Hodler et al. (Am. J. Physiol. **183**, p. 155, 1955).

In the freshwater catfish (*A. nebulosus*): blood 130, gill 55, epithelium of swim bladder 42, kidney 47. In the marine flounder (*P. americanus*): blood 41, kidney 42. In both these species, carbonic anhydrase inhibition (by acetazolamide) elicited a renal effect (Bull Mt. Des. Biol. Lab. IV, part 1, p. 42, 1953), like that in the mammal. In the goosefish (*L. piscatorius*): blood 132, kidney 63. However, in confirmation of previous work and as in the sculpin (vide supra), acetazolamide did not produce its typical renal effect; the CO_2 concentration of urine in 2 goosefish never exceeded 3mM/L after 100 mg/Kg of drug. Thus the role of renal carbonic anhydrase in *marine teleosts* is not clear, and does not appear to be uniform.

Amphibian

The frog has distal tubules all in the ventral plane, and proximal tubules in the dorsal. Dr. E. K. Marshall suggested estimation of carbonic anhydrase in the two segments. *R. climatans* was used. The enzyme was found equally in distal and proximal parts; approximately 30 units/gram. The same concentration was found in the stomach.

Bird

Blood of the herring-gull (*L. argentatus*) had 100-200 units/ml. However, it was noticed that a plot of enzyme activity against volume of diluted blood did not yield a curve like that of the mammal. For the gull (also the chicken) a given increment of blood produced a 2-3 fold greater effect than for mammalian blood. The basis for this is not explained, but it may give a physiological advantage. The supra-orbital gland had 23 units/g; the septal (infra-orbital) gland had 10 units/g. In connection with the finding of K. Schmidt-Nielsen et al. (Am J. Physiol. **195**, p. 321, 1958) that acetazolamide diminished chloride output and secretion from nasal glands, we found that during carbonic anhydrase inhibition, secretion (normally acid to plasma with lower HCO_3^- concentration) became alkaline to plasma.

In most of the cases cited, enzyme activity *in vitro* was "challenged" by acetazolamide at a concentration of 0.1 microgram per ml. In all tests, enzyme activity was reduced by more than 50%. Where there were large amounts of enzyme in erythrocytes, and organs appeared bloody, they were perfused with saline or sea-water prior to analysis.

We thank Mr. Albert Frederick and Mr. Frank Fischer for their

capable and cheerful assistance. The experiments on the goosefish were carried out with Dr. Fredrik Berglund, and those on the herring-gull with Dr. Knut Schmidt-Nielsen.

Distribution of Quinine in the Dogfish (*S. acanthias*)

David P. Rall and Charles G. Zubrod
National Cancer Institute

The distribution of certain weak organic electrolytes between plasma and cerebrospinal fluid in the dog was shown to depend in pKa and lipid solubility of the compound and the pH of the two phases. The presence of acid pericardial fluid in the dogfish prompted an investigation of the distribution of quinine, a lipid soluble weak organic base, between plasma and pericardial fluid, extradural fluid (EDF) and ventricular fluid (VF).

Quinine was determined fluorometrically in a 3% metaphosphoric acid filtrate. In mammalian plasma quinine is 90% bound, however in dogfish plasma about 40% bound. Protein concentrations and paper electrophoretic patterns were obtained for dogfish plasma, EDF and VF. Four samples of plasma contained 2.4-3.4 gm/100ml protein, two samples of EDF contained 1.0-1.7 gm/100 ml. VF was essentially protein-free. The electrophoretic patterns of plasma and EDF were identical, and similar to those previously reported for elasmobranch plasma protein in that albumin was apparently absent.

After quinine injection in dogfish, plasma quinine concentrations were variable and unexpectedly high. Quinine added to blood was quantitatively recovered, but plasma contained only 25% of the blood concentration. Blood was carefully withdrawn from dogfish injected with quinine. It was immediately centrifuged in the cold, and plasma was carefully removed. Blood quinine concentrations were 8-30 times plasma quinine; red cell quinine was 25-40 times plasma quinine; buffy coat quinine was 70-360 times plasma quinine. When 15-60 minutes elapsed before separating cells and plasma, or when the blood was handled so as to cause cell damage or allowed to become warm, the blood/plasma ratio fell to 4, and the cell/plasma ratios fell to 8-12. Plasma quinine concentrations rose 3-6 fold. These observations can account for the high, variable plasma concentrations previously found, and constitute an example of the problems encountered in drug distribution studies. In preliminary, technically satisfactory, experiments quinine was found to be evenly distributed between plasma and VF and EDF, and was concentrated 3-60 times in acid pericardial fluid.

The assistance of Penny Carver is gratefully acknowledged.