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EVIDENCE FOR CARRIER MEDIATED EFFLUX OF CHLORPHENOL RED FROM ISOLATED RENAL TUBULES OF THE FLOUNDER

W. B. Kinter, State University of New York, Syracuse, N. Y.

In two recent studies employing in vitro techniques the efflux (runout) of pre-accumulated organic anions (Diodrast and p-aminohippurate) from renal tissue (goldfish and dog) was accelerated by the presence of low concentrations of competitors in the efflux medium and decelerated by higher concentrations of the same competitors (Kinter and Cline, Amer. J. Physiol., 201:309, 1961 and Farah, et al., J. Pharm. and Exper. Therap., 139:120, 1963). If it were certain that the pre-accumulated anions were leaving from tubular fluid, i.e., not being displaced from intracellular binding sites, then these biphasic effects would constitute strong evidence that the efflux process was more complex than simple diffusion and involved a mobile carrier of the sort generally proposed for the uphill transport producing accumulation. Therefore, to clarify this uncertainty, direct photometric measurement of anionic dye efflux from luminal fluid was undertaken.

Renal tubules isolated from freshly captured flounder were supported on glass wool within a small optical chamber through which Forster's saline medium was perfused. A recording microspectrophotometer with an 8 μ diameter beam of 575 mμ light (absorption peak of chlorphenol red) was used to measure dye concentration in individual tubular lumens (diameter 15-30 μ). During initial perfusion with oxygenated medium containing 2.5×10^{-5} M chlorphenol red, uphill transport into tubular fluid was so rapid that within 25 min. dye concentrations in individual lumens had reached maximal steady values which were 200-2000 times that in the medium.

Control measurements of dye efflux were obtained after a sudden switch to perfusion with any one of the 3 following efflux mediums, none of which would support accumulation: 1) O_2 and 2) N_2 equilibrated medium with zero dye and 3) N_2 equilibrated medium with up to 10^{-4} M dye. Immediately the concentration of dye in luminal fluid began to fall in a strictly exponential manner (plot of log concentration against time gives a straight line for each tubule) and all values for half time of dye efflux were in the range of 25-38 min (mean 33 min). No dye concentration greater than 10^{-4} M (minimum detectable) ever appeared in the tubular cells. Finally, addition of known competitors to efflux mediums 1) and 2) was investigated. Four organic anions were tested, each in a series of experiments which revealed the expected biphasic effect as competitor concentration was raised from 10^{-6} to about 10^{-2} M in succeeding experiments: first mean half-time for chlorphenol red efflux decreased to minimal values, 15 min with 10^{-4} M probenecid or bromcresol green and 9 min with 10^{-3} M p-aminohippurate or Diodrast and second half-time increased above the control value to 48 min with 10^{-2} M probenecid or bromcresol green and more modestly to 15 min with 2×10^{-2} M p-aminohippurate or Diodrast. (Supported by USPHS Grant AM-06479.)