KINETIC STUDY ON THE EFFLUX OF p-HYDROXYBENZOIC ACID FROM HUMAN RED CELLS

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While it is generally accepted that ionised drug molecules are poorly transported across biological membranes, some studies have indicated that the flux due to ionised species may be a significant proportion of the total flux (eg Nishihata et al 1983). The present study was conducted to investigate the contribution of the ionised species of p-hydroxybenzoic acid (PHB) to the total flux across the human red cell membrane by kinetic methods. Work is proceeding on transport studies with salicylate. Rates of efflux of PHB were measured by first equilibrating a red cell suspension with PHB, diluting with buffer, and measuring the appearance of PHB in the buffer. Cells were separated by the filtration method of Dalmark & Wieth (1972) and PHB was assayed by HPLC.
The pH dependence of the efflux rate was determined from red cells suspended in phosphate buffered isotonic saline. Kinetics were first-order. The pH dependence of the efflux rate constant is shown in Fig.1.

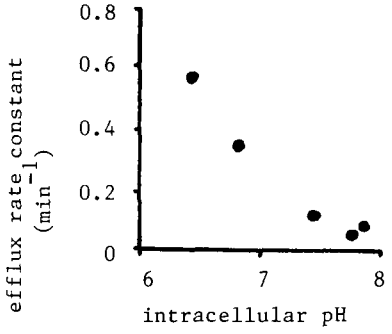

Fig.1 pH dependence of efflux rate constant for PHB

The intracellular pH was estimated from the observed extracellular pH by the equation of Funder and Wieth (1966). These data support the view that only the unionised species is transported to a significant extent. However, a possible interpretation of the data in Fig.1 is that intracellular binding is pH dependent, with the ionised species bound preferentially. Studies on the pH dependence of PHB binding to lysed cells indicated a slight pH dependence which favoured binding of the unionised species, rather than the ionised species. The pH dependence and extent of binding agreed with the results obtained with intact cells. Binding to the membrane fraction was found to be negligible. The ion transport inhibitor DIDS (4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid) did not influence efflux rates.
We conclude that the efflux of PHB from human red cells is due to transport of the unionised species. The temperature dependence of the efflux rate constant (2-40°C) indicated an apparent activation energy of 130 $kJ.mol^{-1}$. This value is considerably greater than the value obtained for salicylate (55 $kJ.mol^{-1}$) by Dalmark and Wieth (1972); it is of the same order of magnitude as their values for anion transport (Cl^-, Br^-, I^-, SCN^-; apparent activation energies 120-150 $kJ.mol^{-1}$). This suggests that the apparent activation energies may be due to the temperature dependence of membrane structure. The low value for salicylate may reflect a specific interaction, related to the membrane stabilising effect of salicylate (Inglot and Wolna, 1968).

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