The effect of *para*-substitution on the rates of enzymic hydrolysis of phenyl acetates*

Several attempts have been made to correlate the influence of substituent groups in substrate molecules with their effects on the rates of enzymic reactions. In contrast to the success that physico-organic theories have had in predicting the course of non-enzymic reactions, little uniformity has been found with biological systems. The difficulty may be that stereochemistry has such a marked influence on enzymic rates; however, the lack of knowledge of the physical factors involved in the formation of an enzyme–substrate complex cannot be minimized.

Cholinesterases and other esterases are capable of hydrolyzing a number of substituted phenyl esters but the observed rates do not appear to be proportional to the HAMMETT substituent constants, which are valid for the non-enzymic hydrolysis of these substances[1]. Recently, it has been found possible to separate the influence of substituents into inductive, resonance and steric contributions[2] and it was of interest to compare these individual factors to the rates of enzymic hydrolyses.

para-Substituted phenyl acetates were used as substrates for human erythrocyte, and plasma cholinesterases, cobra venom cholinesterase and human erythrocyte aromatic esterase[3]. The rates of hydrolysis of 0.1 *M* suspensions of esters were determined manometrically at 37° and pH 7.4 by the method of AMMON[4], the rate of hydrolysis of phenyl acetate being taken as a standard. All determinations were corrected for non-enzymic hydrolysis. When the relative rates were plotted against the substituent constant, a random distribution of points was obtained: when the resonance contribution is neglected and the rates are plotted against the inductive constant, it is found that the points lie upon curves which are similar for each of the four enzymes studied (Fig. 1). It is probable that the very important effects of group size, which have been extensively studied in earlier work[5], are largely responsible for the departure of the observed results from linearity.

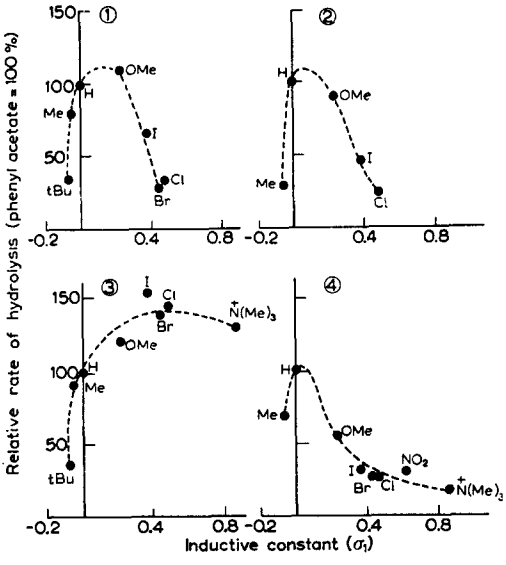

Fig. 1. The effect of inductive substituent constants (σ_I; values of TAFT[2]) on rates of enzymic hydrolysis of *para*-substituted phenyl acetates. Curve 1: human erythrocyte cholinesterase. Curve 2: cobra venom cholinesterase. Curve 3: human erythrocyte aromatic esterase. Curve 4: human plasma cholinesterase. Rate of hydrolysis of phenyl acetate = 100%.

The available data do not suffice to establish whether these observations are of general significance, but further investigations are in progress. Recently, KOSHLAND[6] has pointed out that the template theory of enzyme–substrate interaction will not suffice to explain the phenomena of enzyme specificity. He suggests that in many cases a considerable change in structure occurs at the active site of an enzyme on addition of the substrate which forms a complex. This would be compatible with a large alteration in resonance contribution from the original structures of the substrate molecules but with retention of the inductive effect of substituents. Low temperature spectroscopic studies of enzymes using a range of substrates and competitive inhibitors might be of value in studying this problem.

Department of Biophysics, Medical College of Virginia, Richmond, Va. (U.S.A.) L. A. MOUNTER

[1] L. P. HAMMETT, *Physical Organic Chemistry*, McGraw-Hill Book Co. Inc., New York, 1940.
[2] R. W. TAFT, JR., *Separation of Polar, Steric and Resonance Effects in Reactivity*, in M. S. Newman, *Steric Effects in Organic Chemistry*, John Wiley & Sons, Inc., New York, 1956.
[3] L. A. MOUNTER AND V. P. WHITTAKER, *Biochem. J.*, 53 (1953) 551.
[4] R. AMMON, *Pflügers Arch. ges. Physiol.*, 233 (1933) 486.
[5] V. P. WHITTAKER, *Physiol. Revs.*, 31 (1951) 312.
[6] D. E. KOSHLAND, JR., *Abstracts of the Am. Chem. Soc.*, 132nd Meeting, New York, Sept. 1957, p. 2C.

Received October 23rd, 1957

* This investigation was supported by a research grant CY-3652 from the National Cancer Institute, Public Health Service.