as with fowl plasma, the tissues of rat (Ord & Thompson, 1951), sheep (Davies *et al.* 1952) or rabbit serum (Koelle, 1953) there can be less doubt that the cholinesterases themselves are different.

SUMMARY

1. Two new inhibitors of cholinesterase have been studied, namely, *NN'*-diisopropylphosphorodiamidic anhydride ($[(C_3H_7NH)_2PO]_2O$; bis(diisopropylamino)phosphonous anhydride; DPDA) and the dimethobromide of 1:5-di(*p-N*-allyl-*N*-methylaminophenyl)pentan-3-one (284C51). DPDA is a competitive irreversible inhibitor, selectively inhibiting pseudo cholinesterases, while 284C51 is a competitive reversible inhibitor, selectively inhibiting true cholinesterases.

2. The selectivity of the inhibitors, as expressed by the ratio I_{50} (true): I_{50} (pseudo) is generally high, although this ratio varies considerably among some mammalian species if blood erythrocytes and plasma are the sources of true and pseudo cholinesterases

respectively. It appears that 3×10^{-5} M-DPDA (in contact 30 min. at 38° before addition of substrate) or 10^{-6} to 10^{-5} M-284C51 could be used to inhibit pseudo or true cholinesterase respectively without measurable effect on the other.

3. Some evidence is given suggesting that the apparently typical pseudo cholinesterases of mammalian plasmas may be capable of sub-classification.

4. Further properties of fowl plasma cholinesterase are described, confirming that it is a pseudo cholinesterase in its behaviour towards inhibitors, although the substrate specificity pattern is unlike that of classical pseudo cholinesterase.

We wish to thank Dr A. H. Ford-Moore for supplies of DPDA and Dr Copp of the Wellcome Research Laboratories for the sample of 284C51. Thanks are due also to Dr W. N. Aldridge for certain of his then unpublished data.

Acknowledgement is also made to the Chief Scientist, Ministry of Supply, and to the Senior Representative, Australian Department of Supply, for permission to publish.

REFERENCES

- Adams, D. H. (1949). *Biochim. biophys. Acta*, **3**, 1.
 Adams, D. H. & Thompson, R. H. S. (1948). *Biochem. J.* **42**, 170.
 Adams, D. H. & Whittaker, V. P. (1949). *Biochim. biophys. Acta*, **3**, 358.
 Aldridge, W. N. (1953). *Biochem. J.* **53**, 62.
 Augustinsson, C. B. (1948). *Acta physiol. scand.* **15**, Suppl. 52.
 Augustinsson, C. B. & Nachmansohn, D. (1949). *J. biol. Chem.* **179**, 543.
 Austin, L. & Berry, W. K. (1953). *Biochem. J.* **53**, ix.
 Berry, W. K. (1951). *Biochem. J.* **49**, 615.
 Davies, D. R., Risley, J. E. & Rutland, J. P. (1952). Unpublished data.
 DuBois, K. P., Doull, J. & Coon, J. M. (1950). *J. Pharmacol.* **99**, 376.
 Earl, C. J. & Thompson, R. H. S. (1952). *Brit. J. Pharmacol.* **7**, 261.
 Fulton, M. P. (1952). Private communication.
 Hawkins, R. D. & Mendel, B. (1947). *Brit. J. Pharmacol.* **2**, 173.
 Hawkins, R. D. & Mendel, B. (1949). *Biochem. J.* **44**, 260.
 Koelle, G. B. (1953). *Biochem. J.* **53**, 217.
 Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
 Lineweaver, H. & Burk, D. (1934). *J. Amer. chem. Soc.* **56**, 658.
 Mackworth, J. F. & Webb, E. C. (1948). *Biochem. J.* **42**, 91.
 Mounter, L. A. & Whittaker, V. P. (1950). *Biochem. J.* **47**, 525.
 Myers, D. K. (1952). *Biochem. J.* **51**, 303.
 Ord, M. G. & Thompson, R. H. S. (1951). *Biochem. J.* **49**, 191.
 Sturge, L. & Whittaker, V. P. (1950). *Biochem. J.* **47**, 518.
 Todrick, A. (1952). *Biochem. J.* **52**, xxviii.

APPENDIX

A Note on the Reaction with Inhibitors of Enzymes which are Inhibited by Excess Substrate

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(Received 4 February 1953)

Equations connected with enzyme kinetics are often transformed into linear form, so that experimental data may be examined with the minimum of mathematical labour (cf. Lineweaver & Burk, 1934). Transformations are used not only to determine kinetic constants, but also to establish, among other things, mechanisms of inhibition.

The equations for enzymes which are inhibited by excess substrate cannot be transformed in this manner, but give rise to quadratic or higher order curves. Consequently for some purposes, such as are discussed in the accompanying paper (Austin & Berry, 1953), tests based on linear transformations cannot be used, unless it is possible to work below

the optimum substrate concentration, where linear relationships are a good first approximation. This note mentions some deductions from the higher-order equations which do not appear to have been published.

Lineweaver & Burk (1934) have recapitulated the argument by which it was shown that the relationship between velocity (v) and substrate concentration $[S]$, derived from the simultaneous equations,

$$\begin{aligned} E + S &\rightleftharpoons ES \text{ (active),} \\ ES + (n-1) S &\rightleftharpoons ES_n \text{ (inactive),} \end{aligned}$$

is

$$v = \frac{V[S]}{K_s + [S] + \frac{[S]^n}{K_s}} \quad (1)$$

(V = hypothetical maximum velocity if ES_n were not formed.) The optimum substrate concentration is found when $dv/d[S] = 0$. By differentiating equation 1 it is found that

$$[S]_{\text{opt.}} = \left(\frac{K_s K_s}{n-1} \right)^{1/n} \quad (1a)$$

For non-competitive inhibition the simplest treatment involves considering only the equilibrium

whence the velocity in the presence of inhibitor (v') is

$$v' = v \frac{K_t}{K_t + [I]} \quad (2)$$

It is clear that $dv'/d[S] = 0$ at the same value of $[S]$ that makes $dv/d[S] = 0$; i.e. in the presence of a non-competitive inhibitor the optimum substrate concentration is unaltered.

For competitive inhibition there are three simultaneous equations to solve:

The velocity in presence of inhibitor is now

$$v' = \frac{V[S]}{K_s \left(\frac{K_t + [I]}{K_t} \right) + [S] + \frac{[S]^n}{K_s}} \quad (3)$$

and by differentiating as before, the optimum substrate concentration is

$$[S']_{\text{opt.}} = \left(\frac{K_s K_s K_t + [I]}{n-1} \right)^{1/n} \quad (3a)$$

By comparing equations (1a) and (3a) it is seen that in the presence of a competitive inhibitor the optimum substrate concentration is increased by the factor

$$\left(\frac{K_t + [I]}{K_t} \right)^{1/n}$$

The application of these findings is discussed in the accompanying paper (Austin & Berry, 1953).

REFERENCES

- Austin, L. & Berry, W. K. (1953). *Biochem. J.* **54**, 695.
Lineweaver, H. & Burk, D. (1934). *J. Amer. chem. Soc.* **56**, 658.

Hydrolysis of Benzolcholine Derivatives by Cholinesterase in Serum

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(Received 24 November 1952)

A series of derivatives of benzoylcholine (I) with substituents in ortho, meta, and para positions has been synthesized, and velocity constants of hydrolysis by horse-serum cholinesterase determined; the object of this has been to investigate the influence which the chemical reactivity of a substrate might have on its rate of reaction catalysed by an enzyme. There is already much quantitative information on changes in reactivity that can be expected from the

introduction of substituents into meta and para positions in the benzene ring; the data have been summarized by Hammett (1940), who expressed these changes by the substituent constant S , where $S = \log K - \log K_0$, K being the dissociation constant of the corresponding substituted benzoic acid and K_0 the dissociation constant of unsubstituted benzoic acid. S is positive for an electrophilic substituent, negative for a nucleophilic substituent,