GREAVES, M., SØNDERGAARD, J. S. & MCDONALD-GIBSON, W. (1971). *Br. med. J.*, **2**, 258–260.
HORTON, E. W. (1963). *Nature, Lond.*, **200**, 892–893.
KALEY, G. & WEINER, R. (1968). Prostaglandin Symposium of the Worcester Foundation for Experimental Biology. pp. 321, New York: Interscience Publishers.
KALEY, G. & WEINER, R. (1971). *Ann. N.Y. Acad. Sci.*, **180**, 338–350.
RILEY, J. F. (1959). *The Mast Cells*, p. 39. Edinburgh & London: Livingstone.
SOLOMON, L. M., JUHLIN, L. & KIRSCHENBAUM, M. B. (1968). *J. invest. Derm.*, **51**, 280–282.
SØNDERGAARD, J. S. & GREAVES, M. W. (1971). *Br. J. Derm.*, **84**, 424–428.
VON EULER, U. S. & ELIASSON, R. (1967). *Prostaglandins*, p 139. London: Academic Press.
WELLS, P. W. & EYRE, P. (1970). *Vet. Rec.*, **86**, 173–175.

Isomeric substrate studies with normal and atypical serum cholinesterase

Atypical and usual serum cholinesterase are structurally different and have been separated by chromatography on a DEAE column (Liddell, Lehmann & Silk, 1962). The evidence indicates that the enzymes differ in their amino-acid content. Clark, Glaubiger & La Du (1968) have shown that the pK of the atypical enzyme is lower than that of the usual enzyme. This, together with information obtained from choline affinity studies, has led these workers to suggest a different ionizable group at the binding site (i.e. anionic site) of the atypical enzyme. They also suggest that results from dephosphorylation studies indicate differences between the esteratic sites of the two enzymes.

It was therefore decided to investigate the isomeric substrate specificity of atypical serum cholinesterase to determine whether detectable changes had taken place in the geometry of the esteratic site.

Homozygous atypical and normal human sera obtained from single individuals were diluted 1 : 50 before use. The rates of hydrolysis of acetyl (ACh), butyryl (BuCH) and benzoyl (BzCh) choline and of D-butyryl-α-(D-Bu α-MeCh), L-butyryl-α-(L-Bu α-MeCh), D-butyryl-β- (D-Buβ- MeCh) and L-butyryl-β-(L-Bu β-MeCh)-methylcholine were determined manometrically at 37° in a Warburg apparatus (Beckett, Harper & Clitherow, 1963). In each case, V_{max} was determined over the substrate concentration range 5×10^{-4} to 5×10^{-2}M using diluted serum (1·5 ml) in a total volume of 3 ml. The data were corrected for non enzymic hydrolysis and the K_m and V_{max} values were determined from Lineweaver and Burke double reciprocal plots.

The substrate characteristics, K_m and V_{max} of the substrates studied with the usual and atypical sera are presented in Table 1. As reported generally, the K_m values are consistently lower, and the maximal rates of hydrolysis consistently higher for normal serum as compared with atypical serum.

With the exception of BzCh, the relative rates of hydrolysis of the acylcholines were similar for both sera as shown in Table 2, and the pattern is similar to that obtained for horse serum and purified horse serum cholinesterase by Beckett, Mitchard & Clitherow (1968).

The data presented in this paper are similar to those obtained by Davies, Morton & Kalow (1960) for the hydrolysis of a homologous series of choline esters by usual and atypical human sera. They too showed a wide variation in the ratio atypical K_m: normal K_m and a relatively constant ratio atypical V_{max}: normal V_{max} for all the aliphatic substrates studied; in both studies the rate of hydrolysis of BzCh was relatively faster by atypical serum than for any other substrate. Many workers (Kalow & Staron, 1957; Clark & others, 1968; Erdos, Foldes & others, 1959) have reported significant differences between cholinesterase characteristics obtained with aliphatic and with aromatic choline esters.

Table 1. *Michaelis constants and maximal velocities for the hydrolysis of choline ester iodides by usual and atypical serum cholinesterases.*

Substrate	Usual		Atypical		Ratio	
	K_m	V_{max} μmol	K_m	V_{max} μmol	Atypical K_m	Atypical V_{max}
	m mol /litre	ml serum /min	m mol /litre	ml serum /min	Usual K_m	Usual V_{max}
Acetylcholine	1·35	4·65	6·16	1·17	5·30	0·246
Butyrylcholine	1·09	11·15	2·04	2·32	1·88	0·208
D-Butyryl-α-methylcholine	0·82	9·35	1·66	2·08	2·02	0·217
L-Butyryl-α-methylcholine	0·67	8·01	0·91	1·62	1·36	0·202
L-Butyryl-β-methylcholine	0·72	0·51	1·24	0·11	1·37	0·216
D-Butyryl-β-methylcholine	Rates of hydrolysis too low for measurement					
Benzoylcholine	0·25	1·58	1·05	0·68	4·23	0·430

Table 2. *Relative rates of hydrolysis of choline ester iodides by serum.*

Substrate	Rates of hydrolysis (expressed as % of BuCh)			
	Horse† purified	Horse†	Human usual	Human atypical
Acetylcholine	44·2	46·4	46·5	52·3
Butyrylcholine	100	100	100	100
L-Butyryl-α-methylcholine	81·4	93·7	93·2	90·8
D-Butyryl-α-methylcholine	66·3	78·1	79·7	70·9
L-Butyryl-β-methylcholine	4·5	5·5	5·0	5·0
D-Butyryl-β-methylcholine	Rates of hydrolysis too low for measurement			
Benzoylcholine	15·7	13·0	15·5	34·1

† Data taken from Beckett & others (1968).

The results obtained during the present study support the results of Davies & others (1960) and indicate that the esteratic sites are similar in atypical and usual serum cholinesterase as characterized by their isomeric substrate specificity. The marked changes in the observed K_m values are probably due to altered affinities as a result of a change in the anionic site of atypical serum cholinesterase.

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REFERENCES

BECKETT, A. H., HARPER, N. J. & CLITHEROW, J. W. (1963). *J. Pharm. Pharmac.*, **15**, 362–371.
BECKETT, A. H., MITCHARD, M. & CLITHEROW, J. W. (1968). *Biochem. Pharmac.*, **17**, 1601–1607.
CLARK, S. W., GLAUBIGER, G. A. & LA DU B. N. (1968). *Ann. N.Y. Acad. Sci.*, **151**, 710–722.
DAVIES, R. O., MORTON, A. V. & KALOW, W. (1960). *Can. J. Biochem. Physiol.*, **38**, 545–551.
ERDOS, E. G., FOLDES, F. F., BAART, N., ZSIGMOND, E. K. & SWARTZ, J. (1959). *Biochem. Pharmac.*, **2**, 97–104.
KALOW, W. & STARON, N. (1957). *Can. J. Biochem. Physiol.*, **35**, 1305–1320.
LIDDELL, J., LEHMANN, H. & SILK, E. (1962). *Nature, Lond.*, **193**, 561–562.