The temperature activity relationships of serum cholinesterases

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SYNOPSIS A study has been made of the temperature activity relationships of the serum cholinesterase variants differentiated by means of dibucaine and fluoride inhibition. Using benzoylcholine as substrate, there is a characteristic temperature activity curve for each phenotype, and, for those most sensitive to succinylcholine, this differs radically from that of the normal enzyme.

A true index of serum cholinesterase activity is obtained only at 37°C, as at other temperatures various assumptions and correction factors have to be made, and the results could be misleading.

The means of differentiating the inherited variants of serum cholinesterase (acylcholine acyl-hydrolase, E.C. 3.1.1.8) resulted from the observation that the sera of two patients who had shown a grossly prolonged response to the muscle relaxant, succinylcholine, did not give the usual ratio of hydrolysis rates with benzoylcholine and acetylcholine (Kalow, 1959). These hydrolysis rates were obtained by the spectrophotometric method of Kalow and Lindsay (1955) at 26°C using benzoylcholine and by a manometric assay at 37°C with acetylcholine as substrate. A formula, based on the Arrhenius equation, was given by Kalow and Lindsay to correct benzoylcholine hydrolysis rates to a standard temperature of 26°C and a factor of 1.74 was employed to transform activities at 26°C to 37°C. This formula or similar factors (King, 1965) have been used consistently since then irrespective of the cholinesterase variant being assayed.

It was later demonstrated by Davies, Marton, and Kalow (1960) that what became known as the 'atypical' or dibucaine-resistant enzyme had ratios of activities with various substrates which differed from the 'usual' or normal enzyme. It has since been assumed that this difference in enzyme substrate affinities is the sole factor responsible for the original observation

of different hydrolysis rates and consequently the sole cause of scoline apnoea. Kalow and Lindsay (1955) had noted that the rates of hydrolysis of benzoylcholine and acetylcholine were affected differently by temperature but only reported a temperature coefficient (Q_{10}) of 1.9 for benzoylcholine in the range 12°–26°C.

Methods and Materials

For the present study serum was obtained from patients who had developed a prolonged apnoea after succinylcholine administration, from their relatives, and from laboratory staff. Without allowance for any change in optimum substrate concentration or in pH with temperature, cholinesterase activity was estimated by the method of Kalow and Lindsay (1955) simultaneously with determination of dibucaine number (Kalow and Genest, 1957) and fluoride number (Harris and Whittaker, 1961) using a Unicam SP800 spectrophotometer with temperature control and scale expansion to a slave recorder. This procedure categorized five phenotypes, that is, in the convention of Motulsky (1964), usual $E_1^uE_1^u$, usual atypical heterozygote $E_1^uE_1^a$, usual fluoride-resistant heterozygote $E_1^uE_1^f$, atypical homozygote, $E_1^aE_1^a$, and atypical

fluoride-resistant heterozygote $E_1^a E_1^f$. No example of fluoride-resistant homozygote $E_1^f E_1^f$ has been noted yet in the Glasgow area. Lehmann and Liddell (1969) indicate that this phenotype, comprising genotypes $E_1^f E_1^f$ and $E_1^f E_1^s$, has a frequency of 1 in 120,000, almost as rare as individuals homozygous for the silent gene. These latter of course have practically no serum cholinesterase activity to vary with temperature.

Results and Discussion

The temperature activity relationships of these phenotypes were studied. The mean results as shown in the Figure refer to unit activity at 25°C.

Fig. Temperature-activity curves of serum cholinesterases (benzoylcholine substrate).

The following facts emerge.

The presence of the silent gene does not alter the phenotype-temperature-activity curve, that is, those of $E_1^u E_1^s$ and $E_1^u E_1^u$ are identical, $E_1^a E_1^s$ is the same as $E_1^a E_1^a$ (King and Dixon, 1969), and no doubt the same will apply to $E_1^f E_1^s$ and $E_1^f E_1^f$.

The ratio of activities at 26°C and 37°C for normal serum is 1.78 and the temperature coefficient (Q_{10}) in the range 15°–30°C is 1.90, figures which are in excellent agreement with the values given by Kalow and Lindsay (1955). However, for the atypical homozygote $E_1^a E_1^a$ the factor to correct activities from 26°C to 37°C is 1.08, and the differences originally noted in the hydrolysis rates of benzoylcholine and acetylcholine was therefore due more to the effect of temperature than to differences in enzyme-substrate affinities. With regard to scoline apnoea, however, the latter is the major factor but the temperature anomaly is certainly contributory (King and Dixon, 1969).

Under the assay conditions employed, the optimum temperature for normal serum is 50–51°C, for the usual heterozygotes $E_1^u E_1^a$ and $E_1^u E_1^f$ about 48°C, for genotype $E_1^a E_1^f$ about 36°C, while for the atypical homozygote $E_1^a E_1^a$ progressive inactivation sets an optimum at 32°C. It should be emphasized that this is a reversible inactivation, not a denaturation, since the benzoyl cholinesterase activity at 25°C of neither the usual nor the atypical variants is affected by heating for 60 minutes at 50°C. The assay of activity at two temperatures, either 25° and 37°C or 30°C and 37°C, provides a method of differentiating the usual homozygote and heterozygotes from the $E_1^a E_1^a$ and $E_1^a E_1^f$ variants, ie, those most at risk to succinylcholine. The latter have 25°/37° and 30°/37° ratios of less than 1.3 and less than 1.1 respectively while for the former group the temperature ratios are 1.7 to 2.0 and 1.3 to 1.5. However, it is not possible by this means to differentiate further within these two groups with certainty. It is also predictable that the phenotype $E_1^f E_1^f$ will be found to occupy a position intermediate to these two groups.

With such differences between the variants, for valid results assays must obviously be carried out at a standard temperature. An alternative is to determine the phenotype and employ appropriate temperature correction factors. It is suggested that for routine purposes this could be simplified without incurring a gross error by considering only two groups. In support of this it may be noted that the phenotypes $E_1^u E_1^u$, $E_1^u E_1^a$, and $E_1^u E_1^f$ have respectively Q_{10} (25°–35°C) of 1.76, 1.61, and 1.67, and correction factors from 25°C to 37°C of 1.92, 1.72, and 1.82. This represents a maximum divergence of about 11% in the range 25°–37°C. Again the variants $E_1^a E_1^a$ and $E_1^a E_1^f$ have Q_{10} (25°–35°C) of 1.14 and 1.23 and 25°C to 37°C conversion factors of 1.11 and 1.22 respectively, a divergence of less than 10%.

Reaction Temperature	Standard Temperature		
	25°C	30°C	37°C
15	1.89	2.58	3.62
16	1.80	2.45	3.44
17	1.70	2.32	3.25
18	1.61	2.19	3.08
19	1.52	2.08	2.92
20	1.42	1.94	2.72
21	1.33	1.81	2.54
22	1.24	1.69	2.37
23	1.15	1.57	2.21
24	1.07	1.47	2.06
25	1.00	1.37	1.92
26	0.94	1.28	1.80
27	0.88	1.20	1.68
28	0.82	1.13	1.58
29	0.78	1.06	1.49
30	0.73	1.00	1.41
31	0.69	0.95	1.33
32	0.66	0.90	1.26
33	0.63	0.86	1.20
34	0.60	0.82	1.14
35	0.57	0.78	1.09
36	0.54	0.74	1.04
37	0.52	0.71	1.00
38	0.50	0.68	0.96
39	0.48	0.65	0.92
40	0.46	0.63	0.88

Table I Temperature correction factors for serum cholinesterases (normal $E_1^u E_1^u$)

Reaction Temperature	Standard Temperature		
	25°C	30°C	37°C
15	1.75	1.96	1.95
16	1.61	1.80	1.80
17	1.50	1.67	1.67
18	1.39	1.56	1.55
19	1.30	1.46	1.45
20	1.23	1.38	1.37
21	1.17	1.31	1.30
22	1.12	1.25	1.24
23	1.07	1.20	1.19
24	1.03	1.16	1.15
25	1.00	1.12	1.11
26	0.97	1.09	1.08
27	0.95	1.06	1.05
28	0.93	1.03	1.03
29	0.91	1.01	1.01
30	0.89	1.00	1.00
31	0.88	0.99	0.98
32	0.87	0.98	0.97
33	0.87	0.97	0.97
34	0.87	0.98	0.97
35	0.88	0.98	0.98
36	0.89	0.99	0.99
37	0.90	1.00	1.00
38	0.91	1.02	1.02
39	0.93	1.05	1.04
40	0.96	1.08	1.07

Table II Temperature correction factors for serum cholinesterases (atypical homozygote, $E_1^a E_1^a$)

Temperature correction factors to 25°, 30°, and 37°C, the temperatures recommended (International Union of Biochemistry, 1961, 1965) or commonly used as standard temperatures, for the usual enzyme $E_1^u E_1^u$ and for the $E_1^a E_1^a$ variant are given in Tables I and II. A third table would probably be necessary for the $E_1^f E_1^f$ phenotype. The need to use different tables of correction factors for the cholinesterase variants has even further reaching consequences. If one considers a normal homozygote with serum

activity at the lower limit of normal when assayed at 25°C, it will still be at the lower limit of normal if estimated at 30°C or 37°C. However, serum from an atypical homozygote with the same activity at 25°C would, if assayed at 30°C, indicate a subnormal activity and if estimated at 37°C would only yield a value of about half the lower limit of normal. This explains why some workers (Lehmann, Patson, and Ryan, 1958; Harris, Whittaker, Lehmann, and Silk, 1960), who conducted their estimations at 37°C, have found a better correlation between cholinesterase activity and scoline sensitivity than others who employed lower reaction temperatures (Bush, 1961). It is thus evident that a true and valid index of serum cholinesterase activity can only be obtained if the assay is conducted at 37°C or activity converted to 37°C by means of the appropriate correction factors. Similar, but not identical, anomalies were found in the temperature-activity relationships using acetylthiocholine and butyrylthiocholine as substrates. These will be the subject of a later report.

It is interesting to speculate whether, whatever the function of serum cholinesterase, those patients with the phenotypes $E_1^a E_1^a$, $E_1^a E_1^f$, and probably $E_1^f E_1^f$, will be less affected by a reduction in body temperature than will normal individuals.

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