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Hydrolysis of suxamethonium by different types of plasma

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1. A method, based on the use of the isolated frog rectus abdominis preparation, is described for studying quantitatively the hydrolysis of suxamethonium at low concentrations.

2. Rates of hydrolysis of butyrylcholine, 10 mM, benzoylcholine, 0·05 mM, and suxamethonium, 0·025 mM, by plasma from different species and human plasma with genetic variants of cholinesterase were measured.

3. The rates of hydrolysis of suxamethonium by different types of plasma vary widely. Human plasma with usual cholinesterase and monkey plasma hydrolyse suxamethonium more speedily than do the plasma of cats, dogs and rats, and human plasma with either atypical or fluoride resistant cholinesterase. This is only to a small extent attributable to differences in enzyme concentration and not explained by the presence of an inhibitor.

4. The K_m values for butyrylcholine are very similar for different types of plasma but the K_I values for suxamethonium in the system plasma-butyrylcholine-suxamethonium vary greatly.

5. These results and observations on the inhibition by decamethonium of the hydrolysis of butyrylcholine are consistent with the interpretation that the rates of hydrolysis of suxamethonium, 0·025 mM, obtained with different types of plasma vary because they are a function of two variables, the affinity of the ester for cholinesterase and the stability of the monosuccinyl derivative of the cholinesterase. It seems that human plasma with atypical cholinesterase hydrolyses suxamethonium much slower than does human plasma with usual cholinesterase mainly or solely because of differences in affinity of the ester for the two enzymes. On the other hand, cat plasma appears to hydrolyse suxamethonium much slower than does human plasma with usual cholinesterase mainly because the monosuccinyl derivative of cholinesterase in cat plasma is much more stable than that in human plasma. The reverse might apply for monkey plasma.

6. Inhibition by dibucaine or sodium fluoride of the hydrolysis of benzoylcholine is not a general guide to rates of hydrolysis of suxamethonium by different types of plasma.

Suxamethonium (succinyldicholine; SDCh) is a neuromuscular blocking agent which is hydrolysed by acylcholine acyl-hydrolase (E.C.3.1.1.8; cholinesterase; butyrylcholinesterase) and thus has a brief duration of action. Enzymic hydrolysis

of the ester proceeds in two steps yielding first succinylmonocholine (SMCh) which has, relative to suxamethonium, a low affinity for acetylcholine receptors at motor endplates. The monocholine ester in turn is metabolized at a much lower rate to choline and succinic acid. Inhibition of enzymic hydrolysis enhances the duration of action of suxamethonium at the motor endplate (Brücke, 1956). Using benzoylcholine as substrate for determining the level of cholinesterase activity it has been shown that in man a good correlation exists between the enzyme level in plasma and the duration of apnoea produced by suxamethonium (Kalow & Gunn, 1957). In a few cases, the duration of apnoea is excessively long, but only a proportion of them have a low cholinesterase level as determined with benzoylcholine as substrate (Argent, Dinnick & Hobbiger, 1955). Studying the inhibition by cinchocaine (dibucaine) and other substances of cholinesterase in plasma from subjects in whom the duration of action of suxamethonium had been established, Kalow & Genest (1957) and Kalow & Staron (1957) concluded that there were two genetically controlled variants of cholinesterase, a usual and an atypical form. The two forms could be distinguished by the percentage inhibition of benzoylcholine hydrolysis by 0·01 mM cinchocaine (usually called dibucaine; hence dibucaine number, D.N.). The values of D.N. for usual and atypical forms of cholinesterase in human plasma are 71 and higher and 30 or lower, respectively. Intermediate values represent a mixture of the two enzyme variants; that is, they are obtained with plasma from heterozygous subjects. Subjects with a low value of D.N. for their cholinesterase always have a prolonged duration of action of suxamethonium and Kalow (1959) has shown that the plasma from one such subject metabolized suxamethonium at a much lower rate than did plasma from subjects with a high value of D.N. Since that time, further variants of the cholinesterase in human plasma have been discovered, but their ability to hydrolyse suxamethonium has not been measured (Harris & Whittaker, 1961; Liddell, Lehmann & Silk, 1962; Whittaker, 1968a, b).

The hydrolysis of suxamethonium relative to that of many other cholinesters is slow and thus information on it is limited. This paper describes results obtained in a study of the hydrolysis of butyrylcholine, benzoylcholine and suxamethonium by human plasma with different genetic variants of cholinesterase and an extension of this to plasma of other species.

Methods

Enzyme source

The enzymic hydrolysis of suxamethonium, benzoylcholine and butyrylcholine by various types of plasma was determined, using for each individual substrate whenever possible equiactive plasma concentrations. Enzymic hydrolysis was calculated from initial rates and expressed in μ-moles substrate hydrolysed/ml. plasma per hr. Comparisons of the hydrolysis by plasma and serum obtained from the same blood sample, showed that they were identical within the limits of experimental error. Serum was only used in a few experiments and for simplicity the term plasma will be used throughout. Experiments were generally carried out on freshly obtained samples of plasma but when this was not possible, the plasma was stored at $-15°$ C before use.

Hydrolysis of butyrylcholine

The enzymic hydrolysis of butyrylcholine, 10 mM, was measured by the Warburg technique at 37° C and pH 7·45, using per vessel 3 ml. of plasma diluted in 25 mM $NaHCO_3$ and equilibrated with 95% nitrogen and 5% carbon dioxide. For calculating enzymic hydrolysis corrections were made for non-enzymic release of CO_2.

Hydrolysis of benzoylcholine

The enzymic hydrolysis of benzoylcholine was measured spectrophotometrically at 26° C by the method of Kalow & Lindsay (1955), using a Hilger Watts ultraviolet spectrophotometer, Type 700. All assays were carried out in a 67 mM phosphate buffer, pH 7·4, with 0·05 mM benzoylcholine as substrate. Calculations of enzymic hydrolysis were based on changes in absorption at 240 mμ recorded usually over a 3 min period but over longer periods if enzymic activity was low.

The percentage inhibition of benzoylcholine hydrolysis by 0·01 mM dibucaine (dibucaine number ; D.N. ; Kalow & Genest, 1957) and by 0·05 mM sodium fluoride (fluoride number ; F.N. ; Harris & Whittaker, 1961) were determined under the same conditions. In these experiments the inhibitor and substrate were added together to the cuvette containing the plasma and buffer.

Enzymic hydrolysis of suxamethonium

Suxamethonium, 0·025 mM, was incubated with plasma in 6·7 mM Sørensen's phosphate buffer, pH 7·4, in a stoppered glass tube which was placed in a water bath kept at 37° C and shaken at a rate of 80/min. A 6·7 mM concentration of the buffer was used because it did not interfere with the bioassay of suxamethonium. The final concentration of plasma used in each tube was adjusted so that 50% hydrolysis of suxamethonium was obtained within 10 to 30 min whenever possible. In most instances the concentrations were: human plasma with usual cholinesterase 1%, Cynomolgus monkey plasma 0·25%, Rhesus monkey plasma 0·33%, rat plasma 5%, Patas monkey plasma or human plasma with atypical cholinesterase 10%, and dog or cat plasma 20%. Aliquots of 0·3 ml. were removed from the incubation tube at 3–5 min intervals and mixed with 0·3 ml. of a 0·01 mM solution of the anticholinesterase tetraethyl pyrophosphate (TEPP) in frog Ringer. This, as shown in control experiments, speedily arrested enzymic hydrolysis. After a few minutes' standing at room temperature, the TEPP containing aliquots were diluted to 3 ml. with frog Ringer and their suxamethonium content determined by assay on the frog rectus abdominis preparation.

The assays were carried out on the isolated rectus abdominis muscle of *Rana temporaria,* suspended in 3 ml. oxygenated frog Ringer at room temperature, and contractions, smoothed by a vibrator, were recorded on a smoked drum. During test periods oxygenation was stopped to avoid frothing. Sensitive preparations responded to suxamethonium in a concentration of 0·3 μM and over the range 0·6 to 2·5 μM (0·25 to 1 μg/ml.) the log dose:response curve was linear. The concentration of suxamethonium in each aliquot, tested after tenfold dilution as described above, was assessed by interpolation from a log dose: response curve obtained with standard solutions of suxamethonium on the same rectus muscle. The values of hydrolysis obtained in this way represent values for the conversion of suxamethonium to succinyimonocholine. The succinylmonocholine content of

incubated samples did not interfere with the assay of suxamethonium. This is illustrated in Fig. 1 which shows that succinylmonocholine in a tenfold excess did not affect responses to suxamethonium. Test or standard solutions were assayed at 10 min intervals, and remained in the organ bath for 1, 1·5 or 2·0 min according to the sensitivity of the muscle. A constant time was used throughout an individual experiment. Standard solutions of suxamethonium were made in frog Ringer every 2 hr by dilution from a 0·025 mM stock solution made freshly on the day of the experiment and stored at 4° C to minimize non-enzymic hydrolysis. In addition, the standard solutions contained a final concentration of 0·67 mM Sørensen buffer, pH 7·4 and 0·001 mM TEPP.

The rate of hydrolysis of suxamethonium within the range of 0 to 75% was fairly constant rather than dependent on the amount of ester unhydrolysed and the time required for 50% hydrolysis was chosen for calculating μ-moles suxamethonium hydrolysed/ml. plasma per hr. Values for duplicate tests, obtained with an individual sample of plasma, were usually within 15% or less. Using human plasma with the usual cholinesterase in a final concentration of 1% to 5%, the rate of hydrolysis of suxamethonium was proportional to the plasma concentration. Non-enzymic hydrolysis of the ester was very low, taking 12·7 hr to reach 50%, and only

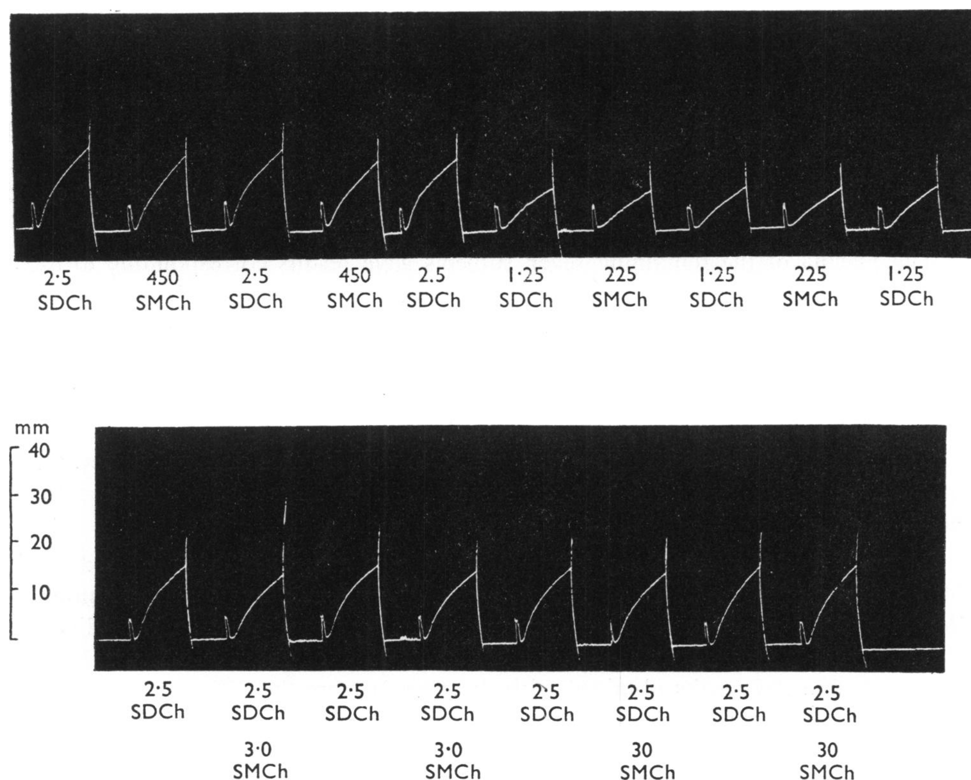

FIG. 1. Comparison between the effects of suxamethonium (SDCh), succinylmonocholine (SMCh) and mixtures of both on the frog rectus abdominis preparation. The figures below each tracing give the molar concentration $\times 10^{-6}$ in the organ bath. The upper record shows that SMCh is approximately 180 times less potent than SDCh. The lower record shows that a twelvefold excess of SMCh does not affect the response to SDCh. The scale applies to both tracings.

in experiments requiring long periods of incubation was it necessary to use a correction for non-enzymic hydrolysis.

Results

Hydrolysis of suxamethonium by plasma of different species

The rates of hydrolysis of butyrylcholine, 10 mM, benzoylcholine, 0·05 mM, and suxamethonium, 0·025 mM, by human and mammalian plasma obtained under the conditions described and expressed in μ-moles substrate hydrolysed/ml. plasma per hr, are summarized in Table 1.

Human plasma with usual cholinesterase. Plasma from thirteen healthy subjects in whom the duration of action of suxamethonium had not been tested but who had normal D.N. values, ranging from 75 to 91, and normal F.N. values, ranging from 52 to 69, hydrolysed suxamethonium at 1·1% of the rate of butyrylcholine, and at 8% of the rate of benzoylcholine.

Human plasma with atypical cholinesterase. Studies of plasma from ten subjects with a history of prolonged apnoea following a single therapeutic dose of suxamethonium, revealed that three of these subjects, referred to in the tables as P.B., N.J.G. and J.G., had D.N. values of 21, 17 and 25, and F.N. values of 17, 24 and 23 respectively. Thus, according to Kalow (1962), they must be considered homozygous for the gene inducing atypical cholinesterase. Genetic studies, to be reported in a separate communication, confirmed this.

It can be seen from Table 1 that the plasma of all three subjects hydrolysed butyrylcholine and benzoylcholine at about 40% of the rate found for plasma of subjects with the usual cholinesterase. The rate of hydrolysis of suxamethonium, however, was approximately 0·02% of the rate of hydrolysis of butyrylcholine and 0·12% of that of benzoylcholine.

The plasma of the remaining seven subjects gave results corresponding to those obtained with plasma containing usual cholinesterase or expected from a mixture of usual and atypical cholinesterase. These results will be presented in a separate paper.

Plasma from a subject homozygous for the fluoride gene. This plasma, kindly provided by Dr. Lehmann, who had first investigated it (Griffiths, Davies & Lehmann, 1966), had a high D.N. value and a low F.N. value and hydrolysed suxamethonium at 0·47% of the rate of butyrylcholine, and at 3·8% of the rate of benzoylcholine.

Plasma of various animal species. Table 1 also shows the rates of hydrolysis of the three choline esters by plasma of cats, dogs, rats and three species of monkey. Whereas the rate of hydrolysis of benzoylcholine by human plasma with different cholinesterase variants was approximately 12 to 15% of the rate of hydrolysis of butyrylcholine the results obtained with the plasma from the different species gave the following values: rat plasma 6%, cat plasma 8%, monkey (three species) plasma 6 to 7% and dog plasma 9%.

Hydrolysis of suxamethonium relative to that of butyrylcholine was as follows: monkey plasma 1·2% (average value for the three species), rat plasma 0·3%, cat plasma 0·1% and dog plasma 0·09%. Thus the turnover of suxamethonium relative to butyrylcholine by monkey plasma and human plasma with the usual cholinesterase is comparable and higher than that by plasma of the other species studied.

TABLE 1. *Hydrolysis of butyrylcholine, benzoylcholine and suxamethonium by different types of plasma*

Type of plasma	Number of subjects or animals	Hydrolysis in μ-moles/ml. plasma per hr			SDCh/BuCh × 100	SDCh/BuCh as % of the ratio for human plasma with usual cholinesterase
		BuCh	BeCh	SDCh		
Man						
Plasma with only usual cholinesterase	13	395±119	55·4±18·3	4·41±1·23	1·14±0·11	100
Plasma with only atypical cholinesterase						
P.B.	1	172	26·8	0·0222	0·0129	
N.J.G.	1	151	18·4	0·0302	0·0200	1·6
J.G.	1	140	24·1	0·0305	0·0218	
Plasma from a subject homozygous for fluoride resistant gene	1	334	41·8	1·58	0·47	41
Cat	12	96·9±35·7	7·31±3·28	0·101±0·035	0·105±0·020	9
Dog	7	280±106	26·1±9·4	0·239±0·088	0·087±0·012	8
Monkey						
Cynomolgus	1	826	60·0	11·5	1·39	
Rhesus	1	611	42·8	8·45	1·38	
Patas	1	41·1	2·25	0·327	0·80	105
	1	21·7	1·35	0·263	1·21	
Rat	6*	124	7·73	0·375	0·303	24

Hydrolysis was determined with butyrylcholine (BuCh), 10 mM, benzoylcholine (BeCh), 0·05 mM, and suxamethonium (SDCh), 0·025 mM. Results represent means ± S.D., unless measurements are on individual samples. * Pooled sample from six rats.

The hydrolysis of suxamethonium relative to that of benzoylcholine also showed wide species differences: dog plasma 0·9%, cat plasma 1·4%, rat plasma 5% and monkey plasma 19%.

Differences between the turnover of suxamethonium relative to butyrylcholine by different types of plasma are illustrated best by expressing them in % of the value obtained with human plasma with usual cholinesterase, as shown in Table 1.

Hydrolysis of suxamethonium by a mixture of human plasma with usual and human plasma with atypical cholinesterase

To investigate whether very low rates of hydrolysis of suxamethonium by human plasma with atypical cholinesterase could be attributed to the presence of an inhibitor, the hydrolysis of suxamethonium by plasma with the usual cholinesterase was measured in the absence and presence of plasma from the three subjects with atypical cholinesterase. Table 2 shows that the rate of hydrolysis of suxamethonium by the former was not significantly affected by the presence in the incubation mixture of 5% plasma from any of the three subjects with atypical cholinesterase.

Inhibition of cholinesterase in different types of plasma by dibucaine and sodium fluoride

In view of the value of assessing inhibition by dibucaine or sodium fluoride of the hydrolysis of benzoylcholine, for differentiating between genetically controlled variants of cholinesterase in human plasma, the effect of these inhibitors on the cholinesterase in the plasma of other species was investigated. The inhibition by 0·01 mM dibucaine of the hydrolysis of benzoylcholine by the different types of plasma was as follows: monkey plasma 92–95%, cat plasma 66–94%, human plasma with usual cholinesterase 75–91%, dog plasma 18–27%, human plasma with a typical cholinesterase 17–25%, and rat plasma 9%. With 0·05 mM sodium fluoride the inhibition was: human plasma with usual cholinesterase 52–69%, human plasma with atypical cholinesterase 17–23%, cat plasma 0–29%, dog plasma 0–23%, monkey plasma 0–11%, and rat plasma 0%. Comparison of the inhibition of benzoylcholine hydrolysis by dibucaine or sodium fluoride with the turnover of suxamethonium relative to that of butyrylcholine by plasma of different species shows no correlation between inhibition and turnover.

Inhibition of cholinesterase in different types of plasma by suxamethonium

Differences in the turnover of suxamethonium relative to that of other cholin-

TABLE 2. *Hydrolysis of suxamethonium (SDCh) by human plasma with usual cholinesterase in the absence and presence of plasma from subjects with only atypical cholinesterase*

	Final concentration	μ-moles SDCh hydrolysed ml. plasma per hr
Plasma A.W.P.	1%	4·21
Plasma A.W.P. + plasma P.B.	1% 5%	4·21
Plasma A.W.P. + plasma N.J.G.	1% 5%	3·93
Plasma A.W.P. + plasma J.G.	1% 5%	4·21

Plasma of subjects P.B., N.J.G. and J.G. contains only atypical cholinesterase and plasma of subject A.W.P. only usual cholinesterase.

esters may at least in part arise from different affinities between substrate and enzyme. Ideally this is studied by measuring the relationship between rate of hydrolysis and substrate concentration, that is, by determination of the K_m value This is technically difficult with suxamethonium by the method used.

An alternative method for getting information on the "affinity" of a substrate with a low turnover is to study its inhibitory effect on the hydrolysis of a substrate with a high turnover. The hydrolysis of butyrylcholine, 10 mM, by different types of plasma, was therefore measured manometrically in the absence and in the presence of different concentrations of suxamethonium. Suxamethonium produced inhibition which was proportional to the concentration of the ester and independent of the order in which suxamethonium and butyrylcholine were added to plasma. In addition, the inhibition remained fairly constant over a 60 min period. For example, when human plasma from ten subjects with only usual cholinesterase was used, suxamethonium, 3 mM, produced on average 55% inhibition when calculated from hydrolysis over 1 hr. The mean values of CO_2 evolved from hydrolysis of butyrylcholine in the absence of suxamethonium during the first and second 30 min periods were 106 ± 12 (S.D.) and 102 ± 18 (S.D.) μl. respectively. In the presence of suxamethonium, 3 mM, $47{\cdot}8 \pm 7{\cdot}7$ (S.D.) and $46{\cdot}6 \pm 8{\cdot}1$ (S.D.) μl. CO_2 were evolved in the first and second 30 min periods respectively. With dog and monkey plasma the results were similar; a fairly constant level of inhibition was maintained over at least 1 hr. In some experiments with cat plasma inhibition became slightly less with time but this never amounted to more than a 10% increase in the rate of hydrolysis over 30 min.

On the basis of these findings the following method was adopted for determining the molar concentration of suxamethonium which produced 50% inhibition (I50) of the hydrolysis of butyrylcholine. Butyrylcholine in a final concentration of 10 mM was added to the diluted plasma, and to mixtures containing plasma and various concentrations of suxamethonium. The CO_2 output for the subsequent 5 to 35 min (and in a few experiments 5 to 65 min) was calculated and corrected for spontaneous hydrolysis of both esters. Figure 2 illustrates the recorded relationship (based on means) between the inhibition of butyrylcholine hydrolysis and suxamethonium concentration for human plasma with either usual or atypical cholinesterase, and the plasma of dogs, cats, monkeys and rats. The mean values for the I50 for different plasmas are summarized in Table 3. In the case of human plasma with atypical cholinesterase, the I50 was obtained by extrapolation from the inhibition produced by 9 mM suxamethonium, since with this concentration of suxamethonium the rate of non-enzymic hydrolysis of suxamethonium already approached that of the enzymic hydrolysis of butyrylcholine. The slopes of the inhibition curves on the whole were similar for all types of plasma, but the I50 values differed greatly, and comparisons between the I50 values for suxamethonium and its rate of hydrolysis, when used in a concentration of 0·025 mM, showed that the two parameters were not linearly related.

Plasma from the subject homozygous with respect to the fluoride resistant gene gave an I50 value of 2·9 mM. This falls within the range observed with human plasma containing the usual cholinesterase. It can be seen from Fig. 2 that the slope of the inhibition curve differed from that seen with human plasma containing the usual cholinesterase, but since only one experiment could be carried out with the plasma of the subject homozygous with respect to the fluoride resistant gene, the significance of the discrepancy between slopes is uncertain.

One factor which will influence the I50 value of suxamethonium for different types of cholinesterase under the experimental conditions is the affinity of butyrylcholine for the enzyme. The K_m values for the hydrolysis of butyrylcholine by different types of plasma were therefore determined. This was done by measuring the rates of hydrolysis of different concentrations of butyrylcholine, and plotting them according to the method of Woolf & Hofstee as described by Dixon & Webb (1964). In this type of plot v is plotted against $\frac{v}{(S)}$, where v is the observed rate of hydrolysis and (S) the substrate concentration. The intercept on the ordinate

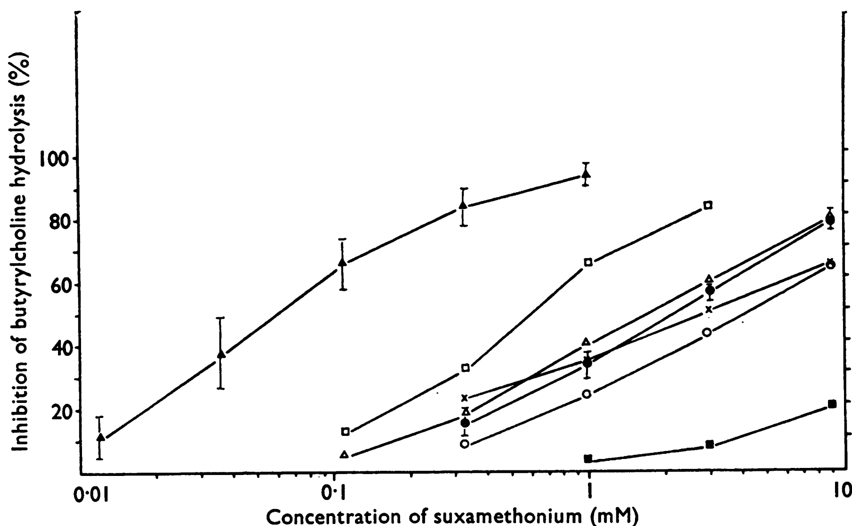

FIG. 2. Inhibition by suxamethonium of the hydrolysis of butyrylcholine, 10 mM, by different types of plasma. Plasma from the different sources is denoted by the symbols: cat, ▲——▲; usual human cholinesterase, ●——●; dog, △——△; monkey, ○——○; rat, □——□; atypical human cholinesterase, ■——■; fluoride resistant human cholinesterase, ×——×. The experimental points represent means except in the case of rat plasma, which was used as a pooled sample (six animals). For cat plasma and for human plasma with only the usual cholinesterase means ± S.D. are shown.

TABLE 3. *Affinities of butyrylcholine (BuCh) and suxamethonium (SDCh) for cholinesterase, assessed with different types of plasma.*

Type of plasma	K_m for BuCh $\times 10^3$	I50 for SDCh $\times 10^3$	K_I for SDCh $\times 10^3$
Man			
Plasma with only usual cholinesterase	0·6	2·3±0·3	0·13
Plasma with only atypical cholinesterase			
Subjects: N.J.G.	1·5	47	6·1
J.G.	2·7	57	11
P.B.	2·7	25	5·3
Cat	0·56	0·063±0·026	0·0033
Dog	1	1·8±0·8	0·17
Rat	0·99	0·62	0·056
Monkey (Cynomolgus)	0·72	6·3	0·43

All calculations are on a molar basis. The K_m values were determined for human plasma with usual cholinesterase and rat plasma using plasma pooled from five subjects and six animals, respectively. In the case of the cat and dog, five and seven individual plasma samples, respectively, were pooled. For monkey plasma and human plasma with atypical cholinesterase individual plasma samples were used. The I 50 values were obtained on individual plasma samples, except for rat plasma, which was a pooled sample from six rats. The number of individual samples of plasma were: for human plasma with only usual cholinesterase, thirteen; cat, eighteen; dog, five.

gives V_{max} and on the abscissa $\frac{V_{max}}{K_m}$. Knowing v, V_{max} and (S), K_m can be calculated.

Table 3 shows that the K_m values for butyrylcholine are very similar for the cholinesterases in plasma of different species, and thus the variations in the I50 for suxamethonium are to a large extent not attributable to variations in the affinity of butyrylcholine for the various types of cholinesterase.

From the observed K_m values for butyrylcholine, and the I50 for suxamethonium, the K_I values, that is, the molar concentration of suxamethonium required for 50% occupancy of the active sites of cholinesterase in the absence of butyrylcholine, was calculated using the equation

$$\frac{v}{v_I} = 1 + \frac{(I)}{K_I\left(1 + \frac{(S)}{K_m}\right)}$$

In this equation, which is based on analyses (equation VIII 16) by Dixon & Webb (1964), v is the velocity of hydrolysis in the absence of inhibitor, v_I the velocity in the presence of inhibitor (suxamethonium), (I) the inhibitor (suxamethonium) concentration, (S) the substrate (butyrylcholine) concentration, and K_m the Michaelis constant for cholinesterase and substrate (butyrylcholine). Using the I50 values and rearranging the equation we obtain

$$K_I = \frac{I_{50}}{1 + \frac{(S)}{K_m}}$$

Table 3 shows in addition to K_m values for butyrylcholine and the I50 values for suxamethonium the K_I values for suxamethonium. It can be seen that the relationship between K_I and I50 value for different plasma are very similar.

Inhibition by decamethonium of the hydrolysis of butyrylcholine by various types of plasma

The degree of inhibition of the enzymic hydrolysis of benzoylcholine by a given concentration of dibucaine or sodium fluoride varies with the types of plasma used.

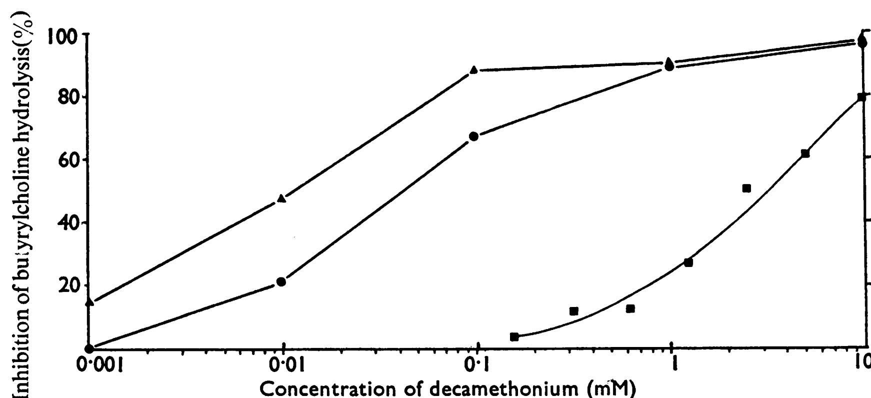

FIG. 3. Inhibition by decamethonium of the hydrolysis of butyrylcholine, 10 mM, by human plasma with either usual or atypical cholinesterase and by cat plasma. Cat, ▲; usual human cholinesterase, ●; atypical human cholinesterase, ■.

This indicates the presence of structural differences between the enzymes involved. Because of similarities in structure between suxamethonium and decamethonium, the inhibitory effect of the latter on hydrolysis of butyrylcholine by selected types of plasma was measured. The experimental conditions used were the same as those described for studies of the inhibition of the enzymic hydrolysis of butyrylcholine by suxamethonium.

The results obtained are shown in Fig. 3 and a comparison between Fig. 3 and Table 3 shows that the I50 values of decamethonium and suxamethonium for human plasma with atypical cholinesterase are 60 and 20 times, respectively, higher than those for human plasma with the usual cholinesterase. The I50 values of decamethonium and suxamethonium for cat plasma on the other hand are 0·3 and 0·03, respectively, relative to those for human plasma with the usual cholinesterase, if the latter are taken as 1. This suggests that the relatively low turnover of suxamethonium by cat plasma and by human plasma with atypical cholinesterase are not explainable in the same way, and that the former cannot be a consequence of a reduced affinity of the ester for the cholinesterase which hydrolyses it.

Discussion

The rates of hydrolysis of suxamethonium in high concentrations by plasma from several species have been measured manometrically by various authors, who usually expressed it as a percentage of the rate of hydrolysis of acetylcholine, also used in high concentrations. Values obtained in this way are: human plasma 6% (Evans, Gray, Lehmann & Silk, 1952), 4% (Tsuji, Foldes & Rhodes, 1955) and 2% (Klupp & Stumpf, 1953); horse plasma 4% (Glick, 1941), 3·4% (Whittaker & Wijesundera, 1952) and 2·0% (Klupp & Stumpf, 1953); and dog plasma 8·1% (Hall, Lehmann & Silk, 1953) and 1·0% (Klupp & Stumpf, 1953). A maximum rate of hydrolysis of suxamethonium by human or horse plasma is obtained with a concentration of the ester of approximately 6mM. In the range 3·2 mM to 25·5 mM suxamethonium hydrolysis follows zero order kinetics, since the enzyme is saturated with substrate, but at concentrations above 6mM some substrate inhibition occurs, as shown by Whittaker & Wijesundera (1952) and Tammelin (1953).

In adult man suxamethonium is usually used in a dose of 0·11 m-mole (45 mg of the dichloride) and if this dose is distributed without hydrolysis or excretion throughout the extra cellular fluid, the final concentration will be 0·008 mM. If the drug is confined to plasma it will be 0·035 mM.

It is therefore of considerable interest to study the enzymic hydrolysis of suxamethonium at low concentrations. This can be done by using labelled suxamethonium (Schmidinger, Held & Goedde, 1966) or by bioassay. As shown by Lüllman & Förster (1953) and Norton & de Beer (1954) suxamethonium produces a contraction of the isolated frog rectus preparation. In our own experiments we found that this preparation can be used to determine suxamethonium quantitatively in a concentration of 0·6 μM or higher. Succinylmonocholine in a tenfold excess did not interfere with assays of suxamethonium, enzymic hydrolysis of suxamethonium was speedily arrested by tetraethyl pyrophosphate and corrections for non-enzymic hydrolysis were very small. On the basis of these observations an assay method was designed which gave reliable quantitative information on the hydrolysis (conversion to succinylmonocholine) of 0·025 mM suxamethonium at 37° C and pH 7·4 by different types of plasma.

The rate of enzymic hydrolysis of suxamethonium, 0·025 mM, by plasma was found to differ with the species and in the case of human plasma with the genetic variant of the cholinesterase. Absolute values for the hydrolysis of suxamethonium/ml. plasma decreased in the order: Cynomolgus monkey plasma; Rhesus monkey plasma; human plasma with usual cholinesterase; human plasma with fluoride resistant cholinesterase; rat plasma; Patas monkey plasma; dog plasma; cat plasma; human plasma with atypical cholinesterase. The relatively low rate of hydrolysis of suxamethonium by human plasma with atypical cholinesterase is in agreement with one result obtained on the same type of plasma by Kalow (1959), and another one by Schmidinger *et al.* (1966). Structural differences between the usual and the atypical form of human cholinesterase are suggested by differences in their sensitivity to inhibitors such as dibucaine, sodium fluoride and decamethonium (Kalow & Davies, 1958). The similarity of the rate constants for inhibition of usual and atypical human cholinesterase by tetraethyl pyrophosphate suggests that the main difference between the two enzymes occurs at the anionic site.

Our results show that the variations between the rates of hydrolysis of suxamethonium by different types of plasma are largely not attributable to differences in enzyme concentration as shown by comparisons between the rates of hydrolysis of suxamethonium and butyrylcholine. Since a concentration of 0·025 mM suxamethonium was used for hydrolysis studies, the question arises whether differences in the degree of enzyme saturation with substrate do account for the observed differences between rates of hydrolysis of the ester. To investigate this the K_I of suxamethonium in the system plasma-butyrylcholine-suxamethonium and the K_m for butyrylcholine were determined for the different types of plasma. Values for the K_m of butyrylcholine ranged from 0·56 mM with cat plasma to 1 mM with dog plasma. The values obtained for human plasma with usual or atypical cholinesterase were similar to those reported by Davies, Marton & Kalow (1960), but in general all values were slightly lower than those reported by Augustinsson (1963). Differences in the ionic composition of the medium probably account for this (Myers, 1952). The K_I values for suxamethonium obtained with different types of plasma varied widely. When results on human plasma with usual cholinesterase are compared with those on human plasma with atypical cholinesterase, it can be seen that the rate of hydrolysis of suxamethonium is inversely related to the K_I value. These findings support the interpretation that at low substrate concentration differences between the rates of hydrolysis of suxamethonium by the two types of human plasma mainly or solely reflect differences in affinity of the ester for the two types of cholinesterase (Kalow & Davies, 1958). In agreement with this is the finding that the affinity of decamethonium for usual human cholinesterase is approximately 50 times higher than for atypical human cholinesterase.

The same interpretation does not hold for the hydrolysis of suxamethonium by cat plasma. By comparison with human plasma containing usual cholinesterase, cat plasma hydrolyses suxamethonium slowly although the K_I value for the ester is very low indeed. Furthermore, the difference between the I50 values of decamethonium for the two types of plasma is small by comparison with the difference between K_I values. The value of K_I is only a measure of apparent affinity and determined by affinity of suxamethonium for cholinesterase and stability of the acylated enzyme (monosuccinylderivative of cholinesterase). Both these factors must be considered as variables, and differences between them will result in dis-

crepancies between the rates of hydrolysis of suxamethonium by different types of plasma. In the case of cat plasma the stability of the acylated enzyme must be considerably greater than that formed in human plasma with normal or atypical cholinesterase. With monkey plasma the reverse—a lower stability of the acylated enzyme—might apply.

Comparison between the rate of hydrolysis of suxamethonium and the percentage inhibition of the hydrolysis of benzoylcholine by dibucaine or sodium fluoride shows that the correlation which applies for usual and atypical forms of human cholinesterase does not apply to cholinesterases in the plasma of animals in general. Our results also show that the rate of hydrolysis of suxamethonium by individual variants of human cholinesterase must be investigated to avoid erroneous conclusions as regards their importance in any considerable prolongation of the action of suxamethonium which might be observed in subjects with such variants.

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REFERENCES

ARGENT, D. E., DINNICK, O. P. & HOBBIGER, F. (1955). Prolonged apnoea after suxamethonium in man. *Br. J. Anaesth.*, **27**, 24–30.

AUGUSTINSSON, K. B. (1963). Classification and comparative enzymology of the cholinesterases and methods for their determination. *Handbuch der Experimentellen Pharmakologie. Ergänzungswerk*, vol. 15, chap. 4, pp. 89–128. Berlin–Göttingen–Heidelberg: Springer–Verlag.

BRÜCKE, F. (1956). Dicholinesters of alpha, omega-dicarboxylic acids and related substances. *Pharmac. Rev.*, **8**, 265–335.

DAVIES, R. O., MARTON, A. V. & KALOW, W. (1960). The action of normal and atypical cholinesterase of human serum upon a series of esters of choline. *Can. J. Biochem.*, **38**, 545–551.

DIXON, M. C. & WEBB, E. C. (1964). *Enzymes*, 2nd ed. London: Longmans.

EVANS, F. T., GRAY, P. W. S., LEHMANN, H. & SILK, E. (1952). Sensitivity to succinylcholine in relation to serum cholinesterase. *Lancet*, **1**, 1229–1230.

GLICK, D. (1941). Some additional observations on the specificity of cholinesterase. *J. biol. Chem.*, **137**, 357–362.

GRIFFITHS, P. D., DAVIES, D. & LEHMANN, H. (1966). A second family demonstrating the homozygote for fluoride resistant pseudocholinesterase variant. *Br. med. J.*, **2**, 215–216.

HALL, L. W., LEHMANN, H. & SILK, E. (1953). Responses in dogs to relaxants derived from succinic acid and choline. *Brit. med. J.*, **1**, 134–136.

HARRIS, H. & WHITTAKER, M. (1961). Differential inhibition of human serum cholinesterase with fluoride. Recognition of two new phenotypes. *Nature, Lond.*, **191**, 496–498.

KALOW, W. (1959). The distribution, destruction and elimination of muscle relaxants. *Anesthesiology*, **20**, 505–518.

KALOW, W. (1962). *Pharmacogenetics. Heredity and the Response to Drugs.* London: W. B. Saunders.

KALOW, W. & DAVIES, R. O. (1958). The activity of various esterase inhibitors towards atypical human serum cholinesterase. *Biochem. Pharmac.*, **1**, 183–192.

KALOW, W. & GENEST, K. (1957). A method for the detection of atypical forms of human serum cholinesterase. Determination of dibucaine numbers. *Can. J. Biochem.*, **35**, 339–346.

KALOW, W. & GUNN, D. R. (1957). The relation between dose of succinylcholine and duration of apnoea in man. *J. Pharmac. exp. Ther.*, **120**, 203–214.

KALOW, W. & LINDSAY, H. A. (1955). A comparison of optical and manometric methods for the assay of human serum cholinesterase. *Can. J. Biochem.*, **33**, 568–574.

KALOW, W. & STARON, N. (1957). On distribution and inheritance of atypical forms of human serum cholinesterase, as indicated by dibucaine numbers. *Can. J. Biochem.*, **35**, 1305–1320.

KLUPP, H. & STUMPF, Ch. (1953). Über Unterschiede in der enzymatischen Spaltung von Dicarbonsäure-bis-cholinestern durch Menschen-, Pferde- und Hundserum. *Enzymologia*, **16**, 189–192.

LIDDELL, J., LEHMANN, H. & SILK, E. (1962). A "silent" pseudocholinesterase gene. *Nature, Lond.*, **193**, 561–562.

LÜLLMAN, H. & FÖRSTER, W. (1953). Über die Wirkungen neuromuskulär blockierender Substanzen auf den Rückenmuskel des Blutegels und den M. Rectus abd. des Frosches. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **217**, 217-224.

MYERS, D. K. (1952). The effect of salt on the hydrolysis of acetylcholine by cholestinerase. *Arch. Biochem.*, **37**, 469–487.

NORTON, S. & DE BEER, E. J. (1954). Excretion of succinyldicholine in urine of cat and mouse. *J. Pharmac. exp. Ther.*, **110**, 392–397.

SCHMIDINGER, S. T., HELD, K. R. & GOEDDE, H. W. (1966). Hydrolysis of succinyldicholine by pseudocholinesterase at low concentrations. *Humangenetik*, **2**, 221–224.

TAMMELIN, L. E. (1953). Succinylcholine iodide (celocurin). A synthetic drug with a curare-like effect. *Acta chem. scand.*, **7**, 185–195.

TSUJI, F. I., FOLDES, F. F. & RHODES, D. H., Jr. (1955). The hydrolysis of succinyldicholine chloride in human plasma. *Archs int. Pharmacodyn. Thér.*, **104**, 146–155.

WHITTAKER, M. (1968a). An additional pseudocholinesterase phenotype occurring in suxamethonium apnoea. *Br. J. Anaesth.*, **40**, 579–852.

WHITTAKER, M. (1968b). Differential inhibition of human serum cholinesterase with *n*-butylalcohol: recognition of new phenotypes. *Acta genet., Basel*, **18**, 335–340.

WHITTAKER, V. P. & WIJESUNDERA, S. (1952). The hydrolysis of succinyldicholine by cholinesterase. *Biochem. J.*, **51**, 475–479.

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