85. Blood Esterases

By **Derek Richter** and **Phyllis Godby Croft**, *Central Pathological Laboratory (L.C.C.) and Mill Hill Emergency Hospital, London, N.W. 7*

(*Received 14 August 1942*)

It has recently been shown that there is a rise in the choline esterase activity of the serum in states of severe emotional tension [Richter & Lee, 1942 *a*, *b*]. In attempting to assess the physiological significance of this observation and apply it for clinical purposes it was necessary to have further information as to the precise nature of the esterases occurring in blood.

Apart from the choline esterase, it has long been known that human serum contains a 'serum lipase', which hydrolyses simple aliphatic esters and glycerides such as methyl butyrate and tributyrin [Rona & Bach, 1920]. The 'serum lipase' has been found also in a number of other animal species, but little is known of it apart from the observation that the name 'lipase' is probably a misnomer, since the enzyme has little or no action on the true fats and there is no evidence that it has anything to do with lipid metabolism [Cherry & Crandall, 1932].

Vahlquist [1935] attributed the hydrolysis of the aliphatic esters, glycerides and choline esters to one and the same enzyme, but this was disputed by Hall & Lucas [1937]. Stedman and his collaborators [1932; 1933; 1935; 1937] showed that the blood esterases behave differently in different species. They found that the serum choline esterase in certain species exerted a considerable hydrolytic action on tributyrin, and a slight action on methyl butyrate; but the evidence for their conclusions was criticized by Shaw [1935].

The considerable divergences of opinion as to the specificity and other properties of the esterases in serum may be due in part to the different sources of material and the variety of experimental conditions used by different investigators. Certain factors, such as the effect of the neutral salt concentration and the inhibition of esterases by bromthymol-blue, which has frequently been used as an indicator in measuring esterase activities by the titration method, must also be considered as possible sources of error [Alles & Hawes, 1940; Vahlquist, 1935]. It is still not clear to what extent the esterases other than choline esterase are able to hydrolyse acetylcholine. It is also uncertain to what extent choline esterase is responsible for the hydrolysis of aliphatic esters and glycerides by serum and corpuscles in the different species which have commonly been used as experimental animals.

In view of these uncertainties a reinvestigation of the esterases occurring in blood has now been made with the object of defining and characterizing them more clearly and devising criteria for distinguishing between them that could be used, in particular, for purposes of clinical investigation.

Experimental methods

Material. Horse blood was collected under sterile conditions in glass flasks and brought immediately from the slaughterhouse to the laboratory. The human blood consisted of venous specimens taken from patients admitted on account of neurotic complaints, but in whom a careful examination had failed to show any significant physical abnormality. Cat, dog and rabbit blood were obtained without the use of anaesthetics from an artery or vein.

Esterase activity. Esterase activities were estimated manometrically with a Warburg apparatus by measuring the CO_2 liberated from bicarbonate buffer by the fatty acids formed by hydrolysis of the esters. The method was similar to that of Jones & Tod [1935] and no correction was applied for the CO_2 retained by buffers present. Serum (0·2 ml.) was diluted immediately before use to 4 ml. with a solution containing 0·03 *M* $NaHCO_3$ and 0·12 *M* NaCl which was kept in a closed bottle in equilibrium with 5 % CO_2 in O_2. The diluted serum was placed in the Warburg cup and the substrate in the side tube. The bath temperature was 37° and the vessels were filled with O_2 containing 5 % CO_2. The substrate for choline esterase was 0·5 ml. of a solution of 2·5 % acetylcholine chloride in 0·9 % NaCl. Methyl butyrate (0·1 ml.) and tributyrin (0·1 ml.) were used as substrates for the other esterases. Since these two esters are only sparingly soluble in water a slight excess of substrate remained undissolved. Preliminary experiments showed that under these conditions the hydrolysis-time curves gave satisfactorily straight lines during the first 30–40 min., and the enzyme activities, which were calculated from the slope, were reproducible to within about 10 %. When eserine or other solutions were used, 0·35 ml. of the required concentration was added to 2·65 ml. of diluted serum. Inhibitors were allowed to stand for 20 min. with the enzyme before adding the substrate. All additions were brought to *p*H 7·4 before use. Esterase activities are expressed as micro-equivalents (μE.) of ester hydrolysed per ml. of serum (or corpuscles) per hour. The abbreviations Ac.ch., Me.but. and Tribut. are used for acetylcholine, methyl butyrate and tributyrin respectively. The choline esterase activities (μE./ml./hr.) may be converted into the usual clinical units [Jones & Tod, 1935] by dividing by the factor 2·36.

Horse serum

Horse serum was examined in the first place since it has received considerable attention from other investigators and it is better understood than the other blood esterase systems. Stedman, Stedman & Easson [1932] and Stedman & Stedman [1935] found that horse serum contains two enzymes, (*a*) an esterase which hydrolyses simple esters such as methyl butyrate or tributyrin, and (*b*) a choline esterase which they believed to be entirely specific for choline esters, though Easson & Stedman [1937] concluded later that this was uncertain and that it might also exercise a small activity on other esters. Shaw [1935] criticized the evidence obtained by Stedman *et al.* [1932] and concluded that 'the esterase prepared from horse serum by the method of Stedman *et al.* [1932] is not choline esterase'. Easson & Stedman [1937] have replied to this criticism. The possibility that a third enzyme, a lipase, may also be present in horse serum was suggested by Stedman, Stedman & White [1933].

In the present investigation the term 'ali-esterase' has been used to indicate an esterase of the type that acts preferentially on the simple aliphatic esters and glycerides.

Separation of esterases by methods of partial destruction. Specimens of horse serum were tested with acetylcholine, methyl butyrate and tributyrin under the conditions described. They showed considerable activity with all three substrates:

Horse serum no.	Esterase activity in μE./ml./hr.		
	Ac.ch.	Me.but.	Tribut.
1	86	264	125
2	100	222	101

A number of procedures were applied to the serum to produce partial destruction of the enzymes: these consisted of (1) ageing by keeping for 14 days, (2) heating to 50–55°, (3) drying with acetone, (4) fractional precipitation with ammonium sulphate, and (5) treatment with adsorbents. When the resulting products were tested again with the three substrates, all these treatments except the fractional precipitation were found to

have produced a significant change in the relative activities towards the three substrates, indicating a partial separation into two enzymes. The resulting enzymic activities, expressed as a percentage of the original activity, are given in Table 1.

Table 1. *Partial destruction of esterases of horse serum*

Serum no.	Treatment	% of original activity		
		Ac.ch.	Me.but.	Tribut.
1	Ageing, 14 days	60	95	100
2	Ageing, 19 days	63	83	98
1	Deminrolit A at *p*H 4	81	12	19
2	Deminrolit A at *p*H 4	100	41	42
1	Fuller's earth at *p*H 4	22	96	56
2	Fuller's earth at *p*H 4	57	93	77
1	Heat 10 min. at 55°	67	11	17
2	Heat 30 min. at 50°	47	34	12
2	Acetone drying	2	6	7
1	$(NH_4)_2SO_4$ precip.	30	31	33
2	$(NH_4)_2SO_4$ precip.	19	18	15

Fractional precipitation by $(NH_4)_2SO_4$ was done exactly according to the directions given by Stedman *et al.* [1932]. Deminrolit A is a specific adsorbent prepared by the Permutit Co.; it was used after preliminary treatment with a small amount of fuller's earth. The serum was shaken with the adsorbents which were then removed by centrifuging.

The activity towards methyl butyrate and tributyrin generally underwent similar changes as a result of the various treatments, indicating that these two substrates were hydrolysed by the same enzyme, an ali-esterase distinct from the choline esterase. The choline esterase was much more resistant to destruction by heating, but less resistant to the effects of acetone drying, than the ali-esterase.

Inhibition by eserine. Eserine sulphate (10^{-5}) produced complete inhibition of the activity towards acetylcholine; but no significant inhibition with methyl butyrate. With tributyrin as substrate there was an inhibition of about 10%. This result was repeated and confirmed many times.

Serum no.	Ac.ch.		Me.but.		Tribut.	
	Alone	Eserine	Alone	Eserine	Alone	Eserine
1	86	0	256	242	100	95
2	107	0	182	186	96	81

It was clear that the ali-esterase had no action on acetylcholine, and conversely that the choline esterase of horse serum appeared to be almost entirely specific in that it showed no measurable activity towards methyl butyrate and only very slight activity towards tributyrin. These results confirm and extend the observations made by the Stedmans and their collaborators and agree entirely with their conclusions.

Human serum

Preliminary experiments with human serum showed that it readily hydrolysed the three esters used in the present study, but it differed from horse serum in that it was relatively more active towards acetylcholine and less active with methyl butyrate. The choline esterase activity varied greatly in different individuals, ranging in the present series from 67 to 279, and it was noticeable that the activity towards methyl butyrate and tributyrin generally varied in parallel with the choline esterase activity. This was shown by the ratios of the activities Ac.ch./Me.but. and Ac.ch./Tribut. Experiments with oxalated plasma gave results identical with those with serum (Table 2).

There was no reason to expect that two dissimilar enzymes such as an ali-esterase and a choline esterase should occur together in such relatively constant proportions. The probability of these figures occurring by chance was found on statistical analysis to be

Table 2. *Esterase activities of different human sera*

No.	Esterase activity in μE./ml./hr.			Relative activities	
	Ac.ch.	Me.but.	Tribut.	Ac.ch./Me.but.	Ac.ch./Tribut.
1	165	44	53	3·7	3·2
2	240	110	59	2·2	4·2
3	67	24	41	2·7	1·6
4	199	97	116	2·0	1·7
5	279	99	207	2·8	1·3
6	234	105	139	2·2	1·7
7	234	103	154	2·3	1·5
8	67	31	52	2·2	1·3
9	90	40	66	2·2	1·4
10	97	49	75	2·0	1·3
11	56	25	36	2·2	1·6
12	123	67	101	1·8	1·2
13	94	41	78	2·3	1·2
14	143	51	91	2·8	1·6
15	98	47	99	2·1	1·0
16	69	26	44	2·6	1·6

Oxalated plasma was used instead of serum in no. 16. The coefficient of correlation between the activities towards Ac.ch. and Me.but. calculated by the formula $\frac{\Sigma xy}{\sqrt{(\Sigma x^2 \times \Sigma y^2)}}$ [Fisher, 1938] was 0·94 and the probable error of the coefficient was 0·02. The correlation was therefore statistically significant.

very small since the correlation coefficient (0·94) was very high and greatly exceeded the probable error (0·02). This gave evidence that the enzymes hydrolysing the three substrates were either closely related or identical.

Attempted differentiation between enzymes. Human serum differed markedly from horse serum in that the same methods which were successful in achieving a partial separation into two enzymes in the horse failed completely with human serum.

The choline esterase of human serum showed little falling off in activity on keeping for periods of a few weeks, but an old specimen of plasma which had been kept for 18 months and still retained about half of the original activity showed no significant change from the normal ratio of activities:

Ac.ch.	Me.but.	Tribut.	Ac.ch./Me.but.	Ac.ch./Tribut.
69	26	44	2·6	1·6

The percentages of the original activities that remained after (1) heating to 55° for 20 min. and (2) treatment with adsorbents were as follows:

Treatment	% of original activity		
	Ac.ch.	Me.but.	Tribut.
Heat 20 min. at 55°	37	37	34
Adsorption on deminrolit A at *p*H 4	40	49	50

Inhibition by eserine. Eserine concentrations higher than about 10^{-6} caused almost complete inhibition of the hydrolysis of all three substrates by human serum, indicating again that they were probably all hydrolysed by the same enzyme, choline esterase.

Serum no.	Ac.ch.		Me.but.		Tribut.	
	Alone	Eserine, 10^{-5}	Alone	Eserine, 10^{-5}	Alone	Eserine, 10^{-5}
1	157	0	71	6	79	6
2	173	5	48	6	43	4
3	173	0	64	0	104	0
4	205	0	85	0	118	0
5	175	0	63	0	103	0

The difference between the systems in human and horse sera in this respect was brought out very strikingly by plotting the inhibition percentage against the eserine

concentration (Fig. 1). All esterase activity was strongly inhibited at very high eserine concentrations, but at intermediate concentrations of about 10^{-5} the horse-serum activity was only slightly inhibited towards methyl butyrate, while the inhibition with human serum was practically complete.

The results with human serum were consistent with the view that one enzyme, a choline esterase, was mainly responsible for the hydrolysis of all three substrates, but the slight irregularities in the Ac.ch./Me.but. and Ac.ch./Tribut. ratios in different subjects

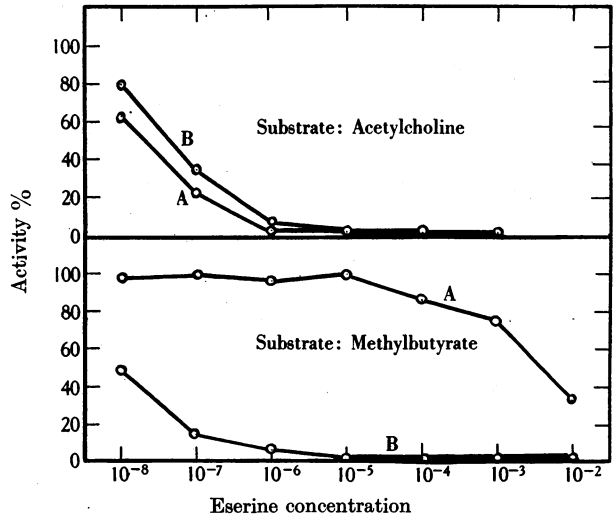

Fig. 1. Inhibition of esterases of horse serum (curves *A*) and human serum (curves *B*) by eserine. Activities expressed as percentage of activity without eserine.

suggested that a relatively small and irregular amount of a second ali-esterase may also occur. These observations agree closely with the conclusions of Vahlquist [1935] and Easson & Stedman [1937], but they disagree with those of Hall & Lucas [1937].

Human red blood corpuscles

Red blood corpuscles which had been washed free from serum with 0·9 % NaCl showed a greater activity than the serum towards acetylcholine and less activity towards tributyrin and methyl butyrate (Table 3).

Table 3. *Human red blood corpuscles*

	Esterase activity in μE./ml./hr.			Relative activities	
No.	Ac.ch.	Me.but.	Tribut.	Ac.ch./Me.but.	Ac.ch./Tribut.
1	156	65	56	2·4	2·8
2	134	43	32	3·1	4·2
3	240	39	54	6·1	4·4
4	348	44	61	7·9	5·7
5	310	58	148	5·3	2·1
6	350	60	91	5·8	3·8
7	360	68	86	5·3	4·2
8	350	65	84	5·4	4·2
9	280	38	61	7·4	4·6
10	370	34	50	10·9	7·4
11	339	128	81	2·6	4·2

The system differed from that in serum in that the activity towards acetylcholine and the aliphatic esters showed considerable independent variation. Treatment with fuller's

earth caused significant alterations in the relative activities towards the different substrates, which gave evidence of a partial separation into two enzymes:

	% of original activity		
Adsorbent	Ac.ch.	Me.but.	Tribut.
Fuller's earth	5	14	11

The presence of two or more esterases was confirmed by the behaviour towards 10^{-5} eserine, which caused complete inhibition of acetylcholine hydrolysis, but no significant inhibition of the hydrolysis of the other esters:

	Ac.ch.		Me.but.		Tribut.	
No.	Alone	Eserine, 10^{-5}	Alone	Eserine, 10^{-5}	Alone	Eserine, 10^{-5}
1	186	0	19	24	38	35
2	216	3	54	52	63	58
3	200	1	26	22	49	45

These experiments gave evidence of the existence in the red blood corpuscles of two enzymes, (*a*) an ali-esterase which hydrolysed simple aliphatic esters, and (*b*) a choline esterase which differed from the serum choline esterase in being completely specific in its action. Any activity of the choline esterase towards methyl butyrate or tributyrin was less than 3 % of the activity towards acetylcholine.

Cat blood

Using the inhibition by 10^{-5} eserine as a criterion for distinguishing between ali-esterases and choline esterases, the esterases occurring in cat serum and corpuscles were investigated (Table 4).

Table 4. *Differentiation between blood esterases by means of eserine*

			Activity in μE./ml. hr.		
Species	Tissue	Esterases	Ac.ch.	Me.but.	Tribut.
Horse	Serum	Choline esterase	107	0	11
		Ali-esterase	0	182	95
Man	Serum	Choline esterase	184	71	78
		Ali-esterase	0	0	0
	Corpuscles	Choline esterase	182	0	0
		Ali-esterase	0	22	45
Dog	Serum	Choline esterase	169	59	65
		Ali-esterase	0	12	6
	Corpuscles	Choline esterase	83	21	30
		Ali-esterase	0	26	24
Cat	Serum	Choline esterase	108	8	25
		Ali-esterase	8	36	90
	Corpuscles	Choline esterase	48	11	40
		Ali-esterase	0	27	69
Rabbit	Serum	Choline esterase	18	36	110
		Ali-esterase	0	97	300
	Corpuscles	Choline esterase	34	9	20
		Ali-esterase	0	83	130

The figures representing ali-esterase activities were obtained with the different substrates in the presence of 10^{-5} eserine: the choline esterase activities represent the additional activity found in the absence of eserine.

The serum choline esterase appeared to be unspecific for acetylcholine when examined in this way, since the activity of the serum towards the aliphatic esters was significantly inhibited by eserine. An ali-esterase was also present.

The corpuscle choline esterase was not specific for acetylcholine; the ali-esterase present in the corpuscles was less active than that in the serum.

Dog blood

With dog serum the hydrolysis of all three substrates was markedly inhibited by eserine, indicating that the serum choline esterase showed considerable activity towards the aliphatic esters. The ali-esterase showed no activity towards acetylcholine.

The esterases present in the corpuscles appeared to consist, as in the cat, of a non-specific choline esterase and an ali-esterase which did not hydrolyse acetylcholine.

Rabbit blood

In rabbit serum there appeared to be a relatively inactive choline esterase, and a very active ali-esterase which showed no activity towards acetylcholine. In the corpuscles two enzymes similar to those in the serum were found.

The figures given in Table 4 should be treated with considerable reserve. Activities of the order of 10 or less approached the experimental error and are therefore doubtful. It should be emphasized that the differentiation between the ali-esterases and choline esterase depended entirely on the assumption that the former was unaffected, while the latter was completely inhibited, by 10^{-5} eserine. This assumption was justified in the experiments with human and horse serum by the inhibition curves (Fig. 1); but in the tissues which showed relatively little choline esterase activity, such as rabbit serum, it may be doubted whether this still holds good. Apart from these limitations, the figures give a general indication of the approximate order of the esterase activities in the serum and corpuscles of a number of species, and they may be useful until more precise methods of differentiating between the different esterases have been worked out in each individual case. A specific inhibitor for the ali-esterase would be of great value for this purpose, but all attempts to find such an inhibitor have hitherto proved unsuccessful. It is possible that in some species more than one ali-esterase may be present.

Inhibition of esterases by quinine and atoxyl

Earlier investigators achieved some success in differentiating between different types of esterases by making use of quinine and atoxyl as inhibitors. Liver esterase, for example, is stated to be inhibited by atoxyl but not by quinine, while 'serum lipase' is inhibited by both [Rona & Bach, 1920; Petow, 1929].

A series of blood systems was now examined by means of these inhibitors to test how far they might be useful for the purpose of the present investigation and to bring the present and earlier observations into line. The inhibitor concentrations were similar to those used by Petow [1929] in the clinical examination of esterases in tissues. The conditions were otherwise the same as have been previously described (Table 5).

Table 5. *Inhibition and acceleration of esterases by atoxyl and quinine*

Species	Tissue	Inhibitor	Substrate Ac.ch.	Substrate Me.but.	Species	Tissue	Inhibitor	Substrate Ac.ch.	Substrate Me.but.
Horse	Serum	Atoxyl	100	100	Cat	Serum	Atoxyl	100	90
		Quinine	21	100			Quinine	32	130
Man	Serum	Atoxyl	53	100		Corpuscles	Atoxyl	120	109
		Quinine	23	228			Quinine	90	130
	Corpuscles	Atoxyl	100	100	Rabbit	Serum	Atoxyl	100	100
		Quinine	70	100			Quinine	53	32
Dog	Serum	Atoxyl	100	100		Corpuscles	Atoxyl	100	120
		Quinine	34	165			Quinine	69	74
	Corpuscles	Atoxyl	167	92					
		Quinine	67	185					

All activities are expressed as percentage of the activity with substrate alone. Concentration of quinine sulphate 0·2 mg./ml. Concentration of atoxyl 0·02 mg./ml.

The most striking result of these experiments was in showing that the behaviour of the choline esterases and ali-esterases towards the two inhibitors depended greatly on the source from which they were derived. In some cases strong inhibitions were found, in others there was no apparent effect, while in a few instances accelerations were observed.

Quinine produced in every case a partial inhibition of the hydrolysis of acetylcholine by choline esterase; but in several cases a marked acceleration with methyl butyrate as substrate was observed.

The accelerating action of quinine on the hydrolysis of methyl butyrate by human serum was previously reported by Vahlquist [1935]. In the present investigation atoxyl was also found to exert an accelerating effect on the hydrolysis of acetylcholine by the choline esterase of dog and cat corpuscles. That the choline esterase, and not the ali-esterase, was concerned in these accelerations was shown by the inhibition obtained on the further addition of 10^{-5} eserine.

	Me.but.			Ac.ch.		
	Alone	Quinine	Quinine + 10^{-5} eserine	Alone	Atoxyl	Atoxyl + 10^{-5} eserine
Human serum	100	228	15	—	—	—
Cat corpuscles	100	130	50	100	120	0
Dog serum	100	165	18	—	—	—
Dog corpuscles	—	—	—	100	167	0

Activities expressed as percentage of activity with substrate alone.

Sulphanilic acid, which resembles atoxyl in molecular configuration, produced no effect when added in the same concentration as atoxyl, in the hydrolysis of acetylcholine by the choline esterase of dog blood corpuscles.

The effects of the various inhibitors and accelerators depended on the substrate used; for example, quinine accelerated with methyl butyrate but inhibited the hydrolysis of tributyrin by choline esterase.

Inhibition by amines

The accelerating action of quinine on the hydrolysis of methyl butyrate by choline esterases raised the question of whether this is a special property of quinine or whether it is a general property of organic bases. The effect of a series of amines on the hydrolysis of methyl butyrate by human and horse serum was therefore tested.

	Human serum	Horse serum
Triethylamine	84	88
Ethylamine	81	85
Methylamine	94	77
Choline	92	97

Amine concentration: 2·5 mg./ml. Activities expressed as percentage of activity with substrate alone.

In every case an inhibition was observed.

Hydrolysis of castor oil by blood esterases

Orientating experiments were carried out to test the lipolytic activity of the serum and corpuscles in man, horse and dog, using castor oil as a substrate, with and without the addition of bile salt as a lipase activator.

A 10 % emulsion (0·5 ml.) of castor oil and water prepared as described by Cole [1933] was placed in the side tube of the Warburg vessel; the main vessel contained 1·5 ml. of serum or corpuscles diluted with 1·5 ml. of bicarbonate buffer. When bile salt was used, 0·4 ml. of a solution containing 50 mg. sodium tauroglycocholate per ml. was added to the main vessel and 1·1 ml. of buffer was used. Some slight activity was noted in the experiments with dog serum; after the addition of bile salt dog corpuscles also showed slight activity, but in general there was little activity and in no case did the activity exceed 5 μE./ml./hr. It was clear that none of the tissues contained a lipase comparable in activity with the ali-esterases and choline esterases that have been described.

Hydrolysis of cholesterol acetate by blood esterases

The hydrolysis of cholesterol acetate by sera and corpuscles was tested under the same conditions as in the experiments with castor oil, with and without the addition of bile salt. The substrate was used as an emulsion which was prepared by shaking an acetone solution containing 50 mg./ml. cholesterol acetate with 30 ml. water and then dialysing the emulsion against water to remove the acetone.

The greatest activity was found with dog corpuscles which, in the presence of bile salt, gave an activity of 12 μE./ml./hr., but none of the tissues showed an activity greater than 1 μE./ml./hr. in the absence of bile salt.

The sterol esterase activity in these tissues was therefore relatively small.

Discussion and Conclusions

The esterase activities of the sera and red blood corpuscles in a number of species have been measured by the manometric method in the presence of NaCl and $NaHCO_3$-CO_2 buffer at 37°. By the use of different substrates, specific inhibitors and various methods of separation, data were obtained as to the specificities and other properties of the esterases occurring in the different tissues. The results differed to some extent from those obtained by other investigators; but they are in general agreement with the main conclusions of the Stedmans, particularly, and they confirm many of their results.

Esterases occurring in blood

Choline esterases. From a physiological point of view choline esterases are characterized chiefly by (*a*) their ability to hydrolyse choline esters, and (*b*) their inhibition by eserine. Eserine also inhibits other esterases at high concentrations, and this is responsible for some confusion in the literature [Ammon, 1935], but at a concentration of 10^{-5} eserine is highly specific as a choline esterase inhibitor. The two criteria of hydrolysing choline esters and eserine inhibition were sufficiently definite to allow the choline esterases to be distinguished sharply from the other esterases occurring in blood. The choline esterases obtained from certain sources appeared to be entirely specific in that they showed no measurable activity towards methyl butyrate or tributyrin; choline esterases obtained from other sources were not specific. The activity of human serum choline esterase, for example, towards methyl butyrate and tributyrin was about 50 % of the activity towards acetylcholine, and the activity towards methyl butyrate could be further increased by the addition of quinine.

The choline esterase of human red blood corpuscles contrasted strongly with serum choline esterase in being far more specific towards acetylcholine. It showed no measurable activity towards methyl butyrate or tributyrin, and under the conditions described any activity towards these substrates was less than 3 % of the activity towards acetylcholine. It showed no acceleration with quinine.

Previous investigators have frequently referred to choline esterase as if it were one specific enzyme, but in the present investigation we have been impressed by the great differences in specificity and other properties of the choline esterases obtained from different sources. This was also observed in certain instances by Alles & Hawes [1940] and by Glick [1941]. It would appear more accurate to describe the choline esterases as a group of enzymes showing considerably divergent properties, rather than to regard choline esterase as a single entity. It is also clear that attempts which have been made to deduce the physiological behaviour of the choline esterases in muscle and nervous tissue from studies of the choline esterases of serum are liable to error on this account.

Ali-esterases. The word 'esterase' is commonly used as a comprehensive term to include the enzymes of all types that hydrolyse esters. The term 'ali-esterase' has been used in

the present work to describe the particular type of esterase that acts preferentially on the simple aliphatic esters and glycerides. The ali-esterases in blood had little or no action on fats, acetylcholine, or cholesterol acetate under the conditions of the present investigation.

Ali-esterases were found in the sera and red blood corpuscles in several species, but they were absent or present in only very small amounts in human serum. The ali-esterases of blood were similar in substrate specificity and in inhibition by surface-active substances to the well-known liver esterase, but they showed characteristic differences in their inhibition by atoxyl and quinine.

Lipases. There is evidence that the leucocytes contain a small amount of a true lipase that hydrolyses fats [Kraut & Burger, 1938]; but orientating experiments, using castor oil under the conditions described, confirmed the conclusion of other investigators that there is normally little or no true lipase present in the serum. Lipases may enter the serum from the pancreas or other organs in pathological conditions: the properties of the lipase then depend on the organ in which it originated, and in some cases this may be inferred from its inhibition by quinine or atoxyl [Petow, 1929; Cherry & Crandall, 1932].

Sterol esterases. Esterases which hydrolyse the esters of cholesterol and other sterols have been described in the serum in several species [Schramm & Wolff, 1940]: they are stated to be distinct from the other esterases which have been described. Preliminary experiments showed that they are very much less active than the ali-esterases or choline esterases and they were therefore not further investigated.

Esterases in the blood in different species

Figures giving the approximate order of activity of the blood esterases in a number of species are summarized in Table 4.

Horse serum. The existence of two esterases, a choline esterase and an ali-esterase, in horse serum was confirmed in a number of different experiments. The choline esterase did not act to any appreciable extent on methyl butyrate, but there was evidence that it acted slowly on tributyrin under the conditions of our experiments.

Human serum. Human serum showed a considerable activity towards methyl butyrate and tributyrin as well as towards acetylcholine. The apparent divergence of our figures from those of Easson & Stedman [1937] in this respect was probably due to the different experimental conditions. A variety of experiments designed to effect a separation into two or more enzymes, including (*a*) heat treatment, (*b*) partial destruction by ageing, and (*c*) treatment with adsorbents, agreed with the results of (*d*) experiments with inhibitors and (*e*) the measurement of the relative activities in different sera, in indicating that one enzyme only is mainly responsible for the activity towards all three substrates. This is not in agreement with the views of Hall & Lucas [1937], but it agrees with Vahlquist [1935] and Easson & Stedman [1937].

It is concluded that there is no evidence for the existence of a 'serum lipase' in normal human serum. Pancreatic lipase, or lipase from other organs, may occasionally occur in pathological sera, but normal serum contains little or no true lipase. Human serum may contain a small and variable amount of an ali-esterase derived from the red blood corpuscles [Richter & Croft, 1942], but the activity of normal human serum towards methyl butyrate and tributyrin is due mainly to the serum choline esterase, which shows considerable activity towards simple aliphatic esters and glycerides. The term 'serum lipase' is misleading and should be abandoned.

The choline esterase of human serum, like other choline esterases, was inhibited by a variety of organic bases; but the activity towards methyl butyrate was characteristically increased by over 100 % by the addition of a small amount of quinine. This accelerating action was not shown with acetylcholine or tributyrin as substrates, or with simpler bases such a triethylamine in the place of quinine.

The activity of human serum choline esterase varied considerably from one individual to another. Previous work has shown that the activity is lowered in conditions of debility and increased in acute emotional states and in other conditions associated with increased activity of the autonomic nervous system [Richter & Lee, 1942 *a*, *b*]. A temporary rise in activity is produced as a result of vigorous exercise [Richter & Croft, 1942].

Human red blood corpuscles. The esterases in blood corpuscles are of particular interest from a clinical point of view, owing to the possibility of their getting into the serum through partial haemolysis in vitro, or as has been observed in conditions such as pernicious anaemia, through haemolysis in vivo [Simon, 1924].

Using the methods that proved most effective in the case of horse serum, namely, (*a*) comparison with three substrates, (*b*) inhibition by 10^{-5} eserine and (*c*) fractional adsorption, it was found that the red blood corpuscles contained two esterases, (*a*) a choline esterase, and (*b*) an ali-esterase. The choline esterase was much more active than the serum choline esterase and differed from it strikingly in that it was highly specific and showed no measurable activity towards methyl butyrate or tributyrin. It also showed no acceleration with quinine. The ali-esterase was relatively inactive: it was not appreciably inhibited by atoxyl.

Previous investigators have considered the possibility of there being a definite distribution of choline esterase between the blood plasma and cells [Stedman & Russell, 1937; Sabine, 1940], but it was found by Richter & Lee [1942*a*] that the serum choline esterase does not come from the corpuscles. The differences in specificity and in the behaviour of these two enzymes towards atoxyl and quinine make the possibility of a distribution between the plasma and corpuscles very improbable.

Cat, dog and rabbit blood. In cat, dog and rabbit blood a choline esterase and an ali-esterase were present in both the serum and the corpuscles. The choline esterase activity of rabbit blood was relatively low, but cat and rabbit blood contained very active ali-esterases in both serum and corpuscles.

Summary

1. The esterase activities of the blood corpuscles and sera in horse, man, dog, cat and rabbit have been estimated manometrically in the presence of NaCl and $NaHCO_3$ at 37°.

2. The properties of the choline esterases and 'ali-esterases' (i.e. those attacking simple aliphatic esters) occurring in these tissues have been examined by the use of specific inhibitors and methods of partial separation.

3. The choline esterases occurring in different species, and in serum and corpuscles of the same species, differ greatly in their properties.

4. The hydrolysis of methyl butyrate and tributyrin by human serum is mainly accounted for by the choline esterase. There is no evidence for the existence of a 'serum lipase' in normal human serum.

5. The choline esterase of human red blood corpuscles differs from the serum choline esterase in being entirely specific for choline esters and in behaving differently towards inhibitors.

6. Quinine inhibits the action of choline esterases on acetylcholine, but it accelerates the action of choline esterases derived from certain sources on methyl butyrate.

7. The hydrolysis of acetylcholine by the choline esterase of dog and cat corpuscles is accelerated by atoxyl.

8. The ali-esterases in blood show no activity towards acetylcholine.

The authors thank Dr Maclay and the Clinical Staff of the Hospital for their generous co-operation and the Rockefeller Foundation for supporting this investigation.

REFERENCES

Alles, G. A. & Hawes, R. C. [1940]. *J. biol. Chem.* **133**, 375.
Ammon, R. [1935]. *Ergebn. Enzymforsch.* **4**, 102.
Cherry, J. S. & Crandall, L. A. [1932]. *Amer. J. Physiol.* **100**, 266.
Cole, S. W. [1933]. Practical Physiological Chemistry, p. 184. Cambridge: Heffer.
Easson, L. H. & Stedman, E. [1937]. *Biochem. J.* **31**, 1723.
Fisher, R. A. [1938]. Statistical Methods for Research Workers, p. 128. Edinburgh: Oliver and Boyd.
Glick, D. [1941]. *Nature, Lond.*, **148**, 662.
Hall, G. E. & Lucas, C. C. [1937]. *J. Pharmacol.* **61**, 10.
Jones, M. S. & Tod, H. [1935]. *Biochem. J.* **29**, 2242.
Kraut, H. & Burger, H. [1938]. *Hoppe-Seyl. Z.* **253**, 105.
Petow, H. [1929]. Oppenheimer's Die Fermente und ihre Wirkungen, **3**, 1502.
Richter, D. and Croft, P. G. [1942]. In the Press.
Richter, D. & Lee, M. H. [1942*a*]. *J. ment. Sci.* **88**, 428.
—— —— [1942*b*]. *J. ment. Sci.* **88**, 435.
Rona, P. & Bach, E. [1920]. *Biochem. Z.* **111**, 166.
Sabine, J. C. [1940]. *J. clin. Invest.* **19**, 833.
Schramm, G. & Wolff, A. [1940]. *Hoppe-Seyl. Z.* **263**, 73.
Shaw, F. H. [1935]. *Aust. J. exp. Biol. med. Sci.* **13**, 251.
Simon, H. [1924]. *Z. ges. exp. Med.* **39**, 407.
Stedman, E. & Russell, W. R. [1937]. *Biochem. J.* **31**, 1987.
Stedman, E. & Stedman, E. [1935]. *Biochem. J.* **29**, 2107.
Stedman, E., Stedman, E. & Easson, L. H. [1932]. *Biochem. J.* **26**, 2056.
Stedman, E., Stedman, E. & White, A. C. [1933]. *Biochem. J.* **27**, 1055.
Vahlquist, B. [1935]. *Skand. Arch. Physiol.* **72**, 133.