CXXXIX. A COMPARISON OF THE CHOLINE-ESTERASE ACTIVITIES OF THE BLOOD-SERA FROM VARIOUS SPECIES.

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It was recently shown by Stedman, Stedman and Easson [1932] that, whereas liver esterase and pancreatic lipase are without appreciable action on esters of choline, there is present in the blood-serum of the horse an enzyme which readily hydrolyses such esters, but which exerts little, if any, action on simple esters such as methyl butyrate. In order to emphasise the specific nature of its activity, the new enzyme was termed choline-esterase. No other sera were examined at the time for the presence of this enzyme owing to the difficulty in measuring its activity in horse-serum with any degree of accuracy without first submitting it to extensive purification. Further experience with the work has, however, now enabled us so to modify the method of estimation that the activity of the choline-esterase present in 1 cc. of serum can be measured with a fair degree of accuracy. It therefore appeared to us to be of interest to examine the blood-sera from a number of species for the presence of this enzyme and, at the same time, to determine the activities of the same sera towards methyl butyrate and tributyrin.

Methods of estimation.

The choline-esterase activities of the sera examined were measured by the method of continuous titration described in our earlier communication. The accuracy of the results so obtained depends largely on the p_H at which the estimations are made. In our original experiments p_H 7.4, using bromothymol blue as indicator, was first employed, but difficulty was experienced with unpurified serum owing to the buffering action of the serum-proteins. The difficulty was overcome to some extent by working at p_H 8.6, using *o*-cresolphthalein as indicator, but this introduced an additional complication owing to the unstable nature of the substrates involved. Whereas the non-enzymic hydrolysis of the choline esters was almost negligible at p_H 7.4, it became relatively large at 8.6 and increased, moreover, rapidly with increase in the p_H of the solution, so that the small fluctuations in alkalinity which are unavoidable during the titration, or a slightly incorrect adjustment of p_H such as is liable to occur when one is employing pigmented serum, caused appreciable variations in the amount of non-enzymic hydrolysis and hence uncertainty regarding that caused by the enzyme. By choosing a p_H intermediate between the above values, namely 8.0, it has been found possible to avoid these difficulties. At this acidity both the buffering power of the serum-proteins and the non-enzymic hydrolysis of the substrate are small, while the colour change of cresol red, which was the

indicator chosen, was particularly marked. The actual estimations were made in the following manner. To 100 cc. of CO_2 -free water previously warmed to 30° and placed in a thermostat at this temperature were added 5 drops of an aqueous solution of cresol red, prepared according to Clark [1925], and 1 cc. of a solution of 2.5 g. of butyrylcholine bromide in 10 cc. of water. When acetylcholine was employed as substrate, 1 cc. of a solution of 2.5 g. of acetylcholine chloride in 10 cc. of water was used. The mixture was brought to p_{H} 8.0, using a standard borate buffer for comparison, by addition of 0.02 *N* sodium hydroxide which had been prepared from metallic sodium and was hence free from carbonate. To the solution so prepared 1 cc. of the serum under examination was added and, after again adjusting the p_{H} to 8.0, the standard alkali was added drop by drop from a burette as required to maintain the p_{H} of the solution at the above value. Burette readings were taken every 5 minutes for a period of 20 minutes. When the serum was pigmented it was found convenient, provided sufficient serum was available, to prepare a borate buffer containing 1 cc. of the serum for use as a standard. It should be mentioned that the titrations were carried out in a thermostat with glass sides, the flasks standing on a platform of opal glass provided with a background of the same material.

The activities of the sera towards methyl butyrate were determined by the same technique, 0.25 cc. of the pure ester being employed as substrate. This was dissolved in the 100 cc. of water before the addition of the serum.

The hydrolysis of tributyrin was followed by the stalagmometric method of Rona and Michaelis [1911] under the conditions (p_{H} 7.9; t 20°) described by Stedman and Stedman [1931].

Results.

The sera used in these experiments were obtained from various sources. Those from a few animals (ox, sheep, pig) were procured from the slaughter house; in certain other instances we are indebted to colleagues for providing us with the material. The blood from which the remainder were prepared was collected by various methods. As far as possible, however, particularly when small animals such as the mouse and pigeon were involved, it was obtained by heart puncture. Immediately after separation from the clot the clear serum was rendered sterile by shaking it with sufficient chloroform to saturate it, a procedure which had been found to exert no deleterious action on the choline-esterase present in horse-serum. Most of the specimens of serum examined were transmitted in this condition from London to Edinburgh and were investigated as rapidly as possible after their arrival. Before carrying out the estimations a slight precipitate, which usually separated during transit, was removed in the centrifuge.

The results are collected in Table I and, for convenience of discussion, are arranged somewhat arbitrarily in six groups. Butyrylcholine was employed in preference to acetylcholine as substrate for estimating the choline-esterase activity since it is hydrolysed more rapidly by the enzyme. With sera of high choline-esterase activity, however, the activity towards acetylcholine was also determined provided sufficient serum was available. In most instances the serum was obtained from one individual animal, but with the smaller animals a mixed specimen from a number of individuals was necessarily employed. Sera from two individuals were examined separately in the case both of the cat and the rabbit; the individual results are therefore quoted. The results given by two different mixed specimens of fowl-serum are also included in Table I since one specimen was examined in a fresh condition without treatment

Table I. *Choline-esterase and esterase activities of various blood-sera.*

Substrate	cc. 0.02 N NaOH required to titrate acid liberated in 20 mins.			Diminution in drop number in 40 mins. Tributyrin
	Butyryl- choline	Acetyl- choline	Methyl butyrate	
A. {	Monkey	8.8	3.45	13
	Man	4.95	2.6	10
	Dog	4.8	2.45	4
	Fox	2.4	—	0
B. {	Horse	5.45	2.0	24
	Guinea-pig	3.75	1.35	72
	Cat	2.15	—	36
	Cat	1.9	—	60
C. {	Pigeon	0.3	—	80
	Goose	0	—	44
	Duck	0.2	—	21
	Tortoise	0	—	—
	Mouse	0.4	—	96
D. {	Pig	0.6	—	0
	Fowl	0.5	—	4
	Fowl	0.7	—	3
E. {	Rat	0.05	—	18
	Rabbit	0.05	—	22
	Rabbit	0.1	—	23.5
F. {	Goat	0.1	—	1
	Ox	0.05	—	0
	Sheep	0.1	—	0
	Ferret	0	—	11
	Frog	0	—	0
	Hedgehog	0	—	0

with chloroform whereas the other had undergone the routine treatment described above. The conditions of estimation chosen were such that the hydrolysis of the substrates proceeded at a constant rate during the period of estimation. It has therefore been considered unnecessary to record the readings taken at 5 min. intervals in the cases of the choline esters and of methyl butyrate, and at 20 min. intervals in the case of tributyrin; only the total titration figures or diminution in drop number are included in the table. In a few instances the activity of the serum towards tributyrin was too great for an estimation to be made with 1 cc. of the serum. In these cases a volume (0.5 or 0.25 cc.) was chosen such that the diminution in the drop number did not exceed 24. Similarly, owing to the high activity of pigeon-serum towards methyl butyrate only 0.25 cc. was used for this estimation. In order to facilitate comparison with the results obtained with other sera, the figures quoted in the table for such cases are those calculated for 1 cc. from the results on the assumption that the amount of hydrolysis is proportional to the concentration of enzyme. With the exception of the figures for tributyrin, for which no correction is necessary, deductions have been made in each case for the non-enzymic hydrolysis of the substrate. These deductions are, however, small and were determined experimentally. For the period of 20 mins. they amount to 0.2 cc. for butyrylcholine, 0.35 cc. for acetylcholine and 0.15 cc. for methyl butyrate. It should be mentioned that the ferrets from which the serum was obtained had been inoculated with distemper virus.

In addition to the experiments recorded in Table I, a comparison has been made of the choline-esterase and esterase activities of human blood-sera from several individuals. The results are given in Table II.

Table II. *Choline-esterase and esterase activities of human blood-sera.*

Substrate	cc. 0.02 N NaOH required to titrate acid liberated in 20 mins.			Diminution in drop number in 40 mins. Tributyrin
	Butyryl- choline	Acetyl- choline	Methyl butyrate	
G. A. H. B.	6.0	3.4	0.15	12
L. H. E.	5.1	—	0.05	10
A. C. W.	4.95	2.6	0.15	10
E. S.	4.65	2.3	0.05	10
J. R.	4.5	—	—	10
J. M. McD.	3.6	—	—	6

DISCUSSION.

The experiments recorded in Table I demonstrate that the specific enzyme, choline-esterase, which we have previously shown to be present in horse-serum occurs also in the blood-sera from a number of animals. There are, however, enormous variations in the concentration of the enzyme in the various sera, and the animals which we have examined can be roughly although somewhat arbitrarily classified into three groups according as their respective sera contain relatively high concentrations, traces, or no detectable amount of the enzyme in question. The first group comprises the following animals, which are named in descending order of the choline-esterase activities of their sera: monkey, horse, man, dog, guinea-pig, fox, cat. Within this group, however, considerable variations in concentration occur, as is illustrated by the figures obtained for the monkey and the cat, which show that the serum from the former animal possesses a content of the enzyme which is four times that of serum from the latter. In the second group, comprising the pig, fowl, mouse, pigeon and duck, a small but definite choline-esterase activity is exhibited by the serum, while in the third and largest group consisting of the tortoise, frog, hedgehog, goose, rat, rabbit, goat, ox, sheep and ferret, no choline-esterase could be detected by the method which we have employed.

The physiological function of choline-esterase is doubtless to destroy acetylcholine, or possibly other esters of choline, and it might be considered, in view of the small amounts of a substance with such powerful physiological properties as acetylcholine which would be expected to gain access to the blood, that a concentration of the enzyme which would suffice for the destruction of such small quantities would not be detectable by the method which we have used for its estimation. Such an argument cannot, of course, be entirely refuted, but it must be emphasised that our method of estimation is much more delicate than it appears to be superficially, the conditions under which the estimations have been carried out having been so chosen as to increase its sensitivity almost to the maximum. Two factors have contributed to this. Firstly, we have employed butyrylcholine in place of acetylcholine as substrate, since the former ester is hydrolysed approximately twice as rapidly as the latter by the enzyme; this can be seen from those results in Table I in which both esters have been employed. Secondly, relatively high concentrations of substrate have been used. While it is impossible at present numerically to assess the effect of the second factor, it can nevertheless be stated that a diminution in the substrate concentration below that which we have employed causes a falling off in the amount of hydrolysis which, at low concentrations, becomes considerable. When it is further borne in mind that our method will measure an amount of enzymic

hydrolysis which is of the same order of magnitude as the non-enzymic hydrolysis which occurs under the conditions of our experiments, it appears exceedingly probable that it is capable of detecting the enzyme when it is present in serum in concentrations possessing physiological significance. It must not, however, be overlooked that our results have been obtained entirely with serum, and that the absence of the enzyme from this does not necessarily imply its absence from the corpuscles. This fact is mentioned since Plattner and his colleagues, in work quoted in our previous paper, have obtained results which they interpret as indicating that the destructive action of blood on acetylcholine is associated with the corpuscles. Galehr and Plattner [1927] have, moreover, examined the action of the defibrinated blood from a number of species upon acetylcholine, using pharmacological methods of estimation, and have obtained an order of activity which cannot be reconciled with our results except on the assumption that the corpuscles also possess activity. Further, Plattner and Bauer [1928] state that acetylcholine is destroyed by frog's blood, whereas we have failed to detect any choline-esterase in frog-serum.

In our previous experiments with horse-serum, it was shown that considerable variations occurred in the choline-esterase contents of sera from different horses. The estimations recorded in Table II now demonstrate that similar variations occur in human serum from different individuals. It has not, unfortunately, been possible to determine, under comparable conditions, the pulse rates of the individuals concerned, and hence no evidence can be presented as to a relationship between this magnitude and the choline-esterase content of the serum. Nevertheless, since the concentration of choline-esterase in the serum must necessarily determine the rate at which any acetylcholine entering the blood is destroyed, the magnitude of this concentration must be regarded as one of the factors which control the activity of the heart in those species in which the enzyme occurs.

The division of Table I into six groups is designed to emphasise the absence of any marked relationship between the choline-esterase and esterase activities of the various sera. A high choline-esterase activity may be associated either with an almost negligible (Group A), or an appreciable (B) esterase activity. On the other hand sera with high esterase contents may exhibit little or no activity towards choline esters (C). The remaining groups, in which there is a small but definite choline-esterase activity (D), or none at all (E and F), associated with small or negligible esterase activities, can obviously throw no light on the point under discussion. The results in Groups A, B and C, particularly when considered in conjunction with our previous results in which it was demonstrated that extensively purified choline-esterase from horse-serum exhibits only a small activity towards methyl butyrate, do, however, appear to indicate that at least two different enzymes, choline-esterase and esterase, are present in many of the sera examined. But it cannot be asserted that choline-esterase itself is entirely without action on simple esters. On the contrary, it appears probable that the small esterase activities exhibited by the sera of Group A may be due to the choline-esterase which these sera contain in relatively large amount. Similarly, it is possible that the small activity of pigeon-serum towards butyrylcholine is due, not to the presence of choline-esterase, but to the action of esterase, of which it contains an abnormally large amount.

A similar lack of relationship between the activities of the various sera towards tributyrin and their choline-esterase and esterase activities is also apparent from Table I. This suggests the presence in certain of the sera of a third enzyme, possibly a lipase. But it is again possible that both choline-

esterase and esterase possess some activity towards tributyrin. In one instance, that of the fox, no hydrolysis of tributyrin occurred, although the serum contained a moderate concentration of choline-esterase. It might be concluded from this that the latter enzyme does not attack tributyrin were it not for the fact that the results for butyrylcholine and tributyrin obtained with human sera (Table II) exhibit a certain parallelism.

SUMMARY.

The blood-sera from a number of animals have been examined for the presence of enzymes which hydrolyse choline esters, methyl butyrate and tributyrin. The results, which have been tabulated, show that little relationship exists among the sera from various species with respect to their relative activities towards the three substrates and it is concluded that at least two enzymes, choline-esterase and esterase, are present in many of the sera. A third enzyme, possibly a lipase, is also probably present in some.

The specificities of choline-esterase and esterase are probably not absolute. A consideration of the results obtained with different sera suggests that, in addition to hydrolysing esters of choline, the former enzyme exerts a relatively minute hydrolytic action on methyl butyrate and possibly also on tributyrin. The serum-esterase may similarly hydrolyse esters of choline to a very small extent; it almost certainly attacks tributyrin.

The choline-esterase content of human serum, which is relatively high, varies considerably with different individuals. Its magnitude is probably an important factor in controlling the activity of the heart.

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