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MUSCULAR ACTIVITY AND CHOLINE ESTERASE

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It was reported recently that the serum choline esterase activity is raised by muscular exercise in man [Richter & Croft, 1942*a*]. This observation suggested that the high choline esterase activities that have been found clinically in thyrotoxicosis and in acute emotional states [Tod & Jones, 1937; Richter & Lee, 1942 *a*, *b*] might be due to the increased neuro-muscular activity commonly seen in these conditions.

The investigation has now been pursued with a view to defining more clearly the relationship between the serum choline esterase and muscular activity. The source of the additional choline esterase which appears in the serum during muscular effort has also been investigated.

Methods

In the majority of experiments the authors served as subjects, but we are particularly indebted to Dr M. S. Jones and Dr R. Scarisbrick for giving us specimens of blood from a number of 'effort syndrome' patients and controls before and after exercise. The study of patients with 'effort syndrome' appeared relevant to the present investigation since in this condition the normal physiological responses to muscular effort (tachycardia, breathlessness, sweating and fatigue) are generally exaggerated; but the patients showed no essential difference from the controls as far as the choline esterase changes were concerned and the experiments on them entirely confirmed the experiments on normal individuals. Apart from the effort syndrome patients, all the experimental subjects were believed to be normal.

Venous blood specimens were taken from the cubital veins. Capillary blood specimens of 1·5–4·0 c.c. were obtained by expressing the blood from an incision made above the finger nail. Care was taken to avoid venous stasis while taking the blood.

Choline esterase estimation. Esterase activities were estimated by the manometric method with a Warburg apparatus under similar conditions to those described by Richter & Croft [1942*b*]. In carrying out the estimation 0·5 c.c. of

a 2·5% solution of acetylcholine chloride in 0·9% NaCl was put in the Warburg cup. The serum (0·2 c.c.) was diluted immediately before use to 4 c.c. 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 oxygen. The diluted serum was put in the main part of the Warburg cup and mixed with the acetylcholine solution after equilibrating at 37°; in the experiments with red blood corpuscles, the centrifuged corpuscles were taken up in a blood pipette and treated in exactly the same way as the serum. The rate of liberation of CO_2 was obtained by plotting the manometer readings against time for the first 45 min. and drawing a line through the points. Estimations were frequently done in duplicate and the two values generally agreed to within 3% with acetylcholine as substrate and 10% with the aliphatic esters. In the experiments with eserine 0·35 c.c. 1/10,000 eserine sulphate was added to 2·65 c.c. diluted serum and the mixture allowed to stand for 20 min. before adding the acetylcholine. The experiments with methyl butyrate and tributyrin were carried out in exactly the same manner except that 0·1 c.c. of the pure substrate was put in the side tube in place of the acetylcholine solution: the total volume was kept the same. The accuracy was greater when the activities were high than when low activities were obtained.

Esterase activities are expressed as micro-equivalents (μE.) of ester hydrolysed per c.c. of serum (or corpuscles) per hr. The activities were corrected for the spontaneous hydrolysis of acetylcholine which corresponded to 16·5 μE./c.c./hr. under these conditions: the spontaneous hydrolysis of methyl butyrate and tributyrin was negligible. The choline esterase activities may be converted into the usual clinical units [Jones & Tod, 1935] by dividing by the factor 2·36.

Results

Effect of muscular exercise on the serum choline esterase. Muscular exercise produced a significant rise in the serum choline esterase activity provided that the exercise was fairly vigorous and lasted not less than 5–10 min. The extent of the rise depended on the amount and nature of the exercise and differed in different subjects. Some typical figures are given in Tables 1 and 2 and in Fig. 1. The change was statistically significant when examined by Fisher's '*t*' test [1938]. In some experiments the serum choline esterase returned almost to normal again within 10 min., but in others it took considerably longer than this. That the rise in esterase activity was due to a choline esterase and not to some other esterase was shown by the complete inhibition by eserine (Table 2). The rise was shown in citrated and oxalated plasma as well as in serum.

Esterase changes in red blood corpuscles. The choline esterase in the red blood corpuscles is generally much more active than that in the serum. When the choline esterase activities were estimated in both serum and centrifuged blood corpuscles, before and after exercise, the rise in the serum was accompanied by

TABLE 1. Serum choline esterase changes with exercise

The subjects were all adult males 60–80 kg. in weight. Subjects P. 1, P. 2, P. 4 and P. 8 were effort syndrome patients. The 'press-ups' in Exp. 3 and Fig. 1 consisted of raising the body from the prone position by extending the arms. The bicycle ergometer was pedalled at a constant rate with a load adjusted so that the work performed was 6000–8000 ft. lb./min. (830–1100 kg.m./min.). Venous blood specimens were taken from the arm in the ergometer experiments; capillary blood was taken in the other experiments. The patients were taken from the Effort Syndrome Unit at Mill Hill Emergency Hospital and they were diagnosed by Dr M. S. Jones. In the experiments with patients oxalated plasma was used instead of serum.

Subject	Exercise	Duration min.	Total work kg.m.	Serum choline esterase μE./c.c./hr.	
D.R.	Raising 4 kg. wt. with right arm 20 times per min.	10	1,120	(*a*) Resting	252
				(*b*) After exercise	320
				(*c*) 10 min. after exercise	282
				(*d*) 15 min. after exercise	254
W.D.	Raising 4 kg. wt. with right arm 20 times per min.	2	167	(*a*) Resting	205
				(*b*) After exercise	212
				(*c*) 20 min. after exercise	205
D.R.	30 'press-ups'	5	5,760	(*a*) Resting	273
				(*b*) After exercise	306
				(*c*) 18 min. after exercise	268
P. 1	Bicycle ergometer	4	3,880	(*a*) Resting	126
				(*b*) After exercise	152
				(*c*) 20 min. after exercise	126
P. 2	Bicycle ergometer	5	4,850	(*a*) Resting	79
				(*b*) After exercise	100
P. 4	Bicycle ergometer	25	24,250	(*a*) Resting	90
				(*b*) After exercise	106
P. 8	Bicycle ergometer	5	4,850	(*a*) Resting	168
				(*b*) After exercise	183
				(*c*) 10 min. after exercise	168

a simultaneous fall in the choline esterase activity of the corpuscles (Table 2). Further, assuming the haematocrit to lie within normal limits, the fall in corpuscle esterase activity corresponded approximately in amount to the rise in the serum. This indicated that the extra choline esterase appearing in the serum as a result of muscular exercise most probably had come from the red blood corpuscles.

The results are not due to a partial haemolysis since haemolysis would not lead to a lowering of the concentration of choline esterase (μE./c.c./hr.) in the remaining red blood corpuscles. In any case the sera showed no trace of haemoglobin when carefully examined by eye, while haemolysis of less than 0·2% of the red blood corpuscles could have been readily detected.

It was noticeable that in four of the subjects the loss of choline esterase from the red blood corpuscles continued in the 10 min. after the cessation of exercise.

Specificity of the choline esterase appearing in the serum after exercise. Human serum contains a choline esterase which hydrolyses methyl butyrate and tributyrin in addition to choline esters. The red blood corpuscles, on the other hand, contain two esterases, (*a*) a choline esterase which is highly specific for choline esters, and (*b*) an ali-esterase which is not inhibited by eserine and which

TABLE 2. Esterase changes in plasma and corpuscles due to exercise on bicycle ergometer

All blood specimens were venous. Subjects P. 3, P. 5 and P. 6 were effort syndrome patients. The subjects were all adult males 60–80 kg. in weight. The figures represent activities (μE./c.c./hr.) with acetylcholine, methyl butyrate and tributyrin as substrates. The figures in brackets represent the activities obtained after the addition of 10^{-5} eserine sulphate. The bicycle ergometer was adjusted so that the work done was approximately 970 kg.m./min. during the first 10 min.: with subjects who were able to continue longer than this the work was then increased to approximately 1400 kg.m./min.

			Plasma μE./c.c./hr.			Blood corpuscles μE./c.c./hr.		
Subject	Duration min.		Ac.ch.	Me.but.	Tribut.	Ac.ch.	Me. but.	Tri-but.
P. 3	3	(*a*) Resting	72 (0)	—	—	294 (0)	—	—
		(*b*) After ex.	91 (0)	—	—	281 (0)	—	—
		(*c*) 10 min. after ex.	95 (0)	—	—	262 (0)	—	—
P. 5	7	(*a*) Resting	192	—	101 (0)	355	—	72
		(*b*) After ex.	204	—	113 (4)	334	—	65
Dr R.	12	(*a*) Resting	55	25	36	355	34	50
		(*b*) After ex.	81	32	46	337	35	54
		(*c*) 10 min. after ex.	74	25	40	334	38	61
Dr J.	13	(*a*) Resting	65	31	64	365	42	68
		(*b*) After ex.	107	50	80	320	31	54
		(*c*) 13 min. after ex.	95	47	71	306	38	61
L.C.	8	(*a*) Resting	62	34 (1)	—	376	41	—
		(*b*) After ex.	80	41 (8)	—	338	38	—
		(*c*) 10 min. after ex.	71	36 (12)	—	340	33	—
H.B.	12	(*a*) Resting	140	57 (0)	—	329	53	—
		(*b*) After ex.	156	59 (4)	—	315	53	—
		(*c*) 10 min. after ex.	145	67 (9)	—	301	34	—
P. 6	8	(*a*) Resting	150 (0)	61 (0)	—	387	42	—
		(*b*) After ex.	176 (0)	78 (12)	—	322	35	—
		(*c*) 5 min. after ex.	160 (0)	66 (5)	—	339	40	—

acts preferentially on simple aliphatic esters and glycerides, such as methyl butyrate and tributyrin [Richter & Croft, 1942 *b*].

The serum and corpuscle choline esterase differ considerably in their specificity in that only the former is active towards the aliphatic esters. It appeared that it should be possible to obtain further evidence as to the source of the additional choline esterase appearing in the serum during exercise by testing with methyl butyrate and tributyrin whether this additional enzyme is of the specific or of the unspecific variety. Any conclusions as to the specificity must be based on the comparison of relatively small differences between two or more estimations, and it was clear that experiments of this kind would be difficult since they must make very considerable demands on the accuracy of the experimental technique: all estimations were therefore carried out in duplicate or triplicate. The figures obtained with methyl butyrate and tributyrin are given in Table 2.

In analysing the figures in Table 2 it should be noted that some of the aliesterase as well as the choline esterase passed from the red blood corpuscles into the serum during muscular exercise. This was shown by (*a*) the decrease in

the activity of the blood corpuscles towards the aliphatic esters and also by (*b*) the appearance in the serum of an esterase which was not inhibited by eserine. The choline esterase and the ali-esterase did not pass at exactly the same rates from the corpuscles into the serum and the two enzymes appeared to shift independently of each other. Exercise increased the rate of hydrolysis by the serum of methyl butyrate and of tributyrin, but this was accounted for by the ali-esterase. When the ali-esterase was taken into account, it was clear that the

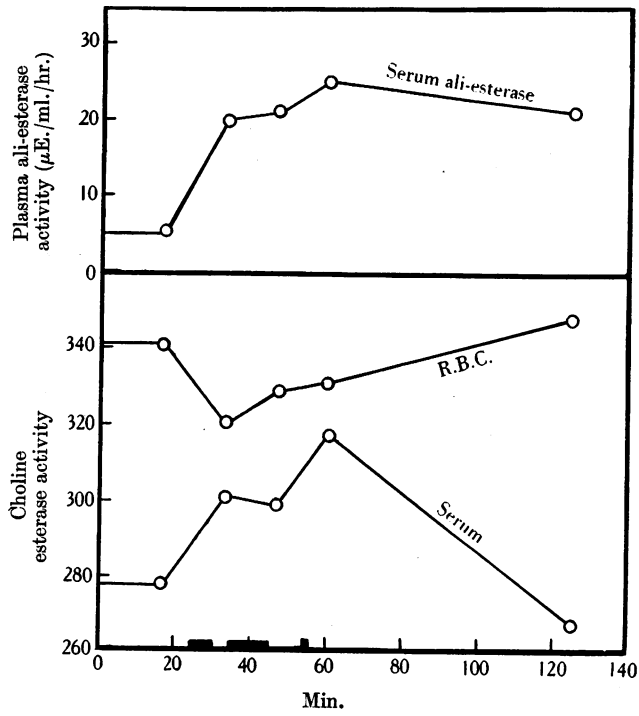

Fig. 1. Esterase changes in blood during exercise. Subject D. R. (72 kg.). Weights (4 kg.) lifted 100 times with right arm (520 kg.m.) at 25–30/min.; 100 steps climbed (6580 kg.m.) at 34–44/min.; ¼ mile run at 54–55 min. Esterase activities in μE./c.c. citrated plasma or packed red blood cells/hr. Methyl butyrate was the substrate for the ali-esterase. Capillary blood.

additional choline esterase appearing in the serum had no significant activity towards the aliphatic esters. This gave evidence that the esterase appearing in the serum during muscular exercise was not of the normal serum type, but was of the specific type, similar to that which occurs in the red blood corpuscles.

Failure to obtain a significant esterase shift after exercise of 5 or more min. duration was observed in only one out of thirty experiments. On this occasion the subject (D.R.) showed a high initial serum choline esterase activity of 321 which was not further increased by vigorous exercise: the ali-esterase activity

was 24. This experiment was done on a particularly cold day before the central heating of the laboratory had been turned on. It appeared likely that the failure of the serum choline esterase to rise with exercise was due to its being already abnormally high before the beginning of the experiment as a result of

TABLE 3. Exercise experiments giving esterase changes with plasma or serum protein and corpuscle haemoglobin concentrations

Haemoglobin estimations were done by the Haldane method: protein by the biuret method. Exercise indicated by asterisk in Exp. 3 consisted of running ¼ mile in about 1 min.

(1) Subject D.R.

				Corpuscles		Whole blood	
Time min.	Work kg.m.	Plasma C.E. μE./c.c./hr.	Plasma proteins mg./100 c.c.	C.E. μE./c.c./hr.	Hb. mg./c.c. cells	C.E. μE./c.c./hr.	Haemato-crit % of r.b.c.
0	—	263	7·9	347	337	141	48
1–10	7820	—	—	—	—	—	—
13	—	288	8·0	329	334	139	50
16–34	Resting	—	—	—	—	—	—
34	—	257	8·0	346	334	139	49

(2) Subject P.G.C.

				Corpuscles		Whole blood	
Time min.	Work kg.m.	Serum C.E. μE./c.c./hr.	Serum proteins mg./100 c.c.	C.E. μE./c.c./hr.	Hb. mg./c.c. cells	C.E. μE./c.c./hr.	Hb. mg./c.c. blood
0	—	121	7·0	291	262	217	134
7–15	8200	—	—	—	—	—	—
17	—	135	7·0	275	262	217	141
20–75	Resting	—	—	—	—	—	—
75	—	116	7·0	301	262	217	138

(3) Subject D.R.

		Serum			Corpuscles	
Time min.	Work kg.m.	C.E. μE./c.c./hr.	Proteins mg./100 c.c.	Ali-esterase μE./c.c./hr.	C.E. μE./c.c./hr.	Hb mg./c.c. cells
0	—	278	7·8	5	341	345
8–13	520	—	—	—	—	—
16	—	301	8·0	20	320	350
18–28	6580	—	—	—	—	—
30	—	299	8·0	21	329	350
37–38	*	—	—	—	—	—
43	—	318	8·3	25	331	345
45–108	Resting	—	—	—	—	—
108	—	268	8·0	21	348	340

(4) Subject patient 9

		Plasma			Corpuscles	
Time min.	Work kg.m.	C.E. μE./c.c./hr.	Proteins mg./100 c.c.	Ali-esterase μE./c.c./hr.	C.E. μE./c.c./hr.	Hb mg./c.c. cells
0	—	162	6·2	—	393	326
0–5	4850	—	—	—	—	—
5	—	174	6·0	—	376	328
5–13	Resting	—	—	—	—	—
13	—	155	6·0	—	385	328

the bodily reaction (neuro-muscular) to the lowered temperature. Since the esterase shift was observed to occur normally in the same subject at other times, the non-appearance of the shift under these conditions did not appear to conflict seriously with the main conclusions.

Corpuscular fluid equilibrium and esterase shift. Some individuals show a transient increase in the mean corpuscular diameter immediately after exercise [Wiechmann & Schürmeyer, 1925; Price-Jones, 1933]. This effect has been attributed to the passage of fluid from the plasma into the corpuscles owing to the increased CO_2 tension of the blood. Although the effect is small and apparently occurs only at exhaustion, it might account for an apparent lowering of the corpuscular esterase content that would be significant in the exercise experiments that have been described.

The passage of fluid from the plasma into the corpuscles would be shown (*a*) by a lowering of the haemoglobin concentration per unit volume of corpuscles, (*b*) by a change in the haematocrit, and possibly (*c*) by an increase in the total plasma protein concentration, if equilibrium with the tissue fluids was not attained. Experiments in which these three factors were measured (Table 3) confirmed the esterase shift but failed to detect any significant change in the mean corpuscular volume. It was further observed that whereas the effect observed by Wiechmann & Schürmeyer and Price-Jones was very transient and disappeared in 10 min. after the cessation of exercise, the loss of esterase from the red blood corpuscles always persisted for 10 or more min. (Table 2). It was concluded that changes in the fluid equilibrium could not account for the esterase shift that was observed in these experiments.

The effect of factors other than exercise on the choline esterase activity

The transfer of choline esterase from corpuscles to serum as a result of muscular exercise might be secondary to some biochemical change occurring in the blood at the same time. The following experiments on the subject D.R. were done to test this possibility, but no significant change in the serum choline esterase was observed.

Adrenaline (1 mg.) was injected and $3\frac{1}{2}$ hr. later a second dose (0·5 mg.) was given. *Ergotamine* (1 mg. 'Femergin') was taken by mouth. *Histamine* (1 mg. of acid phosphate) was injected subcutaneously. The *acid-base balance* of the blood was changed by overbreathing (37·4 l./min. for 10 min.), by underbreathing (3·8 l./min. for 10 min.) and by rebreathing from 5 l. of a mixture of 5% CO_2 in oxygen for 7 min. In the last experiment the pulse rate had risen to 98 and sweating, nausea, giddiness and tremor supervened.

Anoxaemia. Local anoxaemia produced by passing a ligature round the arm so as to effect a temporary stoppage of the circulation, caused a sharp rise in the serum choline esterase. This was also observed by McArdle [1940]. The rise was of the same order of magnitude as that occurring in muscular exercise, but

the effect differed from that occurring during exercise in that the esterase activity of the red blood corpuscles showed no change (Table 4). Tests with aliphatic esters showed that there was also no shift in the ali-esterase, and the additional choline esterase found in the serum was of the unspecific type. The

TABLE 4. Effect of stasis on serum and corpuscle esterase activities

Venous stasis for 10 min. was obtained with a sphygmomanometer cuff round the arm inflated to 100–120 mm. pressure. Venous blood was taken in Exp. 3 and capillary blood (after clamping the brachial artery) in Exps. 1 and 2. The figures represent activities (μE./c.c./hr.) with acetylcholine, methyl butyrate and tributyrin as substrates. The figures in brackets represent the activities obtained after the addition of 10^{-5} eserine.

			Serum μE./c.c./hr.			Blood corpuscles μE./c.c./hr.		
No.	Subject		Ac.ch.	Me.but.	Tribut.	Ac.ch.	Me.but.	Tribut.
1	P.G.C.	(*a*) Resting	117	—	—	278	—	—
		(*b*) After stasis	141	—	—	284	—	—
2	D.R.	(*a*) Resting	282	—	—	346	—	—
		(*b*) After stasis	396	—	—	352	—	—
3	D.R.	(*a*) Resting	252 (0)	94 (2)	153 (0)	370	45	68
		(*b*) After stasis	342 (0)	130 (4)	208 (0)	370	50	68

changes occurring during stasis were clearly different from those during exercise. It was noted that the ratio of blood corpuscles to serum was far greater than normal in the specimens collected during stasis. This suggested that the rise in serum choline esterase during stasis might be explained by the increased passage of fluid through the capillary walls.

Estimations of the serum proteins confirmed an increased concentration as a result of circulatory stasis, sufficient in amount to account for the observed rise in choline esterase activity. The figures (Table 5) contrasted with similar

TABLE 5. Comparison of the effects of exercise and of venous stasis on the serum choline esterase and total serum proteins

Total serum proteins estimated by the biuret method [Harrison, 1937]. Capillary blood in the stasis experiments: venous blood in the exercise experiments. In Exps. 3 and 4 oxalated plasma was used instead of serum.

No.	Subject		Choline esterase μE./c.c./hr.	Increase %	Total serum proteins mg./100 c.c.	Increase %
1	P.G.C.	(*a*) Resting	117	0	7·5	0
		(*b*) After stasis	141	20	8·7	22
2	D.R.	(*a*) Resting	282	0	7·7	0
		(*b*) After stasis	396	40	10·0	30
3	P. 8	(*a*) Resting	168	0	7·1	0
		(*b*) After exercise	183	9	7·2	1
4	P. 6	(*a*) Resting	150	0	6·4	0
		(*b*) After exercise	176	17	6·4	0

estimations made on sera taken before and after exercise, in which there was a rise in choline esterase activity without any corresponding increase in the total serum protein concentration.

Site of the choline esterase shift during exercise

It was not necessary to exercise the whole body in order to produce a large choline esterase shift: a maximal shift could be produced even when the exercise was limited to one arm (Table 1). This made it unlikely that the observed change in the blood choline esterase occurred locally in the exercised muscle, since only a small fraction of the systemic blood could flow at any time through an exercised arm: the local change in the blood traversing the arm would have to be very great to account for the considerable change observed in the whole systemic blood. A further experiment to elucidate this point was carried out by exercising the muscles of one arm and comparing the choline esterase activity of the blood taken from the exercised arm with that of blood from the other arm. The superficial circulation through the skin was reduced to a minimum by immersing the hand in cold water. Blood was taken from the median cubital vein while exercising the flexor and extensor muscles of the left forearm and hand by alternately clenching the fist and extending the fingers with sufficient vigour to produce rapid fatigue:

	Serum C.E. μE./c.c./hr.	Serum proteins mg./100 c.c.	Corpuscle C.E. μE./c.c./hr.
Left arm	282	8·0	318
Right arm	270	7·5	318

(Subject: D.R. Muscles of left forearm and hand exercised for 2 min. before taking blood from the median cubital veins.)

No significant difference was found in the choline esterase activities of the serum or corpuscles of blood taken from the two arms. The very slightly increased activity of the serum from the exercised arm may be attributed to the slight local concentration of the serum proteins due to oedema in the muscle which is known to occur during vigorous exercise.

It is concluded that the shift in the blood choline esterases during muscular exercise does not occur locally in the muscle exercised; it may occur in some visceral organ, or it may occur uniformly throughout the whole of the circulating blood as a result of the chemical changes in the blood due to exercise.

Effect of chemical factors on the blood choline esterase distribution in vitro

In view of the chemical changes in the lactate, potassium and other metabolites during exercise, the effect of a series of substances on the choline esterase distribution between the corpuscles and plasma was tested in vitro. The chemical substances were incubated at 37° with fresh oxalated whole blood and the choline esterase activities of the plasma and corpuscles were estimated (Table 6).

When sufficient neutral salt (NaCl, KCl or Na_2SO_4) was added to make the plasma strongly hypertonic there was an apparent change in the choline

esterase activities of the plasma and corpuscles due to shrinkage of the cells and corresponding dilution of the plasma, but no evidence was obtained of any shift in the choline esterase distribution with any of the chemical factors tested.

TABLE 6. Effect of chemical factors on choline esterases in oxalated whole blood

Oxalated whole blood was incubated at 37° for 20 min. with and without the additions. With glutathione the incubation was for 40 min. During incubation with Na_2SO_4 the corpuscle volume decreased from 43 to 32%. With CO_2 the gas was bubbled through for 10 min. The cholesterol was added in the form of a fine emulsion prepared by shaking a 20% solution of cholesterol with a 20% aqueous solution of lecithin and dialysing for 2 hr. against distilled water to remove the alcohol.

Addition	Conc. mg./c.c.	Plasma μE./c.c./hr.		Corpuscles μE./c.c./hr.	
		Alone	With addition	Alone	With addition
NaH_2PO_4	5	100	102	256	249
Na_2SO_4	20	137	116	315	377
NaCl	15	100	71	256	313
KCl	20	100	81	256	306
CO_2	Sat.	196	192	303	294
Choline Cl	5	137	142	315	314
Na lactate	2	137	140	315	308
Na ascorbate	5	137	140	315	310
Glutathione	10	119	121	270	264
Lecithin	5	176	182	261	261
Lecithin and cholesterol	5	176	182	261	258

It was noted by previous investigators [Alles & Hawes, 1940] and confirmed in this laboratory that when the choline esterase activity of the red blood corpuscles was measured, the same result was obtained whether the cells were previously haemolysed or not. This suggested that the corpuscle esterase might be mainly adsorbed or combined at the surface of the cells.

It appeared possible that the choline esterase might still be mainly associated with the cell membranes after haemolysis, but experiments to test this by haemolysing with distilled water or with ether and carefully centrifuging off the empty cell membranes showed that the whole of the enzymic activity passed with the haemoglobin into the supernatant fluid and no choline esterase activity was shown by the cell membranes.

	Choline esterase (μE./c.c./hr.)		
	Whole cells	Skins	Supernatant solution
Haemolysis by distilled water	340	0	301
Haemolysis by ether	233	0	214

The absence of enzymic activity in the cell membranes did not disprove the view that the enzyme is normally adsorbed in part on the surface, since the membranes are manifestly damaged in the process of haemolysis.

Lysolecithin. If the choline esterase is mainly adsorbed on the cell surface, it appeared that an agent such as lysolecithin which progressively damages the

cell membrane might free the enzyme from the surface and produce an artificial esterase shift. It was found in preliminary experiments that haemolysis of human erythrocytes suspended in serum became evident in about 3 hr. in the presence of 1/10,000 lysolecithin at 37° C. Incubation of the corpuscles under these conditions for 1½ hr., which should be sufficient to damage the cell membranes without actually haemolysing them, produced no significant esterase shift.

	Serum		Corpuscles	
	Incubated alone	With lysolecithin	Incubated alone	With lysolecithin
(1)	182	173	303	302
(2)	176	183	261	266

It appeared likely that the esterase shift was produced in vivo by the mediation of some chemical substance; but normal human erythrocytes gave no change in esterase activity when incubated in vitro with serum taken from a subject immediately after exercise.

Serum (from exercised subject)		Corpuscles	
Incubated alone	With corpuscles	Incubated alone	With serum from exercised subject
297	297	315	329

Incubation of the cells alone with serum produced no esterase shift in periods up to 30 min. There was also no return to normal in the esterase activities of sera and corpuscles collected after exercise on incubation for 2½ hr.

Discussion

Evidence has been obtained that during muscular exercise there is a shift in the choline esterase from the red blood corpuscles to the serum. This occurs in man as a normal physiological process. The shift was observed to occur in a variety of different types of exercise which were not necessarily very strenuous. The time taken for a significant change to occur was as little as 3 min. The decrease in the choline esterase content of the red blood corpuscles amounted in some experiments to 16 or 17%. The percentage rise in the serum choline esterase depended on the initial concentration and the haematocrit; but when the initial serum choline esterase activity was low the rise amounted in some experiments to an increase of 50 or 60%. In addition to the choline esterase shift there also occurred a similar shift in the eserine-resistant ali-esterase, an enzyme which acts preferentially on the simple aliphatic esters and glycerides.

Vahlquist [1935] and Hall & Lucas [1937] reported that muscular exercise is without effect on the serum choline esterase. Croxatto, Huidobro, Croxatto & Salvestrini [1939] reported a rise in the blood choline esterase in cats under Dial anaesthesia on stimulating the motor nerves to a leg, but Trowbridge [1941] failed to verify this.

The apparent discrepancy between our results and those of Vahlquist, who

examined the serum of a man after 'an hour's hard tennis-playing', may be due to too long an interval being left between the exercise and the taking of the blood sample; the serum choline esterase may return to normal within 10–15 min. after exercise and the peak in the rise might easily have been missed. This might apply also to the work of Hall & Lucas. The experiments of Croxatto *et al.* are difficult to interpret since whole blood was used without stating the haematocrit; the choline esterase concentration is different in the serum and corpuscles and it is not clear which was concerned in the change observed.

Source of additional choline esterase. The additional choline esterase found in our experiments in the serum after exercise was shown to come from the red blood corpuscles, since the corpuscles gave a simultaneous fall in choline esterase which was similar in amount to the increase in the serum.

Further evidence of the source of the additional choline esterase in the serum after exercise was obtained by making use of the fact that the corpuscle choline esterase differs considerably from the serum choline esterase in its specificity towards different substrates [Richter & Croft, 1942 *b*]. Experiments with methyl butyrate and tributyrin gave figures which agreed with expectations in showing that the additional choline esterase in the serum after exercise resembles the corpuscle choline esterase rather than the normal serum choline esterase in its specificity.

Mechanism of choline esterase shift. It appeared possible that the choline esterase shift during exercise might be due to the change in acid-base equilibrium between the serum and corpuscles; but alteration of the acid-base equilibrium by vigorous overbreathing, underbreathing or by rebreathing 5% CO_2 in oxygen produced no significant change in the choline esterase. Experiments with adrenaline and histamine showed again that the shift cannot be attributed to the liberation of these substances in the blood during exercise.

Anoxaemia produced by passing a ligature round the arm so as to effect a temporary circulatory stasis caused a rise in the serum choline esterase of the blood taken from the ligatured arm, but this effect was due to the local rise in the total serum proteins through oedema. It was different from the shift occurring during exercise, since there was no corresponding fall in the corpuscle choline esterase activity and no shift in the ali-esterase.

It is unlikely that the choline esterase shift during exercise occurs locally in the active muscles since the blood taken from vigorously acting muscles showed no significant difference in choline esterase from that taken from muscles that were inactive.

Attempts to reproduce the choline esterase shift in vitro by adding chemical substances to oxalated whole blood at 37° gave negative results with lactic acid, sodium acid phosphate and a series of other metabolites which change in concentration during muscular exercise. These experiments gave no clue as to the mechanism of the choline esterase shift.

It is hard to believe that a protein such as the corpuscle choline esterase is able to pass with any degree of ease through the cell walls; it therefore appeared probable that the enzyme is mainly adsorbed or combined at the cell surface, from which it is partially released during exercise. However, the empty cell membranes which were obtained after haemolysis showed no choline esterase activity.

The apparent changes in choline esterase activity of the serum and corpuscles during muscular exercise might be attributed to a diffusible coenzyme or to an inhibitor passing in the reverse direction from the serum into the cells. While these must be considered as formal possibilities, there is no evidence of the existence of any such inhibitor or coenzyme and it is unlikely that a factor of this kind would affect the eserine-resistant ali-esterase in precisely the same way. A shift in the choline esterase is therefore the simpler interpretation of the results.

The changes in the serum and corpuscle choline esterase during exercise are too large to be accounted for as a haemoconcentration effect, and similarly they would appear to be too large to be related to the sudden discharge during exercise of 'non-circulating' blood from the spleen. Adrenaline, which also causes contraction of the spleen, was without effect.

Assuming that our interpretation of the results is correct and that there is a shift of choline esterase and ali-esterase from the corpuscles to the serum, it would appear probable that this change is effected by some chemical metabolite released during exercise which elutes the enzymes adsorbed or combined at the surface of the corpuscles so that they pass into the plasma.

Physiological significance of serum choline esterase. Previous investigators have assigned to the serum choline esterase an important function in destroying acetylcholine entering the blood from the tissues and in so controlling the state of autonomic balance [Stedman & Russell, 1937; Antopol, Tuchman & Schifrin, 1937; Tod & Jones, 1937]. Schütz has attempted to relate the serum choline esterase activity to the state of activity of the central nervous system [1941].

Against these views it should be pointed out that the choline esterase activity of the serum is usually much lower than that of the blood corpuscles or of the tissues, where most of the destruction of choline esters may be presumed to occur. It is therefore unlikely that the changes that have been observed in the serum choline esterase activity can be very significant in affecting the acetylcholine content of the blood.

A number of attempts have been made by previous investigators to make use of the serum choline esterase activity as a clinical test and this work has added to our knowledge of its significance.

High serum choline esterase activities have been observed (*a*) in acute emotional states, (*b*) in thyrotoxicosis [Antopol *et al.* 1937], and (*c*) in 'post-concussional' states [Richter & Lee, 1942 *b*], while low activities have been

observed (*d*) in catatonic stupor [Tod & Jones, 1937], (*e*) in the extreme debility associated with advanced tuberculosis, carcinoma, anaemia and liver disease [McArdle, 1940], and (*f*) after administering narcotics [Ahlmark & Kornerup, 1939; Schütz, 1941]. It is evident that the conditions associated respectively with high and with low serum choline esterase activities in man are contrasted with respect to the prevailing conditions of autonomic and of neuro-muscular activity. A case might be made out for associating the serum choline esterase activity with either of these two factors. In muscular exercise both autonomic and neuro-muscular activity again occur together and are associated with a serum choline esterase rise.

It is clear that further data must be obtained before the signifieance of the serum choline esterase activity can be established; but the results of the present investigation show that muscular activity is one of the factors that must be taken into account in clinical work on the serum choline esterase activity.

Summary

1. The serum choline esterase activity is raised during muscular exercise in man.

2. The choline esterase of the blood corpuscles falls during muscular exercise and this may account for the rise in the serum choline esterase.

3. Some of the ali-esterase in the red blood corpuscles also passes into the serum during muscular exercise.

4. The serum choline esterase activity is not raised by the administration of adrenaline, ergotamine or histamine or by overbreathing, underbreathing or rebreathing the expired air.

5. The serum choline esterase activity is raised during circulatory stasis.

6. The rise in serum choline esterase during circulatory stasis is due to the increase in the concentration of the serum proteins: it is not related to the rise during muscular exercise.

7. The mechanism of the choline esterase shift and the significance of the serum choline esterase activity are discussed.

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