SERUM CHOLINE ESTERASE AND ANXIETY.

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FOLLOWING the work of Dale (1934) and Loewi (1935) on the relationship of choline esterase to the transmission of nervous impulses, a number of studies have been made of the choline esterase activity of the serum in different physiological and pathological conditions. The serum esterase activity varies considerably from one individual to another, but it generally remains very constant in any one individual even over long periods of time; it is believed to be unaffected by changes in diet, exercise, fatigue, or by menstruation. The earlier investigators were unable to correlate the esterase level with any such factor as age, sex, heart rate, blood pressure, weight, or with pathological conditions such as benign or malignant tumours, chronic infections, heart diseases or neurological conditions (v. Verebely, 1936; McGeorge, 1937); they found only a tendency to low esterase activities in acute infections (Hall and Lucas, 1937) and in advanced tuberculosis (Vahlquist, 1935).

In an investigation in which psychopathic patients were included Tod and Jones (1937) and Jones and Stadie (1939) described a number of positive correlations between the serum choline esterase and various clinical conditions. They found a high esterase activity in anxiety states and lowered activity in catatonic stupor, epilepsy, schizophrenia and also in advanced tuberculosis and carcinoma. Antopol *et al.* (1937, 1938) associated high serum esterase activity with thyrotoxicosis and low activities with liver disease, anaemia and hyperpyrexia. Milhorat (1938), in agreement with Jones and Stadie, attributes the low esterase activities in these conditions to the general debility, but McArdle (1940) concludes that impairment of liver function is the primary cause.

The present work was carried out with the object of obtaining further information as to the relationship between the serum choline esterase activity and anxiety.

METHODS.

Capillary blood (1 ml.) was taken from the lobe of the ear and collected in a centrifuge tube, which was spun after standing overnight at room temperature or for 2 hours at 37° C. for the clot to form. The serum choline esterase was estimated as described by Jones and Tod (1935). This method depends on adding the serum to acetylcholine, which is broken down by the enzyme to choline and acetic acid. The acetic acid liberates an equivalent amount of carbon dioxide from the bicarbonate buffer solution in which the reaction is carried out and the carbon dioxide is measured in a Warburg apparatus. In carrying out the estimation 0·5 ml. of a 2·5 per cent. solution of acetylcholine chloride in 0·9 per cent. NaCl was put into the side-tube of the Warburg cup. The serum (0·2 ml.) was mixed with 3·8 ml. of buffer solution containing 0·03 M $NaHCO_3$ and 0·12 M NaCl, which was kept in a closed bottle under oxygen containing 5 per cent. CO_2. The diluted serum (3 ml.) was put in the main part of the Warburg cup, which was filled with 5 per cent. CO_2 in oxygen and shaken in a bath at 37° C. The acetylcholine solution was added to the serum, and manometer readings were taken at intervals of 5 to 10 minutes for about 45 minutes. The choline esterase activity of the serum remained unchanged on keeping for several days in the ice-chest, but the activity of the diluted serum decreased rapidly on keeping and it was therefore necessary to do

the dilution immediately before the estimation. The rate of liberation of CO_2 was obtained by plotting the manometer readings against the time and drawing a line through the points. The enzyme activity is expressed as the volume of CO_2 (cu. mm. at 37° and 760 mm.) liberated by 1 ml. of serum at 37° under the conditions described, in 1 minute. About 7 cu. mm. of this figure was due to the slow spontaneous hydrolysis of acetylcholine under these conditions.

Results.

Normal Controls and Anxiety States.

It appeared desirable to test in the first place whether the observation of Tod and Jones of a raised serum choline esterase level in anxiety states could be confirmed with the case material at our disposal. This consisted of a large heterogeneous group of war neurotics of all kinds at the Mill Hill Emergency Hospital. It included a number of acutely anxious patients of a kind rarely seen in peace time and providing excellent material for an investigation of this sort.

A comparison was made between three groups: (*a*) 12 adults believed to be normal, consisting mainly of doctors and laboratory workers, (*b*) 12 patients with anxiety states, and (*c*) 12 non-neurotic patients, consisting mainly of surgical cases. The anxiety neurotics were kindly selected by Dr. M. S. Jones, and in making this selection particular emphasis was laid on the physical signs of anxiety, such as sweating of the palms of the hands and in the axillae, tremor and tachycardia. The group of surgical patients was included as a further control to eliminate any effect that might be due to living under hospital conditions. The results for the three groups may be summarized as follows:

	Normal controls.	Anxiety states.	Surgical patients.
Mean choline esterase activity	74	104	75
Range	35–112	77–162	46–105
Standard deviation	22	24	20

The mean choline esterase activities of 74 and 75 in the normal and surgical control groups were slightly lower than the mean activity of 83 which Tod and Jones found for their normal group, but agreed closely with the values 71 and 78 found by McArdle (1940) for two similar groups. The difference between the control groups and the patients with anxiety states, with a mean of 104, was very marked. Examined statistically by Fisher's *t* test, which is specially designed for application to small groups (Fisher, 1938), the difference was clearly significant. According to Fisher's formula *t* came to 3·71 and *P* was less than 0·01; this means that the probability was more than a hundred to one that the observed increase in esterase activity in the patients with anxiety states was real and not due to chance.

This result was made still more convincing by the fact that the anxiety neurotics were selected in two batches of 6 patients, and the first batch, containing the first choice of the most anxious patients, gave the very high mean of 112, while the second batch of 6 less anxious patients gave a mean of 97 in serum choline esterase activity. Owing to the large extent of the normal range (35–112) there was considerable overlapping of the individual esterase activities in the normal and anxiety groups, but it was noted that the highest individual esterase activity of 162 units observed in the patients with anxiety states was given by a patient who had previously been judged by Dr. Jones on clinical grounds to be the most anxious patient of the whole group. Several of the anxiety neurotics had esterase levels which came well within the normal range, but considered as a whole the results gave satisfactory evidence of a correlation between high serum choline esterase and anxiety.

Choline Esterase and Plasma Proteins.

The existence of a connection between serum choline esterase and anxiety raises the question of what is the physiological significance of a high serum esterase activity. A high serum esterase activity implies that the serum contains an increased amount of one of the normal protein constituents with enzymic activity. It appeared desirable to know whether any of the other blood proteins are also present in increased amount when the esterase level is high.

The possibility that a high serum esterase activity might be due to the removal of an inhibitor rather than to an increase in enzyme was first tested by seeing whether the activity of a mixture of sera of high and low activities was lower than the mean, which would be expected if an inhibitor were present, or equal to the mean, which would be expected if the amount of enzyme were increased in anxiety :

Serum C.E.	Serum. C.E.	Mean C.E.	C.E. of mixed sera.
A 119	B 46	83	84
C 113	D 63	88	86

The figures for the choline esterase of a 50 per cent. mixture agreed closely with the mean of the activities of the individual sera, making it unlikely that an inhibitor was concerned.

Esterase distribution in serum, plasma and cells.—The presence of an increased amount of esterase in the serum might be due to (*a*) an increased outpouring of the enzyme from the tissues into the blood, or (*b*) an alteration in the ratio of insoluble enzyme in the cells to soluble enzyme in the plasma, in which case a high serum esterase activity should go with a lowered cell esterase level. The figures in Table I show that the latter view is incorrect. The extent of variation in the esterase activity of the red blood corpuscles (140–207) was much less than in the plasma or serum (31–170). Plasma and serum gave identical values within the experimental error. The cell esterase activity tended to increase to a small extent in parallel with the plasma esterase level ; this might be due to the small amount of serum which adhered to the cells, since in order to exclude loss of enzyme by diffusion out of the cells no attempt was made to wash them.

TABLE I.

Patient No.	Choline esterase activity.		
	Plasma.	Serum.	Cells.
1	30	31	140
2	77	—	175
3	89	—	155
4	100	—	192
5	123	125	183
6	126	128	202
7	167	170	207

Cell esterase given in units per ml. centrifuged cells. Cells haemolysed by standing for 1½ hours at 20° with 2 vols. distilled water. Potassium oxalate added to plasma to prevent clotting.

Esterase and serum proteins.—It appeared possible that the high serum esterase level in anxiety might be a haemoconcentration effect due to the sweating, which was extremely marked in several of the anxiety neurotics. This is excluded by the figures in Table II, which show that there is no apparent connection between esterase activity and the concentration of total serum proteins, serum albumin, globulin or the albumin/globulin ratio.

TABLE II.

Subject No.	Serum choline esterase.	Total serum proteins. Per cent.	Serum albumin. Per cent.	Serum globulin. Per cent.	A/G ratio.
1	31	7·30	5·11	2·19	2·34
2	54	6·91	5·02	1·89	2·66
3	77	6·48	4·69	1·79	2·62
4	77	7·50	—	—	—
5	80	6·93	—	—	—
6	82	7·44	5·30	2·14	2·48
7	93	7·03	4·61	2·42	1·91
8	96	6·76	—	—	—
9	100	6·48	4·97	1·51	3·29
10	109	7·37	5·15	2·22	2·32
11	121	6·90	—	—	—
12	128	7·88	5·83	2·05	2·84

Serum proteins estimated by the Micro-Kjeldahl method as described by Harrison (1937). Subjects 1 and 2 were believed to be normal ; the others were neurotic patients.

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Serum esterase and amylase.—The choline esterase activity is much higher in the tissues, and particularly in nervous tissue, than in the blood, and the serum esterase level may be expected to depend on the rate at which it passes out of the tissues into the blood. It appeared desirable to know whether the same conditions that favour a high serum choline esterase level also are the same as those favouring the passage of other enzymes from the tissues into the blood; whether, in fact, the high esterase activity in anxiety is specific for this enzyme or is paralleled by a similar rise in the other blood enzymes.

The serum amylase was selected for investigation together with the choline esterase, and for this purpose the following micro-method of estimating the serum amylase was worked out:

The serum (0·2 ml.) with 5 ml. M/15 phosphate buffer pH 7 and 0·3 ml. 1 per cent. starch solution in 15·4 per cent. NaCl was incubated in a water bath at 37° C. Samples (0·5 ml.) were tested from time to time with 0·02 ml. N/50 iodine solution until the point was reached when 98 per cent. of the starch had disappeared. The amylase activity was calculated as mgm. starch hydrolysed per ml. serum per hour.

The figures showed that the rise in serum choline esterase level was specific and unaccompanied by a corresponding rise in the serum amylase.

TABLE III.

Subject No.	Choline esterase.	Serum amylase.	Subject No.	Choline esterase.	Serum amylase.
1	22	17·3	9	89	10·0
2	29	10·7	10	92	12·0
3	67	8·2	11	99	8·8
4	69	8·8	12	102	4·8
5	75	9·5	13	117	19·2
6	79	5·9	14	112	11·2
7	84	6·6	15	120	7·7
8	88	7·4	16	120	15·0

Serum amylase activity expressed as mgm. starch hydrolysed per ml. serum per hour. Subjects 1, 2, 8 and 13 believed to be normal; the others were neurotic patients.

CHANGES IN SERUM CHOLINE ESTERASE.

Previous investigators have frequently commented on the constancy of the serum choline esterase level in normal individuals. v. Verebely (1936) observed changes of only 15 per cent. in the sera of 100 individuals observed over periods extending from two hours to six months. McGeorge (1937), on the other hand, found that in 20 per cent. of his patients the serum esterase activity was halved or doubled during the period of observation. In the present investigation many instances were observed in which the esterase activity, repeated after varying time intervals, showed no significant change; but other cases were found where considerable changes occurred and these were specially investigated.

From the point of view of the correlation between high serum esterase activity and anxiety it was particularly desirable to know whether an abnormally high serum esterase level in anxiety is (*a*) a constitutional factor which is commonly present in the type of individual who is liable to anxiety, or (*b*) whether it is due to a temporary rise during the patient's illness. This could be tested by seeing whether the esterase activity remains steady or falls when there is an improvement in the patient's condition with a diminution in anxiety.

Prolonged narcosis treatment.—Blood samples were obtained through the courtesy of Dr. R. Fraser from three anxious patients whom he was treating by the prolonged narcosis method. They received 2 ml. "somnifaine" intramuscularly at 12-hourly intervals as a basic narcotic and sufficient sodium amytal or paraldehyde by mouth 4-hourly to maintain 20 hours of sleep per diem for 10 days.

Patient No.	Before narcosis.	During narcosis.			After narcosis.	Change. Per cent.
		1st day.	4th day.	7th day.		
1	99	94	—	99	98	0
2	96	—	—	—	101	5
3	139	—	152	137	139	0

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The figures gave no evidence of any significant effect on the serum choline esterase activity produced by narcosis under these conditions; they serve rather to illustrate the constancy with which similar values may be repeatedly obtained. Improvement in the clinical condition of the patients was uncertain.

Vitamin B_1.—The work of Antopol, Glaubach and Glick (1939), who found that the serum choline esterase activity is raised in vitamin B_1 deficient pigeons, suggested the idea of testing whether the high esterase activities in anxiety neurotics could be lowered by administering considerable doses of vitamin B_1. No significant change in esterase activity was observed; there was also no change in the clinical condition.

TABLE IV.

Patient No.	Days treated.	Serum choline esterase. Before B_1.	After B_1.	Per cent. change.
1	4	125	129	0
2	3	109	101	− 7
3	3	129	130	0
4	3	127	118	− 7
5	3	136	169	+24
6	3	147	153	0

Patients were given " Benerva " tablets containing 5 mgm. vitamin B_1 three times a day.

Changes in anxiety neurotics in hospital.—Twelve neurotics who were found to have high serum esterase activities shortly after admission were tested again before discharge after a period of 4 to 11 weeks in hospital.

TABLE V.

Patient No.	Weeks in hospital.	Serum choline esterase. Initial.	Final.	Per cent. change.
1	8	114	101	− 11
2	8	126	89	− 29
3	4	119	130	+ 9
4	4	117	101	− 14
5	7	134	31	− 77
6	6	134	82	− 39
7	4	120	100	− 17
8	8	135	121	− 10
9	11	131	125	− 5
10	4	136	153	+ 13
11	4	117	118	− 0
12	11	162	170	+ 5
Mean	6·6	129	110	− 15

In 3 of the patients there was a slight rise in activity, in 1 no change, and in 8 a decrease in esterase activity ranging from 5 to 77 per cent. The mean esterase activity of the group was 129 on admission and 110 immediately before remission. Although the difference in the means was not statistically significant in a group of this size ($t = 1{\cdot}9$ and $P = 0{\cdot}09$), the figures suggest a tendency for the high esterase activities in anxious patients to come down towards the normal with the general improvement in their condition and diminution in anxiety during their stay in hospital.

Changes in esterase activity in normal subjects.—Repeated serum esterase estimations were done on three normal individuals over a period of five months to test whether any changes in esterase activity were produced by the minor anxiety-provoking occurrences of normal life. No significant changes were observed as a result of heavy air raids which occurred during this period (one individual showed no change, for example, on the morning after a raid in which a bomb had exploded in his garden and severely damaged his house), nor was any change observed as a result of worry or depression of short duration. On the other hand, a period of

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severe worry lasting for over a week reported by subject "A" was accompanied by a sharp rise in activity from 117 to 166; the esterase activity subsequently returned to normal with the cessation of the worry. In subject "B" a period of severe emotional tension lasting for more than a week, during which she was "extremely upset," was similarly accompanied by a rise in serum esterase activity from 54 to 68 with a subsequent return to normal.

A further observation that might possibly be significant was that the individuals giving the lowest esterase activities in the normal control group appeared less subject to anxiety than those who gave the highest figures. This was illustrated, for example, by the observation that the individual who gave the lowest figure of 35 was uncommonly little affected and complained only of the incidental loss of sleep during severe air raids in which many people in the vicinity were killed, while the individual with the highest normal esterase activity of 112 was observed to be so excessively agitated during air raids that this led in fact to his serum esterase activity being tested.

The available information is still too limited for definite conclusions to be drawn, but these observations suggest that the serum choline esterase tends to rise during periods of severe and prolonged anxiety in normal individuals as well as in anxiety neurotics. It would be surprising if there were a sharp difference between normal and neurotic individuals in this respect.

Discussion.

The present investigation has confirmed the original finding of Tod and Jones (1937) that the serum choline esterase activity is abnormally high in anxiety states. The increase in esterase activity would appear to be specific for this enzyme, since it is not accompanied by a similar increase in the serum amylase or in the serum albumin or globulin.

It is difficult to assess the physiological significance of a high serum esterase activity until the origin of the choline esterase in the serum is known. The esterase activity is normally much higher in the red blood corpuscles than in the serum, and it therefore appeared possible that the high serum activity found in anxiety might be due to a change in the distribution of the enzyme between the plasma and the cells; in that case a high serum activity should go with a relatively low cell esterase activity. Estimations of the cell and plasma activities in the same sample of blood and carried out at the same time showed that this does not occur, but the cell activity tends even to rise when the plasma activity is high. This indicates that the high esterase activities in anxiety are due, not to a change in distribution, but to an increased outpouring of the enzyme from the tissues into the blood. The choline esterase activity is highest in nervous tissue, but the observation of abnormal serum esterase activities in liver disease (McArdle, 1940) suggests that the liver must also be considered as a possible source of the enzyme.

The question of whether the high serum esterase activity in anxiety is due to a temporary rise associated with the emotional tension, or whether it is a constitutional factor which tends to be high in the type of individual who is liable to anxiety, cannot yet be regarded as settled. The available evidence suggests that the former view is probably correct, since (*a*) a fall in esterase activity was observed in 8 out of 12 neurotics during their stay in hospital, and (*b*) two instances were recorded in which there was a temporary rise in esterase activity during periods of severe and prolonged emotional tension.

Summary.

(1) A mean serum choline esterase activity of 104 was given by a group of 12 anxiety neurotics, while mean activities of 74 and 75 were given by groups of 12 normal and 12 surgical controls; the high mean activity given by the anxiety neurotics was statistically significant.

(2) The high esterase activity in anxiety states was not due to the removal of an inhibitor; it was due to an increased outpouring of the enzyme from the tissues. A similar increase was not shown by the serum amylase, serum albumin, serum globulin or total serum proteins.

(3) The serum esterase activity was not affected by treatment with vitamin B_1 or by prolonged narcosis for 10 days under the conditions stated. In 8 out of 12

neurotics with high esterase activities the esterase activity decreased during their stay in hospital.

(4) Temporary rises in serum choline esterase activity were observed in two normal individuals during periods of severe and prolonged emotional tension.

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References.

ANTOPOL, W., TUCHMAN, L., and SCHIFRIN, A. (1937), *Proc. Soc. exp. Biol., N.Y.*, **36**, 46.
ANTOPOL, W., SCHIFRIN, A., and TUCHMAN, L. (1938), *ibid.*, **38**, 363.
ANTOPOL, W., GLAUBACH, S., and GLICK, D. (1939), *ibid.*, **42**, 679.
DALE, H. H. (1934), *Brit. med. J.*, **1**, 835.
FISHER, R. A. (1938), *Statistical Methods for Research Workers*, London, p. 128.
HALL, G. E., and LUCAS, C. C. (1937), *J. Pharmacol.*, **59**, 34.
HARRISON, G. A. (1937), *Chemical Methods in Clinical Medicine*, London, p. 370.
JONES, M. S., and STADIE, W. C. (1939), *Quart. J. exp. Physiol.*, **29**, 63.
JONES, M. S., and TOD, H. (1935), *Biochem. J.*, **29**, 2242.
LOEWI, O. (1935), *Proc. Roy. Soc.*, B, **118**, 299.
MCARDLE, B. (1940), *Quart. J. Med.*, **9**, 107.
MCGEORGE, M. (1937), *Lancet*, **1**, 69.
MILHORAT, A. T. (1938), *J. clin. Invest.*, **17**, 649.
TOD, H., and JONES, M. S. (1937), *Quart. J. Med.*, **6**, 1.
VAHLQUIST, B. (1935), *Skand. Arch. Physiol.*, **72**, 133.
VEREBELY T. VON (1936), *Klin. Wschr.*, **15**, 11.