action of mustard gas (*H*) with ethyl aminoacetate in anhydrous ethanol in the presence of sodium acetate.

2. The reaction of *H* with glycine in aqueous solution at pH 9 yields a water-soluble product with unusual properties.

3. *H* reacts extensively with sodium phosphate in aqueous solution; analyses of the reaction product indicate that it is a sulphonium salt derived from 2 mol. of *H* and containing three phosphate ester groups.

4. It has been found that when *H* reacts with serum proteins in the presence of sodium phosphate at pH 8–9, complexes are formed which contain, in addition to an increased concentration of sulphur, appreciable amounts of esterified phosphate which cannot be removed by electrodialysis.

5. When these complexes are injected intravenously into rabbits they give rise to strongly precipitating antisera.

6. The precipitin reactions between these coupled proteins and the appropriate antisera are inhibited by the reaction product of *H* and sodium phosphate, but not by a number of other derivatives of diethyl sulphide.

7. The complexes formed when *H* reacts with serum proteins at pH 8–9 with no buffer present have been found to be ineffective in producing serum precipitins in rabbits.

Microanalyses of the reaction products of mustard gas and ethyl aminoacetate were carried out by Mrs Barker through the kindness of Dr Leo Marion, Chemistry Division, National Research Council, Ottawa. Carbon and hydrogen contents of the 'thiodiglycol phosphate' were measured by Dr A. Elek, Los Angeles, California.

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Investigations into Cholinesterase Levels in Serum and Cerebrospinal Fluid of Psychotic Patients

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Claims have been made that the level of serum cholinesterase can be correlated with certain psychiatric conditions. This level is said to be raised in states of anxiety and depression, and lowered in catatonic stupor, schizophrenia and epileptic conditions (Jones & Tod, 1937; Jones & Stadie, 1939; Birkhäuser, 1941*a, b*; Richter & Lee, 1942; Butt, Comfort, Dry & Osterberg, 1942; Schütz, 1944).

The data accumulated were quite often inconsistent and did not permit definite conclusions about the role of cholinesterase in disturbances of the nervous tissue. Mendel, Hawkins & Nishikawara (1948) assumed that this might be due to the fact that at the time when these earlier investigations were reported the existence of two enzyme systems

capable of hydrolysing acetylcholine was not known. Only later Mendel & Rudney (1943) and Mendel, Mundell & Rudney (1943) distinguished between the true cholinesterase and the pseudo-cholinesterase and developed methods for measuring the activities of these enzyme systems. The authors claimed that only the true cholinesterase is essential for hydrolysis of acetylcholine *in vivo*.

The investigations reported in this paper were made because of certain therapeutic possibilities envisaged, which might occur when using different substances influencing cholinesterase activity. The following points were of particular interest: (i) Whether there existed any correlation between the values of true cholinesterase and the total brain size

and the number of functioning neurons. (ii) Whether serum true cholinesterase varied in different mental disorders. (iii) What changes of true cholinesterase levels took place in the serum after electrically induced convulsions. (iv) What relationship existed between true and pseudo-cholinesterase in the serum if changes of one of them occurred. (v) Whether a relationship existed between true cholinesterase in the serum and in the cerebrospinal fluid (c.s.f.). (True cholinesterase in the c.s.f. has recently been reported (Reiss & Hemphill, 1948).)

METHODS

The cholinesterase activity was determined manometrically, using Warburg manometers, and the results expressed in μ l. of CO_2 produced by 1 ml. serum or c.s.f. in 30 min. at 37.5° . The substrate concentrations used throughout the determination were: 0.3 M-acetylcholine, 0.06 M-benzoylcholine, and 0.3 M-acetyl- β -methylcholine. The substrate (0.2 ml.) was placed in the side compartment, and the c.s.f. or serum in the main compartment, with 2% bicarbonate solution (0.2 ml.) and distilled water sufficient to make the total fluid content up to 2.0 ml. For true cholinesterase determinations 1 ml. of serum was used, but for determinations with acetylcholine and benzoylcholine 0.1 or 0.05 ml. serum was sufficient. Determinations with c.s.f. required 1.6 ml.; only clear sera or c.s.f. were used. All estimations were made in duplicate, the average error being $\pm 1.4\%$. Blood was collected from the cubital vein. The usual technique for electroshock treatment was followed, a major epileptic fit being induced with standard Ediswan apparatus. Blood samples were taken before and 2, 15 and 60 min. after the onset of the epileptic fit. The same procedure was repeated after several treatments.

RESULTS

Serum true cholinesterase determinations were made on fifty-five male chronic psychotics (aged 59–85 years). The results were divided into three groups, showing those above (a), inside (b) and below (c) the normal range (Table 1). Of these patients 13 (24%) showed serum true cholinesterase values within the normal range, 31 (56%) gave high values and 11 (20%) low values. Twenty-one middle-aged schizophrenics and fifteen mental defectives gave normal values. It is remarkable that the cholinesterase value of the middle-aged schizophrenics is relatively constant over a period of 3 weeks. One 9-year-old mental defective, a hydrocephalic, gave high cholinesterase values (patient no. 6 in Table 3).

Some specimen values of the fifty-five aged male psychotics for true and pseudo-cholinesterase in serum and c.s.f.s. are recorded in Table 2, while Table 3 shows true and pseudo-cholinesterase values of mental defectives. Both tables show that no correlation could be found between the true cholinesterase and pseudo-cholinesterase content of the

serum. There is also no correlation between the cholinesterase content of serum and c.s.f.; the c.s.f. cholinesterase content could be the same whether the serum level was 170 or 5. Tables 2 and 3 show also that low levels of serum or c.s.f. cholinesterase were not related to small brains, as in defectives, or reduced cortical size, as in organic or senile dementia.

The serum cholinesterases of fifteen patients were investigated before and after electroconvulsion treatment. Representative results of five patients are recorded in Table 4. The effect of the electrically induced convulsions on the level of serum true cholinesterase was not the same for each patient. From 1 to 90 min. after shock the true cholinesterase may rise, fall or remain constant. After repeated shocks, however, the serum true cholinesterase level of the patient (taken in the morning, under standard conditions, before treatment) showed a steady decrease. This decrease is greater than spontaneous changes seen in middle-aged schizophrenics in the course of 3 weeks (cf. Table 1).

Only very slight change can be seen in the level of the pseudo-cholinesterase after electroshock treatment. The two enzyme systems react quite independently. True cholinesterase could decrease, while pseudo-cholinesterase showed a rising tendency, and vice versa.

DISCUSSION

No correlation between pseudo- and true cholinesterase was found in any of the investigations described in this paper. This is in agreement with the recent findings of Mendel *et al.* (1948), who investigated these enzyme systems in the plasma of male rats after thyroidectomy and inanition. However, considering the quite different substrates hydrolysed by these two enzyme systems no close biological correlation was to be expected.

The changes produced by electrical convulsions in the true cholinesterase level may be the expression of a regulatory mechanism starting after increased acetylcholine production has taken place. One could assume that two factors are involved in this regulatory process: (1) immediately after an electrically induced fit a great part of the enzyme system is used by the nervous system for the hydrolysis of acetylcholine and disappears from the circulation; (2) new cholinesterase is produced as a compensation, and reaches the circulation. The chronological co-ordination between these two processes is decisive for the kind of change—decrease or increase—in the true serum cholinesterase after electroconvulsion treatment.

Some evidence was contributed by animal experiments. We found that after intravenous injection of 4–8 mg. of acetylcholine into rabbits the true cholinesterase content of the serum 4 min. after injection decreased in four experiments, was unchanged

Table 1. *Serum true cholinesterase of some mental patients*(Cholinesterase activities are expressed in $\mu\text{l. CO}_2$ produced by 1 ml. serum in 30 min.)

Patients and controls	No. investigated	Age		Serum true cholinesterase	
		Range	Average	Range	Mean $\pm \sigma$
Aged psychotics (a)*	31	59-76	66.9	62.8-233	116.2 $\pm$ 43.5
(b)	13	60-77	67.5	26.0-54.3	37.2 $\pm$ 8.6
(c)	11	65-84	75	0-22.4	6.9 $\pm$ 8.1
Middle-aged schizophrenics	21	17-48	34.7	22.4-48.6	31.6 $\pm$ 6.47
Same patients 3 weeks later				22.4-45.4	34.7 $\pm$ 6.69
Mental defectives	15	13-54	26.3	26.4-54.8	37.5 $\pm$ 8.47
Normal controls	11	18-34	28.3	25.5-36.6	31.5 $\pm$ 4.09

* See text.

Table 2. *Serum and cerebrospinal fluid cholinesterases of some male psychotics*(Cholinesterase activities are expressed in $\mu\text{l. CO}_2$ produced by 1 ml. serum in 30 min. ACh., acetylcholine; BCh., benzoylcholine; MCh., acetyl- β -methylcholine.)

No.	Patient	Age	Serum			Cerebrospinal fluid		
			ACh.	BCh.	MCh.	ACh.	BCh.	MCh.
4	H.M.	62	1660	690	170	10.9	4.5	5.6
6	A.E.J.	60	1671	402	167	14.7	5.4	9.0
9	F.W.B.	71	3080	1000	110	11.0	2.1	3.85
21	E.E.A.	72	3530	1457	87	15.4	0.83	5.6
26	E.N.P.	76	1822	1458	63	11.5	2.52	5.54
29	A.J.	69	2450	435	73	12.6	0	4.7
36	T.R.	73	2080	875	37	14.7	1.7	5.7
48	E.A.	78	2240	1012	8.6	15.4	8.1	8.4
50	D.M.	82	1767	662	0	21.0	0.94	9.2
52	W.G.H.	65	2685	1155	8	9.2	0	5.1
53	T.W.	71	1952	840	0	15.9	6.5	10.0

Table 3. *Serum and cerebrospinal fluid cholinesterases of mental defectives*(Cholinesterase activities are expressed in $\mu\text{l. CO}_2$ produced by 1 ml. serum in 30 min. ACh., acetylcholine; BCh., benzoylcholine; MCh., acetyl- β -methylcholine.)

Patient no.	Age	Cerebrospinal fluid			Serum			Diagnosis
		ACh.	BCh.	MCh.	ACh.	BCh.	MCh.	
1	29	11.3	3.3	6.8	2244	830	27.6	Imbecile
2	27	17.5	3.6	6.4	2656	1075	32.2	Imbecile (epileptic)
3	24	16.5	3.95	7.85	2531	1212	49.4	Imbecile
4	24	9.15	—	5.15	2599	1155	39.6	Feeble-minded
5	28	14.3	4.2	6.8	2106	828	30.4	Imbecile
6	9	21.2	5.9	7.3	4606	2118	63.8	Hydrocephalic imbecile
7	13	9.35	2.58	—	2598	1110	36.3	Microcephalic
8	27	10.8	—	5.5	2522	835	29.5	Imbecile
9	20	9.55	4.9	4.7	2560	1080	39.7	Imbecile
10	19	8.6	2.3	3.5	2498	1058	37.9	Imbecile
11	54	11.1	0.7	6.3	2573	950	44.4	Microcephalic imbecile
12	17	9.8	3.4	4.8	2311	1388	30	Idiot
13	19	17.8	4.1	8.5	3773	1642	46.8	Imbecile
14	56	—	—	—	1961	842	26.4	Microcephalic imbecile
15	18	14.1	3.4	6.1	3123	1388	54.8	Imbecile
16	20	7.05	2.42	2.9	2873	1232	37.2	Mongol imbecile

in one, and increased in a sixth (work to be published elsewhere).

In most patients, after one electrically induced convulsion a lowered cholinesterase level could be seen on the next morning, and a further shock at this time would depress the cholinesterase level still

further. We do not know to what extent this level can be depressed, nor do we know as yet whether many fits induced at short intervals would produce a rapid lowering of serum cholinesterase or, on the other hand, stimulate a compensatory mechanism, but we feel that this effect of electroshock is of

Table 4. *Serum cholinesterases of patients before and after electroconvulsion treatment*

(Cholinesterase activities are expressed in $\mu\text{l. CO}_2$ produced by 1 ml. serum in 30 min. ACh., acetylcholine; BCh., benzoylcholine; MCh., acetyl- β -methylcholine.)

Date	Patient	After shock								
		Before shock			After 1-3 min.			After 15 min.		
		ACh.	BCh.	MCh.	ACh.	BCh.	MCh.	ACh.	BCh.	MCh.
12. i. 48	H.K.K.	3518	1362	70	4100	1537	67	3694	1332	62
19. i. 48		3538	1443	75	3813	1774	74	3450	1312	41
26. i. 48		3688	1644	56	3901	1672	51	3682	1708	65
19. i. 48	D.R.D.	2107	939	63	2360	1097	53	2163	1093	42
26. i. 48		2015	908	44	2232	1065	41	2264	1081	54
23. ii. 48		2391	1133	34	2665	1300	20	2478	1327	32
21. vi. 48		—	1080	27	—	—	—	—	—	—
3. v. 48	W.	—	1186	42	—	1180	39	—	—	—
10. v. 48		—	1162	35	—	1195	34	—	1142	32
3. v. 48	R.T.	—	1522	44	—	1583	41	—	1479	39
10. v. 48		—	1242	37	—	1280	32	—	1290	35
24. v. 48		—	1210	34	—	1346	36	—	1232	40
19. i. 48	G.K.W.	3349	1414	66	3593	1350	98	3488	1456	80
26. i. 48		3395	1443	57	3595	1637	70	3195	1356	55
2. ii. 48		2962	1188	42	3625	—	61	3355	1374	51

special interest in view of the recent reports from the Maudsley Hospital in London of the experimental treatment of psychotic illness and depression with anticholinesterase substances (Nevin & Rowntree, 1948).

The abnormally high serum true cholinesterase in more than half the chronic psychotic patients may also be explained on a basis of supply and demand. Comparatively slight activity of nerve cells may call for the production of correspondingly little acetylcholine, while at the same time organs responsible for the production of cholinesterase may produce a minimum amount of the enzyme, which, however, is not required. It may be postulated that this high blood cholinesterase is an expression of a disturbed balance, but it would be premature to say as yet that this is characteristic of a psychosis, but may be an effect of age, more pronounced in psychotic than in normal individuals.

The mental defectives, being both young and not psychotic, can be imagined to have a neural equilibrium more resembling normal, and, as the cholinesterase level does not seem to be related to brain size or quantity of neurons, it is reasonable to expect that the serum cholinesterase would be within the normal range, which has been found to be the case.

The complete disappearance of cholinesterase in some of the oldest patients is probably due to the cessation of the production of an enzyme which has not been required for a long period, or to a general atrophy of the centres of production.

The remarkable absence of correlation between true cholinesterase in the serum and in the cerebrospinal fluid cannot be explained at present.

SUMMARY

1. Of fifty-five aged male psychotics studied, thirty-one had a high, thirteen a normal and eleven a low serum true cholinesterase.

2. Of sixteen mental defectives investigated fifteen gave values within the normal range. A low serum or cerebrospinal fluid cholinesterase level was not related to brain size.

3. No correlation could be observed between true and pseudo-cholinesterase levels, or between serum and cerebrospinal fluid cholinesterase levels.

4. The significance of the true cholinesterase levels in the various groups is discussed.

5. From 1 to 90 min. after electroconvulsive treatment the serum cholinesterase level may be raised, lowered or unchanged, but after repeated shocks there is a decrease in this level.

6. It is suggested that the changes in true cholinesterase levels in electroconvulsion treatment cases may imply a regulatory mechanism in which (i) cholinesterase is at once used by the central nervous system to break down acetylcholine, and therefore disappears from the blood, and (ii) new cholinesterase is produced to enter the blood stream.

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Amino-acid Decarboxylases of Rat Liver

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Two amino-acid decarboxylases are known to occur in the rat's liver: 3:4-dihydroxyphenylalanine (DOPA) decarboxylase (Holtz & Credner, 1942), and cysteic acid decarboxylase (Blaschko, 1942*a*). Data on the activities of these two enzymes in liver extracts from normal rats were required, for comparison with observations on animals deficient in vitamin B₆ (Blaschko, Carter, O'Brien & Sloane-Stanley, 1948). Differences were found in the cysteic acid decarboxylase activities of liver extracts from male and female rats; the present paper contains an analysis of these findings.

Page (1945) has not been able to find any DOPA decarboxylase activity in extracts from rat tissue; experiments reported below were carried out in which his findings were tested.

METHODS

The manometric measurement of decarboxylase activity followed closely the methods already described (Blaschko, 1942*a, b*). Extracts were prepared as follows. The tissues were ground with sand in the cold for 5–10 min., until there was no further change in the appearance of the paste; ice-cold 0.0675 M-sodium phosphate buffer of pH 7.4 (1 ml./g. of tissue) was then added, and the mixture was centrifuged for 5 min. at a relative centrifugal force of about 800 g, to remove the sand and a few pieces of connective tissue and undamaged cells. The opaque viscous supernatant fluid was used. The enzymic activity is expressed as q_{CO_2} , i.e. as μ l. CO₂ formed/mg. fresh wt. of tissue/hr. The substrate used is shown in parentheses, thus: q_{CO_2} (DOPA) or q_{CO_2} (cysteic). In the calculation, the simplifying assumption was made that 1 ml. of extract contained the enzymic activity from 500 mg. of tissue.

For the manometric experiments, each flask contained 1.6 ml. of extract; 0.4 ml. of either water or 0.01 M-substrate was added from the side bulb immediately after the initial readings. Readings were taken for 30 min., after which time the contents of each flask were acidified by adding 0.3 ml. of 3N-H₂SO₄ from the central well, in order to

determine the amount of CO₂ retained in the solution as bicarbonate. The substrates used were either L-3:4-dihydroxyphenylalanine or L-cysteic acid. All experiments were carried out at 37.5° in an atmosphere of N₂. The enzymic activities of the extracts were calculated from the initial linear rates of formation of CO₂, corrected for retention.

Adult white rats of the Wistar strain were used throughout.

RESULTS

3:4-Dihydroxyphenylalanine decarboxylase activity of liver extracts

Extracts from eighteen normal rats' livers were tested; the results are given in Table 1. The mean value of q_{CO_2} (DOPA) was 0.69; there was no correlation of the enzymic activity with either sex or age of each rat. These experiments show that, under the

Table 1. 3:4-Dihydroxyphenylalanine decarboxylase activities of rat livers

Body wt. (g.)	Sex	q_{CO_2} (DOPA) (μ l. CO ₂ /mg. fresh liver/hr.)
—*	—	0.438
—*	—	0.794
—	♂	0.575
155	—	0.820
165	—	0.820
170	♀	0.690
173	—	0.540
180	—	0.641
185	—	0.887
215	—	0.875
220	♀	0.820
230	—	0.706
250	—	0.643
270	♂	0.563
283	♂	0.590
305	♂	0.610
Mean		0.69
S.E. of mean		± 0.03

* Each determination made with the pooled livers of two rats.