(14) *Cushing's disease*. Four cases showed a blood chromogen level near the upper limit of the normal range (average 215 colour units/ml.).

(15) *Addison's disease*. In one mild case the chromogen level was moderately below the normal average (98 colour units/ml.).

(16) *'Hypertensive' and 'arteriosclerotic' heart disease with cardiac failure*. In the heart muscle either the total chromogen or the concentration of adrenaline proper was abnormally high in 78% of 32 cases. In one case the myocardial chromogen was abnormally low after therapeutic sympathectomy, which may be comparable with the disappearance of chromogenic material from the cat's heart after denervation, as observed by Cannon & Lissák [1939].

(17) *Unexplained sudden cardiac death* of a young athlete. The chromogen concentration of the heart muscle was excessively high (1622 colour units/g. against an average of 698 in 21 normal hearts).

(18) *Ageing and arteriosclerosis*. The average chromogen concentrations of heart, kidneys and arterial walls were found to be lowest in early childhood. They increased gradually and reached a maximum in senile age. Particularly high chromogen concentrations were found in arteriosclerosis (138 specimens).

(19) *Adrenal tumours*. In two cases with adrenal tumours, abnormally high chromogen concentrations were found in arterial walls and kidneys.

SUMMARY

1. The results obtained with the method of Shaw [1938] for the assay of 'adrenaline' in blood and tissues (adrenal glands, liver, spleen, kidney, brain, heart) are due to the presence in these tissues not only of adrenaline itself, but also of adrenaline-like substances which contain a catechol nucleus, and of ascorbic acid. Thus, although the method is not specific for adrenaline, it can be used for investigations of adrenal and adrenergic function in a broader sense.

2. The results of numerous determinations carried out under various experimental and clinical conditions are in general accord with existing knowledge or concepts regarding adrenal and adrenergic physiology and pathology.

3. Contrary to the author's earlier view, it is no longer held that the low denominator of the 'specific ratio' obtained by Shaw's method for many tissues is due to the presence of adrenaline in combination with cortical hormones. This ratio is low when adrenaline-like compounds or ascorbic acid are present.

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Studies on Cholinesterase

3. SPECIFIC TESTS FOR TRUE CHOLINESTERASE AND PSEUDO-CHOLINESTERASE

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In the animal body two esterases capable of hydrolysing acetylcholine exist: a true cholinesterase, acting exclusively on certain choline esters, and a non-specific enzyme, which also hydrolyses a variety of non-choline esters [Mendel & Rudney, 1943]. The non-specific or pseudo-cholinesterase, purified from horse serum and from dog pancreas [Mendel & Mundell, 1943], exhibits its maximum activity at

high concentrations of acetylcholine (above 300 mg./100 ml.), and is far less active at low substrate concentrations; the specific or true cholinesterase, on the other hand, found in red blood cells and brain tissue, displays its greatest activity at low substrate concentrations (below 3 mg./100 ml.) and is strongly inhibited at high concentrations of acetylcholine.

These differences in activity make it possible to decide whether an enzyme solution contains mainly pseudo- or true cholinesterase. The activity is measured towards 0.06 *M* and 0.0006 *M* acetylcholine: a ratio (activity at 0.06 *M*/activity at 0.0006 *M* acetylcholine) of 3, as found with horse serum, indicates a preponderance of pseudo-cholinesterase; conversely, a ratio of 0.3, obtained, for example, with erythrocytes, points to the predominance of true cholinesterase.

To measure quantitatively the activities of true and pseudo-cholinesterase, new tests based on the following facts are used:

(1) Acetyl- β -methylcholine (*dl*-acetyl- β -methylcholine chloride—'Mechoyl'—Merck) is hydrolysed by true but not by pseudo-cholinesterase.

(2) Benzoylcholine is hydrolysed by pseudo- but not by true cholinesterase. The benzoylcholine chloride used was prepared according to Fourneau & Page [1914] and Baer [1942], and was further purified by refluxing with absolute acetone. Both preparations had m.p. 206°.

(3) Neither acetyl- β -methylcholine nor benzoylcholine is hydrolysed by the common esterase, an enzyme capable of hydrolysing glycerides and aliphatic esters [Easson & Stedman, 1937].

EXPERIMENTAL

(a) *Specificity of substrate.* Table 1 gives the results of experiments in which preparations of true and pseudo-cholinesterase were allowed to act on acetyl- β -methylcholine and on benzoylcholine. All

Table 1. *Hydrolysis of acetyl- β -methylcholine and benzoylcholine*

Type of 'cholinesterase'	Hydrolysis (μ l. CO ₂ evolved in 20 min.) of		
	Acetylcholine 0.02 <i>M</i>	Acetyl- β -methylcholine 0.03 <i>M</i>	Benzoylcholine 0.006 <i>M</i>
Pseudo-cholinesterase:			
Horse serum, purified preparation (<i>Q</i> _{Ach} 52,000)	162	1	41
Dog pancreas, purified preparation (<i>Q</i> _{Ach} 430,000)	148	1	37
True cholinesterase:			
Human red blood cells, purified preparation	90	76	0
Dog brain (<i>nucleus caudatus</i>)	99	79	0

All the results given above and in subsequent tables are corrected for non-enzymic hydrolysis.

measurements of enzyme activity were carried out manometrically, as previously described [Mendel & Rudney, 1943]. These results show that acetyl- β -methylcholine is hydrolysed very slowly, if at all, by pseudo-, but readily by true cholinesterase. Benzoylcholine, on the other hand, is not hydro-

lysed at all by true cholinesterase, but fairly rapidly by pseudo-cholinesterase. Fortunately, neither of these two esters is hydrolysed by the common esterase. When guinea-pig plasma, which contains both cholinesterases as well as common esterase, is allowed to act on benzoylcholine or acetyl- β -methylcholine in the presence of 10⁻⁵ *M* eserine (a concentration completely inhibiting both cholinesterases, but not interfering with the action of the common esterase), no hydrolysis of either acetyl- β -methylcholine or benzoylcholine occurs (Table 2).

Table 2. *Effect of eserine on the hydrolysis of various substrates by guinea-pig plasma*

Conc. of eserine <i>M</i>	Hydrolysis (μ l. CO ₂ evolved in 10 min.) of			
	Tri-butyrin 0.003 <i>M</i>	Acetylcholine 0.06 <i>M</i>	Acetyl- β -methylcholine 0.03 <i>M</i>	Benzoylcholine 0.006 <i>M</i>
0	1560	321	28	118
10 ⁻⁵	1510	0	0	0

All results represent the activity of 0.5 ml. plasma.

(b) *Estimation of true and pseudo-cholinesterase in blood.* Tables 3 and 4 show that enzyme concentration and reaction velocity are directly proportional under the experimental conditions selected.

Table 3. *Hydrolysis of acetyl- β -methylcholine (0.03 *M*)*

Haemolysed human erythrocytes (ml.)	μ l. CO ₂ /10 min.
0.05	7.0
0.1	13.8
0.2	28.2
0.3	42.0

Table 4. *Hydrolysis of benzoylcholine (0.006 *M*)*

Horse plasma (ml.)	μ l. CO ₂ /10 min.
0.05	12.8
0.1	26.0
0.2	52.6
0.3	76.8

On the basis of the observations of Tables 1-4, quantitative estimations of true and of pseudo-cholinesterase in blood were made with acetyl- β -methylcholine and benzoylcholine respectively. For comparison, the activity of the blood was also measured with the old method, with acetylcholine (0.06 *M* and 0.0006 *M*) as substrate. The procedure adopted was as follows:

Freshly drawn oxalated blood was used, and care was taken to avoid haemolysis. The blood was centrifuged for 15 min. in a haematocrit at 2000 r.p.m. The blood cells were washed twice with 0.9% NaCl and then brought to

the original blood volume with water. 0.2 ml. of this solution, in a total volume of 3 ml., was used for the estimation of blood-cell activity with acetyl- β -methylcholine and benzoylcholine. For the measurement of the pseudo-cholinesterase in plasma, 0.1–0.2 ml. of plasma, in a total volume of 3 ml., proved sufficient, while 1 ml. of plasma, in a total volume of 6 ml., was required to measure the activity of the true cholinesterase, since the plasma of several species contains only small amounts of this enzyme. 0.03 *M* acetyl- β -methylcholine and 0.006 *M* benzoylcholine

however, disclose the unexpected fact that plasma II, in spite of its greater activity at low concentrations of acetylcholine, contains far smaller amounts of true cholinesterase than plasma I. The apparent discrepancies in these results are easily understood when the action of the pseudo-cholinesterase, present in greater amounts in plasma II than in plasma I, is taken into account: the pseudo-cholinesterase, being capable of exerting some effect even

Fig. 1.

Fig. 1. Hydrolysis of acetyl- β -methylcholine. $\times$ — $\times$ dog plasma; $\bullet$ — $\bullet$ human plasma; $\square$ — $\square$ purified human blood cells; $\circ$ — $\circ$ horse plasma.

Fig. 2.

Fig. 2. Hydrolysis of benzoylcholine. $\times$ — $\times$ dog plasma; $\bullet$ — $\bullet$ human plasma; $\circ$ — $\circ$ horse plasma.

were used in all experiments. These substrate levels were well above the optimum found for any one species. Some of the activity-substrate concentration curves obtained with plasma and cells are shown in Figs. 1 and 2.

RESULTS

Table 5 shows that wide variations in the activity of the true cholinesterase in plasma remain unnoticed when acetylcholine is the substrate. Since

Table 5. Cholinesterase activity of 1 ml. dog plasma

	(μ l. CO_2 evolved in 20 min.)				
	Acetyl- β -methylcholine 0.03 <i>M</i>	Benzoylcholine 0.006 <i>M</i>	Acetylcholine		Ratio (a)/(b)
			(a) 0.06 <i>M</i>	(b) 0.0006 <i>M</i>	
Dog I	112	376	682	476	1.4
Dog II	54	803	1438	577	2.5

plasma II is more active than plasma I both at high and at low concentrations of acetylcholine, it would appear that plasma II contains larger amounts of both pseudo- and true cholinesterase. The presence of more pseudo-cholinesterase in plasma II is confirmed by the measurements with benzoylcholine. Tests with acetyl- β -methylcholine,

at low concentrations of acetylcholine, adds its activity at this substrate level to that of the true cholinesterase, and thereby renders estimations of the true cholinesterase illusory.

The experiments in Table 6 show the distribution of the two enzymes in plasma and cells of various species and disclose the following facts: (1) The blood of some species (ox, sheep) contains no pseudo-cholinesterase (Exps. 6, 7). (2) True cholinesterase is present in the blood of all species investigated (Exps. 1–11). (3) In most species the true cholinesterase is located mainly in the red blood cells, the plasma containing smaller amounts of the enzyme (Exps. 1–7). (4) Scarcity or lack of true cholinesterase in the blood cells of certain species (cats, birds) is compensated for by the presence of a comparatively larger amount of true cholinesterase in the plasma (Exps. 8–11).

The constant presence of true cholinesterase in blood suggests that this enzyme performs an essential function in the blood stream. By virtue of its ability to deal with low concentrations of acetylcholine, the true cholinesterase in all probability plays a major part in regulating the amount of acetylcholine in the circulation. Pseudo-cholinesterase, being absent from the blood of certain

Table 6. *Activity of plasma and cells contained in 1 ml. of blood*

		$\mu\text{l. CO}_2$ evolved in 20 min.						
Exp.	Source of blood	Acetyl- β -methylcholine 0.03 <i>M</i>		Benzoylcholine 0.006 <i>M</i>		Acetylcholine		Ratio (a)/(b)
		Plasma	Cells	Plasma	Cells	(a)	(b)	
						0.06 <i>M</i> Plasma	0.0006 <i>M</i> Plasma	
1	Man: B.M. ♂	17	848	411	0	1116	472	2.4
	H.R. ♂	16	769	409	0	1075	414	2.6
	D.M. ♀	11	761	288	0	805	288	2.8
	D.M. 2 months later	12	—	289	—	801	275	2.9
2	Horse: (i) ♂	24	169	261	0	833	241	3.5
	(ii) ♀	15	160	231	0	844	227	3.7
3	Dog: (i) ♀	37	150	224	0	409	234	1.7
	(ii) ♂	33	92	485	0	868	348	2.5
4	Guinea-pig: (i) ♂	49	224	287	0	635	336	1.9
	(ii) ♂	57	190	293	0	773	502	1.5
5	Rabbit	37	67	48	0	102	64	1.6
6	Ox	Trace	425	0	0	Trace	Trace	—
7	Sheep	31	149	0	0	32	94	0.3
8	Cat: (i) ♂	54	32	102	0	404	242	1.7
	(ii) ♀	27	16	83	0	365	183	2.0
9	Chicken	179	Trace	28	0	416	279	1.5
10	Duck	26	Trace	15	0	108	101	1.1
11	Pigeon: (i) ♀	37	0	—	—	503	361	1.4
	(ii) ♂	43	0	—	—	582	422	1.4

species, is apparently not indispensable in this respect; moreover, it could play only a minor rôle in controlling the level of acetylcholine in the blood, since it is unable to hydrolyse low concentrations of this substrate efficiently.

SUMMARY

1. Measurements of cholinesterase activity in which acetylcholine is used as substrate give an inaccurate picture of the levels of true cholinesterase

and pseudo-cholinesterase in any mixture of the two enzymes.

2. Methods for quantitative estimations of true cholinesterase and pseudo-cholinesterase based on the use of acetyl- β -methylcholine and benzoylcholine as substrates are described.

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Bacterial Reduction of Tetrathionate

(A REPORT TO THE MEDICAL RESEARCH COUNCIL)

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We have previously reported that certain intestinal organisms have the power of reducing tetrathionate quantitatively to thiosulphate [Pollock, Knox &

Gell, 1942]. The present paper gives the experimental evidence for this reduction and discusses some of the problems which it has suggested.