The Distribution of Cholinesterase Types in Mammalian Tissues

BY M. G. ORD AND R. H. S. THOMPSON

Department of Chemical Pathology, Guy's Hospital Medical School, London, S.E. 1

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Much work has been carried out in recent years in order to characterize more fully the mammalian cholinesterases, but, with certain exceptions referred to below, this has been largely limited to a study of these enzymes in brain, red blood cells and plasma. This work has amply demonstrated that fundamental differences in specificity and in reaction kinetics exist between the cholinesterases present in these three tissues (Alles & Hawes, 1940; Glick, 1941; Richter & Croft, 1942; Bissegger & Zeller, 1943), and Mendel and his colleagues have described 'true' and 'pseudo' cholinesterases which hydrolyse respectively acetyl- β -methylcholine and benzoylcholine (Mendel & Rudney, 1943; Mendel, Mundell & Rudney, 1943).

Selective inhibition of these different types of cholinesterase has been described for a number of substances: Hawkins & Gunter (1946) reported inhibition of the pseudo-cholinesterase by the dimethylcarbamate of (2-hydroxy-5-phenylbenzyl)-trimethylammonium bromide (Nu 683). A more recent prostigmine analogue, the *N*-*p*-chlorophenyl-*N*-methylcarbamate of *m*-hydroxyphenyltrimethylammonium bromide (Nu 1250), inhibits the 'true' enzyme (Hawkins & Mendel, 1949). Di-*isopropyl* fluorophosphonate (DFP) has also been shown to be a selective inhibitor of the pseudo-cholinesterase (Mazur & Bodansky, 1946; Mendel & Hawkins, 1947), while $\beta\beta'$ -dichlorodiethyl-*N*-methylamine hydrochloride (DDM) selectively inhibits the true cholinesterase (Thompson, 1947; Adams & Thompson, 1948). The degree of inhibition of these cholinesterase types has also been studied for certain of the polyphosphate esters, notably hexaethyl tetraphosphate and tetraethyl pyrophosphate (Brauer, 1948; Dubois & Mangun, 1947).

Turning to *in vivo* work, attempts have been made to correlate the signs of toxicity produced by injection of DFP with cholinesterase levels in the blood and in the brain (Mazur & Bodansky, 1946; Freedman, Willis & Himwich, 1949). In view of the early signs of intoxication by this compound (salivation, lachrymation, diarrhoea and muscular twitchings), it would be of interest to know more about the interaction of DFP and other cholinesterase inhibitors with the enzymes present in tissues other than brain and blood. In the first place it is desirable to know whether the two types of cholinesterase described in serum and red blood cells respectively are present also

in other tissues, and if so in what relative amounts, or whether further types of cholinesterase with different kinetic and other properties are present in these sites.

Information concerning this distribution is scanty. Mendel & Mundell (1943) have described a pseudo-cholinesterase prepared from dog pancreas, while Langemann (1944), using (1) the influence of substrate concentration on hydrolysis rate, and (2) degree of inhibition by percarine, caffeine and papaverine as his criteria for differentiation, has concluded that human brain and skeletal muscle contain only the true enzyme, while human ovary contains only a pseudo-cholinesterase. Sawyer & Hollinshead (1945) showed that cat peripheral nerve fibres and ganglia contain both true and pseudo-cholinesterases in approximately equal concentrations; they also found that after section and degeneration of the preganglionic fibres the true cholinesterase of the ganglion and of the degenerating preganglionic fibres is lost at a more rapid rate than the pseudo-cholinesterase, a fact which they interpret as minimizing the physiological significance of the latter enzyme in synaptic transmission.

Plattner & Hinter (1930), Gilman, Carlson & Goodman (1939) and others have studied the total cholinesterase activity of a wide range of animal tissues, but no attempt to compare the cholinesterase types in a series of different tissues, using the more recently developed methods of differentiation, was made until the work of Sawyer & Everett (1947). These workers briefly reported on the relative rates of hydrolysis of acetyl- β -methylcholine and benzoylcholine by a number of rat tissues, and showed that liver and certain glandular tissues hydrolyse benzoylcholine more rapidly than acetyl- β -methylcholine; they did not, however, report on the use of any differential inhibitors in these various sites, nor on the cholinesterase types in unstriated muscle, apart from uterus.

The experiments described below represent the initial stages of a more extended comparison of the types of cholinesterase activity of different mammalian tissues. In view of the differences in blood esterases which exist among animals of different species, we have studied, so far, only the tissues of the rat and, in a few instances, of man. We have compared the relative rates of hydrolysis of acetylcholine and of Mendel's 'specific' substrates, acetyl-

β -methylcholine and benzoylcholine, by various rat tissues, and have studied the effect of mixed substrates on these esterases; we have also examined the sensitivity of the cholinesterases of these different tissues to inhibition by DFP.

A preliminary report on this work was given to the Biochemical Society on 16 December 1949 (Ord & Thompson, 1950).

EXPERIMENTAL

Materials. White rats weighing from 100 to 200 g. were used. On account of the sex differences, which have previously been noted by other workers in the cholinesterase levels of various tissues (Birkhäuser & Zeller, 1940; Beveridge & Lucas, 1941; Sawyer & Everett, 1947), only male animals have so far been examined. They were killed by decapitation and exsanguination, after which the carcasses were perfused with 0.9% NaCl introduced through the inferior vena cava and the descending aorta; perfusion was continued until the effluent fluid was no longer visibly blood stained.

The tissues under investigation were then removed, weighed, minced with scissors and finally homogenized in 0.025M-NaHCO₃ in a high-speed homogenizer of the type described by Folley & Watson (1948). The dilution of the tissue sample in the final homogenate varied according to the activity of the tissue under examination.

The following tissues have been studied: brain, skeletal muscle (diaphragm, quadriceps femoris), suprarenal gland, stomach, liver, lung, heart (auricle and ventricle), salivary gland (submaxillary), Harderian gland, intestinal mucosa and muscle, and skin. In preparing the intestinal mucosa and muscle the ileum was removed, opened longitudinally, washed free from food debris with 0.9% NaCl, and the mucosa scraped from the muscle layer with a sharp scalpel. Skin was taken from young rats weighing from 8 to 10 g., and was prepared and sliced by the method described by Thompson & Whittaker (1944).

Human tissues were obtained either post-mortem or from material removed by surgical operation. Adherent blood was removed by blotting with filter paper, after which the tissue was finely minced with scissors and washed repeatedly in 0.9% NaCl before homogenizing in 0.025M-NaHCO₃.

Estimation of esterase activity. Ammon's (1933) adaptation of the Warburg manometric technique was used. All measurements were made in duplicate at 38° and at pH 7.4, and were corrected for non-enzymic hydrolysis of the substrate. Esterase activity, measured over 0–30 min., is expressed as μ l. of CO₂/g. of tissue (wet wt.)/hr., except where otherwise stated.

Substrates. (1) Acetylcholine chloride (ACh) (British Drug Houses, Ltd.); (2) Acetyl- β -methylcholine chloride (MCh) (Savory and Moore, Ltd.); (3) Benzoylcholine chloride (BCh), prepared by Dr A. H. Ford-Moore, Experimental Station, Porton. Each substrate was dissolved in 0.025M-NaHCO₃ immediately before use, and a sufficient volume of the solution added to the reaction mixture to bring the final concentration to 0.015M for ACh and BCh, and 0.03M for MCh. Rough determinations of the Michaelis curves have shown that these concentrations lie upon the flat part of the curves obtained with the pseudo-cholinesterase, or are in excess of the peak concentrations for the true

enzyme. Successive 10 min. readings over the first 30 min. showed either a steady rate of CO₂ production, or at most only a very slight falling off.

Inhibitor. Di-isopropyl fluorophosphonate (DFP) was kindly provided by the Experimental Station, Porton. This was dissolved in 0.025M-NaHCO₃.

RESULTS

The relative esterase activities of homogenates of different tissues from adult male rats towards ACh, BCh and MCh are shown in Table 1. This table includes the results of at least two experiments on each tissue, and the individual figures across each line refer to a direct comparison of the three substrates in a single experiment. With each tissue further experiments have been done using only a single substrate. The levels of ACh hydrolysis for a given tissue obtained from different rats have in general agreed well; in the case of ventricle, for example, twelve separate experiments on different animals gave a mean value of 3630 μ l. of carbon dioxide/g./hr., with a standard deviation of 515. As would be expected, a greater degree of variation has been found among the results obtained with BCh and MCh for those tissues showing only slight activity towards these substrates.

We have confirmed the wide variations in the rates of hydrolysis of ACh by different tissues, a finding which is to be expected when the differences in innervation and cellular make-up are considered. Particularly high values have been found for heart auricle; intestinal mucosa and muscle, Harderian gland and heart ventricle have also shown high activity. In this connexion it is of interest that Antopol, Glaubach & Glick (1939) have shown that the total cholinesterase content of the auricles of rabbit heart is also considerably greater than that of the ventricles.

It will be seen that the relative rates of hydrolysis of BCh and MCh by these various tissues also differ strikingly. For comparative purposes the rates of hydrolysis of these 'specific' substrates are also expressed in Table 1 as percentages of the rate of hydrolysis of ACh by the given tissue. When expressed on this basis it will be noted that the tissues studied appear to fall into three groups: group A, tissues which hydrolyse MCh very much more rapidly than BCh, e.g. brain, skeletal muscle and suprarenal gland; group B, tissues which hydrolyse MCh and BCh at approximately equal rates, e.g. stomach, liver, lung and salivary gland; group C, tissues which hydrolyse BCh very much more rapidly than MCh, e.g. heart ventricle and auricle, intestinal muscle and mucosa, Harderian gland and skin.

The mean MCh/BCh ratio for these three groups is > 10, 0.9 and 0.25 respectively. Rat serum, which has been shown by Mundell (1944) to contain both true and pseudo-cholinesterases, has a MCh/BCh ratio of 2.1.

Table 1. *Relative cholinesterase activities of rat tissues towards ACh,† BCh and MCh*

Tissue	Activity (μ l. of CO ₂ /g./hr.)			Activity as percentage of ACh activity		Ratio MCh/BCh			
	ACh	BCh	MCh	BCh	MCh				
Brain	5250 } 5570 } 5880 }	5535	455 } 236 } 346 }	346	4040 } 4240 } 4453 }	4245	6	75	12
Quad. femoris	114 } 245 } 188 } 376 }	231	0 } 21 } 28 } 17 }	16	89 } 151 } 121 } 269 }	158	7	69	10
Diaphragm	728 } 528 }	628	0 } 0 }	0	334 } 350 }	342	0	54	>54
Suprarenal gland	3180 } 1900 }	2540	93 } 0 }	47	1240 } 747 }	994	2	39	20
Stomach	2060 } 1010 }	1535	467 } 267 }	367	462 } 155 }	309	24	20	0.8
Liver	706 } 925 } 1105 }	912	184 } 198 } 253 }	212	219 } 140 } 200 }	186	23	20	0.9
Lung	992 } 703 }	848	228 } 202 }	215	174 } 176 }	175	25	21	0.8
Salivary gland	1890 } 1288 }	1589	609 } 369 }	489	656 } 391 }	523	31	33	1.1
Heart ventricle*	3300 } 3900 }	3600	1105 } 995 }	1050	315 } 300 }	308	29	9	0.3
Heart auricle*	11600 } 9960 }	10780	2880 } 3920 }	3400	1040 } 820 }	930	32	9	0.3
Intestinal muscle*	3840 } 3500 }	3670	1620 } 1690 } 1240 }	1517	590 } 685 } 571 }	615	41	17	0.4
Intestinal mucosa*	5320 } 7500 }	6460	3130 } 3380 }	3255	468 } — }	468	50	7	0.1
Harderian gland	6060 } 6360 }	6210	3180 } 3320 }	3250	515 } 288 }	401	52	6	0.1
Skin*	3500 } 2560 }	3030	830 } 694 }	762	358 } 230 }	294	25	9	0.4
Serum	950 } 842 }	896	136 } 88 }	112	308 } 162 }	235	12	26	2.1

* Activity measured over 0–15 min.

† Acetylcholine chloride, ACh; benzoylcholine chloride, BCh; acetyl- β -methylcholine chloride, MCh.

These findings are consistent with one of two possibilities: first, these three groups of tissues might each contain a characteristic cholinesterase capable of hydrolysing both BCh and MCh in addition to ACh, although at different rates and therefore each showing a different MCh/BCh ratio; such an esterase, by hydrolysing both 'specific' substrates, would differ fundamentally from those studied in brain, red cells, plasma and pancreas. An alternative possibility is that the tissues of the three groups each contain varying amounts of two different enzymes, both of which can hydrolyse ACh, while one can hydrolyse BCh but not MCh, and the other MCh but not BCh, i.e. that the groups each contain varying amounts of the true and pseudo-cholinesterases, in Mendel's sense.

Summation experiments with mixed substrates were therefore carried out on tissues from groups B and C. From Table 2 it will be seen that for each of the tissues studied the carbon dioxide output in the presence of both substrates amounts to 81–102 % of the sum of the carbon dioxide outputs in the presence of each substrate separately. Since the concentration of each substrate was adequate for maximum rate of enzymic activity over the period studied, the summation occurring in the presence of both substrates suggests that two enzymes are concerned, one hydrolysing BCh exclusively or almost exclusively, and the other MCh.

In order that the rate of simultaneous hydrolysis of two substrates by two related enzymes should equal the sum of the rates of hydrolysis of each sub-

strate separately, it must be assumed that the two enzyme systems function independently of each other, and that neither substrate inhibits the activity of the other enzyme. Butyrylcholine, which has been shown to be a substrate for pseudo-cholinesterase (Stedman & Stedman, 1935; Nachmansohn & Rothenberg, 1945), has been found to act as a competitive inhibitor of the true enzyme (Cohen, Kalsbeck & Warringa, 1949), and Aldridge (1949) has stated that benzoylcholine shows a similar inhibitory action on the true cholinesterase in washed red cells of goat blood, although the action is only about one-third as great as that exerted by butyrylcholine. Although inhibitory effects may account for the finding that only 80–90 % of the arithmetical sum of the rates of hydrolysis of the separate esters was found for some of the tissues studied, such effects clearly do not interfere with the demonstration that complete or almost complete summation of activity occurs with these two substrates under our conditions.

substrate was tipped in was maintained constant, and amounted to 15 min. at room temperature followed by 10 min. in the thermostat while reaching temperature equilibrium. The values given in Table 3 are derived from at least three experiments on any given tissue for a given substrate. From these results an estimate has been made of the concentration of DFP (I_{50} concentration) required to produce 50 % inhibition of esterase activity under standardized conditions *in vitro* (Table 4).

It will be seen from Table 4 that the hydrolysis of BCh by the tissues of group *B*, and to a lesser extent by those of group *C*, is inhibited by very much lower concentrations of DFP than those required to produce an equal degree of inhibition of the hydrolysis of MCh. It must be stressed that for any given tissue the hydrolysis of the various substrates was carried out under identical conditions, so that in each case the system contained similar amounts of inert protein which might theoretically combine with DFP and so reduce the effective concentrations available

Table 2. *Hydrolysis rates of mixed substrates by rat tissues*

	Hydrolysis rate ($\mu\text{l. CO}_2/\text{g./hr.}$)				
	MCh (0.03 m)	BCh (0.015 m)	(MCh + BCh)		'Summation' (%)
			Found	Calc.	
Salivary gland	336	340	616	676	91
Liver	214	226	369	440	84
Lung	199	154	291	353	83
Stomach	214	200	360	414	87
Ventricle	256	1510	1795	1766	102
Intestinal mucosa	1360	5540	6900	6900	100
Intestinal muscle	1050	1920	2400	2970	81

An attempt was also made to carry out and interpret summation experiments using ACh + BCh and ACh + MCh. In every case the rate obtained with either pair of substrates was greater than that obtained with the specific substrate alone, but less than with ACh alone, i.e. no evidence of summation was observed. The results suggested therefore that each of the specific substrates is hydrolysed by an enzyme which also hydrolyses ACh, but owing to the difficulty in reaching an accurate interpretation these experiments were not pursued.

Since, as mentioned above, it has been shown by earlier workers that DFP is a selective inhibitor of the pseudo-cholinesterase of human plasma, experiments have been carried out with this compound in the hope of obtaining further information on the nature of the enzymic hydrolysis of MCh and BCh in these various tissues. The percentage inhibitions of enzymic activity towards ACh, BCh and MCh caused by the addition *in vitro* of varying concentrations of DFP to the various tissue homogenates are shown in Table 3. In these experiments the time of incubation of the homogenate with the inhibitor before the

for producing inhibitory effects. If the hydrolysis of BCh and MCh is carried out by one enzyme it is unlikely that there would be this degree of disparity in sensitivity to DFP by one and the same enzyme preparation. The findings suggest rather that the hydrolysis of BCh by tissues of groups *B* and *C* is brought about by an enzyme of the 'pseudo' type, highly sensitive to inhibition by DFP, whereas the hydrolysis of MCh by these tissues is brought about by a different, relatively DFP-insensitive enzyme, of the type found also in brain and skeletal muscle.

It is of interest that for the groups *B* and *C* preparations the I_{50} value for the hydrolysis of ACh in each instance closely approximates to the value for BCh hydrolysis, even in the case of salivary gland and lung where BCh and MCh are hydrolysed at approximately the same rate (Table 1). Further, when the hydrolysis of ACh is considered it will be noted that salivary gland, lung, ventricle and Harderian gland are all considerably more sensitive than brain or diaphragm to inhibition by DFP. There is also a striking difference in the sensitivity of MCh hydrolysis to DFP, ventricle and Harderian gland being

Table 3. *Inhibition by DFP of the hydrolysis of ACh, BCh and MCh by rat tissues*

(ACh, 0.015M; BCh, 0.015M; MCh, 0.03M. Activity measured over 0-30 min.)

Concentration of DFP ($\times 10^{-8}\text{M}$)			Acetylcholine															
			0.36	0.54	0.9	1.35	1.8	3.6	4.5	5.4	7.2	9.0	13.5	18.0	45	90	135	180
Tissue			Percentage inhibition															
Brain	—	—	—	—	—	—	—	—	—	—	30	35	—	57	79	87	94	
Diaphragm	—	—	—	—	—	—	—	—	—	—	47	52	61	74	97	—	—	
Lung	27	36	54	—	76	—	—	—	—	—	—	—	—	—	—	—	—	
Salivary gland	22	27	40	—	63	—	80	—	—	—	—	—	—	—	—	—	—	
Ventricle	—	—	32	50	67	83	91	96	98	100	—	—	—	—	—	—	—	
Harderian gland	—	—	17	20	33	43	—	73	—	94	—	—	—	—	—	—	—	

Concentration of DFP ($\times 10^{-8}\text{M}$)			Benzoylcholine										
			0.18	0.36	0.54	0.9	1.35	1.8	3.6	4.5	5.4	7.2	9.0
Tissue			Percentage inhibition										
Lung	—	15	26	42	58	69	—	92	—	—	—	—	
Salivary gland	24	30	—	55	66	71	—	92	—	—	—	—	
Ventricle	—	—	—	32	50	69	—	95	99	—	99	—	
Harderian gland	—	—	—	20	33	46	—	77	—	—	98	—	

Concentration of DFP ($\times 10^{-8}\text{M}$)			Acetyl- β -methylcholine												
			1.8	3.6	4.5	5.4	7.2	9.0	13.5	18.0	45	54	72	90	135
Tissue			Percentage inhibition												
Brain	—	—	—	—	—	23	36	—	50	—	—	76	93	95	—
Diaphragm	—	—	—	—	—	21	32	44	63	—	—	95	—	—	—
Lung	—	—	—	—	—	—	25	33	—	49	67	76	—	—	—
Salivary gland	—	—	—	—	—	21	28	35	53	—	61	—	—	—	—
Ventricle	32	42	—	52	—	68	—	—	—	—	—	—	—	—	—
Harderian gland	12	23	—	39	50	60	—	—	—	—	—	—	—	—	—

Table 4. *Concentration of DFP required to produce 50% inhibition of esterase (I_{50})*

(Concentration of substrates: ACh, 0.015M; BCh, 0.015M; MCh, 0.03M)

Group	Tissue	Wt. of tissue (mg./bottle)	I_{50} concentration ($\times 10^{-8}$ M)		
			ACh	BCh	MCh
A	Brain	30	30.0	—	37.0
	Diaphragm	150	12.0	—	27.0
B	Salivary gland	150	1.3	0.8	42.0
	Lung	500	0.9	1.1	42.0
C	Ventricle	210	1.4	1.4	5.0
	Harderian gland	100	3.5	2.2	7.1

very much more sensitive than the tissues of groups A and B. While it is essential to exercise caution in drawing conclusions from the comparative effects of a single inhibitor on preparations from different tissues, it should be noted that in the case of ventricle the bottles actually contained a greater weight of tissue than in the case of salivary gland, and yet the I_{50} concentration for MCh hydrolysis by ventricle is only one-eighth that for lung, while brain, with only 30 mg. present in each bottle, again falls into the more insensitive group. On present evidence, however, we do not feel justified in concluding that these results indicate that ventricle and Harderian gland

contain a different esterase for the hydrolysis of MCh from that possessed by the tissues of groups A and B.

DISCUSSION

In addition to Sawyer & Everett's (1947) demonstration of the ability of glandular tissues and of uterus to hydrolyse BCh, Augustinsson (1948) has reported that guinea pig small intestine shows approximately equal activity towards both MCh and BCh, while McNaughton & Zeller (1949) have stated that guinea pig parotid gland is a typical source of the pseudo-cholinesterase. The presence of a high BCh-

hydrolysing activity in heart muscle, however, does not appear to have been demonstrated before; indeed Nachmansohn & Rothenberg (1945) have claimed that benzoylcholine is not hydrolysed by the esterase present in the apex of ox-heart muscle. Although we have not studied the apex alone, we have demonstrated a very high rate of hydrolysis of BCh by both rat ventricle and auricle.

When the relative cholinesterase activities of different tissues towards ACh, BCh and MCh are compared (Table 1) one striking fact emerges, namely, that by contrast with brain, skeletal muscle and suprarenal gland, those tissues which we have studied and which receive only post-ganglionic autonomic innervation exhibit an activity towards BCh either equalling or exceeding that towards MCh. From this limited survey of different tissues it would seem that in the case of the rat those tissues in which acetylcholine exerts a nicotine-like action (i.e. in which it is concerned with transmission to another neurone or to a striated muscle cell), contain predominantly the true cholinesterase, while those in which it exerts a muscarine-like action contain both true and pseudo-cholinesterases in such amounts that the hydrolysis rate of BCh either equals or exceeds that of MCh.

The inclusion of brain as a tissue in which acetylcholine exerts a nicotine-like action may be questioned on the grounds that many of the central actions of acetylcholine are sensitive to atropine. Feldberg (1945), however, has pointed out that atropine sensitivity should not be regarded as the sole test for differentiation of the actions of acetylcholine, and concludes that its central effects should be considered together with its actions on peripheral ganglia and motor end-plates.

The question at once arises as to whether the pseudo-cholinesterase in these tissues is concerned physiologically with transmission processes, or with some other metabolic function unconnected with acetylcholine and conduction, and only brought into prominence when the cell structure is disorganized in homogenized preparations. The evidence we have at our disposal is at present inadequate to answer this question. In connexion with the physiological roles of these tissue esterases it is worth recalling that acetyl- β -methylcholine exhibits mainly muscarine-like actions, a fact which may be relevant to our finding that this compound is more rapidly hydrolysed by 'nicotinic' than by 'muscarinic' tissues. Further, butyryl choline has been found by Adams (1949) and Adams & Whittaker (1949) to be hydrolysed rapidly by the pseudo-cholinesterase of plasma, but hardly at all by the true cholinesterase of erythrocytes, and Chang & Gaddum (1933) showed that while butyrylcholine had only negligible pharmacological activity on rabbit intestine and on rabbit blood pressure it was highly active on the

frog rectus abdominis and in eserinizied leech muscle preparations. These observations suggest, therefore, that butyrylcholine exhibits mainly a nicotine-like action, which would accord with our finding of high pseudo-cholinesterase activities in the 'muscarinic' sites. If it is legitimate to relate the types of action of these choline esters to the relative amounts of the two types of esterase present in different tissues, it would suggest that the pseudo-cholinesterase in these autonomically innervated tissues is situated, even in intact preparations, in such a way as to be able to inactivate circulating butyrylcholine, and not intracellularly in such a way as to be inaccessible to circulating substances or to substances released at or near the cell surface.

By contrast with this wide distribution of the pseudo-cholinesterase in the rat, it has been claimed by Gunter (1946) that in the ox and sheep a number of glandular tissues are unable to hydrolyse benzoylcholine, but no other properties were examined to determine whether any further differences could be detected among the various tissue esterases present in these species. The observations of Sawyer & Everett (1947) and of ourselves in rats, may, however, be of significance in human physiology and pathology, since in preliminary experiments with human tissues we have found an active pseudo-cholinesterase in certain areas of the brain and also in uterus, ureter and jejunal muscle and mucosa.

SUMMARY

1. A survey has been made of the relative rates of hydrolysis of acetylcholine, benzoylcholine and acetyl- β -methylcholine by various different rat tissues.
2. It has been found that benzoylcholine and acetyl- β -methylcholine are hydrolysed at approximately equal rates by stomach, liver, lung and submaxillary gland, while in the case of heart auricle and ventricle, intestinal muscle and mucosa, Harderian gland and skin, benzoylcholine is hydrolysed more rapidly than acetyl- β -methylcholine.
3. Summation experiments, and observations on the degrees of inhibition produced by di-*isopropyl* fluorophosphonate (DFP), indicate that these tissues contain two cholinesterases, one DFP-sensitive and the other relatively DFP-insensitive, i.e. that they contain both true and pseudo-cholinesterases in Mendel's sense.
4. With acetylcholine as substrate, salivary gland, lung, ventricle and Harderian gland are much more sensitive than brain or skeletal muscle to *in vitro* inhibition by DFP.
5. The possible significance of these results in connexion with the nicotine-like and muscarine-like actions of choline esters and with the effects produced by cholinesterase inhibitors is discussed.

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REFERENCES

- Adams, D. H. (1949). *Biochim. biophys. Acta*, **3**, 1.
 Adams, D. H. & Thompson, R. H. S. (1948). *Biochem. J.* **42**, 170.
 Adams, D. H. & Whittaker, V. P. (1949). *Biochim. biophys. Acta*, **3**, 358.
 Aldridge, W. N. (1949). Private communication.
 Alles, G. A. & Hawes, R. C. (1940). *J. biol. Chem.* **133**, 375.
 Ammon, R. (1933). *Pflüg. Arch. ges. Physiol.* **233**, 486.
 Antopol, W., Glaubach, S. & Glick, D. (1939). *Proc. Soc. exp. Biol., N.Y.*, **42**, 280.
 Augustinsson, R. B. (1948). *Acta physiol. scand.* **15**, Suppl. 52.
 Beveridge, J. M. R. & Lucas, C. C. (1941). *Science*, **93**, 356.
 Birkhäuser, H. & Zeller, E. A. (1940). *Helv. chim. Acta*, **23**, 1460.
 Bissegger, A. & Zeller, E. A. (1943). *Helv. physiol. pharmacol. Acta*, **1**, C86.
 Brauer, R. W. (1948). *J. Pharmacol.* **92**, 162.
 Chang, H. C. & Gaddum, J. H. (1933). *J. Physiol.* **79**, 255.
 Cohen, J. A., Kalsbeck, F. & Warringa, G. P. J. (1949). *Ned. Tijdschr. Geneesk.* **93**, 1741.
 Dubois, K. P. & Mangun, G. H. (1947). *Proc. Soc. exp. Biol., N.Y.*, **64**, 137.
 Feldberg, W. (1945). *Physiol. Rev.* **25**, 596.
 Folley, S. J. & Watson, S. C. (1948). *Biochem. J.* **42**, 204.
 Freedman, A. M., Willis, K. & Himwich, H. E. (1949). *Amer. J. Physiol.* **157**, 80.
 Gilman, A., Carlson, R. I. & Goodman, L. (1939). *J. Pharmacol.* **66**, 14.
 Glick, D. (1941). *Nature, Lond.*, **148**, 662.
 Gunter, J. M. (1946). *Nature, Lond.*, **157**, 369.
 Hawkins, R. D. & Gunter, J. M. (1946). *Biochem. J.* **40**, 192.
 Hawkins, R. D. & Mendel, B. (1949). *Biochem. J.* **44**, 280.
 Langemann, K. (1944). *Helv. physiol. pharmacol. Acta*, **2**, C17.
 McNaughton, R. A. & Zeller, E. A. (1949). *Proc. Soc. exp. Biol., N.Y.*, **70**, 165.
 Mazur, A. & Bodansky, O. (1946). *J. biol. Chem.* **163**, 261.
 Mendel, B. & Hawkins, R. D. (1947). *Biochem. J.* **41**, xxii.
 Mendel, B. & Mundell, D. B. (1943). *Biochem. J.* **37**, 64.
 Mendel, B., Mundell, D. B. & Rudney, H. (1943). *Biochem. J.* **37**, 473.
 Mendel, B. & Rudney, H. (1943). *Biochem. J.* **37**, 59.
 Mundell, D. B. (1944). *Nature, Lond.*, **153**, 557.
 Nachmansohn, D. & Rothenberg, M. A. (1945). *J. biol. Chem.* **158**, 653.
 Ord, M. G. & Thompson, R. H. S. (1950). *Biochem. J.* **46** (in the Press).
 Plattner, F. & Hintner, H. (1930). *Pflüg. Arch. ges. Physiol.* **225**, 19.
 Richter, D. & Croft, P. G. (1942). *Biochem. J.* **36**, 746.
 Sawyer, C. H. & Everett, J. W. (1947). *Amer. J. Physiol.* **148**, 675.
 Sawyer, C. H. & Hollinshead, H. (1945). *J. Neurophysiol.* **8**, 137.
 Stedman, E. & Stedman, E. (1935). *Biochem. J.* **29**, 2563.
 Thompson, R. H. S. (1947). *J. Physiol.* **105**, 370.
 Thompson, R. H. S. & Whittaker, V. P. (1944). *Biochem. J.* **38**, 295.

The Virulence-enhancing Factor of Mucins

1. A BIOLOGICAL ASSAY OF VIRULENCE-ENHANCING ACTIVITY

By H. SMITH

Microbiological Research Department, Experimental Station, Porton, Wilts

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Nungester, Wolf & Jourdonais (1932) discovered that a marked increase in the virulence for mice of strains of *Staphylococcus aureus*, *Streptococcus pneumoniae* (Type II) and *Strep. haemolyticus* could be effected by injecting intraperitoneally a suspension of the organisms in 5% (w/v) hog gastric mucin. About the same time Miller (1933) found that the same procedure would reduce the minimum lethal dose for mice of strains of *Neisseria meningitidis* by 10^{-6} .

Since that time the virulence-enhancing power of mucin (review: Olitzki, 1948) has been used ex-

tensively, particularly in serum and chemotherapeutic testing, for the production of experimental infection in several different test animals; relatively small numbers of bacteria of many different species have been used. The intratracheal route of infection for the production of pneumonias has been used with a success equal to that of the more widely exploited intraperitoneal route. Most workers have used dried commercial hog gastric mucin, mainly but not solely the 'Granular Mucin 1701W' of Wilson Laboratories, Chicago, U.S.A. Anderson & Oag (1939) and Tunnicliffe (1940) are in agreement on the activity of