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DISTRIBUTION OF ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE IN THE MYENTERIC PLEXUS AND LONGITUDINAL MUSCLE OF THE GUINEA-PIG INTESTINE

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Abstract—Manometric analyses of the hydrolysis of various choline esters and the effects upon it of selective anticholinesterases showed that in blood-free homogenates of plexus-containing longitudinal muscle sheets detached from guinea-pig small intestines two enzymes were present, a typical acetylcholinesterase (AChE) and a typical butyrylcholinesterase (BuChE). Homogenization did not release into solution all the AChE or BuChE, as demonstrated by the finding that after centrifugation activity was present not only in the supernatant fractions but also in the sediments. The mechanical removal of Auerbach's plexus led to a 76·3 ± 3·3 per cent decrease in AChE content but reduced the BuChE content only by 10·6 ± 9·3 per cent, as assessed by the hydrolysis of DL-acetyl-β-methylcholine and *n*-butyrylcholine, respectively. Since DL-acetyl-β-methylcholine is also hydrolysed slowly by BuChE the decrease in AChE content could be even greater, by another 10 per cent. In the ascending and transverse colon the contents of AChE and BuChE in the plexus-containing longitudinal muscle were lower than those found in the small intestine. The loss of Auerbach's plexus reduced the AChE content by 57·4 ± 3·0 per cent, without change in BuChE content. Small regional differences in the content of AChE but not of BuChE were detected in the longitudinal muscle of the small intestine.

SINCE acetylcholine is the humoral transmitter at the pre- and post-ganglionic endings of the vagus in the small intestine, the nature and distribution of enzymes present for its destruction at these sites are of interest. Apart from the histochemical findings of Koelle,[1,2] Gerebtzoff and Bertrand[3] and Donhoffer,[4] there are only a few data about the nature and quantitative distribution of cholinesterases in the different layers of the gut. Manometrically, a high acetylcholine-destroying activity has been found in the mucosa[5,6] and in homogenates of the whole intestine, which included the mucosa.[7] Goutier-Pirotte and Goutier[6] reported that only 4 per cent of the total "non-specific" cholinesterase (butyrylcholinesterase) of the small intestine was present in the longitudinal muscle.

In this paper results are presented which show that in the guinea-pig the Auerbach's plexus-containing longitudinal muscle layer of the small intestine, detached by the technique described by Ambache [8] and Ambache and Freeman,[9] contains an acetylcholine hydrolase (acetylcholinesterase; AChE; EC 3.1.1.7) and an acylcholine acyl hydrolase (butyrylcholinesterase; BuChE; EC 3.1.1.8). The effect of removing the plexus on the AChE and BuChE content of the longitudinal muscle in the small

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intestine and colon, and studies on the regional variation in enzyme activity in the plexus-containing longitudinal muscle of the small intestine are also reported.

A preliminary account of these experiments has been presented at a meeting of the Physiological Society.[10]

METHODS

Albino guinea-pigs weighing 600–1000 g were used throughout, in order to obtain large enough tissue samples. The animals were killed by cervical fracture and the intestines and mesentery were perfused with 0·3–0·5 l. of Locke solution at ca. 35° from a cannula in the anterior mesenteric artery; a venous outlet was provided by an incision in the posterior vena cava. To drain the lumen of the gut during the perfusion, the ileum was intubated just above the ileocaecal valve. The perfusion was stopped when the fluid emerging from the cut vein was clear and the vessels were free of any visible trace of blood. The small intestine and the ascending and transverse colons were excised; their lumen was cleaned by repeated flushing with Locke solution.

Small intestine. The longitudinal muscle was separated as a broad sheet with Auerbach's plexus adherent, as described by Ambache[8] and Ambache and Freeman.[9] As found by Paton and Zar,[11] Auerbach's plexus sometimes breaks away during this separation and the longitudinal muscle is thus rendered plexus-free. When the sheet was detached by pulling, it was therefore scanned, usually centimetre by centimetre, in oblique light to determine whether it was visibly plexus-containing or not; the contrasting appearance of the plexus-free and plexus-containing muscle is illustrated in Fig. 1. Substantial amounts of plexus-free tissue could be obtained only from a limited number of guinea-pigs. The separated plexus-containing sheets were dropped into an ice-cooled beaker containing 3–4 ml of Locke solution and kept there until collection from the whole gut of one animal was complete. Samples of plexus-free tissue were collected in a separate cooled beaker.

Ascending and transverse colon. The proximal 50–60 cm, consisting of some 30 cm of ascending and 30 cm of transverse colon, were used, discarding the 40 cm beyond the sigmoid flexure. In the ascending colon the longitudinal "taenial bands", 1–1·5 mm wide, are visible on either side of the mesenteric attachment. These bands are 3–5 mm apart and appear to be mainly fibro-elastic; unlike the taeniae of the caecum they are palpably hard and cannot be dissected off. The two bands merge some 20–30 cm below the caecal junction and are absent from the transverse colon.

The tissue collected for study consisted of longitudinal muscle sheets detached, by the procedure described above, from the whole circumference of the gut in the transverse colon, but in the ascending colon only from the non-diverticulated, anti-mesenteric, "intertaenial" arc of the circumference, i.e. after exclusion of the taenial bands by means of two longitudinal incisions, one along the "outer", anti-mesenteric edge of each band. Upon detachment, the first 2–3 cm of the sheet usually contained some circular muscle, but with further traction this was soon left behind.

Since Auerbach's plexus in the colon was less obvious to the naked eye than in the ileum, each colon sheet was viewed under a dissecting microscope. Auerbach's plexus appeared as a grey network; in some experiments, its presence or absence was confirmed by staining parallel samples with 0·01 % methylene blue in 0·9 % NaCl solution, and by electrical or nicotine-stimulation in an organ bath. Plexus-free areas were

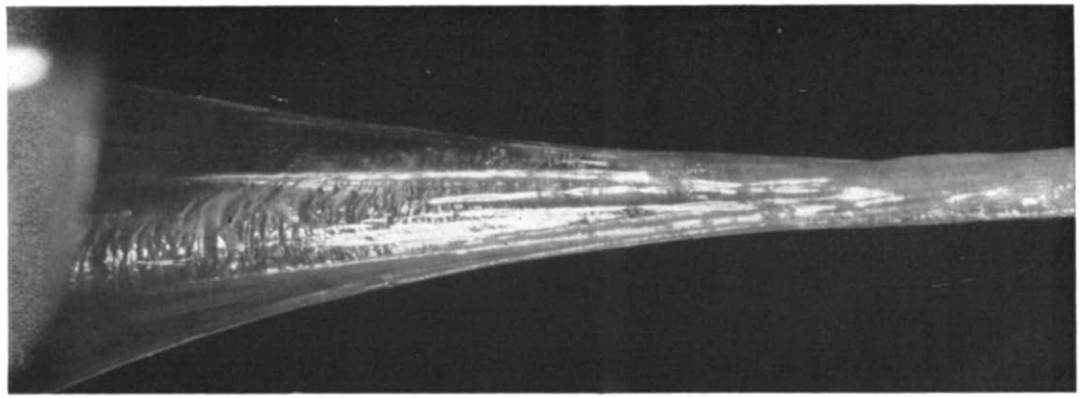

FIG. 1. Detached longitudinal muscle sheet from guinea-pig ileum; at the lower end, Auerbach's plexus is visible in the middle portion of the sheet but is absent from the edges.

dissected away from plexus-containing areas in each sheet, and the two types of tissue were pooled separately in ice-cooled beakers of Locke solution; it was necessary to pool tissue from two to five guinea-pigs for each experiment.

Homogenates. The tissue was blotted gently between sheets of Whatman No. 1 filter paper, weighed and transferred to a homogenizer. For every 100 mg tissue, 1 ml of distilled-deionized water was added before grinding, either by hand in a Griffiths tube with a ground glass pestle or in a high-speed micro-homogenizer (Sorvall "Omni-mixer", Type OM 1220, used with 5 ml nylon chamber inserts), in which it was possible to process as little as 0·5 ml. The homogenates or supernatants and sediments obtained from them were stored at −15°, occasionally for several days, before assay.

Cholinesterase activity was determined on the following three preparations:

(a) Whole homogenates (≡ 100 mg tissue/ml), which after thawing were further ground with acid-washed sand in a mortar.

(b) The supernatant fraction (one experiment) obtained by centrifugation at 1000 rev/min for 5 min of a whole homogenate which had been diluted with 3 parts of 25 mM $NaHCO_3$.

(c) The supernatants and sediments of homogenates (≡ 100 mg tissue/ml), which after preparation had been centrifuged for 5 min at 3300 rev/min and then stored at −15°. After thawing, the sediments were further ground with acid-washed sand.

Enzyme activities were determined by the Warburg manometric method at 37°, with a gas phase of 95% N_2 + 5% CO_2, using as substrates acetylcholine chloride, 10 mM; propionylcholine perchlorate, 10 mM; DL-acetyl-β-methylcholine chloride, 20 mM; and *n*-butyrylcholine iodide, 10 mM. The volumes of supernatants, whole homogenates or sediments used per flask were adjusted according to their enzymic activity, to represent at most 50 mg tissue, and the total volume of each flask was made up to 3 ml with 25 mM $NaHCO_3$. The CO_2 output from 5 to 35 min after the addition of substrate to the enzyme was recorded and corrected for non-enzymic CO_2 production and tissue blank. Values for hydrolysis of the substrates used were based on the means of duplicate assays except when stated otherwise, and expressed as μl CO_2/100 mg wet tissue/hr.

Regional variation of enzyme activity in the small intestine. In six guinea-pigs the excised small intestine was divided into equal thirds and separate pools of plexus-containing tissue were then collected from the upper, middle and lower thirds. In order to assess regional variation in the dry/wet weight ratio, in four experiments the separate pools of plexus-containing tissue from the three regions of the small intestine (and also from the colon) were weighed before and after heating at 100° in an oven, to a constant weight.

Anticholinesterases. The enzymic hydrolysis for acetyl-, propionyl-, *n*-butyryl- and DL-acetyl-β-methylcholine was determined both in the absence and in the presence of the selective inhibitor of butyrylcholinesterase[12] *iso*-OMPA (tetramono*iso*propyl pyrophosphortetramide). In these experiments *iso*-OMPA, 50 μM, was added to the enzyme 20 min before addition of the substrate, and after the flasks had been placed into the water-bath and equilibrated with 95% N_2 + 5% CO_2. To obtain selective inhibition of acetylcholinesterase, the anticholinesterase BW 284C51 (1:5 bis(4-allyldimethylammonium phenyl)-pentan-3-one dibromide)[13] was added to the enzyme 5 min before the addition of substrate.

RESULTS

Guinea-pig small intestine

Characterization of the cholinesterases present in blood-free homogenates of plexus-containing (PC) longitudinal muscle sheets. The rates of hydrolysis of acetylcholine, propionylcholine and the selective substrates[14] for acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), i.e. DL-acetyl-β-methylcholine and *n*-butyrylcholine respectively, were studied in the absence and in the presence of the anticholinesterase *iso*-OMPA, 50 μM. Table 1 shows the results obtained with the supernatant fraction of a centrifuged PC-homogenate (Expt 1, representative of two such experiments on material from different animals) and with an uncentrifuged whole homogenate of PC-tissue (Expt 2). It can be seen that *iso*-OMPA, an inhibitor with a higher affinity

TABLE 1.

	ACh	PrCh	BuCh	MCh
Expt 1. Supernatant obtained by centrifugation for 5 min (1000 rev/min)				
Control	700	1381	1420	173
+ 50 μM *iso*-OMPA	248	188·5	54·5	129
Inhibition (%)	65	86	96	25
Expt 2. Whole homogenate from another guinea-pig				
Control	1553	2927	2787	594
+ 50 μM *iso*-OMPA	780	407	76	453
Inhibition (%)	50	86	97	24

Characterization of the cholinesterases in plexus-containing longitudinal muscle sheets of guinea-pig small intestines, as shown by the effect of pre-incubation with 50 μM *iso*-OMPA on the rates of hydrolysis, expressed as μl CO_2/100 mg wet tissue/hr, of 10 mM acetylcholine (ACh), propionylcholine (PrCh) and *n*-butyrylcholine (BuCh), and 20 mM DL-acetyl-β-methylcholine (MCh).

for BuChE than for AChE,[12] changes the rate of hydrolysis from *n*-butyrylcholine > propionylcholine > acetylcholine to acetylcholine > propionylcholine > *n*-butyrylcholine. This is only possible if the homogenates contain a mixture of AChE and BuChE. Such a conclusion is supported by results obtained with the anticholinesterase BW 284C51, which has a higher affinity for AChE than for BuChE.[12] These results are illustrated by Fig. 2.

If DL-acetyl-β-methylcholine were hydrolysed only by AChE and *n*-butyrylcholine only by BuChE, then the rates of acetylcholine hydrolysis by AChE in Expts 1 and 2 of Table 1 would amount to 310 and 1000 μl CO_2/100 mg wet tissue/hr, respectively; the corresponding values for BuChE would be 390 and 553, respectively. The relative rates for the hydrolysis by AChE of acetylcholine–propionylcholine–*n*-butyrylcholine would be 1:0·42:0·01 (Expt 1) and 1:0·44:0·01 (Expt 2). For BuChE these values would be 1:3·14:3·57 in Expt 1 and 1:4·5:5 in Expt 2.

However, it is known that the selectivity of *n*-butyrylcholine for BuChE is not absolute and that this ester is also hydrolysed slowly by AChE; a similar reservation

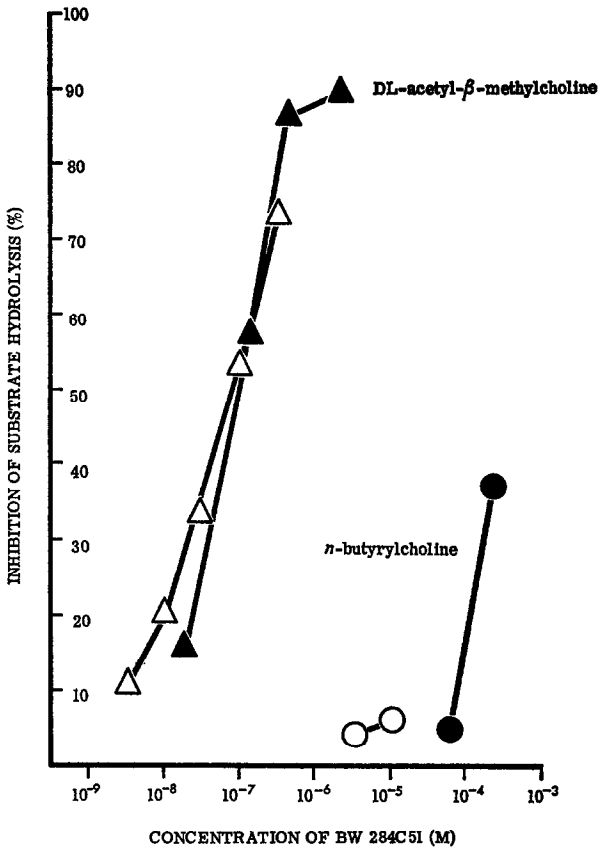

FIG. 2. Inhibition by BW 284C51 of the hydrolysis of DL-acetyl-β-methylcholine, 20 mM, (△ and ▲) and of *n*-butyrylcholine, 10 mM, (○ and ●) by two samples of a whole homogenate of plexus-containing longitudinal muscle from the guinea-pig small intestine.

applies to the use of DL-acetyl-β-methylcholine as a selective substrate for AChE.[14] Assuming that 10 per cent of the hydrolysis of DL-acetyl-β-methylcholine is attributable to BuChE, as indicated by the results presented in Fig. 2, and that the hydrolysis of *n*-butyrylcholine after inhibition by *iso*-OMPA represents a hydrolysis by AChE, the rates of ACh hydrolysis in Expts 1 and 2 by AChE can be recalculated as 292 and 907 μl CO_2/100 mg wet tissue/hr, respectively, and the corresponding values for BuChE as 408 and 646. The relative rates for the hydrolysis by AChE of acetylcholine–propionylcholine–*n*-butyrylcholine then become 1:0·76:0·20 (Expt 1) and 1:0·52:0·10 (Expt 2). For BuChE the values become 1:2·84:3·32 in Expt 1, and 1:3·80:4·18 in Expt 2.

The true values obviously lie between those obtained by the two methods of calculation, both of which clearly demonstrate that the homogenates contain a mixture of a typical AChE with a typical BuChE and that DL-acetyl-β-methylcholine and *n*-butyrylcholine are acceptable substrates for quantitative comparisons between the contents of AChE and BuChE in different homogenates since at least 90 per cent of the hydrolysis of DL-acetyl-β-methylcholine is attributable to AChE and an even higher selectivity applies to *n*-butyrylcholine.

Differences in cholinesterase content of blood-free longitudinal muscle sheets, attributable to the presence or absence of Auerbach's plexus. Acetylcholinesterase. A highly significant difference ($P < 0{\cdot}001$) in the AChE content of plexus-free (PF) relative to plexus-containing (PC) longitudinal muscle obtained from the same animal, was found in six out of seven experiments, when assessed by the hydrolysis of 20 mM DL-acetyl-β-methylcholine. In the six guinea-pigs, as shown in Table 2, the ratio AChE in PF-tissue: AChE in PC-tissue varied from 17 to 39 per cent, with an average of 23·7 per cent. In the seventh guinea-pig, omitted from the Table, the ratio was 81 per cent; this was taken to indicate that Auerbach's plexus had not been successfully removed.

Table 2 also shows that the reduction in AChE content due to removal of plexus was easily detectable in whole homogenates and in supernatants obtained from them and not related to the degree of tissue break-up prior to assay.

TABLE 2.

Substrate:	Acetylcholinesterase 20 mM DL-acetyl-β-methylcholine			Butyrylcholinesterase 10 mM *n*-butyrylcholine		
	Plexus			Plexus		
Guinea-pig No.	Present	Absent	% PF/PC	Present	Absent	% PF/PC
1*	469	182	39	—	—	—
2*	473	81	17	2118	2350	111
3†	317	78	25	2716	2154	79
4†	420	94	22	2641	2971	112
5†	373	82	22	2597	2017	78
6‡	641	111	17	4166	2792	67
Mean	448·8	104·6	23·7	2847	2456·8	89·4
S.E. of mean	± 45·4	± 16·3	± 3·3	± 345·8	± 182	± 9·3

Cholinesterase activities, expressed as μl CO_2/100 mg wet tissue/hr, of plexus-containing (PC) and plexus-free (PF) longitudinal muscle from the small intestines of individual guinea-pigs.

* Supernatant of hand ground homogenate centrifuged at 3300 rev/min for 5 min.

† Summed activities of centrifuged supernatants and sediments of hand ground homogenates.

‡ Uncentrifuged homogenate prepared in a high speed micro-homogenizer.

The results from guinea-pigs Nos. 3–5 given in Table 2 represent the sum of the enzyme activities found in the separated supernatant and sediment fractions of the homogenates. In these experiments the mean AChE activities (±S.E.) of the PC-homogenates, expressed in μl CO_2/100 mg wet weight/hr, were 203 ± 23 and 167 ± 46 for the supernatant and sediment, respectively. The corresponding values for PF-homogenates were 64 ± 8 and 21 ± 3. Thus, the AChE activity of the supernatant fractions represented only 55 per cent of the total activity in the PC-homogenates and 75 per cent in the PF-homogenates.

Butyrylcholinesterase. Table 2 shows that in homogenates for which the PF/PC activity ratio for AChE was low, the PF/PC ratio for BuChE was quite variable. The mean effect brought about by plexus removal was a decrease in BuChE activity of only 10·6 per cent, which was statistically of low significance ($P > 0{\cdot}1 < 0{\cdot}2$). BuChE also differs from AChE in so far as it is less firmly associated with the coarser particles of the homogenates. The mean BuChE activities (±S.E.), expressed in μl CO_2/100 mg wet weight/hr, of tissues from guinea-pigs Nos. 3–5 (Table 2) were 1890 ± 172 and 761 ± 138 for the supernatant and sediment, respectively, of these PC-homogenates. The corresponding values for PF-homogenates were 1988 ± 244 and 392 ± 54. Thus, the BuChE activity of the supernatant represented 71 per cent of the total activity in the PC-homogenates and 84 per cent in the PF-homogenates.

Regional variation in cholinesterase content of the small intestine. The tissues used for the experiments described so far had been taken from different regions of the intestine, depending on which part yielded PF-tissue, and in any given experiment the PC- and PF-homogenates were therefore not from adjacent parts of the muscle. To examine whether there were regional differences in the enzyme content of the intestine, the AChE and BuChE contents in three defined regions of the small intestine of six animals were investigated. Whole homogenates of PC-tissue were prepared from the proximal, middle and distal thirds of the small intestine from each animal. Table 3 gives the AChE and BuChE contents of each of these eighteen regional samples. It can

TABLE 3.

Guinea-pig no.	Proximal Third		Middle Third		Distal Third		Overall Mean	
	AChE	BuChE	AChE	BuChE	AChE	BuChE	AChE	BuChE
1	532·5	2794	514·5	3546·5	501·5	4159*		
2	624	3004	867·5	4844*	619·5	3876		
3	645	2390*	591·5	2146	330·5	2215		
4	287	2400*	408	2647	317	2444		
5	324	2500	304	2720	166	2400*		
6	520	2590	390	2420*	338	2440		
Mean	488·7	2619·6	512·6	3053·9	378·7	2922	460	2865
S.E. of mean	± 61·5	± 96·3	± 8·2	± 405·8	± 64·7	± 350	± 40·5	± 176
Mean/100 mg dry weight)	3768	20197	3697	22050	2720	20991	3395	21079

AChE activity (expressed as μl CO_2/100 mg wet tissue/hr; substrate; 20 mM DL-acetyl-β-methylcholine) and BuChE activity (expressed as μl CO_2/100 mg wet tissue/hr; substrate: 10 mM *n*-butyrylcholine) of whole homogenates of plexus-containing tissue prepared from the proximal, middle and distal thirds of the small intestine in individual guinea-pigs.

Each value is the mean of duplicate samples, except those with asterisks. In 70 per cent of the assays the enzyme activities of duplicate samples differed by 5 per cent or less from their mean and the greatest difference observed in any one single experiment was 13 per cent of the corresponding mean. The means/100 mg dry weight are based on the means/100 mg wet weight and the dry to wet weight ratios, determined in separate experiments. The ratios were: Proximal third: 0·1297; middle third: 0·1385; and distal third 0·1392.

be seen that the mean AChE activity calculated on a wet weight basis is distinctly lower in the distal third of the ileum (P relative to proximal and middle third $> 0{\cdot}2 < 0{\cdot}3$). If the AChE activity is recalculated per 100 mg dry weight (lowest row of Table 3) the difference between the AChE activity of the distal third of the ileum and those of the proximal and middle third becomes even greater than that obtained in calculations based on a wet weight.

The regional differences of BuChE activity, calculated per 100 mg wet tissue, are less than those of AChE and remain negligible if the activity is expressed per 100 mg dry weight.

Cholinesterase content of longitudinal muscle sheets from the guinea-pig ascending and transverse colon.

The enzyme activity, assessed by the hydrolysis of the selective substrates for AChE and BuChE, of homogenates of blood-free PC-sheets of the longitudinal muscle obtained from the transverse colon and from the anti-mesenteric intertaenial arc of the ascending colon was lower than that found with PC-sheets of the blood-free longitudinal muscle of the small intestine. As can be seen by comparing the means

TABLE 4.

Guinea-pig pool no.	Animals per pool	Acetylcholinesterase 20 mM DL-acetyl-β-methylcholine PC	PF	% PF/PC	Butyrylcholinesterase 10 mM n-butyrylcholine PC	PF	% PF/PC
1	2	259	110	43	617	563	91
2	5	222	119	54	737	935	127
3	4	449	164	37	1430	1269	89
4	4	349	140	40	1091	1041	95
5	4	268	96	36	914	937	103
Mean		309·4	125·8	42·6	957·8	949·0	101
S.E. of mean		± 40·5	± 11·8	± 3·0	± 123·9	± 114·1	± 6·8

Cholinesterase activities, expressed as μl CO_2/100 mg wet tissue/hr, of uncentrifuged homogenates of plexus-containing (PC) and plexus-free (PF) longitudinal muscle pools obtained from ascending and transverse colons of several guinea-pigs.

given in Table 4 with those in Table 2, the AChE activity per 100 mg wet tissue was 309·4 ± 40·5 in the colon as against 448·8 ± 45·4 found in the small intestine ($P > 0{\cdot}002 < 0{\cdot}01$). The corresponding values for the BuChE activity were 957·8 ± 123·9 in the colon and 2847 ± 345·8 ($P < 0{\cdot}001$) in the small intestine. The dry/wet weight ratio in the colon was 13·4%, i.e. within the range found in the small intestine, and recalculation of enzyme activities in terms of dry weight did not affect the difference found between colon and small intestine.

The removal of Auerbach's plexus led to a marked decrease in the AChE content but did not alter the BuChE content of the PF- with respect to the PC-homogenates (Table 4). The recorded mean loss of 57·4 ± 3·0% in AChE content on plexus-removal was highly significant ($P < 0{\cdot}001$) but less pronounced than the 76·3% loss found in the small intestine; the level of significance for the difference in loss of

AChE between the muscles of the small intestine and those of the colon was $> 0{\cdot}002 < 0{\cdot}01$.

DISCUSSION

The studies show that when Auerbach's plexus is present the blood-free longitudinal muscle of the small intestine of the guinea-pig contains two cholinesterases, which according to their substrate specificity are a typical acetylcholinesterase and a typical butyrylcholinesterase.[14] Removal of the plexus by stripping reduces the AChE content by 76 per cent. Thus AChE is predominantly associated with Auerbach's plexus, which is in agreement with histochemical findings by Koelle[1,2] in other species. The residual AChE (24 per cent) is undoubtedly overassessed since the substrate used for AChE determinations was DL-acetyl-β-methylcholine, which is not absolutely selective for AChE and is hydrolysed also by BuChE,[14] though very slowly. The error induced by this would represent up to 10 per cent of the activity of PF-tissue since "denervation" only marginally reduces BuChE content. Therefore, the denervation really lowers AChE content by 76–86 per cent. Preliminary pharmacological tests on plexus-free (PF) longitudinal muscle from the guinea-pig ileum showed that BW 284C51, in a concentration which in homogenates produces 50 per cent inhibition of AChE and only 4 per cent inhibition of BuChE, potentiated 2–3 fold the contractile response to exogenous acetylcholine. This however does not prove a physiological role for AChE located in muscle fibres, since residual AChE might not be associated with the muscle fibres themselves but located in "pinched off" parts of the Auerbach's plexus, e.g. in varicosities embedded in muscle fibres. To establish this, electronmicroscopic studies of PF-tissue are indicated. As far as the location of BuChE is concerned our findings are not in agreement with those of Koelle's;[1,2] using a histochemical technique, he consistently observed marked BuChE activity in the plexus as well as in muscle, whereas according to our results (denervation decreased BuChE content only by 10·6 per cent) only a small fraction of the BuChE is located in Auerbach's plexus. This discrepancy might well reflect a species difference since the selective inhibition of BuChE affects responses to neuronal stimulation differently in different species.[15]

The reduction in AChE achieved by the removal of plexus in the PF-longitudinal muscle was somewhat less pronounced in the colon than in the small intestine. Whether this really means that the muscle of the colon itself contains a fair amount of AChE needs further investigation for the reasons stated above. No evidence could be obtained for any association between BuChE and the plexus in the colon.

The cholinesterases in the tissue studied do not readily go into solution and the enzymic activity is dependent on the method of preparation of samples for assay. Keeping the method of preparation of homogenates standardized as much as possible, a regional difference was found to exist in AChE content, whether activity was calculated on a wet or on a dry weight basis. With BuChE the regional variations were smaller than those observed with AChE. Regional variations in AChE content could only account for a small part of the recorded effects of denervation on AChE content; however, in the case of BuChE they could be the explanation for some of the observed scatter of results.

Extension of the studies on guinea-pigs to other species seems desirable. We attempted to obtain PF-tissue from rabbits, but owing to the firmness with which Auerbach's plexus remained attached to the longitudinal muscle we have been so far

unsuccessful. In a single experiment on a rabbit PC-homogenate of the longitudinal muscle of the small intestine, the AChE activity was found to be 842 μl CO_2/100 mg wet weight/hr, nearly double the mean activity of guinea-pigs, and the BuChE activity was 3596 μl CO_2/100 mg wet weight/hr, as determined with 20 mM DL-acetyl-β-methylcholine and 10 mM *n*-butyrylcholine, respectively.

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