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SYNTHESIS OF ACETYLCHOLINE IN THE WALL OF THE DIGESTIVE TRACT

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Acetylcholine is continuously released from the wall of the stomach and intestine, and this release occurs independently of the activity of their extrinsic nerves. Until recently it has been the general inclination to attribute this acetylcholine metabolism to the nervous structures of the myenteric and submucous plexus. But the recent finding that the release of acetylcholine continues even after these structures have been paralysed by either curare or cocaine throws serious doubts on this conception. It is possible that the acetylcholine metabolism in the wall of the digestive tract is, to a great extent at least, non-nervous in origin, comparable to that in the human placenta and intimately linked with the choline and lipin metabolism in this tissue (Feldberg & Lin, 1949*a*).

Another view generally held is that the released acetylcholine exhibits only motor functions in the intestine. One can easily trace this view back to the early findings of Le Heux (1918–19), and Magnus (1920) of a continuous release of choline from the intestinal wall and to their conclusion that choline is the hormone of intestinal movements. This view was transferred to acetylcholine when this substance also was found to be continuously released from the intestinal wall. On the other hand, Wright, Jennings, Florey & Lium (1940) and Florey, Wright & Jennings (1941) considered the possibility that the continuous release of acetylcholine might provide a stimulus for the continuous secretion of succus entericus. Their view was based on the findings that the secretion is not only abolished by atropine but enhanced by small amounts of eserine.

In the present paper an examination has been made of the distribution of choline acetylase, the enzyme responsible for the synthesis of acetylcholine, along the length of the wall of the small intestine and in its different layers.

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A few experiments were carried out on the stomach and the different parts of the large intestine. From an accurate knowledge of such a distribution it should be possible to obtain information on both problems, that of the origin and that of the function of the acetylcholine metabolism of the wall of the digestive tract.

METHODS

Guinea-pigs, rabbits, cats, kittens, dogs and an old male monkey (*Macacus rhesus*) of 11·4 kg. were used. The guinea-pigs, rabbits and kittens were stunned by a blow on the neck, the head cut off and the trunk allowed to bleed from the severed carotid arteries. The cats and dogs were bled during ether-chloroform anaesthesia, the thorax was opened and the heart cut out. Immediately afterwards the small intestine and any other organ required were removed. In the experiment on the monkey the small intestine was removed without previous bleeding from the living animal under nembutal anaesthesia. The lumen of the intestine was washed out with tap water and the intestine afterwards kept in cold saline solution. In order to remove the mucus often adherent to the mucosa, pieces of intestine were slit open and the inner surface carefully cleaned by gently rubbing over it with a finger, keeping the preparation in ice-saline solution which was replaced several times during the cleaning. The necessity for this procedure became clear in the course of the experiments.

In cats and dogs, five layers of the small intestine were separated. Starting from the lumen they will be referred to as: (1) glandularis mucosa or gl. mucosa, (2) muscularis mucosa or m. mucosa, (3) submucosa, (4) circular muscle layer, and (5) longitudinal muscle layer (with serosa attached to it).

The first two layers represent the mucosa; its m. mucosa consists in these animals of a thick sheath of smooth muscle fibres. The term gl. mucosa refers to that part which is easily scraped off from the m. mucosa with a knife. The scraped-off tissue consisted mainly of the glandular tissue. Usually the scraping off was done from a fresh piece of intestine superficially dried between filter papers and the scraped-off material at once brought into ice-cold acetone. In a few experiments the whole piece of intestine was first immersed in ice-cold acetone, left there for a few minutes and the gl. mucosa then scraped off in the acetone.

The submucosa consists, in cats and dogs, of a tough membranous sheath of connective tissue which is easily removed *in toto*. Its dry weight amounts to about 15–20 % of that of the whole wall.

In order to separate the two layers of muscularis externa the piece of intestine was often slipped over a glass rod before the outer longitudinal muscle was stripped off with a needle according to the method described by Magnus (1920). The serosa was not removed and is therefore included in the layer termed longitudinal muscle.

The pieces of intestine or its respective layers were cut into fine pieces in ice-cold acetone and then ground in a mortar for final maceration with fresh acetone. The acetone was filtered off by suction. From the dried material saline extracts were prepared using 1·5 ml. for each 50 mg. dried tissue. The saline extracts were centrifuged and the supernatant fluid only used for incubation. The procedure for setting up a sample was as follows: 1·5 ml. saline extract, corresponding to 50 mg. dried tissue, were incubated for 1 hr. in a volume of 4·5 ml. The remaining 3 ml. consisted of 1·5 ml. of saline solution and 1·5 ml. of a solution containing the following constituents: 3 mg. choline, 4·5 mg. cysteine (solution made up immediately before use), 0·4 mg. ATP–P (corresponding to 8 mg. of the Ba–ATP which is converted to the sodium salt before use), 12 mg. Na-citrate, 3 mg. NaF, 6 mg. KCl, 4 mg. $MgCl_2$, 0·5 mg. eserine sulphate, 0·3 ml. standard phosphate buffer solution (pH 7–7·2). This method has been previously described (Feldberg & Mann, 1946).

For optimal synthesis of acetylcholine sufficient activator or coenzyme A must be present in the synthesizing medium. Samples were therefore frequently set up with additional activator prepared from acetone-dried tissue of rabbit or guinea-pig brain by extraction with saline solution (1·5 ml./50 mg. dried tissue), centrifuging the extract and boiling the supernatant fluid containing the activator. After filtration and cooling 1·5 ml. of the boiled extract were added to each sample,

instead of the 1·5 ml. of saline solution. The term activator, and not coenzyme A, is used throughout this paper because no purification was carried out.

In some experiments the acetylcholine content of the fresh intestinal tissue was determined. For this purpose the acetylcholine was extracted with HCl and then boiled. For each g. tissue about 2 ml. 1/3 N-HCl and 1 ml. phosphate buffer solution containing 0·1 mg. eserine sulphate were used. The tissue was ground with some sand in this mixture and then boiled for a short time. Before being assayed for acetylcholine it was cooled and neutralized.

The acetylcholine assay was carried out on the eserinized frog rectus muscle with the precautions necessary to eliminate the effects of substances present in the extracts which sensitize the frog rectus muscle to acetylcholine (Feldberg & Mann, 1945–6).

RESULTS

Choline acetylase was found to be present in the wall of the small intestine and in all its layers with the exception of the membranous submucosa. In the course of the experiments it soon became clear that the values obtained for some tissues might be too low on account of lack of activator or of presence of an inhibitor.

Lack of activator. This was responsible for the low values obtained with extracts of the muscularis externa, the longitudinal as well as the circular muscle layer. These extracts synthesized often two to three times more acetylcholine when additional activator had been added to the synthesizing medium. With extracts of the gl. and m. mucosa, the effect of additional activator was usually less pronounced. With extracts prepared from the whole wall the results varied according to species. Synthesis by extracts of rabbit's intestine was little if at all affected, whereas the synthesis by extracts of the wall of the small intestine of the other species increased somewhat by additional activator. This is seen from the results given in Table 1. In later experiments, if not otherwise stated, additional activator was therefore added to all samples.

Presence of an inhibitor. Hsu & Chang (1943) mention the presence of an inhibitor for the synthesis of acetylcholine in the fresh mucosa of the dog's small intestine. We found that the inhibitor is also present in extracts of the acetone-dried tissue but only of the gl. mucosa. This was seen when the extracts of acetone-dried mucosa of the dog's small intestine were examined separately and together. Gl. mucosa alone synthesized 2 μg./g., and m. mucosa alone 65 μg./g.; but m. mucosa synthesized only 2·5 μg./g. when incubated with an equal amount of gl. mucosa. Next the inhibiting effect of an extract of gl. mucosa was examined on extract of rabbit brain which synthesized 1025 μg./g./hr. acetylcholine. In the presence of an equal amount of gl. mucosa in the incubation medium this value was reduced to 105 μg./g./hr.

No inhibition was produced by the extracts from acetone-dried m. mucosa or muscularis externa. The inhibition observed with extracts from the whole wall must therefore originate from the gl. mucosa. In rabbits the inhibitor was found along the whole length of the small intestine, in guinea-pigs in the duodenum and jejunum only. The following figures are mean values of three

experiments (Exps. 6, 7 and 8 of Fig. 1) with guinea-pig's intestine. The saline extracts of the acetone-dried tissue of duodenum, upper jejunum, lower jejunum, upper ileum and lower ileum synthesized 193, 120, 130, 175 and 220 μg./g./hr. acetylcholine respectively. These extracts were, in addition, incubated together with extract of acetone-dried brain. Brain (1 g.) plus intestinal tissue (1 g.) synthesized 610, 780, 890, 1060 and 1030 μg. acetylcholine. If we subtract from

TABLE 1. Effect of additional activator on synthesis of acetylcholine in extracts from the wall of the small intestine. Figures represent μg. acetylcholine synthesized per g. acetone-dried tissue in 1 hr. without (*a*) and with (*b*) additional activator

	Guinea-pig		Rabbit		Monkey		Dog		Cat		Kitten	
Layers	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
Whole wall	170	200	73	103	20	35	80	125	35	50	40	48
	350	410	80	90	75	113					35	50
			100	95								
			100	97								
			100	110								
			113	125								
			126	125								
			126	150								
			133	155								
Muscularis externa		—		—	14	37	50	70		—		—
					35	95						
					30	60						
Longitudinal muscle		—		—		—	23	60	15	32		—
							25	53	25	35		
							45	100				
Circular muscle		—		—		—	3	15	20	35		—
							9	18	13	25		
							27	56				
Mucosa		—		—		—		—		—	31	25
M. mucosa		—		—	4	13	68	70	13	18		
							80	120	20	15		
									10	14		
Gl. mucosa		—		—	200	230	42	78	20	13		
							55	80	25	35		
							115	160	25	30		
							100	130				

these values those for the synthesis of the respective intestinal tissues we would obtain per g. brain alone a synthesis of 417, 660, 760, 850 and 840 μg. respectively. These figures have to be compared with 850 μg./g., the amount synthesized on incubation of brain extract alone. Thus only the extracts from the ileum did not inhibit the synthesis in brain extracts. In the presence of extract from duodenum, upper and lower jejunum it was depressed by 51, 28 and 11% respectively. In a similar single experiment with rabbit intestine (Exp. 6, fig. 2) the inhibition produced by the five corresponding extracts of intestinal tissue amounted to 55, 70, 55, 40 and 70 %.

The inhibitor was found to be heat sensitive. Extracts brought to boiling-point lost their inhibiting effect. For instance an extract from dog's gl. mucosa which reduced the synthesis of acetylcholine in rabbit's brain extract from

600 to 260 μg./g. increased it to 700 μg./g. after it had been boiled. The increase results from the presence of activator in gl. mucosa extracts, which only becomes evident after the inhibitor has been destroyed.

The strong heat-sensitive inhibitor would naturally interfere with the determination of choline acetylase activity in extracts of gl. mucosa, but it is possible to account for this factor by determining the inhibition produced by extracts of gl. mucosa on the synthesis of acetylcholine in brain extracts. For instance, in the previously mentioned experiment duodenal extract synthesized 193 μg./g. acetylcholine, but inhibited its synthesis in brain extract by 51%. Assuming a similar inhibition to have occurred in the duodenum extract itself the value of 193 μg./g. would represent about half the actual enzyme activity. It is not certain, however, if we are really justified in making this assumption.

TABLE 2. Inhibition by extracts of glandularis mucosa of the synthesis of acetylcholine in brain extracts

Animal and region of gl. mucosa	μg. acetylcholine synthesized in 1 hr. at 37° C. in extract from: 1 g. dried intestine	1 g. dried brain	1 g. dried intestine plus 1 g. dried brain	Percentage inhibition (−) or percentage augmentation (+)
Dog (duodenum)	90	940	840	−20
	115	940	880	−19
	145	1000	560	−58
	57	320	440	+13
	146	900	1050	0
	80	900	1050	+ 8
	60	670	1000	+40
	130	1300	1500	+ 5
Dog (jejunum)	55	940	1000	0
	55	940	950	− 5
	105	1000	770	−33
	37	320	460	+33
	120	900	1020	0
Dog (ileum)	50	940	770	−23
	110	940	880	−18
	125	1000	1070	− 5
	32	320	400	+15
	180	900	980	−11
Cat (duodenum)	35	1000	680	−35
Cat (ileum)	30	1000	850	−18

Fortunately, the inhibitor is not derived mainly from the tissue of the gl. mucosa, but from its secretion, part of which adheres to the mucosa in the form of mucus. When the surface of the mucosa is carefully cleaned so that the attached mucus is removed as completely as possible, the extracts of the acetone-dried gl. mucosa were found no longer to contain the inhibitor, or contained it in small amounts only which usually did not interfere greatly with the determination of choline acetylase activity. For instance, in only five of the twenty experiments of Table 2 was the depression of synthesis in brain extracts greater than 20 % when they were incubated with extracts of cleaned mucosa; in nine of the twenty experiments there was either no effect or an augmentation.

This augmentation is again explained by the presence of activator in gl. mucosa extracts.

It is thus possible to assess the synthesizing power of gl. mucosa when the necessary precautions are taken. This has been done in all later experiments. If appreciable amounts of inhibitor are, nevertheless, found in the extracts the values for the synthesis of acetylcholine have either to be discarded or the required corrections to be made.

A heat-sensitive inhibitor of the synthesis of acetylcholine has previously been described by Comline (1946) in acetone-dried spleen tissue. He assumes that the inhibitor is an enzyme which destroys the activator or the co-enzyme. As the inhibitor of the gl. mucosa is present in its secretion and heat sensitive it might also be an enzyme which could destroy the co-enzyme as well as the choline acetylase.

Species differences. The highest values were obtained with extract from the tissue of the guinea-pig's small intestine. In some experiments nearly 500 μg./g./hr. acetylcholine were formed. The usual values were between 150 and 300 μg./g./hr.; in a few experiments the values were even lower. With dog and rabbit small intestine the values were between 60 and 140 μg./g./hr. The intestinal wall of the one monkey examined synthesized in the ileum 135 and in the jejunum 35 μg./g./hr. acetylcholine. Both these extracts contained some inhibitor and in about equal amounts. Extracts from the small intestine of the few cats and kittens examined synthesized between 15 and 50 μg./g./hr. There was practically no inhibition of synthesis with the cat's extracts, but those of the kitten intestine inhibited the synthesis in brain extract by 15–35%.

TABLE 3. Comparison of content of acetylcholine and of choline-acetylase in the wall of the small intestine of different species

Species	μg. acetylcholine synthesized in 1 hr. per g. acetone-dried tissue	μg. acetylcholine contained in 1 g. fresh tissue
Cats	15–50	1·2–3·0
8-day kitten	48	8·0
11-day kitten	27	10·4
17-day kitten	32	7·1
Monkey (jejunum)	35	5·2
Monkey (ileum)	130	11·0
Dog	60–140	1·4–3·0
Rabbit	60–140	2·3–2·6
Guinea-pig	150–480	6–10

Comparison between content and synthesis of acetylcholine. The acetylcholine content is not always a reliable measure for the acetylcholine metabolism in the intestinal wall as seen from the results of Table 3. For instance, the results obtained with kitten intestine show that a high acetylcholine content may be associated with a low concentration of choline acetylase. On the other hand, a low content apparently can be taken as a sign of a low acetylcholine metabolism in the wall of the digestive tract.

Distribution of choline acetylase along the length of the small intestine in guinea-pigs and rabbits

The existence of a gradient in the concentration of choline acetylase in the wall of the small intestine would be of interest in connexion with the theory of Alvarez of a 'metabolic gradient' responsible for the unidirectional movements of the intestinal peristaltic waves. In a number of papers Alvarez and his co-workers (see Alvarez, 1940) claim to have found evidence for a metabolic gradient in the small intestine, but its existence is anything but proved. Since tone and movements of the muscle layers are probably under the influence of the continuously released acetylcholine, a relation of such a gradient with the acetylcholine metabolism would appear plausible. However, no such gradient was found for the choline-acetylase distribution along the length of the small intestine.

The guinea-pig small intestine has a thin wall and is usually less than 150 cm. long. The whole length has therefore frequently been used and cut into pieces of 20–30 cm. length which were dried separately with acetone. The rabbit small intestine is longer and its wall is thicker, so that sections from different regions only have been used. In Figs. 1 and 2 the distribution of choline-acetylase along the length of the small intestine is plotted. In order to obtain comparable curves the variations in length of the individual intestines are eliminated, the abscissae indicate the length of each piece of intestine and its distance from the pylorus not in cm. but in percentage of the total length of the small intestine. The actual length in cm. is given, however, for each curve. Similarly, in order to eliminate the variations in the synthesizing power of the individual intestines the ordinates refer to the synthesis as percentages of the highest values obtained for each experiment and not to the μg./g./hr. acetylcholine synthesized; but again the highest value for each experiment is given in this form.

In seven of the nine experiments of Fig. 1 with guinea-pig intestine the distribution curve of choline-acetylase shows a trough in the region of the jejunum; the maximal variations in these experiments amount to 51, 45, 51, 52, 51, 63 and 32% respectively. In other words, the tissue from both ends of the small intestine synthesized about twice as much acetylcholine as that obtained from the jejunum. Exps. 5 and 9 do not follow this pattern, but in Exp. 5 it is at least discernible.

The observed variations are greater than those inherent in the method. For instance, one guinea-pig small intestine was divided into three equal pieces which were put together end to end and cut again into six sections, each of which was dried separately with acetone. Extracts from these samples synthesized 285, 326, 330, 330 and 330 μg./hr./g. dried tissue of acetylcholine respectively. In another experiment the guinea-pig small intestine was cut

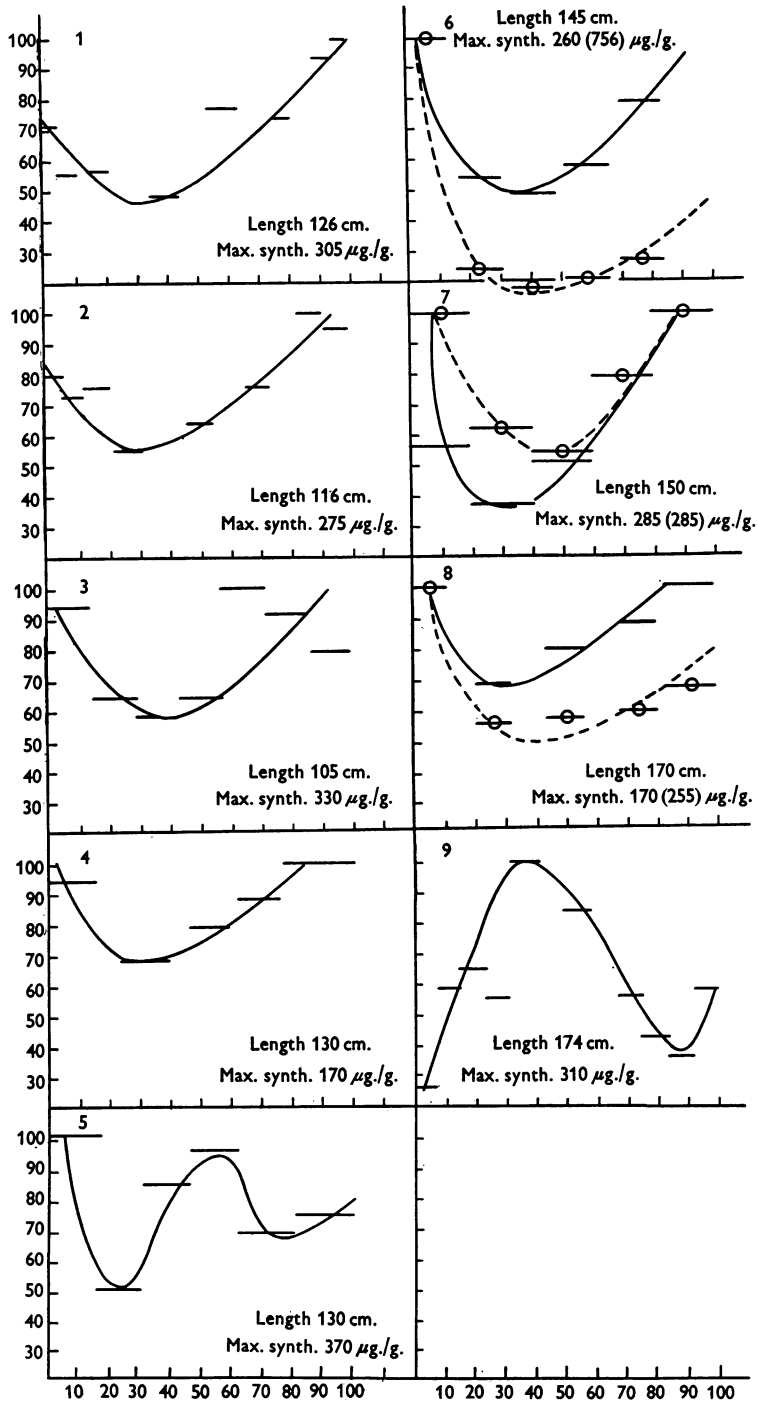

Fig. 1. Distribution of choline-acetylase along the length of guinea-pig small intestine. For details see text.

into sixty pieces which were distributed in six samples. Their values for the synthesis varied between 160 and 200 μg./g. acetylcholine.

The observed variations are also not accounted for by the presence of inhibitor in the extracts. In the dotted curves of Exps. 6–8 this factor was eliminated by using values which were corrected for the presence of inhibitor

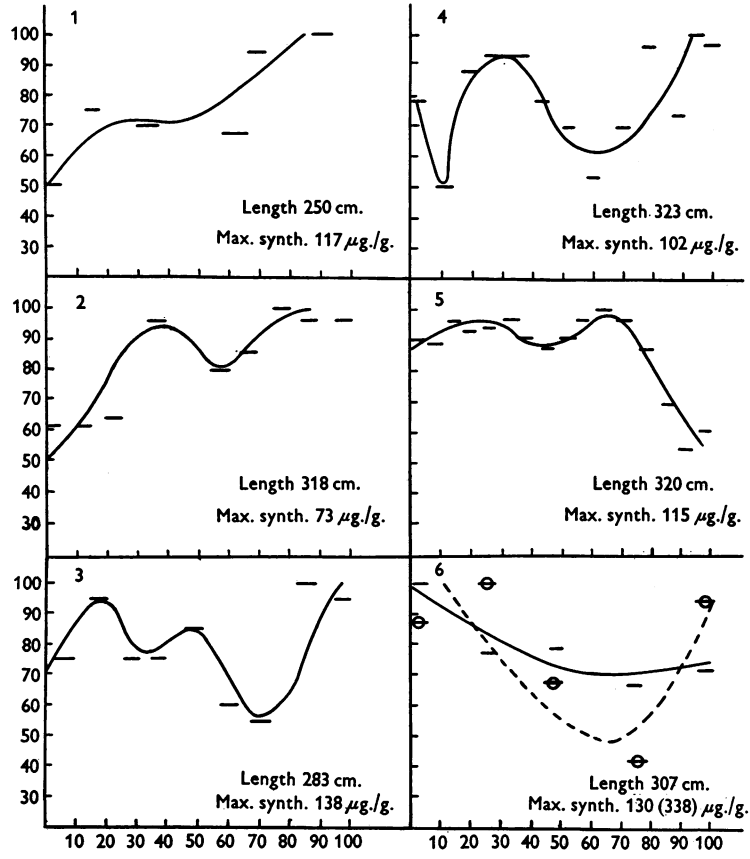

Fig. 2. Distribution of choline-acetylase along the length of rabbit small intestine. For details see text.

by the procedure already described. The shape of the curves remained unchanged. The values in brackets represent the highest corrected value in μg./g. acetylcholine synthesized.

The results from six rabbit small intestines, given in Fig. 2, show no uniform pattern; each experiment gives a different curve. But the dotted curve of Exp. 6, which was obtained with values corrected for the presence of inhibitor, shows a trough which is situated nearer the ileum than in the corresponding experiments with guinea-pig intestine.

Distribution of choline-acetylase in the different layers of the small intestine

Whether the layers were removed from a piece of intestine after it had been immersed in acetone for a few minutes, or from a fresh piece, a loss of choline-acetylase activity of about 10–20% always occurred. This was evident from the fact that extraction of the wall *in toto* gave a higher value than that calculated by addition of all the values obtained with the different layers and taking their thickness into account. The cause for this loss has not been ascertained.

In the rabbit and guinea-pig the separation of the various layers is not easy, but there is no difficulty in scraping off the inner layer which consists mainly of gl. mucosa and perhaps some of the thin m. mucosa. As seen from the results of Table 4 this scraped-off inner layer synthesizes acetylcholine. At the time of these experiments the presence of an inhibitor in mucosa extracts was not known. The finding that this layer had a lower ability to synthesize acetylcholine than the external muscular layer may therefore not represent the true distribution of choline-acetylase in the intestinal wall.

TABLE 4. Comparison of the synthesis of acetylcholine (μg./g./hr.) in acetone-dried mucosa and muscularis externa of guinea-pig and rabbit small intestine

	Guinea-pig					Rabbit		
Mucosa	75	80	160	180	210	20	21	52
Muscularis externa	100	110	370	360	480	50	80	55

In the dog the separation of the various layers is easy. Only the membranous submucosa was found to be devoid of choline-acetylase. The distribution of the enzyme in the remaining four layers is seen from the results of Table 5, in which the position along the intestine of each piece for which a value was obtained is given. The position of a value indicates that the tissue is from the region within the 10% length referred to; it does not mean that the whole 10% length has been used for the sample.

When no additional activator had been added to the samples, the external muscular layers showed extremely low values, whereas those for the other layers were of the same order as those obtained with additional activator. This fact has been already discussed. When additional activator had been added the main results were as follows:

(1) The highest values for choline-acetylase were found with extracts from gl. mucosa.

(2) Usually gl. mucosa from duodenum and lower ileum gave higher values than gl. mucosa from the rest of the intestine.

(3) M. mucosa synthesized per g. less acetylcholine than gl. mucosa from the same piece of intestine but as much as, and sometimes even more than, the two layers of the muscularis externa.

TABLE 5. Synthesis of acetylcholine (μg./g./hr.) in layers of dog small intestine. (G = gl. mucosa; M = m. mucosa; C = circular muscle layer; L = longitudinal muscle layer.)

		Length in percentage of small intestine									
		Duodenum									Ileum
Exp. no.	Layer	1–10	10–20	20–30	30–40	40–50	50–60	60–70	70–80	80–90	90–100
1	G	105	—	55	—	70	—	—	70	50	110
	M	20	—	35	—	40	—	—	63	33	70
	C	17	—	27	—	30	—	—	36	45	17
	L	83	—	65	—	48	—	—	80	65	80
2	G	145	—	—	—	—	87	—	—	—	125
	M	70	—	—	—	—	65	—	—	—	90
	C	87	—	—	—	—	55	—	—	40	40
	L	75	60	40	65	45	40	70	73	65	60
3	G	220	—	—	—	—	—	—	—	—	—
	M	75	—	—	—	—	—	—	—	—	—
	C	75	—	—	—	—	—	—	—	—	—
	L	120	85	80	90	70	—	—	80	105	105
4	G	80	—	—	120	—	—	—	—	180	—
	M	68	—	—	75	—	—	—	—	85	—
	C	80	—	—	70	—	—	—	—	100	—
	L	70	—	—	110	—	—	—	—	23	—
5	G	145	—	—	—	—	—	—	—	—	—
	M	65	—	—	—	—	—	—	—	—	—
	C	110	—	—	—	—	—	—	—	—	—
	L	42	—	—	—	—	—	—	—	—	—
6	G	60	—	—	—	—	—	—	—	—	—
	M	45	—	—	—	—	—	—	—	—	—
	C	30	—	—	—	—	—	—	—	—	—
	L	50	—	—	—	—	—	—	—	—	—
7	G	130	—	—	—	—	—	—	—	—	—
	M	120	—	—	—	—	—	—	—	—	—
	C+L	70	—	—	—	—	—	—	—	—	—
8	C	30	—	—	15	—	—	—	25	—	—
	L	95	—	—	53	—	—	—	50	—	—
9*	G	100	60	50	—	—	—	26	70	—	60
	M	62	45	52	—	—	—	35	53	—	53
	C	15	18	—	—	9	—	—	—	—	—
	L	8	8	—	—	10	—	—	—	—	—
10*	G	140	115	110	—	130	—	75	85	55	65
	M	80	68	95	—	120	—	140	95	115	90
11*	M	80	67	62	95	125	—	130	—	118	80
12*	M	50	38	35	48	—	—	42	—	35	52

* Without additional activator in the samples.

(4) The choline-acetylase of the muscularis externa was not confined to, and often not particularly concentrated in, the longitudinal muscle layer to which the nerve cells of the myenteric plexus are said to adhere when this layer is stripped off. In fourteen of nineteen experiments in which the two layers of the muscularis externa were examined separately from the same piece of intestine the longitudinal muscle synthesized per g. more acetylcholine than the circular muscle but in the other five experiments the reverse was true.

The table shows also that the individual variations of the values are so great that with the exception of the high concentration at the end parts of the gl.

mucosa there are no characteristic and regular differences between the various layers or the various regions of the small intestine. This is seen perhaps even more strikingly in Table 6, where the results are arranged in three columns, the two end parts and the rest of the small intestine, and where only the mean values are given. They are calculated from those intestinal pieces of Exps. 1–6 of Table 5 for which all four layers have been examined.

TABLE 6. Synthesis of acetylcholine (μg./g./hr.) in layers of small intestine of the dog. Mean values from Exps. 1 to 6 of Table 4

(G, M, C and L as in Table 4)

	Regions in percentage of length of small intestine		
Layers	Duodenum (1–10)	Jejunum-ileum (10–80)	Lower ileum (80–100)
G	125	60	114
M	57	56	70
C	67	43	50
L	73	49	57

TABLE 7. Contribution in mg. by the different layers to 1 g. of wall of the dog's small intestine

	Weight of layer in mg./g. wall			
Layers	Duodenum	Jejunum	Ileum	Lower ileum
Gl. mucosa	250	266	223	196
M. mucosa	425	318	283	262
Mucosa	675	584	506	458
Circular muscle	226	315	374	381
Longitudinal muscle	99	101	120	161
Muscularis externa	325	416	494	542

Since the thickness of the layers varies along the length of the intestine the foregoing results do not indicate the contribution each layer makes to the acetylcholine synthesizing power of the whole wall. In Table 7 the acetone-dried weight of each layer is given in mg./g. of wall which, however, does not include the membranous submucosa. It will be seen that the longitudinal muscle layer contributes least to the wall, although its thickness increases somewhat from duodenum to ileum; that the circular muscle is two to three times thicker than the longitudinal muscle; that the gl. and m. mucosa make up about two-thirds of the thickness of the wall in the duodenum but less than half in the ileum; and that this change is mainly accounted for by variations in the thickness of the m. mucosa. From the same samples which were used for calculation of Table 7, the mean values are given in Table 8 for the synthesis of acetylcholine. This enables us to assess the contribution of each layer to the acetylcholine synthesizing power of the whole wall.

TABLE 8. Distribution of choline acetylase in layers of dog small intestine

Layers	μg. acetylcholine synthesized per hr. per g. layer D	J	I	LI	μg. acetylcholine synthesized per hr. in layer per g. wall D	J	I	LI	Percentage distribution of choline acetylase in wall D	J	I	LI
Gl. mucosa	112	98	98	118	28·0	26·1	21·9	23·1	39	38	32	32
M. mucosa	53	59	57	80	22·5	18·8	16·1	21·0	31	28	23	28
Mucosa					50·5	44·9	38·0	44·1	70	66	55	60
Circular muscle	61	52	66	29	13·8	16·4	24·7	23·1	19	24	36	15
Longitudinal muscle	76	76	50	80	7·5	7·0	6·0	21·0	11	10	9	25
Muscularis externa					21·3	23·4	30·7	44·1	30	34	45	40
Whole wall					71·8	68·3	68·7	74·0				

(D = duodenum; J = jejunum; I = ileum; LI = lower ileum.)

The mucosa contributes about 70% in the duodenum and about 60% in the ileum to the choline-acetylase content of the wall. The gl. mucosa contributes slightly more than the m. mucosa and contains usually more choline-acetylase than any other layer, i.e. between 32 and 39% of the total amount although it represents only 20–25% of the tissue of the wall. The longitudinal muscle layer, despite its occasionally high choline-acetylase concentration, contributes least, i.e. only between 9 and 25%. A higher contribution, between 15 and 36%, is made by the much thicker circular muscle layer, although its choline-acetylase concentration is often lower than that of the longitudinal muscle layer.

In Exp. 7 of Table 5 the muscularis externa was not separated into its two layers. Leaving the membranous submucosa again out of the calculation, 1 g. acetone-dried wall consisted of 31% muscularis externa, 35% gl. mucosa and 34% m. mucosa. One gram wall synthesized per hr. 108 μg. acetylcholine, of which 21·7 μg. came from the muscularis externa, 40·8 from the m. mucosa and 45·5 μg. from the gl. mucosa. The mucosa thus contributed as much as 80% to the synthesizing power of the wall.

Two experiments on cats were carried out (see Table 9). Although the choline-acetylase concentration in the whole wall is much lower than in the dog's intestine its distribution in the different layers shows the same trend. Again, the two layers of the mucosa contribute between 60 and 70% to the choline-acetylase content of the wall and the longitudinal muscle layer contributes least. This muscle in cats is about six to seven times thinner than the circular muscle, so that even in the first experiment in which the longitudinal muscle synthesizes per g. tissue over six times as much acetylcholine as the circular muscle, its contribution is less than that of the circular muscle layer.

In the two kittens examined the mucosa and muscularis externa, as far as these layers could be separated by scraping off the inner layer, synthesized about the same amounts of acetylcholine per g. acetone-dried tissue. The scraped-off mucosa made up between 30 and 40% of the total wall.

TABLE 9. Synthesis of acetylcholine in layers of cat small intestine

Exp. and region	Layer	Percentage contribution of layer to wall	μg. acetylcholine synthesized in 1 hr.	
			Per g. layer	Per g. wall
Exp. 1 (jejunum)	Gl. mucosa	18	20	3·6
	M. mucosa	24	18	4·3
	Circular muscle	51	5	2·6
	Longitudinal muscle	7	32	2·2
Exp. 2 (duodenum)	Gl. mucosa	30	35	10·5
	M. mucosa	36	20	7·2
	Circular muscle	31	35	10·9
	Longitudinal muscle	3	35	1·1
Exp. 3 (ileum)	Gl. mucosa	32	30	9·6
	M. mucosa	27	13	3·5
	Muscularis externa	41	14	5·7

In the monkey the different layers were examined in two pieces of ileum using slightly different procedures for each piece. From the one both layers of mucosa were scraped together in acetone and dried separately from the remaining tissue (submucosa *plus* muscularis externa). From the other piece the gl. mucosa was scraped off and dried separately before the preparation was placed in acetone. The m. mucosa was then scraped off and also dried separately. The results, which are given in Table 10, show that the mucosa contained about 80% of the choline-acetylase of the wall, and that it was mainly localized in the gl. mucosa in which the concentration reaches the highest level.

TABLE 10. Synthesis of acetylcholine in layers of monkey ileum

Layer	Percentage contribution of layer to wall	μg. acetylcholine synthesized in 1 hr.			
		Per g. layer		Per g. wall	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2
Mucosa	66	125	—	82·5	—
Gl. mucosa	35	—	230	—	80·5
M. mucosa	31	—	13	—	4·0
M. externa	34	65	37	22·1	12·6

Distribution of choline-acetylase in other tissues

In the rabbit no choline-acetylase was detected in the acetone-dried tissue of the oesophagus, the thin wall of the caecum or the urinary bladder.

In the guinea-pig the enzyme was absent in the tissue of the oesophagus and uterus. The one urinary bladder examined synthesized 40 μg./g./hr. acetylcholine as compared to 360 μg./g./hr. (the highest value) for the small intestine in this animal. Low values were obtained for the stomach and caecum, whereas the values for the colon were of the same order as those for the small intestine. Table 11 gives the results of four experiments.

In one experiment pieces from the cardia, fundus and pyloric region of the stomach were examined separately. The inner layer was scraped off in acetone

and dried separately. It consisted mainly of gl. mucosa and m. mucosa, although it was not possible with this method to give a very sharp separation. The outer layer, however, consisted mainly of muscularis externa. The same procedure was adopted for pieces of the duodenum, caecum and colon. As seen from the results given in Table 12, both layers synthesized acetylcholine, and, with the exception of the colon, contained the enzyme in about equal concentrations.

TABLE 11. Synthesis of acetylcholine in different parts of guinea-pig digestive tract

Tissue	μg./g./hr. acetylcholine synthesized in saline extracts of acetone-dried tissue			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Oesophagus	0	0	0	—
Stomach	50	—	29	51
Small intestine (highest value)	360	460	86	—
Caecum	90	—	19	—
Colon	320	310	98	—

TABLE 12. Synthesis of acetylcholine in μg./hr./g. acetone-dried tissue and per square cm. area of wall of guinea-pig digestive tract

	Acetylcholine (μg./hr./g.)		mg. acetone-dried tissue per cm.² wall		Acetylcholine (μg./hr./cm.²)	
	Mucosa	Musc. ext.	Mucosa	Musc. ext.	Mucosa	Musc. ext.
Oesophagus	0	0	5·3	3·2	0	0
Stomach (cardia)	20	16	12·3	2·0	0·246	0·032
Stomach (fundus)	33	27	12·0	2·4	0·396	0·065
Stomach (pyloric)	30	50	10·8	6·1	0·324	0·305
Duodenum	75	110	7·6	3·2	0·570	0·352
Caecum	24	18	2·3	8·6	0·055	0·155
Colon	140	70	2·8	4·3	0·392	0·301

In the colon its concentration in the inner layer, the mucosa, was double that in the outer layer. The synthesis of acetylcholine has also been expressed so as to show the contribution each layer makes to the choline-acetylase content of the whole wall. With the exception of the caecum the mucosa contributes more than the muscularis externa. The greatest differences in the two layers are found in the cardia and fundus of the stomach and in the caecum; they result from the great variation in thickness of the two layers in these regions.

In the dog some choline-acetylase is present in the wall of the oesophagus, more is present in the wall of the stomach and large intestine but not as much as in the wall of the small intestine. The distribution of choline-acetylase in the different layers is shown in Table 13 which gives mean values from three experiments. In assessing these results it has to be realized that it is not possible to separate in the stomach the glandular tissue from the m. mucosa as completely as in the small intestine. The term gl. mucosa refers, with regard to the stomach, only to a superficial layer of mucosa which can be easily scraped off and which in the fundus consisted of 13–20 and in the pyloric region of 25–30% of the whole mucosa. It was also not possible to obtain a clear separation of the longitudinal and circular muscle layer from the stomach and large intestine.

For these regions the terms longitudinal and circular muscle refer to outer and inner parts of the muscularis mucosa only. In spite of these limitations, however, the results of Table 13 show clearly that the wall of the stomach and large intestine synthesize less acetylcholine than does that of the small intestine, and that in the stomach and large intestine, unlike in the small intestine, the enzyme is not concentrated in the glandular layer of the mucosa. In fact, the superficial layer of the stomach mucosa is almost devoid of the enzyme and the gl. mucosa of the large intestine contains small amounts only.

TABLE 13. Synthesis of acetylcholine in layers of dog digestive tract

(G = gl. mucosa; M = m. mucosa; C = circular muscle layer; L = longitudinal muscle layer; W = whole wall.)

	Synthesis of acetylcholine in μg./hr./g. acetone-dried layer								
	G	M	C	L	W	G	M	C	L
Oesophagus	8·5		10		9·4	3·8		5·6	
Stomach (fundus)	3·3	66	63	13	53·0	0*	18	23	12
Stomach (pylorus)	1·5	5	43	55	26·0	0*	2	17	7
Small intestine	112·0	62	70	67	87·0	34	24	22	7
Large intestine	18·0	22	68	39	49·0	3	7	29	10

* Less than 0·3 μg.

Comparison of choline-acetylase concentration and density of nerve cells in the muscularis externa of the guinea-pig digestive tract

When the longitudinal muscle layer is stripped off from the different parts of the digestive tract of a guinea-pig the nerve cells of the myenteric plexus adhere to this layer in the oesophagus and stomach but to the circular muscle layer in the small and large intestine, as shown by Irwin (1931). By means of appropriate sectioning and staining methods Irwin was able to count the number of these nerve cells fairly accurately along the whole length of the alimentary tract. In Table 14 his figures are given and compared with the

TABLE 14. Comparison of acetylcholine synthesis and number of nerve cells per sq.cm. of muscularis externa in the digestive tract of the guinea-pig

	Acetylcholine (μg./hr./cm.²)	Nerve cells per cm.²	Ratios	
			Synthesis	Nerve cells
Oesophagus	0·000	1350	0	0·4
Stomach (cardia)	0·032	3500	1·0	1·0
Stomach (fundus)	0·065	7000	2·0	2·0
Stomach (pyloric)	0·305	18000	9·5	5·1
Duodenum	0·352	10000	11·0	2·9
Ileum	0·440	7500	13·8	2·1
Caecum	0·155	4500	4·8	1·3
Colon	0·304	18000	9·5	5·1

values obtained in the experiment of Table 12 for the acetylcholine synthesis of the muscularis externa. Taking the figures obtained for the cardiac region of

the stomach as 1, the ratios for the synthesis of acetylcholine can be compared with those for the number of nerve cells. This has been done in the last two columns of Table 14. The ratios show striking discrepancies as well as similarities. A doubling of nerve cells in the ileum is associated with a nearly fourteen-fold increase in synthesizing power; a three-fold increase in number of nerve cells in the duodenum with an eleven-fold increase in synthesizing power; and a five-fold increase in nerve cells in the pyloric part of the stomach and in the colon with a nine-and-a-half-fold increase in synthesizing power. On the other hand, there is some parallelism. The synthesizing power and the number of nerve cells increase from oesophagus to the pyloric part of the stomach, whereas the caecum with its relatively low power of synthesis shows on comparison an even lower ratio for the number of nerve cells.

DISCUSSION

Site of acetylcholine metabolism. The distribution of choline-acetylase in the various layers of the intestinal wall provides new evidence in favour of the assumption that the nerve cells of the myenteric and submucous plexus are not the site of the acetylcholine metabolism. In dogs and cats the gl. mucosa yielded very high values for the synthesis of acetylcholine, in fact often higher values than those from any other layer of the intestinal wall; but the gl. mucosa contains no nerve cells, since the cells of the submucous plexus are situated in the submucosa. We do not know if they adhere to the membranous submucosa or to the lower surface of the m. mucosa when this layer is stripped off: if to the submucosa, the absence of choline-acetylase in this layer and its presence in the m. mucosa would be further proof that the enzyme is not located in the nerve cells.

In dogs and cats the cells of the myenteric plexus are said to adhere to the longitudinal muscle when it is stripped off from the circular muscle layer. Dikshit (1938), in fact, had found that slices from longitudinal muscle *plus* serosa synthesized per g. tissue six to ten times as much acetylcholine as slices from circular muscle. He therefore looked upon the cells of the myenteric plexus as the site of the acetylcholine metabolism. With our method of synthesis the differences were smaller. Per g. tissue, synthesis by the longitudinal muscle layer rarely exceeded three times that of the circular muscle; usually the differences were less than 50% and in some experiments circular muscle tissue actually synthesized more than that of the longitudinal muscle. In addition, this layer is much thinner so that, per unit area intestinal wall, it contained usually less choline-acetylase than the circular muscle layer. It is then difficult to see how the cells of the myenteric plexus can be the sole or even the main site of the acetylcholine metabolism in the muscularis externa.

The answer is not so clear with regard to the experiments in which a comparison was made between the number of cells of the myenteric plexus and the

concentration of choline-acetylase per unit area muscularis externa of the guinea-pig intestine. In the stomach both tissue constituents increased to the same extent from cardia to pylorus. There was also a certain agreement in the changes which occurred with the two constituents from small intestine to caecum and colon. They were found to be low in the caecum but high in the colon and small intestine; on a more quantitative basis, however, the parallelism broke down. A doubling in the number of nerve cells was associated in one instance with a two-fold, in another with a fourteen-fold increase in choline-acetylase concentration.

In evaluating these results we must remember, however, that the values for the number of nerve cells and for the content of choline-acetylase were obtained by different authors; the measurements of the areas may therefore not be comparable in the two sets of experiments. Nevertheless, some of the discrepancies were too great to be accounted for in this way. Some correlation between density of nerve cells and concentration of choline-acetylase in the different regions is to be expected, even if the nerve cells are not the site of the acetylcholine metabolism, because both cell constituents are probably associated with motor activity: the nerve cells with the peristaltic reflex, the acetylcholine metabolism with maintenance of tone and rhythmic movements.

Even if the nerve cells are not the site of the acetylcholine metabolism this does not exclude nervous structures in general. The choline-acetylase of cholinergic nerves is, as far as we know, located in the axon and not necessarily in the cell body; therefore regions with an excessive supply of non-myelinated cholinergic nerve fibres should be particularly rich in choline acetylase. Quantitative figures on the number of nerve cells are no precise indication of the density of nerve fibres, although regions rich in nerve cells are probably also rich in non-myelinated nerve fibres. So far as this is the case, the distribution of the choline-acetylase in the various layers does not suggest an intimate association with nerve fibres.

If we assume a non-nervous origin of the acetylcholine metabolism in the intestinal wall it is probably closely linked to the choline metabolism, the choline in turn being derived from the large amounts of lecithin present. On this assumption the high content of choline-acetylase in the gl. mucosa becomes understandable, since this layer must be particularly rich in lipins. There are previous findings which stress the preponderant role of the mucosa in choline and acetylcholine metabolism, although hitherto no distinction has been made between glandular layer and m. mucosa. According to Abderhalden & Paffrath (1925) the choline output from the mucosa of the cat intestine is ten times greater per g. tissue than from the muscularis externa. Feldberg & Solandt (1942) later found that from the perfused rabbit small intestine more choline oozes out into its lumen, that is, from the mucosa, than was obtained from its venous effluent. Dikshit (1938) observed that, per g. tissue, the mucosa of the

cat and dog small intestine synthesized about 50% more acetylcholine than the muscularis externa with serosa attached to it.

An acetylcholine metabolism of non-nervous origin is not confined to the digestive tract. It certainly occurs in the human placenta, has been suggested for the auricle (Abdon, 1945; Burn & Vane, 1949; Bülbring & Burn 1949) and may be responsible for the high content of acetylcholine found in the cornea of several animals (v. Brücke, Hellauer & Umrath, 1949).

If nervous elements are not the main site of the acetylcholine metabolism the problem naturally arises: What are the structures containing the enzyme? The problem is as unsolved as that of the origin of the different hormones in the gastric and intestinal mucosa. The fact that the thin wall of the guinea-pig small intestine is so much richer in choline-acetylase than that of the other animals examined, suggests perhaps that the enzyme is not present in the smooth muscle fibres or in the gland cells. But it is dangerous to speculate about this problem without further experimental evidence. We have to realize that similar species differences are found for the choline-acetylase content in brain (Feldberg & Mann, 1946) and a different proportion of supporting tissue does not appear to be its sole explanation.

Even if the spontaneous release of acetylcholine is not associated with nervous structures some of the choline acetylase in the intestinal wall must be nervous in origin because of its cholinergic parasympathetic nerve supply. The extent of this contribution may vary in the different layers and sections of the intestine. Evidence for a nervous participation is found in the increased output of acetylcholine during vagus stimulation from the stomach (Dale & Feldberg, 1934) and small intestine (Bunting, Meek & Maaske, 1935). A nervous component may also account for the particularly high concentration of choline-acetylase in the gl. mucosa of the duodenum because the parasympathetic innervation to the secretory cells appears to be confined to this part of the intestine. Wright *et al.* (1940) showed that vagus stimulation augments secretion in the duodenum but not in the jejunum and ileum.

Function of acetylcholine metabolism. It is reasonable to conclude that the choline acetylase in the various layers is responsible for the continuous release of acetylcholine which occurs in the wall of the digestive tract. The fact that with the exception of the membranous submucosa all layers of the wall of the small intestine contain the choline acetylase suggests different physiological functions of the enzyme. From its presence alone no specific function could be deducted. We require the supporting evidence of the actions of acetylcholine, eserine and atropine on the different layers of the intestinal wall. A special physiological function, if attributed to the acetylcholine metabolism in the intestinal wall, should be imitated by acetylcholine, intensified by eserine and abolished by atropine. These considerations have to be kept in mind when discussing the function of the acetylcholine metabolism in the layers of the intestinal wall.

In the *muscularis externa* the choline-acetylase is associated with motor activity, but that does not necessarily mean with the peristaltic reflex which is a reaction of the intestinal wall to increased pressure. It is certain, however, that increased motility of the smooth muscles will facilitate the reflex. The acetylcholine continuously released in the muscularis externa presumably acts in this way and thus provides the necessary background of tone and rhythmic activity in the muscle, necessary for initiation of the reflex. This assumption is supported by the fact that acetylcholine as well as eserine, on the isolated preparation, do not produce real peristalsis but increased tone and rhythmic contractions. In our previous experiments (Feldberg & Lin, 1949*b*) on the isolated rabbit intestine, the peristaltic reflex could often only be initiated regularly by the increased pressure when the bath fluid contained a low concentration of eserine, thus allowing a higher acetylcholine concentration to develop in the intestinal wall and thereby providing a more advantageous background of increased tone and rhythmic activity.

In the *muscularis mucosa* the presence of choline-acetylase must also be associated with motor activity. This muscle layer plays apparently a much greater role in the propulsion of stomach and intestinal contents than has formerly been assumed (Forssel, 1923; Cole, 1928; Barclay, 1933; Gordon & Singleton, 1939). If the choline-acetylase in the muscularis mucosa were responsible for a continuous release of acetylcholine in this layer, thereby producing motor activity, acetylcholine and eserine should have stimulating actions on this muscle as they have on the muscularis externa. Recently, Holton (1949) has shown that a preparation of dog small intestine from which the muscularis externa had been stripped off contracts to eserine and acetylcholine.

The presence of choline acetylase in the *glandular mucosa* may be associated with motor and secretory functions and possibly even with absorption. Muscle fibres of the m. mucosa extend into the tips of the villi, and of necessity are extracted together with the gland cells when the gl. mucosa is scraped off and dried in acetone. The following results suggest that the choline-acetylase in the mucosa may be associated with the movements of the villi. An intravenous injection of acetylcholine causes a single quick and tonic contraction of the villi (Beznak, 1936) and eserine injected intravenously or applied locally initiates automatic movements of the villi which continue for a long time (Verzar & v. Kokas, 1927). Atropine, on the other hand, inhibits the movements of the villi (Hambleton, 1914; King & Arnold, 1922). Acetylcholine, as well as eserine, provokes also strong secretion of succus entericus in the small intestine and even desquamation of the epithelium (Verzar & v. Kokas, 1927; Wright *et al.* 1940). These actions are abolished by atropine. It is therefore probable that the choline-acetylase and the continuous release of acetylcholine in the gl. mucosa provides a physiological stimulus for continuous

secretion of succus entericus. In this connexion it is interesting to note that eserine does not produce gastric secretion and that the superficial layers of the gastric mucosa were found to be free from choline acetylase. Its presence in the stomach mucosa, therefore, appears to be solely connected with motor activity of its strong m. mucosa. Wright, Jennings & Florey (1938) were also unable to provoke secretion from the cat colon with eserine which, however, augmented the secretory response to stimulation of the nervi erigentes. Again, relatively little choline-acetylase was found to be present in the gl. mucosa of the dog large intestine. There is thus some parallelism between the effect of eserine and concentration of choline-acetylase in the gl. mucosa in different sections of the digestive tract.

There remains the possibility that the acetylcholine release plays a role also in absorption. According to Fraser (1946) choline facilitates fat absorption, and Beznak (1936) observed that acetylcholine is a strong stimulus for increased lymph flow from the thoracic duct. This effect was attributed to vaso-dilatation in the pre-capillary arteries and capillaries of the villi, and possibly to increased permeability of the capillary wall. Such vascular changes may be required for absorption.

SUMMARY

1. Synthesis of acetylcholine has been studied in saline extracts of acetone-dried tissue of the wall of the digestive tract with the object of obtaining information about the origin and function of the acetylcholine metabolism in this organ.

2. Per g. acetone-dried tissue the wall of the small intestine synthesized the following amounts of acetylcholine in μg./hr.: guinea-pig, 150–480; rabbit and dog, 60–140; cat and kitten, 15–50; monkey, 35–135.

3. A high acetylcholine content of the wall is not necessarily a sign of a great concentration of choline-acetylase. Kitten small intestine contained as much acetylcholine per g. tissue as that of the guinea-pig.

4. With the exception of the membranous submucosa all layers of the wall of the small intestine contained choline-acetylase although in varying concentrations. This was shown in the cat and dog.

5. A heat-sensitive inhibitor for the synthesis of acetylcholine was found in the acetone extracts of mucosa, derived probably from the mucus adherent to the mucosa. When its surface was carefully cleaned before drying the inhibitory effect was small.

6. The layer of the mucosa on top of the muscularis mucosa, the glandular mucosa, usually gave the highest values for synthesis of acetylcholine, particularly the gl. mucosa from the duodenum and lower ileum. This layer is supposed to be free from nerve cells.

7. Per g. acetone-dried tissue muscularis mucosa synthesized less acetylcholine than gl. mucosa but as much as, and sometimes even more, than the two layers of muscularis externa.

8. The choline-acetylase of the muscularis externa was not confined to, and often not particularly concentrated in, the layer of the longitudinal muscle to which the nerve cells of the myenteric plexus are stated to adhere when this layer is stripped from the circular muscle.

9. The concentration of choline-acetylase in the various layers does not indicate the contribution each layer makes to the acetylcholine-synthesizing power of the whole wall because the thickness of the layers differs and varies along the length of the small intestine. If this factor be taken into account, the two layers of mucosa are shown to contribute about 60–80% to the choline-acetylase content of the wall. The gl. mucosa contributed usually more than any other layer, up to 40%. The thin longitudinal muscle layer, despite its occasionally high choline-acetylase concentration, contributed least, between 9 and 25%.

10. No choline-acetylase was detected in rabbit oesophagus, caecum and urinary bladder or in guinea-pig oesophagus and uterus. Low values were found in guinea-pig stomach, caecum and urinary bladder, and in dog oesophagus, stomach and large intestine. The values for guinea-pig colon, on the other hand, were as high as those for its small intestine.

11. Unlike the small intestine, the stomach and large intestine of the dog contain, in the superficial layer of its mucosa, little or no choline-acetylase.

12. A comparison of choline-acetylase concentration and density of nerve cells in the muscularis externa of guinea-pig alimentary tract was made. The concentration of both tissue constituents changes in the same direction in the different sections, but on a more quantitative basis the parallelism breaks down.

13. The distribution of choline-acetylase in the different parts and layers of the wall of the digestive tract provides further evidence in favour of the theory that the enzyme does not originate mainly from the cells or nerve fibres of the myenteric and submucous plexus, but, like the choline-acetylase of human placenta, is non-nervous in origin.

14. The presence of choline-acetylase of non-nervous origin is probably the basis for the continuous release of acetylcholine in the various layers of the wall of the digestive tract. The possible functions of this acetylcholine metabolism are discussed, taking into consideration the known actions of acetylcholine, eserine and atropine on the various layers. It is concluded that the continuous release of acetylcholine in the muscularis externa provides a background of tone and rhythmic movements of the muscles necessary for initiation of the peristaltic reflex by increased pressure from the lumen. In the mucosa the continuous release of acetylcholine also exerts motor functions. It may stimulate the whole sheath of the muscularis mucosa as well as provide a stimulus for movements of villi. In addition, it probably represents a physiological stimulus for continuous secretion of succus entericus. It may possibly also play a role in absorption.

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