J. Physiol. (1951) 113, 483–505

EFFECTS OF GANGLION-BLOCKING SUBSTANCES ON THE SMALL INTESTINE

By W. FELDBERG

From the National Institute for Medical Research, Mill Hill, London, N.W. 7

(*Received* 23 *September* 1950)

In 1946 Ambache suggested that a number of substances which contract the intestinal preparation owe this effect, in part, to stimulation of the nervous structures of the myenteric plexus in the intestinal wall, i.e. to stimulation of nerve cells or nerve fibres. Emmelin & Feldberg (1947) were unable to confirm some of his findings; moreover, they argued that his interpretation was in some places faulty. The question was recently reopened by Ambache (1949), who reaffirmed his original conclusions and considered the arguments brought forward against his view to be invalid, because he was able to show that some of the substances used as controls by Emmelin & Feldberg (i.e. thought to be pure muscle stimulating substances on the intestine) stimulated the cells of the superior cervical ganglion in the cat. A re-examination of the problem was therefore indicated.

Ambache (1949) questions whether there is any substance which acts on the intestine purely by stimulating smooth muscle, and suggests that those substances commonly believed to act only in this way also have an action on the nervous apparatus in the intestinal wall. If he is correct it would be very difficult to devise convincing control experiments or to assess the mode of action of any drug on the intestinal wall. The position is not as hopeless as that. It will be shown that the histamine response of the intestinal wall is accounted for solely by its smooth muscle fibre stimulating action, and not at all by release of acetylcholine from nerve fibres excited at their endings. The latter mode of action was suggested by Ambache as being analogous to the action of histamine on the sensory nerve endings in human skin, whereby it elicits the axon reflex responsible for the red flare of the triple response.

However, if histamine acts in the intestinal wall purely as a muscle stimulating substance, two further observations made by Ambache become invalid as evidence for a nervous component of a drug action on the intestine, since both were also obtained with histamine: these are the potentiation of a drug response by eserine and the accelerating effect of a drug on the synthesis of acetylcholine in the intestinal wall.

Emmelin & Feldberg ascribed the potentiation of a drug response by eserine to summation of the response with the effect of the accumulating acetylcholine. This explanation still remains the only satisfactory one to explain the potentiation of the 'non-nervous' histamine response. Ambache found that the synthesis of acetylcholine in pieces of intestine incubated with eserine is accelerated by histamine and $BaCl_2$. There is no necessity to assume that a substance which accelerates the synthesis of acetylcholine under the conditions of Ambache's experiment, must act on the nervous apparatus, particularly since it is not certain that the enzyme responsible for the synthesis, choline acetylase, is mainly located in the nervous elements in the intestinal wall (Feldberg & Lin, 1949*b*, 1950). Furthermore, substances like KCl accelerate synthesis of acetylcholine, even in saline extracts of acetone-dried tissue. Apart from the fact that there is no cogent reason why substances acting on the nervous apparatus should affect the synthesis, Emmelin & Feldberg failed to confirm Ambache's observation, a failure attributed by him to deviation from his experimental procedure. This question also had to be re-examined.

Ambache had further supported his theory by showing that prolonged cooling of a piece of intestine, thereby destroying its nervous elements, reduced its sensitivity to various drugs. Emmelin & Feldberg were able to confirm this result for barium, and, to a certain extent, for potassium; they emphasized the need for more evidence before such observations could be regarded as proof of a ganglion stimulating action of these ions on the intestine.

There is one obvious method by which it can be convincingly established that a substance has a ganglion-stimulating action on the intestine, namely by showing that its action is reduced or abolished when the ganglion cells are paralysed or put out of action in some other way. The changes produced by cooling the intestine or treating it with large, paralysing doses of nicotine are difficult to interpret, because these procedures cause additional, unspecific changes in the sensitivity of the intestine. However, a number of substances are now available, which are known to paralyse autonomic ganglia. They have been examined to find out how far they can be used as specific ganglionic inhibitors on the intestine. Of the substances used, D-tubocurarine, tetraethylammonium and hexamethonium, the last proved suitable for this purpose. Its strong paralysing action on autonomic ganglia has been described by Paton & Zaimis (1949).

METHODS

Guinea-pig's and rabbit's ileum preparations were set up in a 15 ml. bath at 34–35° C. with oxygen bubbling through the bath. The bath fluid contained per litre 9 g. NaCl, 0·42 g. KCl, 0·24 g. $CaCl_2$, 1 g. dextrose, 0·5 g. $NaHCO_3$, and 0·005 g. $MgCl_2$. The changes in length of the preparation were recorded with a frontal writing lever (magnification about 3:1) and, in addition, observed through the transparent walls of the tank with the naked eye. The substances employed to make the intestine contract were always used in doses which produced submaximal contractions. They were usually kept in the bath for 30 sec., then washed out and, if not otherwise stated, given at

3–4 min. intervals. The ganglionic inhibitors, cocaine and paralysing doses of nicotine, were allowed to remain in the bath for longer periods (up to 20 min.). When the bath fluid was changed during this time, for instance, when testing a drug during this condition, they were again added at once to the refilled bath.

All values for histamine refer to the base, the phosphate salt being used. The values for acetylcholine, choline, acetyl-β-methylcholine, pilocarpine, refer to the chloride, those for nicotine to the hydrotartrate, and those for eserine to the sulphate.

Synthesis of acetylcholine. Two experiments were performed with barium. Care was taken to follow the description given by Ambache (1946). For each experiment two guinea-pigs were killed by a blow on the neck and bled. The small intestines were removed, rinsed through the lumina with bicarbonate-free Locke solution, and divided into strips of 13–14 cm. length, which were distributed alternately between two beakers. In addition a few cm. length of both intestines were used to determine the acetylcholine content of the intestinal tissue. The intestinal strips were slit open longitudinally, again washed, dried between filter-paper, weighed, and then transferred into two wide test-tubes containing 6 ml. bicarbonate-free Locke solution and 0·2 ml. eserine sulphate 1 in 200. The tubes were incubated for 40 min. in a water bath at 37° C., oxygen bubbling through the samples. To one sample 2 mg. barium chloride was added at the beginning, and at 10 min. intervals thereafter, 0·05 ml. of a 1 % solution of $BaCl_2$, $2H_2O$. After incubation, synthesis was stopped by acidifying the samples with N/3-HCl and boiling them; immediately before boiling, 2 mg. barium was added to the barium-free sample, and 0·1 ml. 1 % $NaSO_4$ added to both in order to precipitate the barium. The assay of acetylcholine was carried out on the frog rectus muscle.

RESULTS

Experiments on the guinea-pig's ileum preparation

Histamine after cocaine

If the histamine contractions of the guinea-pig's intestine were to result in part from stimulation of nerve endings in the intestinal wall, they should be reduced by concentrations of cocaine which paralyse the nervous structures in the intestinal wall. Feldberg & Lin have shown that this is not the case. Cocaine has several actions on the intestine. In weak concentrations it may stimulate the gut, and render it more sensitive to histamine and acetylcholine; the nervous structures may already be paralysed; usually, however, slightly stronger concentrations are required for this purpose. When this happens the contractions to nicotine are abolished, but histamine retains its full effect, or may even produce a stronger contraction (Fig. 1*a*, *b*). A further increase in the concentration of cocaine depresses the muscle fibres as well. At this stage the histamine response is also depressed and this depression increases with increasing concentrations of cocaine. As seen from Fig. 1*c*, the concentrations of cocaine required for this action are not much greater than those required for paralysis of the nervous elements. When paralysing concentrations of cocaine are washed out there often follows a period in which the histamine responses are increased before the excitability of the intestine returns to normal (Fig. 1*c*). This effect corresponds to the increased excitability observed in the presence of weak concentrations of cocaine in the bath.

The fact that cocaine concentrations, which paralyse the nervous structures,

do not reduce the histamine response is evidence against a nervous component in the action of histamine on the intestine. The histamine contractions on the intestine can thus be regarded as purely muscle-stimulating effects.

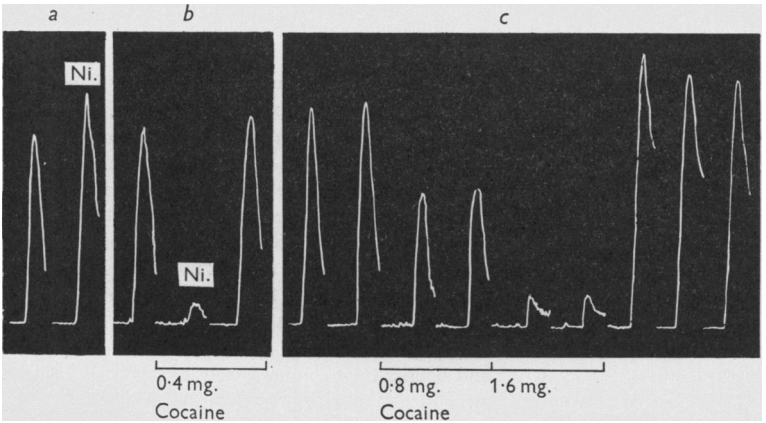

Fig. 1. Guinea-pig's ileum preparation in 15 ml. bath. Effect of different concentrations of cocaine on the response to 0·1 μg. histamine (unmarked) and to 30 μg. nicotine (Ni).

Nicotine paralysis

After large paralysing doses of nicotine, Ambache found the sensitivity of the intestinal preparation to acetylcholine so greatly diminished that a previously active dose of acetylcholine no longer made the gut contract, and doses 10 times greater did not produce the full, original contraction. This was explained by the assumption that the nerve cells after nicotine no longer responded to acetylcholine. It would follow that the contractions of an untreated intestinal preparation to small doses of acetylcholine would have to be looked upon as being mainly the result of a ganglionic, nicotine-like action. This is not correct. Emmelin & Feldberg have shown that after nicotine the sensitivity of the preparation is reduced to all substances examined, not only to $BaCl_2$, acetylcholine, pilocarpine and 2268F, for which Ambache had found a stimulating action on sympathetic ganglia, but to histamine as well, which lacks this action.

The reduced sensitivity of the intestine to histamine during the nicotine paralysis was described by Emmelin & Feldberg, but was not illustrated and, therefore, it may not have been taken into account by Ambache (1949). As shown in a figure since published (Feldberg, 1950), the response to histamine may be reduced to an even greater extent than that to acetylcholine.

The depression does not affect only the longitudinal but also the circular muscle layer, as seen by the effects of nicotine on the peristaltic reflex. Paralysis of the nervous structures abolishes the peristaltic waves of contraction which normally occur in the guinea-pig's intestinal preparation when the gut is

distended by filling the lumen with saline solution, but the longitudinal muscle still contracts to the stimulus of distension, and similarly the circular muscle exerts a tonic resistance against the filling. During the nicotine paralysis, however, the longitudinal muscle no longer contracts when the pressure in the lumen is raised and the atonic condition of the circular muscle is seen by abnormal filling and distension of the intestine (see Fig. 13, p. 42 of Feldberg & Lin, 1949*a*).

The depression by nicotine may be a direct action of nicotine on the muscle fibres or more likely an after-effect of the strong contraction. Another possibility would be to assume that nicotine first stimulates nerve cells in the intestinal wall, some of which give rise to short adrenergic neurons, thus releasing adrenaline, which might persist in the tissue spaces for some time and be responsible for the observed depression. Against this view there is the observation of Munro (1951), who found that the caecal end of the ileum of some guinea-pigs contracts to adrenaline, but that this contraction is also depressed by nicotine.

In many preparations nicotine exerted some depressant effect, even if given in small amounts (15–30 μg./15 ml.), and for a short time only. After washing out the nicotine, the responses to histamine were reduced for a few minutes and spontaneous contractions, if present before the nicotine, disappeared; this after-depression was sometimes followed by a short period of increased excitability to histamine. These changes in excitability were not regularly obtained even on the same preparation with repeated administration of nicotine.

Assay of ganglion inhibiting substances on the intestine

D-Tubocurarine, tetraethylammonium and hexamethonium paralyse the ganglion cells of the myenteric plexus and, therefore, abolish the stimulating action of small doses of nicotine without reducing the histamine response. For D-tubocurarine, this nicotine antagonizing action has been described by Feldberg & Lin (1949*a*); for tetraethylammonium by Collins (1948), who, however, did not attribute the effect specifically to ganglionic paralysis; while Paton & Zaimis (1949) showed that hexamethonium prevented the peristaltic reflex.

There is a certain quantitative relation between the stimulating action of nicotine and the antagonizing action of the inhibitors. When the response to a given dose of nicotine, say 30 or 40 μg., has been abolished, an increase in the dose of nicotine will again elicit a response. Similarly a nicotine response can be progressively reduced by increasing the doses of inhibitor.

After washing out the inhibitor, the ganglion cells gradually acquire their original responsiveness to nicotine, full recovery being attained within about 10 min.

Apart from their ability to paralyse ganglion cells in the intestinal wall, the

ganglion inhibitors increase the excitability of the muscle fibres. This effect is greatest with tetraethylammonium and smallest with hexamethonium. Like cocaine, these ganglionic inhibitors augment the histamine response. For D-tubocurarine this has been described by Schild & Rocha e Silva (1949), for tetraethylammonium by Collins (1948). In addition, the inhibitors, if given in sufficiently high concentration, may produce spontaneous, small, but relatively regular contractions, This has so far been described for tetraethylammonium only (Collins, 1948); for hexamethonium the effect is seen in Fig. 9 at *b*. With D-tubocurarine this effect usually occurred only with concentrations of 1 in 15,000 or stronger. It is demonstrated in a figure published elsewhere (Feldberg, 1950, Fig. 3, p. 289). These concentrations, although eliciting spontaneous movements, diminished the histamine response (see p. 491).

The relative potency of the three ganglionic inhibitors has been assayed by comparing their ability to reduce the nicotine response. The following procedure was adopted. Histamine was given for 30 sec. every fourth minute and interpolated, after each fourth histamine response, by a dose of nicotine (10–60 μg. for 60–90 sec.). The inhibitors were given before the third histamine response and removed with the nicotine. In this way, sufficient time was allowed for the after-effects of nicotine and of the inhibitors to wear off before a new nicotine response was tested. In addition, any changes in excitability of the intestinal muscle occurring during the course of an experiment were recorded and could then be taken into account in the assay. Independent of such changes in sensitivity to histamine, the response to nicotine often increased in the course of several hours' experiment and, therefore, the dose of nicotine was usually reduced in the second half of the assay. The concentrations of inhibitors used were such that the nicotine response was only partially abolished. If no great variations took place in the histamine responses, the reductions in the nicotine responses were compared directly with each other. This was the usual procedure and the one adopted in the experiment of Fig. 2, in which the histamine responses have been omitted from the tracing. When the histamine responses varied, each nicotine response was expressed as a percentage of the last preceding histamine response and the percentages were compared with each other. It was rarely necessary to adopt this procedure; usually the result was the same whichever method of comparison was adopted.

In the experiment of Fig. 2 the potency of hexamethonium was first compared with that of D-tubocurarine by their relative ability to reduce the response to 40 μg. of nicotine; later in the experiment the dose of nicotine was reduced to 30 μg. and hexamethonium was compared with tetraethylammonium. In this and other experiments it was found that the ganglion blocking activity of 20 μg. hexamethonium iodide corresponded to that of between 45 and 50 μg. D-tubocurarine chloride and of about 120 μg. tetraethylammonium iodide.

Drug responses after D-*tubocurarine*

The addition of 500 μg. D-tubocurarine to the 15 ml. bath practically abolished the strong responses produced by 20–60 μg. nicotine; sometimes a small effect persisted, consisting either in increased activity only or in a small tonic contraction. Contractions similar to, or weaker than those produced by the nicotine, but elicited by parasympathomimetic drugs, such as acetylcholine, choline, acetyl-β-methylcholine and pilocarpine were partially affected

by this dose of D-tubocurarine. This is illustrated in Fig. 3. In general it was found that the contractions produced by very small doses of acetylcholine were reduced to a greater extent than those by large doses, as seen from the results of Table 1; contractions produced by doses of acetylcholine greater than 0·5 μg. were only slightly reduced, or remained unaffected.

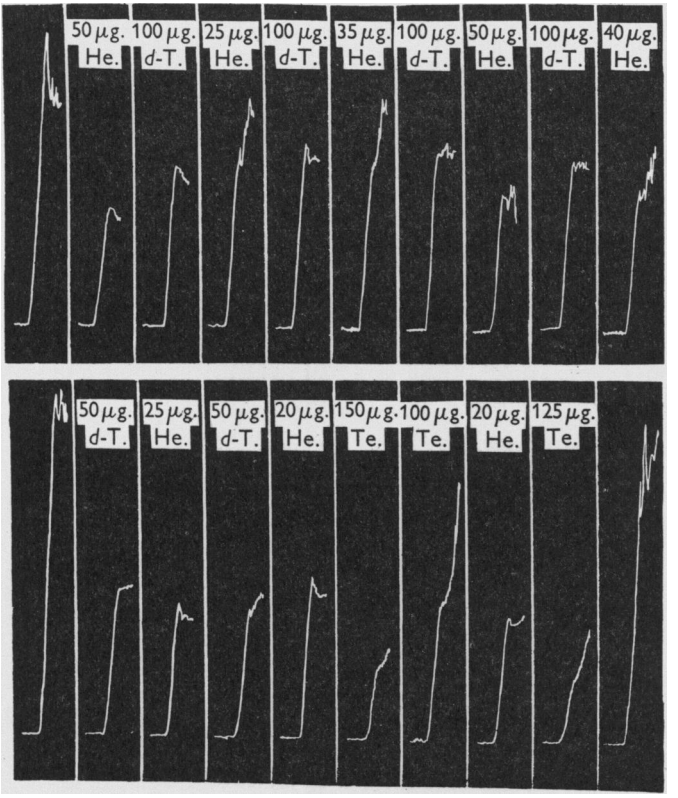

Fig. 2. Guinea-pig's ileum preparation in 15 ml. bath. Assay of ganglionic inhibitors. Contractions in response to 40 μg. (upper tracing) and later to 30μg. (lower tracing) of nicotine in the absence (unmarked) or presence of different amounts of hexamethonium (He), D-tubocurarine (*d*–T), or tetraethylammonium (Te) respectively. Four histamine responses between each nicotine effect have been omitted from the tracing (for details see text).

In Table 1 is given the percentage reduction produced by 500 μg. D-tubocurarine on the contractions of the various drugs examined on nine preparations. The average height of the tracings of the last two contractions immediately before, and of two to three contractions obtained during the action of D-tubocurarine were measured from the smoked drum record at the end of the experiment. The table gives for each drug the doses administered, the average

TABLE 1. Percentage reduction produced by 500 μg. D-tubocurarine of the contractions of the guinea-pig's ileum to various drugs: (*a*) μg. or mg. of drug added to bath; (*b*) mean height of contractions in cm. before D-tubocurarine, measured from tracing; (*c*) percentage inhibition produced by D-tubocurarine.

No. of Exp.	Acetylcholine (μg.)			Acetyl-β-methyl-choline (μg.)			Nicotine (μg.)			Histamine (μg.)			Choline (mg.)			Pilocarpine (μg.)			Barium chloride (mg.)		
	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)
1	0·2	7·2	22	—	—	—	30	7·2	89	0·2	6·5	0									
2	0·15	6·4	45	—	—	—	20	8·8	87	0·15	6·6	15									
										0·15	6·3	0									
3	0·25	7·7	53	0·25	7·2	44	30	6·6	95	0·15	8·3	14	0·45	7·3	45	3	8·6	55	0·6	4·2	83
	0·25	8·4	48							0·5	6·9	1	0·6	9·2	48				1·0	11·0	74
																			1·0	9·3	69
4	0·25	8·3	24	0·3	9·2	24	50	10·0	97	1·25	7·0	14	—	—	—	6·25	8·8	50	1·25	10·7	68
										0·25	8·0	2									
5	0·05	2·7	63	—	—	—	—	—	—	0·35	7·1	1									
	0·5	7·2	24							0·07	3·5	26									
										0·07	2·0	15									
6	0·02	1·6	69	—	—	—	—	—	—	0·4	7	0									
	0·05	2·8	64							0·075	3·7	26									
	0·05	4·0	70							0·05	1·9	5									
	0·1	5·2	61																		
	0·2	8·6	16																		
	0·5	8·5	11																		
7	0·04	3·2	36	0·1	4·3	23	—	—	—	0·2	5·9	0									
	0·1	6·7	46	0·2	6·3	22															
	0·4	9·2	16	0·4	9·2	29															
8	0·05	3·6	33	0·1	4·5	56	30	9·2	92	0·15	4·4	0									
	0·075	7·3	22	0·2	8·2	22				0·1	6·3	0									
	0·2	8·6	23	0·4	10·5	18															
9	0·1	3·8	29	—	—	—	50	6·8	93	—	—	—	—	—	—	2	3·4	50			
	0·25	6·9	32													5	6·3	16			

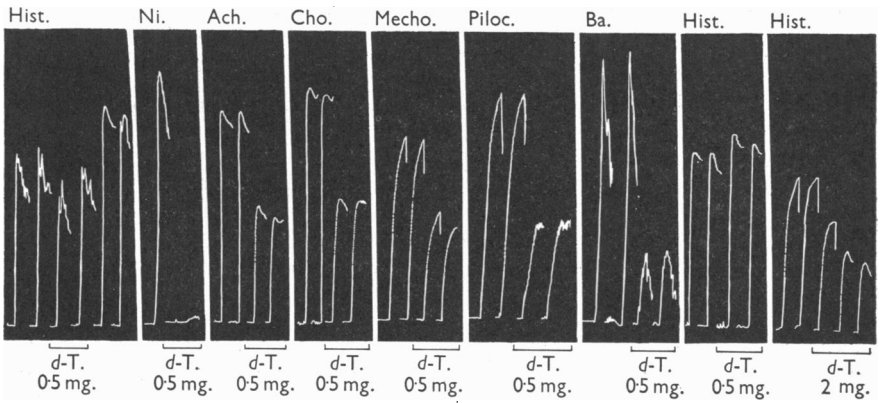

Fig. 3. Guinea-pig's ileum preparation in 15 ml. bath. Effect of D-tubocurarine (*d*-T.) on contractions caused by 0·25 μg. histamine (Hist), by 50 μg. nicotine (Ni), by 0·25 μg. acetylcholine (Ach), by 0·6 mg. choline (Cho), by 0·25 μg. acetyl-β-methylcholine (Mecho), by 6·25 μg. pilocarpine (Piloc), and by 1·25 mg. barium chloride (Ba).

heights on the tracings of the normal contractions and the percentage reduction produced by the D-tubocurarine. It will be seen that this reduction varied for the same drug in different preparations, but that, on the whole, all parasympathomimetic drugs were affected to approximately the same degree. On the other hand, the $BaCl_2$ contractions were reduced to a greater extent. The 500 μg. D-tubocurarine affected the histamine responses slightly only, and the effect varied even on the same preparation. Sometimes there was a reduction of up to 26 %, sometimes there was augmentation. Both effects might be seen in the course of a single administration of D-tubocurarine. For instance, at the beginning of the experiment of Fig. 3 the histamine responses were reduced, but when the D-tubocurarine was washed out they were augmented. In other experiments it was observed that D-tubocurarine sometimes augmented the first but depressed the subsequent histamine responses. When the D-tubocurarine was given several times in the course of an experiment, it might at one time reduce and at another time augment the histamine responses. For instance, at the end of the experiment of Fig. 3, the 500 μg. D-tubocurarine no longer depressed the histamine responses as it did at the beginning, but augmented them.

The relatively large reduction in the responses of the parasympathomimetic drugs cannot be attributed to the ganglion paralysing action of D-tubocurarine. In that case it should be much smaller with acetyl-β-methylcholine, which only has a weak stimulating effect on autonomic ganglia. Furthermore, when the concentration of D-tubocurarine was increased two- to fourfold, the histamine responses were also always greatly reduced. This is illustrated at the end of the experiment of Fig. 3. Histamine, however, possesses no stimulating action on autonomic ganglia.

Lastly, it has been found that paralysis of the ganglia in the intestinal wall by other ganglionic inhibitors either had no effect, or had a smaller effect than D-tubocurarine, on the responses to the various parasympathomimetic drugs.

The effect of D-tubocurarine on the responses to parasympathetic substances and to histamine resembles the action of atropine and is best explained by a weak atropine-like action of D-tubocurarine on the intestine.

Drug responses after tetraethylammonium

The results obtained with tetraethylammonium were essentially the same as those obtained by Collins (1948). The histamine responses were usually augmented, the responses to small doses of nicotine abolished, those to $BaCl_2$ greatly reduced, but those to the parasympathomimetic substances were only moderately depressed and this depression was not regularly obtained even in the same preparation on repeated testing.

The reduction of the acetylcholine or pilocarpine response, when it occurred in the presence of 1–2 mg. of tetraethylammonium, was smaller than that

produced by 500 μg. of D-tubocurarine. For instance, in Exp. 3 of Table 1, the reduction in the response to 6·25 μg. of pilocarpine, produced by D-tubocurarine, was about 50 %, while that produced by 1·5 mg. tetraethylammonium was only 23 %. Even the small reductions produced by tetraethylammonium may not be wholly accounted for by removal of a ganglionic stimulating action, because hexamethonium, which is a stronger ganglion blocking agent, was less effective in this respect.

In several experiments doses of tetraethylammonium (100–200 μg.) were used, which greatly reduced the responses to 25–50 μg. of nicotine. These doses of tetraethylammonium usually reduced the acetylcholine responses slightly, but sometimes not at all. In two experiments, however, they almost abolished them; again it remained doubtful whether this was the result of paralysis of the ganglion cells, because in the same experiment a dose of hexamethonium, which depressed the nicotine response as much as did tetraethylammonium, did not affect the acetylcholine responses. Nor did larger doses of hexamethonium exert any effect. The result is illustrated in Fig. 4.

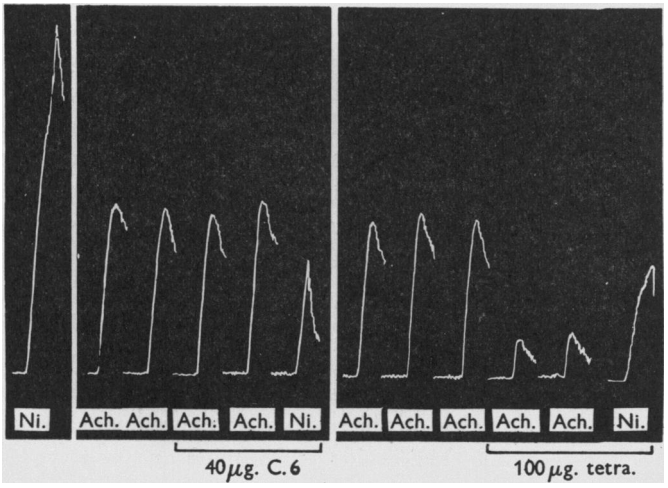

Fig. 4. Guinea-pig's ileum preparation in 15 ml. bath. Effect of 40 μg. hexamethonium (C6) and of 100 μg. tetraethylammonium (Tetra) on responses to 40 μg. nicotine (Ni) and to 0·06 μg. acetylcholine (Ach).

Drug responses after hexamethonium

Parasympathomimetic drugs. In spite of the fact that hexamethonium is a much stronger blocking agent than tetraethylammonium for autonomic ganglia, and not only for those in the intestinal wall but for sympathetic ganglia as well (Paton & Zaimis, 1949), it affects the responses to parasympathomimetic drugs to a smaller extent than tetraethylammonium. On nine preparations the effect of 40–400 μg. hexamethonium was tested 23 times on

the responses to 0·05–0·15 μg. acetylcholine. They were augmented 8 times, remained unchanged 7 times and were reduced 8 times. In four of these the reduction was between 4 and 10 %, in three between 10 and 16 %, and in one 21 %. The effect might vary on the same preparation, so that in the course of an experiment augmentation and reduction were observed with the same dose of hexamethonium and of acetylcholine. In a few experiments in which the acetylcholine responses were reduced, the pilocarpine responses remained unchanged. This is illustrated in Fig. 5. Thus, stimulation of the ganglion cells either does not contribute at all to the contractions produced by the parasympathomimetic drugs on the guinea-pig's intestine, as is seen with pilocarpine, or contributes to a slight extent only, and even then not regularly, to the response, as is seen with acetylcholine.

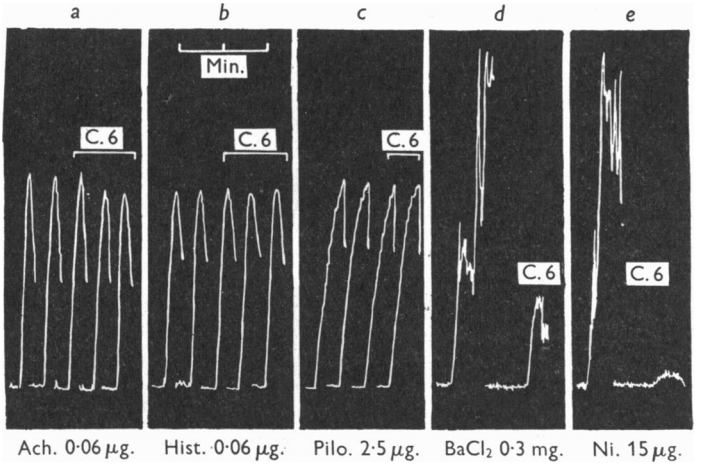

Fig. 5. Guinea-pig's ileum preparation in 15 ml. bath. Effect of 100 μg. hexamethonium (C6) on responses: (*a*) to 0·06 μg. acetylcholine; (*b*) to 0·06 μg. histamine; (*c*) to 2·5 μg. pilocarpine; (*d*) to 0·3 mg. $BaCl_2$; (*e*) to 15 μg. nicotine.

Eserine. Acetylcholine is continuously released in the intestinal wall, and, when its destruction is prevented by eserine, causes contractions. Again, it has been found that ganglionic stimulation contributes only to a slight extent to these eserine contractions. When a few μg. of eserine are added to the bath and kept there for a few minutes, contractions like those seen in Fig. 6 are produced, and when eserine is given in this way at 20–30 min. intervals, comparable, if not identical, responses are obtained.

In the experiment of Fig. 6, 4 μg. of eserine were given for 4 min. every 20 min. in the absence (*A* and *C*) and in the presence (*B* and *D*) of hexamethonium, added to the bath 10 min. before the eserine. The only difference seen in the tracings of *A* and *B* is the longer latency in the presence of the

ganglionic inhibitor. This delay in onset was frequently observed. Another frequent occurrence, illustrated by a comparison of *C* and *D*, was a reduction in the number and size of the troughs. The troughs are associated with contractions of the circular muscle layer. In the absence of hexamethonium, these contractions often spread in the form of peristaltic waves over part or the whole of the preparation, leading to emptying of the lumen. In the presence of the ganglionic inhibitor, strong contractions of the circular muscle layer

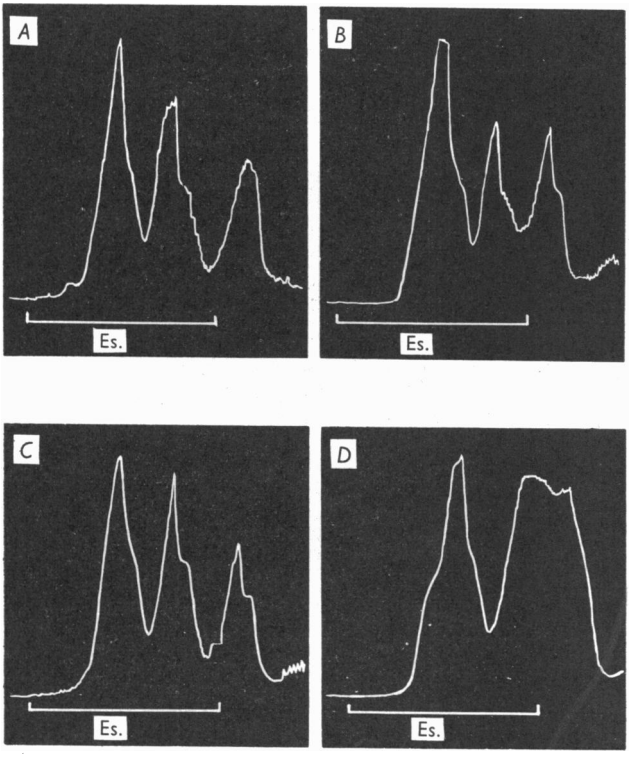

Fig. 6. Guinea-pig's ileum preparation in 15 ml. bath. Effects of 4 μg. eserine sulphate for 4 min. (Es); at *A* and *C* in the absence, at *B* and *D* in the presence of 200 μg. hexamethonium.

also occurred after eserine, but they did not spread over the preparation in the form of peristaltic waves.

Potassium. Hexamethonium depressed the response to potassium in all experiments, but the degree of depression varied even in the same preparation on repeated testing.

When the same dose of KCl is given several times, the response may vary greatly. This sometimes made it difficult to assess the effect of hexamethonium. Nevertheless, a certain reduction in the KCl response in the presence of hexa-

methonium was recognizable in most experiments. Good effects of this kind are seen in the two experiments of Fig. 7. In the first experiment, the response to 10 mg. KCl consisted of an immediate, quick contraction of the longitudinal muscle, followed by abrupt lengthening of the muscle, whilst the KCl was still in the bath. Strong contractions of the circular muscle occurred during the lengthening and were certainly partly responsible for it. In the presence of hexamethonium the contraction of the longitudinal muscle by KCl was reduced, the subsequent lengthening not so abrupt, and the contractions of the circular muscle were absent. When the dose of KCl was increased by 40 % in the presence of hexamethonium, the full original contraction of the longitudinal muscle was again obtained, but no contraction of the circular muscle occurred, and the longitudinal muscle remained relatively well contracted until the KCl was washed out. In the second experiment, 15 mg. KCl produced, after an

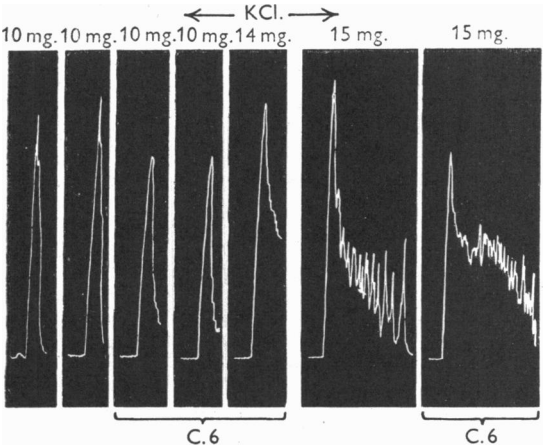

Fig. 7. Guinea-pig's ileum preparation in 15 ml. bath. Effect of 200 μg. hexamethonium on KCl contractions. Two experiments on different preparations.

initial shortening, strong activity of the circular muscle layer, producing large excursions on the tracing. In the presence of hexamethonium the contraction of the longitudinal muscle was again reduced and the activity of the circular muscle nearly abolished.

Barium. Of all substances examined $BaCl_2$, apart from nicotine, was the only one whose effect on the intestine was regularly and greatly diminished by hexamethonium.

Emmelin & Feldberg (1947) found that the responses to repeated administration of the same dose of $BaCl_2$ vary greatly. This may be due to the fact that barium causes after-effects in the sensitivity of the intestinal muscle like those produced by nicotine. The difficulty could be overcome, to a certain extent, by having intervals between each administration of 10 min. or longer. The $BaCl_2$ responses consisted of shortening of the preparation, followed by strong

activity of the circular muscle layer. The effect of hexamethonium on these barium responses was nearly as great as its effect on the nicotine contractions. This result is illustrated in Fig. 5*d*. The main action of small doses of barium on the intestinal preparations is thus ganglionic in origin.

Effect of hexamethonium on spontaneous contractions

A variety of spontaneous contractions are seen on the guinea-pig's ileum preparation. Some of them, which are definitely ganglionic in origin, have a characteristic appearance. In the course of an experiment, particularly when

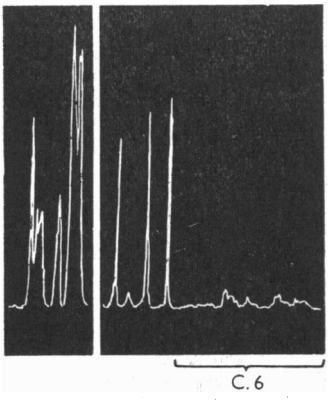

Fig. 8. Lower end of guinea-pig's ileum preparation in 15 ml. bath. Effect of 200μg. hexamethonium on spontaneous contractions.

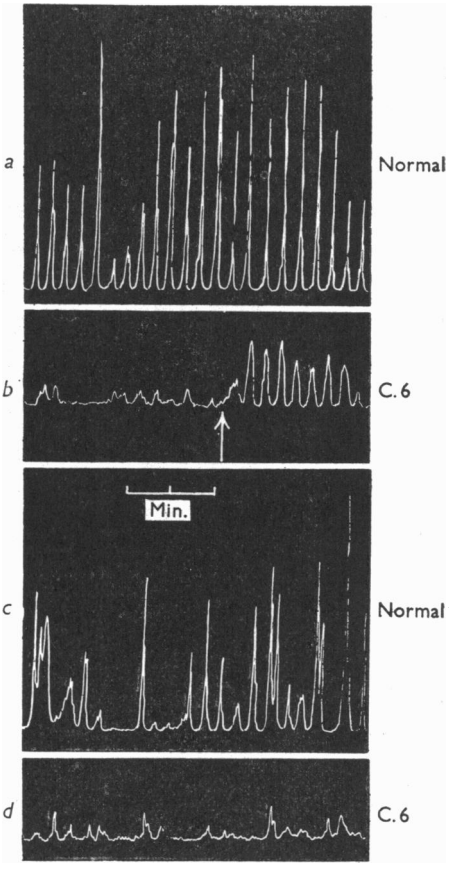

Fig. 9. Guinea-pig's lower ileum preparation in 15 ml. bath. Effect of hexamethonium (C6) on spontaneous contractions: *a* and *c*, without C6; *b*, 100 μg. and from arrow 400 μg. C6; *d*, 50 μg. C6.

Fig. 9.

the preparation has not been subjected to the action of drugs for some time, there may occur groups of single, spike-like contractions or contractions of a composite character. The spike-like contractions usually occur after a small, slow contraction and are terminated by abrupt lengthening of the preparation, which does not affect the slow contraction. Thus the spikes are often seen on

top of a well-defined, slow contraction, which forms a little triangular base or foot. When the spikes occur in groups, not all individual slow contractions give rise to a spike. These details are seen in Fig. 8. Hexamethonium abolishes the spikes, which are thus ganglionic in origin, but leaves the small, slow contractions unimpaired (Figs. 8, 9 and 10).

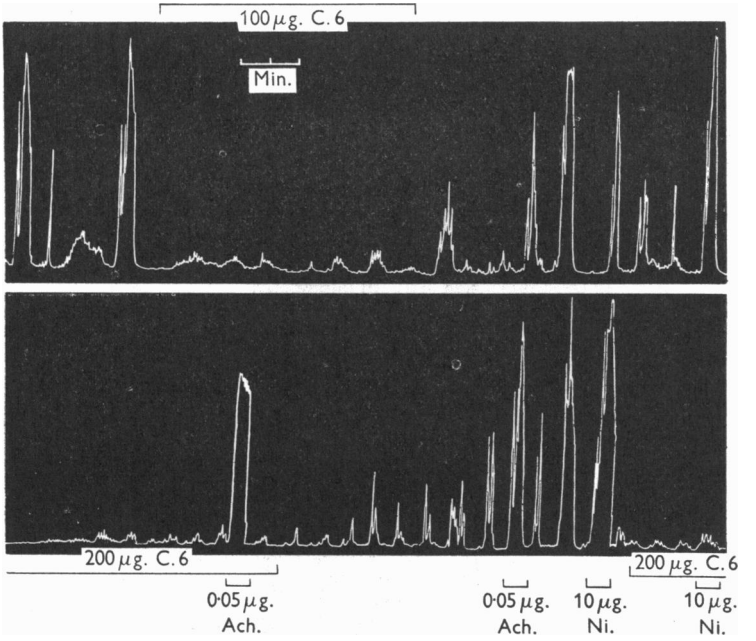

Fig. 10. Guinea-pig's lower ileum preparation in 15 ml. bath. Effect of hexamethonium on spontaneous, and on acetylcholine and nicotine contractions.

Typical composite contractions are seen at the end of Fig. 9 and in Figs. 8 and 10. They are associated with strong contractions of the circular muscle layer which occur during, and are in part responsible for, the sudden lengthenings of the preparation. The composite contractions are abolished by hexamethonium, and are therefore also ganglionic in origin (Figs. 8, 9 and 10)

The two types of contraction of ganglionic origin just described occur frequently in preparations from the very distal ileum, about 1–2 cm. away from the caecum. The experiments of Figs. 9 and 10 are from such preparations, and show how these contractions, like nicotine contractions, are abolished by hexamethonium but reappear some time after it has been washed out.

On the other hand, the slow changes in tone of the longitudinal muscle, which are seen periodically in preparations from all parts of the ileum, are non-ganglionic in origin and occur in the presence of hexamethonium. The same applies in general to the weak motor activity which occurs in many preparations

and can consist of slow or quick, small contractions. Figs. 9 and 10 show such activity in the presence of hexamethonium. In some instances, however, hexamethonium abolished part of this activity. As this effect was not obtained regularly, and as the activity can diminish or stop spontaneously, the possibility that ganglionic stimulation sometimes participates in it is neither proved nor excluded.

As mentioned previously hexamethonium, when given in strong concentrations, may itself produce regular, rhythmic contractions. They are much slower contractions than the sharp, spike-like contractions of ganglionic origin (see Fig. 9*b*).

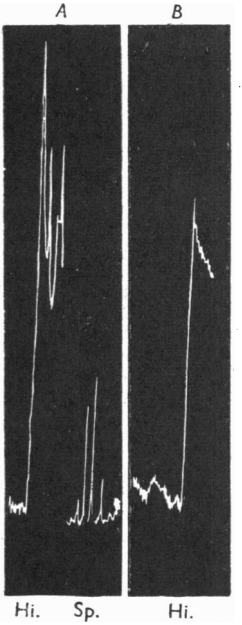

Fig. 11. Guinea-pig's ileum preparation in 15 ml. bath. Effect of 100 μg. hexamethonium (at *B*) on contraction to 0·06 μg. histamine, when strong spontaneous contractions of ganglionic origin are present (Sp).

Hexamethonium proved a useful tool when assaying histamine on the intestine, in which the presence of ganglionic contractions rendered the assay difficult. In this condition the smooth shortening usually caused by histamine may suddenly be speeded up by a further sharp contraction of ganglionic origin. The response, therefore, varies according to whether such a ganglionic component is present or not. The addition of 100 μg. hexamethonium each time the bath is refilled, removes the ganglionic component and thus facilitates the assay. Fig. 11 shows a typical interference of ganglionic contractions in the histamine response, and its removal by hexamethonium; a similar result is seen for acetylcholine in Fig. 10.

Experiments on the rabbit's ileum preparation

On this preparation the effect of one ganglionic inhibitor only, hexamethonium, has been examined in order to find out if it affects the responses to nicotine, acetylcholine, KCl and $BaCl_2$ to the same extent as on the guinea-pig's ileum. The results differ from those obtained on the guinea-pig's ileum, in that the responses to acetylcholine are not affected at all, while those to KCl, on the other hand, are affected to a greater extent. In addition, stronger concentrations of hexamethonium are required to paralyse the ganglion cells.

Spontaneous contractions. Hexamethonium does not affect the large, regular, rhythmic contractions of the longitudinal muscle which are characteristic of the rabbit's ileum preparation. Superimposed on these, however, short, stronger contractions may occur at irregular intervals. These contractions

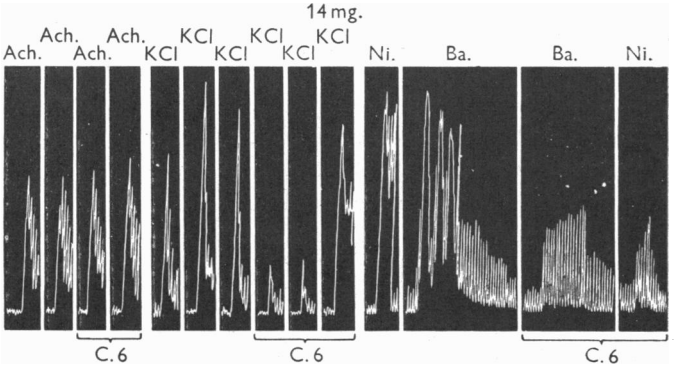

Fig. 12. Rabbit's ileum preparation in 15 ml. bath. Effect of 600 μg. hexamethonium (C6) on contractions produced by 0·2 μg. acetylcholine (Ach), 10 mg. KCl, 25 μg. nicotine (Ni) and 1 mg. $BaCl_2$ (Ba).

were sometimes abolished by the hexamethonium (600 μg.). But as this result could not be obtained regularly, even on the same preparation, no definite conclusions could be made about their origin.

Nicotine. Hexamethonium reduces or abolishes nicotine contractions but larger doses than those effective in the corresponding experiments on the guinea-pig's ileum are usually required. The response to 10–40 μg. nicotine was almost abolished by 600 μg. of hexamethonium (Fig. 12).

Acetylcholine. Whereas the response to small doses of acetylcholine on the guinea-pig's ileum was slightly reduced in about a third of the experiments, this was not so on the rabbit's ileum. There was only once, in one preparation, a very small reduction of the response to 0·2 μg. of acetylcholine. Subsequent repeated testing with hexamethonium (600 μg.) no longer reduced the acetylcholine responses on this preparation, the only effect being a slight augmentation of the first two or three acetylcholine responses after washing out the

hexamethonium. In four other preparations hexamethonium (600 μg.) never reduced the acetylcholine (0·2–0·4 μg.) responses, although it practically abolished much stronger nicotine contractions (Fig. 12). Thus, on the rabbit's ileum a 'nicotine-like' effect does not contribute to the acetylcholine contractions.

Potassium chloride. Hexamethonium affects the KCl contractions of the rabbit's ileum more than those of the guinea-pig's ileum. The effect is therefore easily demonstrable, in spite of the fact that the contractions to the same dose of KCl often vary greatly on repeated administration. In the absence of hexamethonium the KCl contractions are associated with strong contractions of the circular muscle layer, which may spread from the stomach end like a peristaltic wave over the whole length of the preparation, interrupt the shortening of the preparation, and produce on the record a trough followed by renewed shortening. These circular contractions are abolished by hexamethonium, and the shortening also is either greatly reduced or abolished. The effect of hexamethonium on the KCl response is sometimes as strong as its effect on the nicotine and barium contractions, as illustrated in the experiment of Fig. 12. This figure shows, further, that by increasing the dose of KCl by 40 % a strong, and maintained contraction can again be obtained in the presence of hexamethonium.

Barium chloride. The first effect of a small dose of $BaCl_2$ often consists of an increase in the amplitude of the rhythmic contractions only, which may remain partly augmented after the $BaCl_2$ has been washed out. With larger doses of $BaCl_2$ there is intense shortening of the preparation, followed after 10–15 sec. by strong contractions of the circular muscle. These contractions may spread as peristaltic waves from the stomach end over part or the whole of the preparation, and may cause emptying of the contents of the lumen; they account for the troughs seen in Fig. 12 during the barium effect. Alternatively, the circular muscle layer may remain tonically contracted until the $BaCl_2$ is washed out, which usually leads to a period of greatly increased rhythmic contractions. In fact it has been noted that a preparation previously relatively inactive will exhibit, after repeated administration of $BaCl_2$, pronounced rhythmic activity for a long time.

Hexamethonium thus abolishes more or less completely both the contractions of the circular muscle layer and the shortening of the preparation produced by small doses of $BaCl_2$, the only remaining effect being an increase in the amplitude of the rhythmic contractions (Fig. 12).

Effect of barium chloride on synthesis of acetylcholine

Ambache's observation that $BaCl_2$ accelerates the synthesis of acetylcholine in pieces of guinea-pig's intestine, incubated in bicarbonate-free, oxygenated Locke solution, was not confirmed even when his experimental technique was followed in all details. The results are given in Table 2; they confirm those of

Emmelin & Feldberg. In Exp. 1 of Table 2 the acetylcholine yield from the barium-free sample was as great as that from the barium-treated sample; in Exp. 2 it was even greater. This difference is explained by the individual variations which occur in the acetylcholine yield from different samples of incubated pieces of the same intestine and which, according to Emmelin & Feldberg, probably also account for the effects attributed by Ambache to histamine and barium.

TABLE 2. Effect of barium chloride on synthesis of acetylcholine in incubated strips of guinea-pig's small intestines

No. of Exp.	Weights of guinea-pigs (g.)	Content of acetylcholine (μg./g. tissue)		
		Initial content	After 40 min. incubation	
			Without barium	With barium
1	400 400	7·2	11·0	11·0
2	700 800	9·0	14·0	11·5

DISCUSSION

The experiments described in the present paper were performed for two reasons: (1) to test the validity of recent arguments of Ambache (1949) against certain of the conclusions drawn by Emmelin & Feldberg (1947), and (2) to seek by new methods further evidence about his original suggestion (1946) that a ganglionic component may be involved in the contractions produced by various drugs. To some extent evidence in favour of this suggestion was obtained.

As far as the first point is concerned, the results of Emmelin & Feldberg have been confirmed and the arguments brought forward against their conclusions shown to be invalid. The following is a short summary of the main facts concerned.

(1) The greatly reduced sensitivity to acetylcholine of the intestinal preparation after large paralysing doses of nicotine is not due to removal of a ganglionic stimulating action of acetylcholine, as claimed by Ambache, but is the result of a reduced sensitivity of the muscle fibres, since the preparation is then less sensitive to histamine as well.

(2) There is no evidence for the assumption made by Ambache that the histamine contractions of the intestine are in part due to an effect on nerve endings. Against this assumption stands the observation that the histamine contractions are not reduced by cocaine in doses which abolish equally strong nicotine contractions.

(3) If the histamine contractions are fully explained by its action on the smooth muscle fibres, the potentiation of drug responses by small doses of eserine cannot be regarded, as is done by Ambache, as evidence for the participation of a nervous component in the mode of action of a drug, because the histamine response is also potentiated by eserine.

(4) The failure of Emmelin & Feldberg to confirm that barium accelerates the synthesis of acetylcholine in incubated strips of intestine is not due to differences in method, as suggested by Ambache. In the present experiments his method has been followed in all details, but barium produced no acceleration of the synthesis of acetylcholine.

(5) The fact that a substance is known to have a stimulating action on the cat's superior cervical ganglion does not justify the conclusion, made by Ambache, that the contractions it produces on the intestinal preparation must also, at least partly, be ganglionic in origin. Pilocarpine stimulates the superior cervical ganglion in the cat, but so far no evidence has been obtained for a ganglionic component in the pilocarpine contractions of the guinea-pig's intestine. This naturally does not exclude the possibility that, given in sufficiently large amounts, pilocarpine may stimulate the ganglion cells of the myenteric plexus; it only shows that no ganglionic component contributes to the contractions produced by small doses of pilocarpine.

Not only is it unjustifiable to infer an action on the gut from an action on the superior cervical ganglion, but to anticipate similar mechanisms in the guts of different species is also dangerous. For instance, in a certain number of experiments performed on the guinea-pig's intestine a slight ganglionic component was found to contribute to the acetylcholine contractions, but this was never found on the rabbit's intestine. On the other hand, in the rabbit ganglionic stimulation was found to contribute to a much greater extent to the potassium contractions of the intestine than in the guinea-pig.

When assaying the effects of ganglion-blocking substances on the intestine it is necessary to be certain that no other effects are produced, otherwise wrong conclusions may easily be drawn. The unspecificity of the nicotine paralysis has been dealt with; D-tubocurarine was also found to have, apart from its ganglion-blocking effect, a depressant action on smooth muscle fibres resembling a weak atropine-like effect. On the other hand, the results obtained with hexamethonium appeared to be specific for ganglionic paralysis. A reduction of an intestinal contraction by hexamethonium was therefore taken as evidence for removal of a ganglion-stimulating effect.

In making such a general conclusion one must be aware, however, of the danger involved when trying to establish a kind of 'pharmacological histology', i.e. of attributing the action of a drug to a specific histological structure on account of the fact that its action is influenced by another drug. In the case of hexamethonium it would be difficult to explain the results in any way other than by ganglionic paralysis. We do not actually know whether all ganglionic stimuli are blocked to the same extent by hexamethonium; the block, although it affects such divergent stimuli as nicotine and barium, is certainly not absolute but can be overcome by increasing the dose of a given ganglion stimulator. Therefore the possibility exists that some of the drug responses remaining after

hexamethonium are still partly ganglionic in origin. These reservations have to be kept in mind when the following conclusions are made.

Ganglionic stimulation did not account for any part of the pilocarpine contractions of the guinea-pig's intestine, whereas in several cases such a mode of action was responsible, to a small extent at least, for the acetylcholine contractions of the intestine of the guinea-pig, but not of the rabbit. Ganglion stimulation, on the other hand, contributed regularly to the contractions produced by potassium; this contribution was small on the intestine of the guinea-pig but large on that of the rabbit. In both intestines ganglion stimulation accounted for the main response to small doses of barium. To this extent the results agree with Ambache's original conclusions. Furthermore, it was shown that certain characteristic, spontaneous contractions of the intestine are ganglionic in origin.

The fact that, at least in the guinea-pig, ganglion stimulation contributed to a small extent, in a number of experiments, to the acetylcholine contractions, is of interest in connexion with the continuous release of acetylcholine in the intestinal wall. Evidence has recently been brought forward (Feldberg & Lin, 1949*b*, 1950) that this spontaneous release may be non-nervous in origin. It may well be that the acetylcholine so released provides not only a physiological stimulus for excitation of the smooth muscle fibres and gland cells, but also for the ganglion cells; alternatively, it may lower their threshold for excitation by other means. The effect, however, is not pronounced because paralysis of the ganglion cells by hexamethonium did not greatly influence the eserine response. It caused only a slight delay in onset of the eserine contractions of the longitudinal muscle without affecting their strength, but it diminished the contractions of the circular muscle layer which occurred at the height of the eserine response.

The most surprising result was the fact that the barium contractions resulted to a great extent from stimulation of the ganglion cells of the myenteric plexus. In this action barium resembled nicotine more than any other drug so far examined on the intestine, although it apparently lacked the paralysing action on ganglion cells. The ganglion-stimulating effect of the barium was unexpected because, in the assay of spasmolytic drugs on the intestine, barium is commonly used as a means of producing atropine-resistant muscle contractions. In view of the new finding that the contractions to small doses of barium are mainly, even if not wholly ganglionic in origin, this test for spasmolytic drugs needs reinvestigation.

In preliminary experiments, it was indeed shown that the contractions to barium are more resistant to atropine than those to acetylcholine, although they are reduced by atropine to a certain extent. This relative atropine resistance of the barium contractions can be explained on the assumption that when acetylcholine is released from the nerve-endings in close contact with the

muscle fibres, it is less affected by the atropine than when applied to the outside of the preparation in the bath fluid. There is, however, another possible explanation which, although anything but proved, would correlate a number of divergent facts. The increased release of acetylcholine from the stomach and intestine on vagal stimulation could be accounted for by release from cholinergic preganglionic fibres only; the postganglionic fibres have never been shown to be cholinergic and, indeed, it may be postulated that they liberate on excitation an atropine-resistant substance. Vogt (1949) has given evidence in favour of such an assumption. He found that vagus stimulation to the frog's stomach caused an increased release of an atropine-resistant, smooth muscle stimulating substance, which he thinks is identical with the 'P' substance of von Euler & Gaddum (1931). On this assumption, stimulation of the nerve cells of the myenteric plexus by barium would naturally also cause the release of the atropine-resistant substance, and the atropine resistance of the barium effect, as well as of vagus stimulation, would be explained. Only further experiment can decide how far this explanation is valid.

SUMMARY

1. There is no evidence that the histamine contractions of the guinea-pig's small intestine preparation are in part the result of stimulation of nerve endings in the intestinal wall. The contractions are not reduced by cocaine in concentrations which abolish equally strong nicotine contractions.

2. When assaying ganglion-blocking substances on the ileum preparation, care has to be taken to find out if these substances have additional side effects. For instance, the reduced sensitivity of the intestinal preparation to acetylcholine after *large paralysing doses of nicotine* is not the result of ganglion paralysis because, in this condition, the sensitivity to histamine is reduced as well. Similarly, the reduction in the responses to parasympathomimetic drugs found after D-tubocurarine is probably not the outcome of its ganglion-blocking effect. In strong concentrations D-tubocurarine appears to have, in addition, a weak atropine-like effect on the intestine. On the other hand, the effect of hexamethonium in reducing the responses to various substances seems to be entirely the result of ganglion paralysis.

3. The potency of ganglion-blocking agents has been assayed on the guinea-pig's ileum by comparing their effects in reducing the response to a given small dose of nicotine. In order to exclude errors due to changes in sensitivity of the muscle fibres, each nicotine response is interpolated by three histamine responses, so that changes in sensitivity of the muscle fibres are recorded and can be taken into account. With this procedure it was found that, weight for weight, the ganglion-blocking potency on the myenteric plexus of hexamethonium iodide was about 6 times as strong as tetraethylammonium iodide, and about 2–2½ times as strong as D-tubocurarine chloride.

4. Blocking the ganglia by hexamethonium did not reduce the pilocarpine contractions of the guinea-pig's ileum preparation; therefore ganglion stimulation did not contribute to these.

5. Blocking the ganglia by hexamethonium did not reduce the acetylcholine contractions on the rabbit's ileum preparation, but slightly reduced them in a number of experiments on the guinea-pig's ileum preparation. Ganglion stimulation, therefore, sometimes contributed to a small extent to the acetylcholine contractions of the guinea-pig's ileum.

6. Blocking the ganglia by hexamethonium regularly reduced the potassium contractions on the ileum preparation. The effect was small on the guinea-pig's intestine and great on that of the rabbit. Ganglionic stimulation, therefore, contributed to the potassium contractions of the ileum in a degree which varied with the species.

7. Blocking the ganglia by hexamethonium greatly reduced the barium contractions of the guinea-pig's and rabbit's ileum preparation; these contractions were therefore mainly, although not wholly, ganglionic in origin.

8. Blocking the ganglia by hexamethonium made it possible to differentiate between spontaneous contractions of ganglionic and non-ganglionic origin. Those of ganglionic origin were characterized by quickness of contraction and relaxation, and appeared either as single spikes superimposed on small, slow, non-ganglionic contractions, or as composite contractions.

9. No evidence could be found that barium accelerates the synthesis of acetylcholine in incubated strips of guinea-pig's intestine.

REFERENCES

Ambache, N. (1946). *J. Physiol.* **104**, 266.
Ambache, N. (1949). *J. Physiol.* **110**, 164.
Collins, O. A. (1948). *J. Pharmacol.* **94**, 244.
Emmelin, N. & Feldberg, W. (1947). *J. Physiol.* **106**, 482.
v. Euler, U. S. & Gaddum, J. H. (1931). *J. Physiol.* **72**, 74.
Feldberg, W. (1950). *Proc. Roy. Soc.* B, **137**, 285.
Feldberg, W. & Lin, R. C. Y. (1949*a*). *Brit. J. Pharmacol.* **4**, 33.
Feldberg, W. & Lin, R. C. Y. (1949*b*). *J. Physiol.* **109**, 475.
Feldberg, W. & Lin, R. C. Y. (1950). *J. Physiol.* **111**, 96.
Munro, A. F. (1951). *J. Physiol.* **112**, 20*P*.
Paton, W. D. M. & Zaimis, E. J. (1949). *Brit. J. Pharmacol.* **4**, 381.
Schild, H. O. & Rocha e Silva, M. (1949). *J. Physiol.* **109**, 448.
Vogt, W. (1949). *Arch. exp. Path. Pharmak.* **206**, 1.