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THE REACTIONS OF PLEXUS-FREE CIRCULAR MUSCLE OF CAT JEJUNUM TO DRUGS

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Studies on the behaviour of enteric plexus-free preparations of the small intestine were first carried out by Magnus (1904*a–c*). He found that when the longitudinal muscle of the cat's small intestine is stripped off the underlying circular coat, the ganglion cells of the myenteric plexus of Auerbach adhere to the longitudinal layer and the circular muscle can be freed from ganglia. Magnus found that these ganglion-free circular muscle preparations did not contract rhythmically when suspended in Locke's solution and concluded that spontaneous contractions normally observed in intestinal muscle are neurogenic in origin. All later workers, however, observed spontaneous activity in the completely ganglion-free circular muscle (Gunn & Underhill, 1914; Alvarez & Mahoney, 1922; Evans & Underhill, 1923; Gasser, 1926; Eura, 1927; Van Esveld, 1928).

Magnus also used the ganglion-free preparations of circular muscle to study the site of action of certain drugs, including nicotine, eserine and barium. These pharmacological experiments have been repeated by Gasser (1926) and by Van Esveld (1928). Although these three workers used similar methods in preparing ganglion-free intestinal strips, their results disagree both with regard to spontaneous activity of the ganglion-free preparations and their reactions to nicotine. Magnus and Gasser found that nicotine does not cause contraction of the ganglion-free strips, and concluded that stimulation of the intestinal muscle by nicotine is due entirely to an action on the ganglion cells of the myenteric plexus. This view is now generally accepted, but is contradicted by the findings of Van Esveld who found that nicotine stimulates the ganglion-free preparations. Van Esveld's findings, which he checked by a careful histological search for ganglion cells, have received surprisingly little attention.

Magnus's (1905) studies on ganglion-free preparations are also largely responsible for the current notion that barium has a direct stimulant action on the intestinal smooth muscle fibre. He reached this conclusion because he observed that barium always stimulated the ganglion-free intestinal strip even when other drugs which ordinarily stimulated the preparation failed to elicit a response. Van Esveld confirmed Magnus, and showed that denervated and innervated preparations were equally sensitive to barium. More recently, Ambache (1946) has thrown doubt on these views by suggesting that barium normally does not act directly on muscle fibres but on the nervous elements present in the intestine. Ambache's view has received support by the work of Feldberg (1950) and Toh (1951) who, however, suggest that barium has in addition a direct stimulant action on the intestinal muscle.

In view of these discrepant results and of the conclusions drawn from them, we have re-investigated the reactions of plexus-free preparations of the cat's jejunum, checking the absence of ganglion cells by serial histological sections. We have confined our studies, for the present, to acutely denervated preparations and the effects on these of nicotine, barium, eserine and acetylcholine.

METHODS

Adult cats were anaesthetized with ether, and the whole length of the small intestine removed into Tyrode solution or Krebs & Henseleit's (1932) solution at room temperature. Two kinds of preparations were used: muscle strips and tubular segments.

Muscle strip preparations

Ganglion-free circular muscle strips (strip a). Strips of circular muscle were prepared by a method similar to that used by Gunn & Underhill (1914), Gasser (1926) and Van Esveld (1928). The intestine was left undisturbed in the Tyrode solution for about 30 min to allow peristaltic activity to decrease, after which a piece of jejunum 1 cm long was slipped over a glass rod. The rod was of a thickness sufficient to keep the gut in a slightly distended condition. The longitudinal coat was peeled off with a fine needle, elevating about 4 mm strips with each sweep. In this way the great majority of the ganglion cells of the myenteric plexus are removed with the longitudinal muscle. In order to ensure that no ganglion cells remain on the surface of the circular muscle this was either scraped with the sharp edge of a scalpel blade, or the superficial third of the circular muscle was discarded by making a superficial incision along the mesenteric border and stripping off the outer part of the circular muscle from the remainder. The intestine was then removed from the glass rod, gently inverted and replaced on the rod with the mucous membrane outermost. An incision was made in the mucous membrane along the mesenteric border and the cut edges separated a little. The cut was extended through the submucosa leaving the remaining circular muscle intact. The submucosa was stripped off the muscle by gentle traction with a forceps. Blood vessels passing from one layer to the other were cut. Finally a strip of circular muscle, about 4 mm in width, surrounding the mesenteric border was discarded owing to the possibility of ganglion cells being embedded in the substance of the muscle in this region. In this way a circular muscle preparation is produced which is entirely free of ganglion cells of both the myenteric and submucous plexuses. This is illustrated in Pl. 1, which shows a transverse section of a segment of intact cat's jejunum and a similar section through one of the ganglion-free specimens.

Circular muscle strips free of myenteric plexus (strip b). The longitudinal muscle with the myenteric plexus was removed in the same way as in the preceding specimen. The mucous membrane, containing the submucous plexus, was left undisturbed.

Circular muscle strips without submucous plexus (*strip c*). The mucous membrane and submucosa were removed.

Circular muscle strips with myenteric and submucous plexuses intact (*strip d*). A strip of intact intestine orientated to record circular muscle contraction.

Strips of longitudinal muscle containing myenteric plexus (*strip e*). A length of jejunum was slipped over a glass rod and a strip of longitudinal muscle about 7 mm wide and 2–4 cm long was peeled off as described above. Pl. 2 shows that in such a preparation the myenteric plexus remains attached to the longitudinal muscle, whereas the circular muscle becomes free from ganglion cells.

Strip *a* was used most extensively for studying the behaviour of ganglion-free circular muscle. Strip *c* was used in preference to strip *d* when studying the reactions of the innervated circular muscle, because it was found that removal of the mucous membrane with submucosa containing the submucous plexus rendered the preparations more sensitive to drugs, but without otherwise changing their responses.

In order to study the reactions of different strips under comparable conditions three strips were suspended in the same isolated organ bath (volume 150–250 ml.) and usually two organ baths, each containing three strips, were used simultaneously.

In the earlier experiments Tyrode solution was used as bath fluid, but in later experiments Krebs & Henseleit's solution was substituted. It was our impression that the latter gave more consistently responsive preparations. The fluid was kept at 37° C and aerated with a mixture of 95% O_2 and 5% CO_2. The contractions of each strip were recorded by a light frontal writing lever, the weight of which was adjusted to produce a tension of either 4 or 10 g/cm^2. In each case the tension required was calculated from measurements of the length and weight of the muscle strip. The magnification was eightfold in the circular muscle records and threefold in the longitudinal muscle records. The drug to be examined was allowed to be in contact with the preparations for 5 min followed by a rest period of 10 min. Usually all four drugs were examined on the same set of three strips. The drugs were applied in ascending doses, the order varying in different experiments.

Tubular segments (*a modified Trendelenburg method*)

Trendelenburg (1917) studied the volume changes within the lumen of the guinea-pig's intestine in response to raising the intralumen pressure. In the cat's jejunum, with its much more powerful circular muscle, it was found more satisfactory to record pressure rather than volume changes in response to the stimulus of raising the intralumen pressure to 20 or 40 cm H_2O. The apparatus used is illustrated in Fig. 1. A segment of ganglion-free and normal jejunum, each about 4 cm in length, were studied together in the same organ bath filled with Tyrode solution. The ganglion-free segment was prepared as follows: a 5 cm length of jejunum was ligated at each end and moderately distended with Tyrode solution at room temperature. The longitudinal muscle coat was stripped off the gut over its whole circumference using a fine needle. In order to remove any traces of myenteric plexus which might still be attached to the circular muscle the whole surface of the latter was firmly scraped with the sharp edge of a scalpel blade. A length of about 0·5 cm at each end, including the region of the ligatures was then discarded. In this way a preparation of circular muscle was obtained freed of the myenteric plexus but with the mucous membrane and submucous plexus intact.

The normal and ganglion-free preparations were then connected at each extremity to glass tubes. The caudal ends were connected to separate mercury manometers and a common burette (Fig. 1). The tubes leading from the cranial ends were fitted with screw clamps. The whole system was filled with Tyrode solution. The intestinal segments were washed out with Tyrode solution introduced through the burette and this procedure was repeated at intervals throughout the experiment. The intralumen pressure in both segments was raised simultaneously by elevating the burette. Artery forceps were then applied to exclude the burette from the recording systems. The method of recording was similar to that used by Schild, Fitzpatrick & Nixon (1951).

The course of the experiments was as follows: the intralumen pressure was raised to 20 or 40 cm H_2O and the response of the intestine observed for 2½ min, after which a drug was injected into the bath. After a further period of 2½ min the intralumen pressure was lowered, the bath fluid replaced and an interval of 10 min allowed. The duration of each experiment varied between 3 and 6 hr.

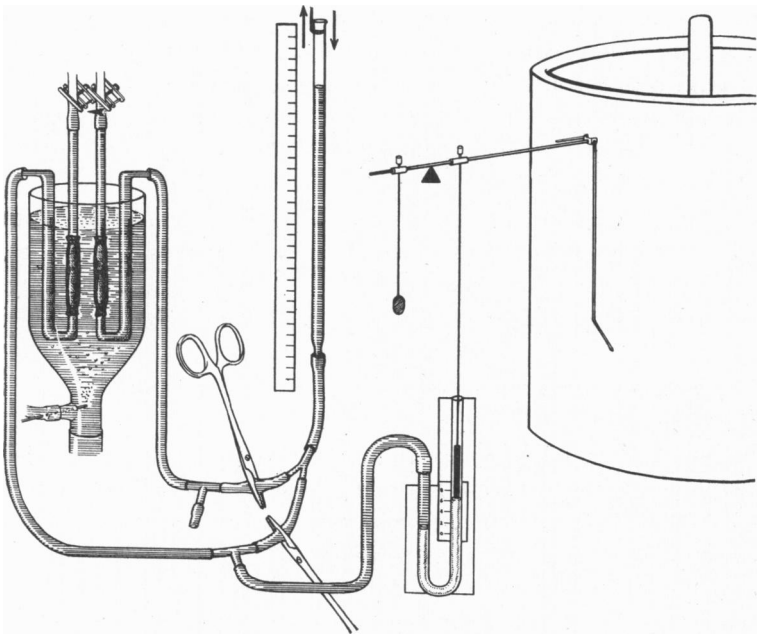

Fig. 1. Diagram of apparatus used in recording intralumen pressure of two intestinal segments. Only one recording manometer is shown.

Histological controls

At the end of each experiment the ganglion-free muscle strips or tubular segments were immersed in a fixative consisting of 20% (v/v) commercial formalin and 2% (v/v) pyridine in tap water in which they were allowed to remain for 3 weeks or longer.

With strip preparations (strip *a*) frozen sections of 40 μ thickness of the entire segment were prepared. These were stained with Bielschowsky-Gros silver and then dehydrated, cleared in creosote and mounted in Canada balsam. The whole extent of all the sections was then examined systematically for ganglion cells. Each preparation entailed an examination of fifteen to twenty sections. All the thirteen ganglion-free strips (strip *a*) from which responses had been obtained were examined in this way. In none were ganglion cells found.

Each tubular segment examined histologically was cut into pieces of approximately 1 cm length and half the gut circumference in width. The pieces were flattened with the muscle uppermost and frozen sections 40 μ thickness were taken beginning from the outer surface. The first few sections were incomplete owing to the impossibility of avoiding some obliquity of sectioning. These were not discarded, but the sectioning was continued until six complete sections of 40 μ thickness had been obtained. All sections were stained and examined for ganglion cells as above. Owing to the great labour involved we have made such histological checks only in thirteen out of a total of the twenty-five tubular segments in which the myenteric plexus had been removed. Ten of these contained no ganglion cells and the other three contained respectively, 61, 28 and 17 ganglion cells of the myenteric plexus over a length of about 4 cm intestine examined. In contrast a 4 cm strip of normal jejunum contains approximately 7000 ganglion cells.

RESULTS

Attempts to reduce variability of response

All previous workers have found the responses of ganglion-free strips of the intestine to drugs to be extremely variable; we made the same observation in our strips and tubular segments; some preparations did not react to the drugs to be examined, whereas others gave a response under the same conditions. The following procedures were adopted in an endeavour to reduce the trauma involved in removal of the ganglion cells and thus perhaps reduce this variability:

(1) The longitudinal muscle and as much of the myenteric plexus as remains attached to it was peeled off, but no attempt made to remove isolated ganglion cells from the circular muscle layer.

(2) The jejunum was gradually cooled and then rendered ganglion-free at 4° C.

(3) The ganglion-free preparations were kept for 24 hr in the ice chest at 4° C before use.

(4) Segments of jejunum were stripped of myenteric plexus *in situ* in the living animal under pentobarbitone sodium anaesthesia and with full aseptic conditions. Five days later the jejunum was removed in the usual way and the responses of the ganglion-free strips studied. This procedure was adopted merely to allow the tissue to recover from the injury, and not with the object of studying the effects of degeneration of the intramuscular nerve fibres and endings.

By none of these modifications has it been possible consistently to eliminate the variability of response to drugs. Throughout the investigation it was found that certain ganglion-free specimens failed to respond to one or more drugs, although they were apparently prepared in the same way as other preparations which gave satisfactory responses. Some ganglion-free specimens failed to respond to any drug throughout an experiment, while others became responsive to some, but not necessarily all drugs during the course of an experiment lasting several hours. Yet others were most responsive at the commencement of an experiment. For this reason a large number of experiments were required with each drug and the presence of a response in a proportion of specimens was considered to be more significant than its occasional absence.

The activity of tubular segments in response to raising the intralumen pressure

Trendelenburg (1917) has shown that raising the intralumen pressure by a few millimetres of water induces in the guinea-pig ileum a series of rhythmic contractions propagated in an aboral direction. He termed this the 'peristaltic reflex' and attributed it to a nervous mechanism. Feldberg & Lin (1949), in

the rabbit and guinea-pig, have shown that these rhythmic waves of contractions resulting from distension of the lumen can be abolished by D-tubocurarine and cocaine. Paton & Zaimis (1949), using the rabbit, found that they were abolished by the ganglion-blocking drugs, hexamethonium and pentamethonium. In the cat's small intestine Trendelenburg had considerable difficulty in demonstrating the peristaltic reflex owing to the strong spontaneous tonus of this preparation. Bayliss & Starling (1901) reported that the rhythmic contractions of the cat's small intestine, in response to distension

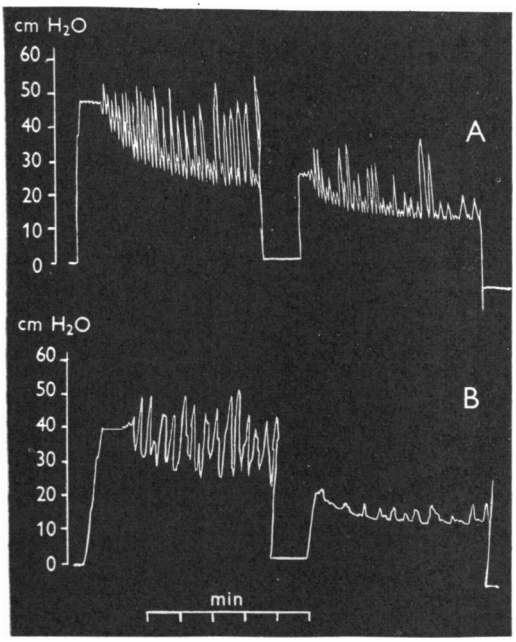

Fig. 2. Cat jejunum in Tyrode's solution. Tubular segment method. Response of normal (A) and myenteric denervated (B) preparations to rise in intralumen pressure. In this instance the ganglion-free preparation responded with rhythmic contractions.

with a balloon, remained localized in contrast to the propagated peristaltic activity observed in the dog and rabbit. Paton & Zaimis (1951) found that the paralysing action of the methonium salts could not be demonstrated on the cat's intestine preparation.

In the present experiments, using higher distension pressures than Trendelenburg and recording the changes in pressure rather than in volume, there was no difficulty in eliciting a series of co-ordinated contractions of the circular muscle of the cat's jejunum. When the intralumen pressure was raised rapidly to 20 or 40 cm H_2O the great majority of the innervated preparations responded immediately or after a latent period of up to 1 min, with a series of

contractions at a frequency of about 8–10 per min and an amplitude of up to 30 cm H_2O (Fig. 2). These contractions were frequently quite regular, but we found it impossible to determine by inspection whether they were propagated as in Trendelenburg's experiments. These rhythmic contractions occurred also in preparations freed of myenteric plexus but much less regularly. They were obtained in only seven out of a total of twenty-three preparations. Fig. 2 illustrates the contractions in one of those seven ganglion-free preparations, and Fig. 3 the failure to elicit such contractions by raising the intralumen pressure in one of the other tubular segments. The contractions of the ganglion-free preparations when present were usually slower, less frequent, and smaller in amplitude than those of the normal.

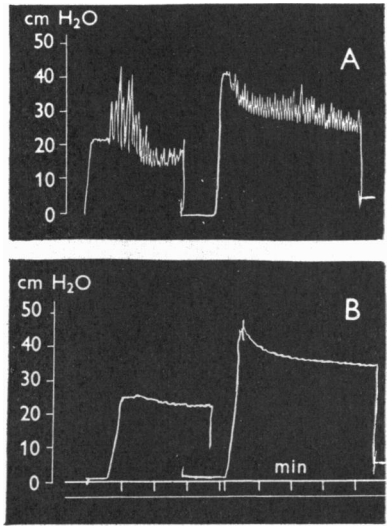

Fig. 3. Cat jejunum in Tyrode's solution. Tubular segment method. Response of normal (A) and ganglion-free (B) preparations to rise in intralumen pressure. An instance of failure of the ganglion-free preparation to respond.

In some instances ganglion-free preparations which did not intially contract in response to raising the intralumen pressure did so later in the experiment. This suggests that lack of rhythmic activity in some of these preparations is due to trauma rather than to the removal of the nervous elements.

Spontaneous activity in isolated strips

Circular muscle strips freed of myenteric and submucous plexus (strip *a*) frequently exhibited spontaneous contractions apparently similar to those occurring in the innervated controls (Fig. 4). It was found that in preparations which had previously been treated with barium the contractions were much more pronounced and continued to be so for a long time after the barium had

been washed out. A similar effect of barium has been observed on the innervated rabbit intestine by Feldberg (1951). A characteristic and regular feature of the ganglion-free preparations was that they became progressively contracted in the course of an experiment lasting several hours, whereas this did not occur in normal controls. An analogous phenomenon was observed in segments of intestine, the myenteric plexus of which had been removed in the living animal *in situ*; this spasm became in fact more prominent with

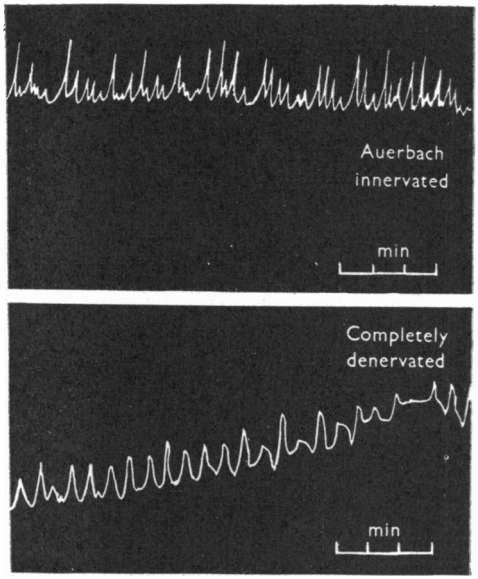

Fig. 4. Cat jejunum in Krebs & Henseleit's solution. Strip method. Spontaneous rhythmic activity in innervated (strip *c* of Pl. 1) and completely ganglion-free (strip *a* of Pl. 1) strips.

time (Evans, 1953, in preparation). At biopsy these segments were always found to be in a state of spasm. Circular muscle strips which had been freed of submucosa and mucous membrane (strips *a* and *c*) were generally more active than those containing submucosa and mucous membrane (strips *b* and *d*).

Nicotine

Stimulating effect of nicotine. Our findings agree with those of Van Esveld in showing that a proportion of the ganglion-free preparations are stimulated by nicotine. Stimulation occurred in twelve of twenty-five ganglion-free tubular segments and in three of nine muscle strips (strip *c*). This effect is illustrated for a ganglion-free tubular segment in Fig. 5. In this specimen there occurred no rhythmic contractions in response to raising the intralumen pressure. Yet addition of nicotine (10^{-5}) produced a rapid transient contraction comparable in strength to the contraction in the innervated control. More

frequently, nicotine produced a weaker contraction in the ganglion-free than in the normal tubular segments; an instance of this is illustrated in Fig. 6. The nicotine response was obtained more frequently in ganglion-free tubular segments than in the ganglion-free strip preparations. The latter, as a rule, showed only feeble responses.

Effect of barium on nicotine contractions. The sensitivity of ganglion-free preparations to nicotine was greatly enhanced by the presence of barium in the bath fluid. This sensitizing effect manifested itself in three ways:

(*a*) Many preparations not responding to nicotine alone could be induced to contract by the previous addition of barium. Table 1 compares the number of ganglion-free specimens giving a contraction with nicotine alone, with the number responding to nicotine in the presence of barium. With both the tubular segments and the muscle strips (strip *a*) the number giving a positive response is appreciably increased by barium.

TABLE 1. Comparison of the number of ganglion-free preparations stimulated by nicotine alone and by nicotine in the presence of barium

	Tubular segment method	Strip method
Nicotine alone		
Stimulated	12	3
Total	25	9
Nicotine + barium		
Stimulated	20	9
Total	24	15

(*b*) Ganglion-free preparations that responded feebly to nicotine alone gave a brisk response in the presence of barium. Fig. 6 illustrates this effect in a ganglion-free tubular segment. Nicotine (10^{-5}) alone induced only a feeble response, whereas the same concentration in the presence of barium produced a transient pressure rise of about 30 mm H_2O. A similar potentiation occurred in ganglion-free strips. Sensitization also occurred in the absence of any visible stimulation by barium itself as shown in Fig. 7, which also demonstrates that the effect of barium was reversible.

(*c*) The threshold-stimulating dose of nicotine in the innervated preparations of the cat's intestine was of the order of 4×10^{-7}. In the absence of barium the threshold for the ganglion-free preparation was usually somewhat higher, but in the presence of barium, the threshold in the innervated and ganglion-free preparation was the same.

Inhibiting effect of nicotine. Three different types of inhibitory effects of nicotine on the small intestine have been described in the intact animal and in isolated preparations and been attributed to different mechanisms:

(*a*) Stimulation of postganglionic sympathetic neurones through the action of nicotine on the mesenteric ganglia or relaxation of the smooth muscle fibres

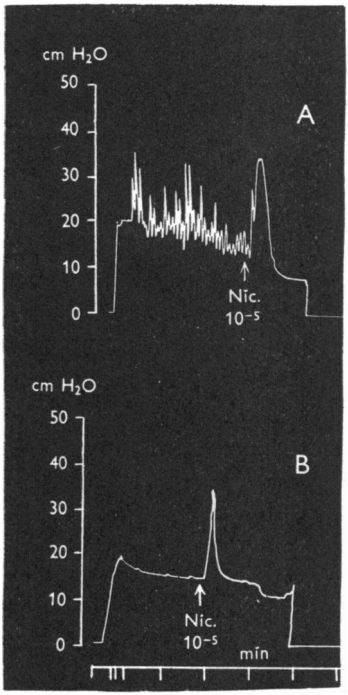

Fig. 5.

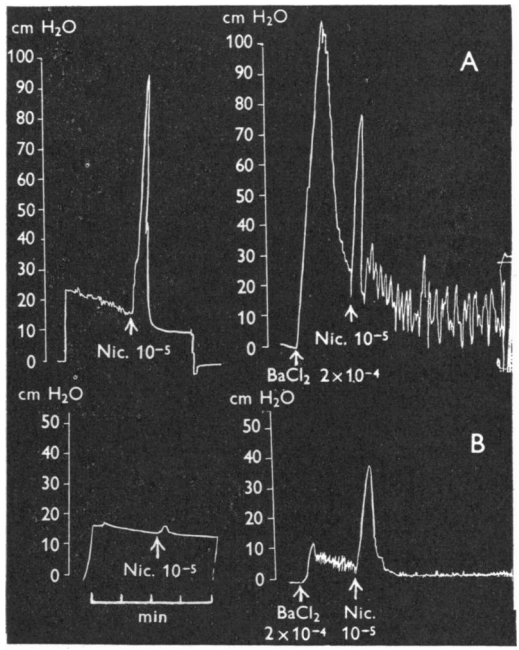

Fig. 6.

Fig. 5. Normal (A) and myenteric denervated (B) cat jejunum in Tyrode's solution. Tubular segment method. Stimulation by nicotine in a specimen which was entirely free of ganglion cells of myenteric plexus.

Fig. 6. Cat jejunum in Tyrode's solution. Tubular segment method. Normal (A) and myenteric denervated (B) preparations. Comparison of the response to nicotine alone and to nicotine in the presence of barium. The barium was added to the bath fluid before the pressure was raised.

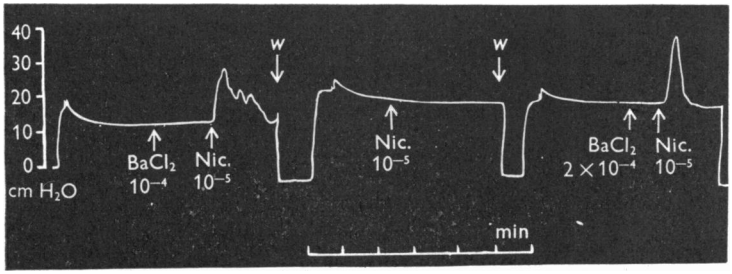

Fig. 7. Cat jejunum in Tyrode's solution. Tubular segment method. Reversible sensitization to nicotine by barium in a myenteric denervated specimen. In this instance barium itself had no visible action. The segment was washed out at *w*.

by adrenaline released through the action of nicotine on the adrenal medulla. This mechanism cannot operate in isolated intestinal preparations since the sympathetic ganglion cells innervating the intestine are situated outside the viscus (Langley & Dickinson, 1889).

(*b*) Paralysis following stimulation. This is readily shown in isolated intestinal preparations and is attributed to different mechanisms by various authors.

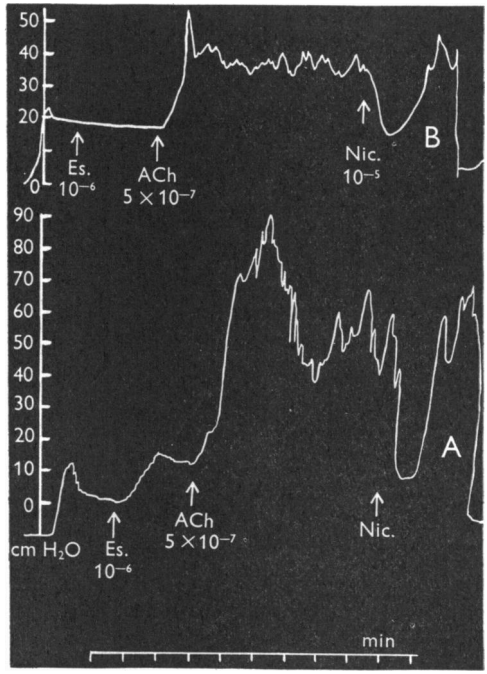

Fig. 8. Cat jejunum in Tyrode's solution. Tubular segment method. Nicotine (10^{-5}) applied in the presence of eserine and acetylcholine produces a marked inhibition in both the normal (A) and ganglion-free (B) preparations.

(*c*) Transient inhibition by nicotine, not preceded by stimulation, occurring in the absence of the sympathetic ganglion cells situated outside the viscus. Langley & Magnus (1905) observed that after degeneration of 'nearly all' the post-ganglionic sympathetic fibres to the intestine in the anaesthetized cat, nicotine produced inhibition which was 'apparently not a wit less than normal'. However, they did not exclude the possibility of nicotine releasing adrenaline from the adrenal glands. Magnus (1905) observed transient inhibition by nicotine, usually followed by stimulation, in isolated strips of intestine. This is also shown in one of the illustrated experiments (Fig. 40) of Van Esveld's paper (1928). Ambache & Edwards (1951) and Ambache (1951) have demon-

strated that a purely inhibitory effect of nicotine may be produced in isolated intestinal preparation from kittens in the presence of atropine and in those from rabbits in the presence of botulinum toxin. They ascribe this effect of nicotine to an action on ganglion cells of the myenteric plexus which are cell bodies of adrenergic neurones and thus when stimulated induce relaxation of the gut.

The paralytic effect of nicotine following stimulation (type 2) was usually pronounced in the innervated preparations but in the ganglion-free preparations it occurred only occasionally and was then relatively inconspicuous. On the other hand, the transient inhibitory action of nicotine (type 3) occurred in both the innervated and ganglion-free specimens. This effect was particularly pronounced in preparations stimulated to contract by the previous addition of eserine and acetylcholine. Fig. 8 shows that this inhibition occurs equally in the normal and ganglion-free preparation. It cannot therefore be due to an action on inhibitory ganglion cells.

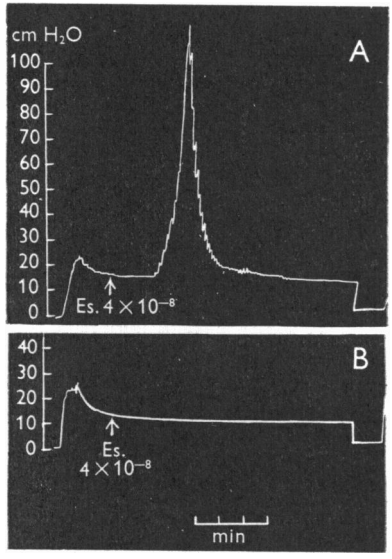

Fig. 9. Cat jejunum in Tyrode's solution. Tubular segment method. Effect of eserine on normal (A) and ganglion-free (B) preparations. (A) responded with a series of powerful contractions culminating in spasm and followed by relaxation. The myenteric denervated preparation (B) failed to respond.

Eserine

Magnus (1905) used very high concentrations of eserine (1:1000, or more) and found that on ganglion-free preparations they produced rhythmic contractions, whereas pilocarpine, barium and strophanthin produced a tonic contraction. He concluded that eserine has some peripheral action on the

muscle cell but that his experiments did not exclude the possibility of a further action of eserine on the myenteric plexus. Van Esveld obtained stimulation with much weaker concentrations of eserine (of the order of 10^{-5}) in only two of eighteen ganglion-free preparations; Gasser also seems to have used the same weak concentrations and obtained a positive effect in five of eighteen such preparations.

In the present experiments only weak concentrations of eserine were used, the highest concentration being 4×10^{-6}. Such concentrations of eserine produced in all innervated preparations after a short latent period a series of rhythmic contractions, accompanied by a pronounced rise in the base-line and frequently culminating in a powerful spastic contraction. On the other hand, stimulation with these concentrations of eserine occurred only once in a ganglion-free preparation where eserine (4×10^{-7}) induced after a latency of 3·5 min a set of rhythmic contractions without rise of base-line. In all the other twenty-two ganglion-free preparations examined these concentrations of eserine had no stimulating effect. This difference between the innervated and ganglion-free preparation is shown in Fig. 9.

Although these weak concentrations of eserine usually produced no contractions in the ganglion-free preparations they regularly caused marked potentiation of the effect of acetylcholine (see p. 391).

Barium

In the normal preparations barium produced effects similar to those of eserine. The latent period with barium was usually shorter than with eserine but both produced the appearance of a co-ordinated set of contractions culminating in a spasm of the circular muscle.

In contrast to eserine, barium regularly stimulated the ganglion-free tubular segments and strips and in the same concentrations (threshold about 10^{-4}) as the innervated preparations (Fig. 10). The character of the response to barium differed in ganglion-free preparations: it induced rhythmic movements in a previously quiescent ganglion-free preparation, but it failed to produce the powerful contraction culminating in the spasm which is so characteristic of the response in the innervated preparation. This difference is shown in Figs. 10 and 11 in strip preparations and tubular segments respectively.

These results confirm Van Esveld in showing that ganglion-containing and ganglion-free strips of circular muscle are stimulated by barium at the same threshold concentration. On the other hand, the complex effect, akin to a peristaltic contraction produced by barium in the innervated tubular segments, does not occur after removal of the myenteric plexus.

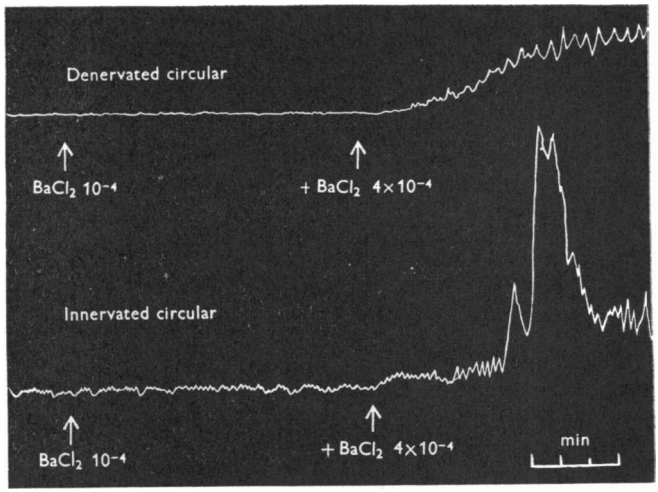

Fig. 10. Cat jejunum in Krebs & Henseleit's solution. Comparison of the character of the response and threshold to barium in an innervated (strip *c* of Pl. 1) and completely ganglion-free (strip *a* of Pl. 1) circular muscle strip.

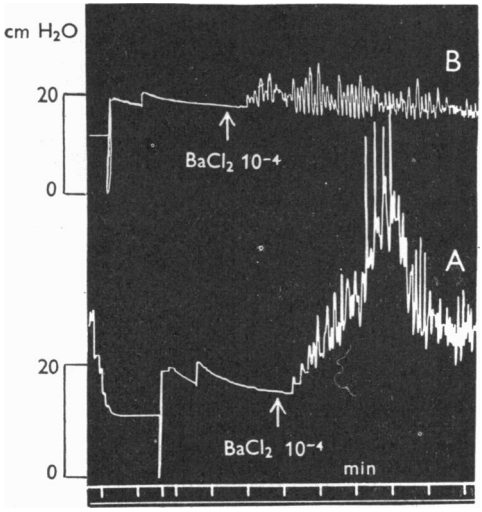

Fig. 11. Cat jejunum in Tyrode's solution. Tubular segment method. Effect of barium. The normal preparation (A) responded with powerful contractions resulting in marked increase in the base-line. The myenteric denervated preparation (B) responded with rhythmic movements without increase in base-line.

Acetylcholine

Both Gasser and Van Esveld found that acetylcholine stimulated ganglion-free strips of circular muscle fairly regularly. Van Esveld (1928) compared the sensitivity of these strips with those of longitudinal muscle containing myenteric plexus and found that the latter were 100–1000 times more responsive to the drug.

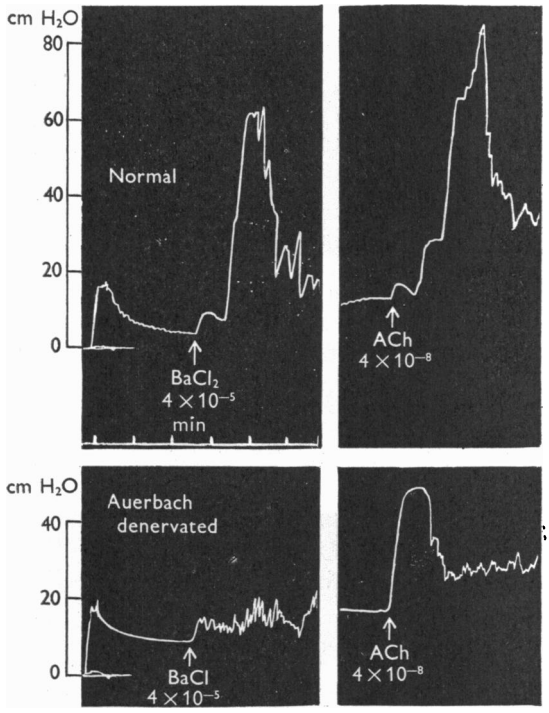

Fig. 12. Cat jejunum in Tyrode's solution. Tubular segment method. Comparison of the effects of barium and acetylcholine in normal and myenteric denervated preparations.

In our experiments most ganglion-free preparations responded to acetylcholine with a tonic contraction which differed both from the transient contraction elicited by nicotine and the stimulation of rhythmic activity produced by barium. Fig. 12 shows a comparison between the effects of barium and acetylcholine in the same preparation.

As a rule the ganglion-free tubular segments and strip preparations of circular muscle were less sensitive than the corresponding innervated preparations. This is demonstrated in Table 2 in which threshold concentrations are compared. Only in a few preparations which had received barium earlier in the experiment were the thresholds approximately equal.

The sensitivity of the ganglion-free preparations was, however, greatly increased by eserine. In its presence the thresholds of the ganglion-free and innervated preparations were approximately equal. This is illustrated in Table 3.

These experiments thus confirm Van Esveld in showing that the denervated preparations are, on the whole, less sensitive than the innervated to acetylcholine, the only exceptions being those which had previously received eserine or barium.

TABLE 2. Comparison of threshold concentrations of acetylcholine alone in normal and ganglion-free preparations

Specimens	Normal (N)	Ganglion-free (GF)	Ratio: GF/N	Method
16 A, B	10^{-8}	$>10^{-6}$	>100	Tubular segment
16 C, D	2×10^{-9}	$>10^{-6}$	>500	,,
17 A, B	2×10^{-9}	5×10^{-7}	250	,,
17 C, D	10^{-8}	10^{-7}	10	,,
18 A, B	2×10^{-8}	5×10^{-7}	25	,,
18 C, D	2×10^{-8}	5×10^{-7}	25	,,
20 A, C	5×10^{-8}	$2{\cdot}5\times10^{-7}$	5	Strip
21 F, E	2×10^{-6}	$>2\times10^{-6}$	> 1	,,
21 C, B	5×10^{-7}	$>2\times10^{-6}$	> 4	,,
22 C, B	2×10^{-8}	4×10^{-9}	$\frac{1}{2}$*	,,
23 I, H	2×10^{-8}	$>2\times10^{-6}$	>100	,,
24 C, B	8×10^{-9}	10^{-6}	125*	,,
24 A	—	8×10^{-9}	1*	,,
24 F, E	3×10^{-8}	4×10^{-6}	130*	,,
24 D	—	2×10^{-7}	7*	,,
25 C, B	3×10^{-8}	3×10^{-8}	1*	,,
25 F, E	3×10^{-8}	$>3\times10^{-6}$	>100*	,,

* Barium applied earlier in the experiment.

TABLE 3. Comparison of threshold concentrations of acetylcholine in the presence of eserine in normal and ganglion-free preparations

Specimen	Eserine concn.	Acetylcholine concn. Normal	Acetylcholine concn. Ganglion-free	Ratio: GF/N	Method
16 A, B	10^{-6}	5×10^{-8}	$2{\cdot}5\times10^{-7}$	5	Tubular segment
16 C, D	10^{-6}	5×10^{-8}	$2{\cdot}5\times10^{-7}$	5	,,
17 A, B	10^{-6}	2×10^{-8}	2×10^{-8}	1	,,
17 C, D	10^{-6}	2×10^{-8}	2×10^{-8}	1	,,
18 A, B	10^{-6}	$>10^{-7}$	10^{-7}	<1	,,
20 A, C	4×10^{-7}	5×10^{-8}	5×10^{-8}	1	Strip

The strips of longitudinal muscle with myenteric plexus attached (strip *e*) were uniformly highly sensitive to acetylcholine; in several instances concentrations of 4×10^{-9}, and on one occasion 8×10^{-10} gave a good contraction. This high sensitivity is remarkable since the peeling off of longitudinal muscle strips, only about 100 μ thick, from the underlying circular muscle necessarily involves considerable trauma. It would seem, therefore, that the relative insensitivity of ganglion-free circular muscle is due to the absence of Auerbach's plexus rather than to the trauma inflicted.

DISCUSSION

The method originally introduced by Magnus of preparing ganglion-free intestinal circular muscle provides the most direct way of studying the site of action of drugs in the intestine. The purely pharmacological methods of using antagonistic drugs such as the ganglion-blocking agents as a means of studying the site of action of active drugs are open to the objection that these antagonists may have some action on the muscle as well as on the ganglion cells. Similarly, deductions based on the use of ganglion-stimulating drugs such as nicotine are open to the objection, confirmed during this investigation, that nicotine has a direct action on the muscle cells.

The method of Magnus has a number of limitations which prevent it from furnishing a complete picture of the site of action of drugs. In the first place, as pointed out already by Magnus, this method cannot be used to determine with certainty whether a drug has an action on ganglion cells in addition to its action on smooth muscle. However, some information can be obtained by comparing the threshold dose and nature of the response in normal and ganglion-free preparations. Secondly, although Magnus's method provides smooth muscle devoid of ganglion cells, the intramuscular nerve fibres and their endings are intact and these may be acted upon by drugs. This objection may be countered by carrying out the stripping processes *in vivo* and allowing sufficient time for complete degeneration of the nerve fibres. In unpublished experiments of this kind we found it impossible to produce a denervation of the whole circumference of the intestine. Subsequent histological examination showed that some ganglion cells remained attached at the mesenteric border and these preparations cannot therefore be used as tubular segments, but it may be possible to obtain in this way chronically denervated strips of part of the circumference.

Another disadvantage in the use of ganglion-free preparations is the inconsistency of their response to drugs, and this is probably the reason for the divergent conclusions reached by different authors. This inconstancy is difficult to explain. The normal preparations behave much more regularly, and it is therefore unlikely that the irregularities are explained on the assumption that the inherent pattern of drug receptors in the intestinal smooth muscle varies from animal to animal and in different preparations from the same intestine. It is possible to assume, however, that in the process of stripping the myenteric plexus some drug receptors become more easily deranged than others. Such a hypothesis could explain why the proportion of ganglion-free preparations responding to different drugs varied in our experiments. For example, in our series, nineteen out of thirty-four preparations failed to respond to nicotine alone, whereas only four of twenty-nine preparations failed to respond to barium. Yet one of the barium-refractory preparations responded to nicotine.

Our findings show that stimulatory as well as inhibitory effects of *nicotine* are obtainable in ganglion-free preparations of intestinal circular muscle of cats. This is in agreement with the results obtained by Van Esveld, but at variance with the conclusions of Magnus and Gasser that nicotine acts, in the intestine, only on ganglion cells. The finding that nicotine contracted about 45% of the ganglion-free preparations shows that its stimulating action on intestinal preparations cannot be explained wholly by an effect on ganglion cells. The proportion of ganglion-free preparations which contracted to nicotine increased even further, to about 75%, in the presence of barium. The mechanism of this sensitizing effect of barium is at present unknown. It exerted a similar effect on the acetylcholine response. Further, in ganglion-free preparations barium initiated or enhanced rhythmic activity. This latter effect may be related to the potentiating action on nicotine and acetylcholine contraction and be of a non-specific nature, the barium rendering the ganglion-free muscle more active and thus more sensitive to the two drugs. The sensitizing effect of barium, however, occurred in some preparations in which barium itself did not produce visible effects.

Nicotine also produced transient inhibitory effects in ganglion-free intestinal circular muscle, particularly when these had been stimulated to contract by eserine and acetylcholine. This inhibition is therefore not ganglionic in origin. The same conclusion cannot be drawn for the paralysis which follows the stimulatory action of nicotine for this paralysis was not obtained in ganglion-free preparations and may therefore be ganglionic in origin.

The conclusion that nicotine has a stimulatory and inhibitory effect on intestinal preparations which is not ganglionic in origin is supported by evidence from other muscle preparations. The ureter of the pig contains no ganglion cells (Hryntschak, 1925), yet it is contracted by small and relaxed by large doses of nicotine (Macht, 1916). On the isolated horn of the virgin rabbit uterus, which is also believed to be free of ganglion cells (Reynolds, 1945), both stimulatory and inhibitory effects of nicotine have been observed (Kehrer, 1907; Fardon, 1908; Ogata, 1921).

There remain three possible sites of action of nicotine in ganglion-free intestinal preparations: (i) the remaining post-ganglionic fibres and nerve endings of the myenteric plexus, (ii) the 'terminal reticulum' and 'sympathetic ground plexus', (iii) the plain muscle fibre.

The possibility must be considered that nicotine acts on the post-ganglionic fibres of the myenteric plexus in view of the fact that nicotine stimulates not only ganglion cells but also nerve fibres or endings, for instance in the skin and mesentery (Coon & Rothman, 1940; Brown & Gray, 1948).

The 'terminal reticulum' has been described by Stöhr (1932, 1934) and Reiser (1933) and the 'sympathetic ground plexus' by Boeke (1940). These do not degenerate after removal of the ganglion cells. There is doubt as to

their nervous nature (Johnson, 1925; Nonidez, 1936, 1937) and recent investigations on the cat's intestine by one of us (Evans, 1953, in preparation) suggest that the 'reticulum' is in reality a sheath for the post-ganglionic nerve fibres, serving the same function as the Schwann sheath of somatic nerve fibres.

Although the nerve endings and the nerve nets cannot be excluded as the site of action of nicotine on the ganglion-free intestinal preparations, as well as on those other muscle preparations mentioned above in which nicotine has stimulatory and inhibitory actions, they could not provide an explanation for the nicotine effects on the chick amnion. This preparation consists of a layer of smooth muscle covered by epithelium and has repeatedly been shown to be entirely free of nervous elements (Verzar, 1914; Peterfi, 1913; Baur, 1928; Ferguson, 1940; Pierce, 1933). Langley (1905) and Baur (1928) found that nicotine increased the rhythmic contractions of the amnion and Baur found that higher concentrations of nicotine (1:50,000) produced pure inhibition. It is therefore likely that the stimulatory and inhibitory actions of nicotine on ganglion-free preparations resides in the smooth muscle fibre itself.

The finding that ganglion-free circular muscle requires for contraction higher concentrations of *acetylcholine* in the bath fluid than the innervated muscle does not necessarily imply an intrinsic reduction in the acetylcholine sensitivity of the ganlion-free preparation, because in the presence of eserine in weak concentration both preparations become equally sensitive to acetylcholine. Although eserine may have a direct sensitizing action it is more likely that in these concentrations its action is due to inhibition of cholinesterase. This would mean that, in the absence of eserine, the acetylcholine of the bath fluid has to pass a zone of cholinesterase activity which is more potent in the ganglion-free preparation than in the normal in order to reach the receptors in the circular muscle. It has been shown by Koelle (1951) that cholinesterase in intestinal muscle is mainly intracellular and concentrated chiefly at the muscle fibre membranes; there is thus the possibility that the mechanical process of stripping off the longitudinal muscle and the myenteric plexus causes injury of the superficial muscle fibres of the circular coat, whereby their intracellular cholinesterase becomes more accessible for acetylcholine destruction. In this way a potent zone of cholinesterase activity would be created in the superficial part of the circular muscle. It is unlikely that the effect which barium exerts on the acetylcholine sensitivity of ganglion-free preparation is effected by rendering the intracellular cholinesterase again less accessible to acetylcholine, since barium has a similar potentiating effect on the nicotine response of the ganglion-free preparations.

The finding that the stimulatory action of weak concentrations of *eserine* on the circular muscle is dependent upon the presence of the myenteric plexus was surprising, although it had been previously observed by Gasser and Van Esveld. The slow onset of the effect suggests that eserine acts by inhibition of

cholinesterase activity. In that case we are forced to assume that in the normal preparation there is a continuous release of acetylcholine in the circular muscle layer and that this release comes to an end when the myenteric plexus is removed. Presumably the nerve fibres which originate from the myenteric plexus are cholinergic; they may have the ability to synthesize and release acetylcholine continuously as long as they are in continuity with the parent cells, but lose this property when they become separated.

The presence of the myenteric plexus can thus profoundly, and in different ways, modify the action of a drug. It is doubtful that a clear-cut distinction between drugs acting on the myenteric plexus and on plain muscle can always be maintained. Many drugs may produce effects on both, and the presence of the myenteric plexus may modify the action of drugs in several ways: (1) ganglion cells may have a lower threshold than the muscle cells, and be the only ones directly affected by low concentrations of drug; (2) the threshold of ganglion cells may not be very different from that of muscle cells, but their stimulation by the drug causes a propagated disturbance with more far-reaching effects; (3) the presence of the intact nerve plexus may influence and perhaps increase the sensitivity of plain muscle to drugs (Van Esveld's view); (4) direct stimulation of plain muscle by the drug may initiate impulses which are propagated by the plexus.

Rhythmic activity in normal and ganglion-free preparations. The movements induced in the small intestine as a result of distension of the lumen are of two main types: (*a*) Continuous rhythmic activity which remains more or less localized and which serves to mix the intestinal contents and to promote absorption. The character of these movements varies considerably in different species; in some the rhythmic activity consists of segmentation as described by Cannon (1902), in others mainly of pendulum movements. These rhythmic movements involve both muscle coats and are presumed to be myogenic in origin for they occur in ganglion-free preparations as well as when the ganglion cells have been paralysed by nicotine (Bayliss & Starling, 1899; Trendelenburg, 1917; Thomas & Kuntz, 1926; Alvarez, 1937). (*b*) Intermittent peristaltic contractions which travel along the intestine and which serve to propel its contents. Bayliss & Starling (1899) used the term 'peristaltic contraction' for the more complex wave of activity which occurs intermittently in response to distension. They described this response as follows: 'Shortly after putting in the bolus the contractions of the segment of intestine immediately above the bolus undergo increasing augmentation until the intestine at this point enters into a strong tonic contraction. This presses the bolus onwards and as the bolus moves the ring of constriction follows it up....'

The rhythmic activity we observed in innervated tubular segments of the cat's intestine in response to raising the intralumen pressure occurred also in about a third of the ganglion-free preparations. This must, therefore, be at

least partly myogenic in origin. These tubular segments were, however, unsuitable for recording the progress of peristaltic waves and thus for discriminating between peristaltic and rhythmic myogenic activity. Nevertheless, certain contractions could be shown to be ganglionic in origin. Barium produced, in innervated preparations, a series of contractions culminating in spasm resembling the sequence of events described by Bayliss & Starling in the initial phase of a peristaltic contraction. These spastic responses were absent in the ganglion-free preparations and were, therefore, probably brought about by an effect of barium on the myenteric plexus. The similar spastic contraction produced by eserine were also dependent on the integrity of the myenteric plexus.

SUMMARY

1. The drug responses of ganglion-free circular muscle preparations of cat jejunum have been investigated. The ganglion cells of the myenteric plexus of Auerbach were removed by stripping off the longitudinal muscle coat to which they adhere. The submucous plexus of Meissner's plexus was removed by detaching the submucosa and mucous membrane from the circular muscle. The completeness of removal of the ganglion cells was checked histologically.

2. Two kinds of ganglion-free preparations were used: (i) circular muscle strips suspended in Tyrode or Krebs & Henseleit's solution; (ii) tubular segments the intralumen pressure of which was recorded. The behaviour of tubular segments was studied by a modification of the Trendelenburg method and consisted of recording changes not of volume but of pressure within the intestinal lumen. By using higher distension pressure than Trendelenburg the method is applicable for the cat's intestine with its very thick circular muscle coat.

3. Rhythmic contractions of ganglion-free circular muscle, in the absence of drugs, were recorded with both preparations and are therefore myogenic in origin.

4. Nicotine stimulates ganglion-free preparations (confirming Van Esveld). Subliminal concentrations of barium sensitizes these preparations to the stimulant actions of nicotine. In ganglion-free preparations which have been stimulated by eserine and acetylcholine, addition of nicotine produces a transient inhibition preceding stimulation. It is concluded that both stimulations and primary inhibition by nicotine can occur in the absence of ganglion cells.

5. Eserine, in the low concentrations used (4×10^{-6} or less), failed to stimulate ganglion-free preparations. These doses regularly stimulated circular muscle preparations containing myenteric plexus.

6. Barium stimulated both ganglion-containing and ganglion-free circular muscle at similar threshold concentrations, but the character of the response in the two preparations differed. In the ganglion-free preparation it induced

only a series of rhythmic contractions with little or no rise in base-line, whereas in the innervated preparations barium caused powerful contractions culminating in spasm. It is concluded that the action of barium is partly ganglionic, partly muscular.

7. Acetylcholine stimulated both normal and ganglion-free preparations, but the threshold in the latter was generally higher. In the presence of eserine and barium, however, the thresholds were approximately the same. Strips of longitudinal muscle containing myenteric plexus were very sensitive to acetylcholine.

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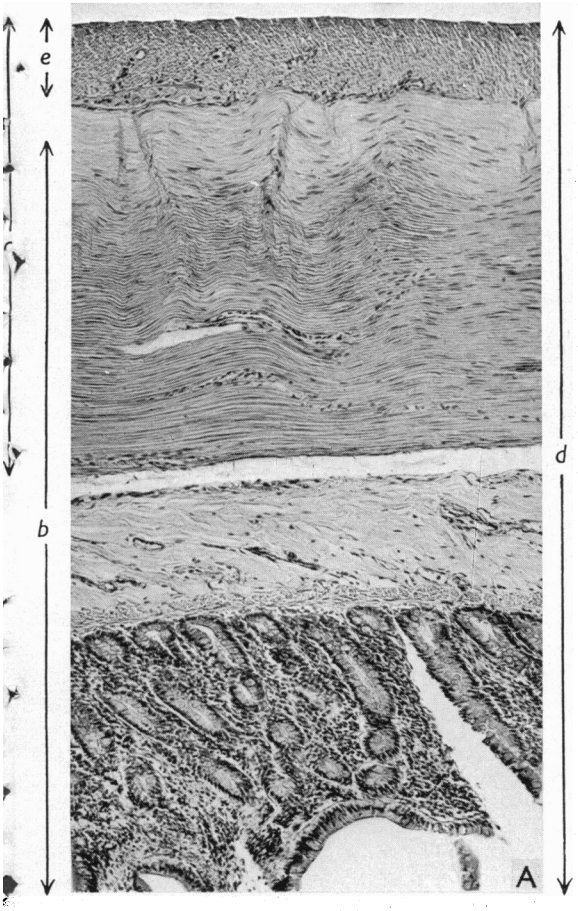

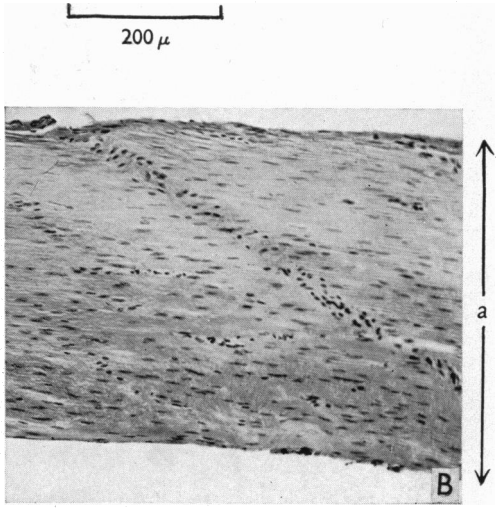

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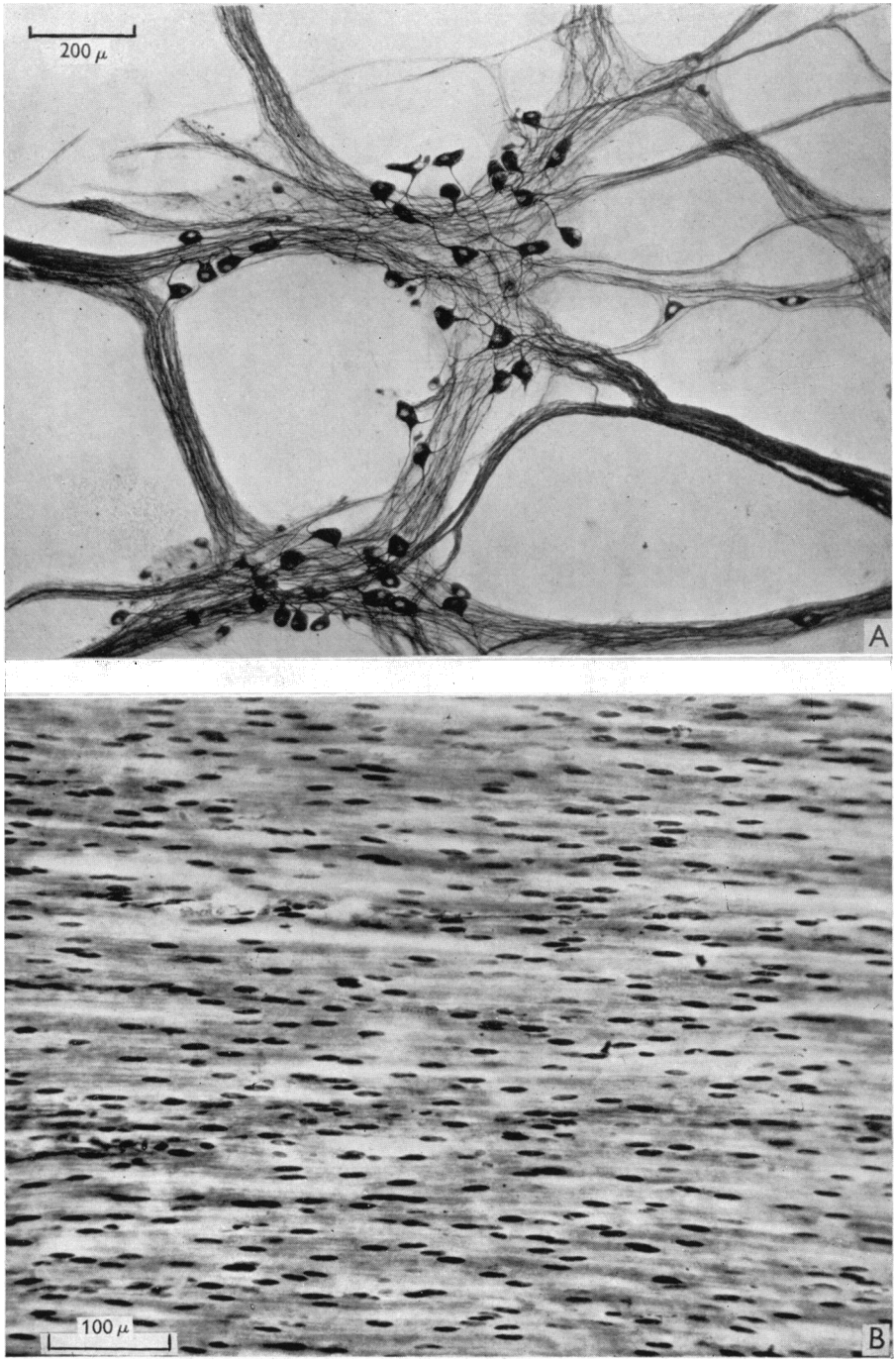
200 μ
A
100 μ
B

EXPLANATION OF PLATES

PLATE 1

A. Transverse section of intact cat jejunum indicating the layers present in the different strips described in the text. Haematoxylin and eosin.

B. Transverse section of ganglion-free circular muscle strip (preparation *a*). Haematoxylin and eosin.

PLATE 2

A. The entire thickness of the stripped longitudinal muscle coat with myenteric plexus attached. Bielschowsky-Gros method. The muscle cells are unstained.

B. Tangential section, 40 μ thick, through outer surface of circular muscle coat showing absence of ganglion cells. Myenteric plexus had been removed by peeling off the longitudinal muscle coat and scraping the superficial surface of the circular muscle. Bielschowsky-Gros method.