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THE INHIBITORY ACTION OF NICOTINE ON THE RABBIT COLON

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The response to nicotine of intestinal preparations *in vitro* is usually a contraction due to stimulation of the parasympathetic cholinergic neurones in Auerbach's plexus. Ambache (1951) and Ambache & Edwards (1951) found that an inhibitory effect of nicotine could be demonstrated if these intrinsic cholinergic motor neurones were first put out of action, either by *Clostridium botulinum* toxin (rabbit small intestine) or by atropine (kitten small intestine and stomach). This effect could be blocked by nicotine in ganglion-cell-paralysing doses and by hexamethonium. In the ileum of the rabbit an inhibitory effect of nicotine could not be produced in the presence of atropine because the motor effects of nicotine were not abolished by atropine unless high, and probably non-specific, concentrations of this drug were used. In contrast we have found previously, in experiments on preparations of the rabbit colon, that nicotine could produce inhibition in this region of the alimentary canal. By selecting suitable dose levels of nicotine we could obtain either pure inhibitory responses (with low doses), or pure motor responses (with high doses), or mixed inhibition and contraction; cholinergic blocking agents were not required in these experiments.

Nicotine is known to produce in other organs peripheral effects which are apparently due to the release of catechol amines, in particular, acceleration of the heart (Hoffman, Hoffman, Middleton & Talesnik, 1945; Kottegoda, 1953*a*), constriction of blood vessels (Kottegoda, 1953*b*; Hilton, 1954; Burn & Rand, 1958; Strömblad, 1959) and dilatation of bronchial smooth muscle (Hawkins & Paton, 1958). Ambache (1951) attributed the inhibition with nicotine in the intestine to a release of catechol amines from the nerve endings of adrenergic neurones in Auerbach's plexus, since it could be blocked by ephedrine. In other tissues chromaffin cells have been suggested as an alternative source of the catechol amines. Since nicotine-induced inhibition can be demonstrated easily in the colon, we have used this preparation for further investigations of the mechanism of nicotine action and, in particular, to decide whether chromaffin cells, rather than adrenergic neurones, might be the source of

the inhibitory amines. The results do not support the view that chromaffin cells are responsible for this effect.

METHODS

Rabbits of either sex were killed by a blow on the neck and bled. Preparations were made, as described by Garry & Gillespie (1955), of the rabbit colon complete with its extrinsic sympathetic (lumbar colonic) and parasympathetic (pelvic) nerve supplies, each of which could be stimulated separately. Magnus preparations of colon and ileum, without dissected lengths of the extrinsic nerves, were also used. The preparations were suspended in Krebs's saline solution at 36° C in a 200 ml. isolated organ bath, and the contractions of the longitudinal muscle were recorded on a smoked drum with a light gimbal isotonic lever (magnification 3 ×); the tension on the preparations was 0·5 g. The composition of the bath fluid was as follows: (g/l.) NaCl, 6·92; KCl, 0·35; $CaCl_2$, 0·28; KH_2PO_4, 0·16; $MgSO_4.7H_2O$, 0·29; $NaHCO_3$, 2·1; glucose, 2.

To make mucosa-free preparations the colon was first converted into a flat strip by a longitudinal cut along the antimesenteric border, and the preparation was pinned out on cork, mucosal side upwards. The line of cleavage between the submucosa and the underlying muscle layer was easily identified and the mucosa and submucosa were stripped off with fine forceps, preferably in the direction of the circular muscle. When suspended in the organ bath such preparations displayed the rhythmic activity usual in normal preparations, with the added advantage of a more stable base line; they responded well to electrical stimulation of the extrinsic sympathetic and parasympathetic nerves.

Two methods were used for staining the chromaffin cells in the gut. First, the chromaffin reaction, unmodified. The piece of intestine was opened out, pinned flat on to a cork board with quills, and then fixed for 24 hr in Müller's fluid containing 10% formalin. The material was then 'post-chromed' for 3 days in a 2·5% aqueous solution of potassium bichromate, after which it was washed, dehydrated, embedded in paraffin and sectioned at 7 μ. The sections were subsequently lightly counter-stained with haemalum. The second method was that of Sevki, as described by Adams-Ray & Nordenstam (1956). The preliminary treatment was the same as above but, after embedding in paraffin, the tissue was sectioned and the sections stained by a modified Giemsa method.

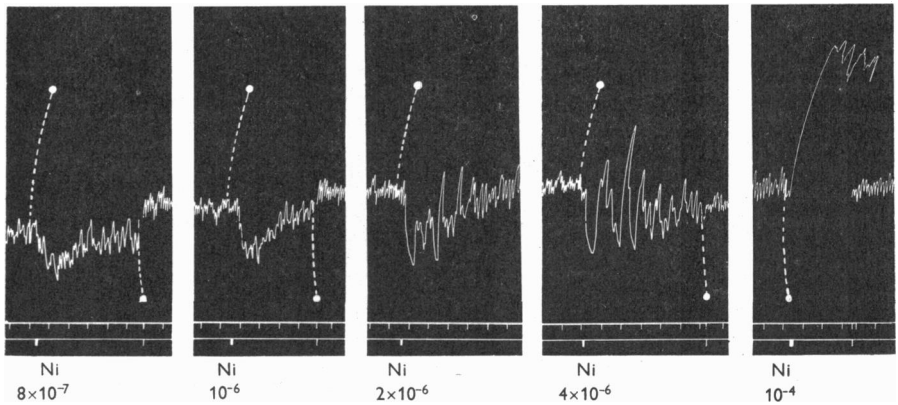
The following drugs were used: adrenaline hydrochloride, acetylcholine chloride, atropine sulphate, dimethylphenyl-piperazinium iodide (DMPP), hexamethonium bromide (May & Baker), nicotine hydrogen tartrate, reserpine (Serpasil; Ciba), and choline 2:6 xylyl ether bromide (TM 10). Concentrations refer to the salts.

RESULTS

The action of nicotine and DMPP. It is well known that the response to nicotine of the rabbit's small intestine is always a contraction. In the colon the type of response varies with the concentration of nicotine. With low concentrations the first response is always inhibition. When the concentration of nicotine is increased the response becomes first biphasic, with inhibition followed by contraction, and finally purely motor. These results are shown in Text-fig. 1. The concentration of nicotine which will produce pure inhibition varies from one preparation to another, but is usually between 10^{-6} and 10^{-5}. As Ambache & Lessin (1955) have shown, in the ileum the ganglion-stimulating drug DMPP can also cause

inhibition. In the colon the effect is neither greater nor more readily elicited than with nicotine and the drug was not used extensively.

Effect of atropine. Nicotine in concentrations greater than 10^{-5} causes the colon to contract. It is possible that in these concentrations it also stimulates the inhibitory mechanism, but this is masked by the strong contraction arising from the stimulation of the cholinergic ganglion cells in Auerbach's plexus. If the effects of stimulation of these motor neurones could be blocked at the nerve–muscle junction by atropine, then it should be possible to reverse the normal motor response by uncovering the co-existing inhibition. This expectation was fulfilled, as is shown in Text-fig. 2. The response at *A* to nicotine 10^{-5} before atropine was a contrac-

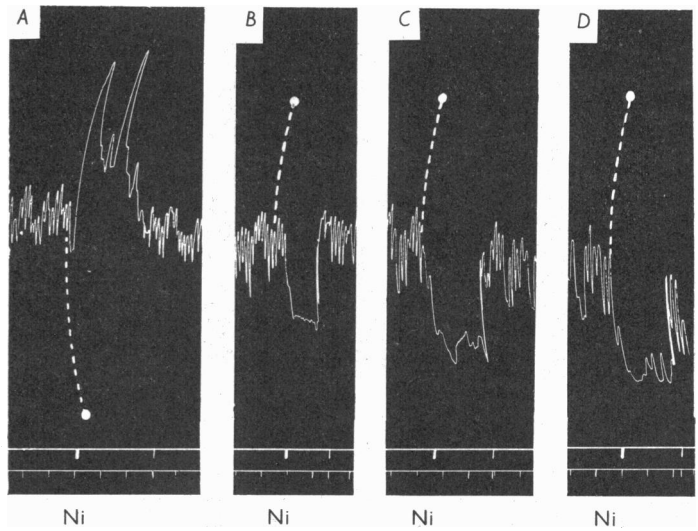


Text-fig. 1. Rabbit colon preparation (*in vitro* in this, as in all subsequent experiments). Curved dotted lines indicate addition of nicotine (Ni), or washing. Nicotine in low concentrations elicits an inhibitory, and in high concentrations a motor, response. In all text-figures time marker = 30 sec.

tion; at *B* in the presence of atropine 10^{-4}, nicotine 10^{-5} produced inhibition. The concentration of atropine used was that which was necessary just to block the response to stimulation of the parasympathetic (pelvic) nerves, on the assumption that these nerves have as their final pathway the same motor neurones as are stimulated by nicotine. Atropine in such high concentrations, besides blocking 'muscarinic' effects, also reduced 'nicotinic' effects slightly (Text-fig. 2). Thus, small doses of nicotine, which before atropine produced inhibition, were ineffective after atropine. However, larger doses of nicotine, previously eliciting contractions, now had a reverse effect, i.e. inhibition. Fortunately, after the atropine was washed out, its anti-muscarinic blocking action persisted for some hours, as was shown by the block of the motor response to pelvic nerve stimulation. In contrast, the antagonism by atropine of nicotine inhibition disappeared fairly quickly, as shown for example in Text-fig. 2 by the

increasing effectiveness of nicotine in producing inhibition, 20 min and 40 min respectively after the removal of atropine.

In most experiments atropine was used in this way to demonstrate the inhibitory effect of nicotine. Atropine sulphate 10^{-4} or 2×10^{-4} was added to the bath fluid and left in contact with the preparation until the response to stimulation of the pelvic nerve was blocked. The atropine was then removed and nicotine in a concentration of 10^{-5} added, to produce inhibition. This method reliably demonstrated inhibition and avoided the necessity of determining in each preparation the exact dose of nicotine, which would, acting alone, produce inhibition.



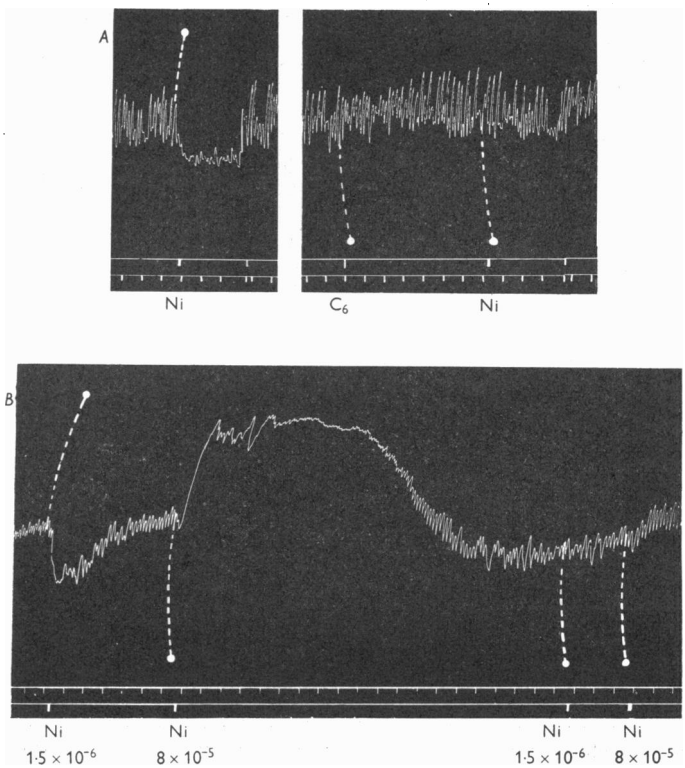
Text-fig. 2. Reversal by atropine of the nicotine response in the rabbit colon. At Ni, nicotine 10^{-5}. *A*, before atropine; *B*, in the presence of atropine 10^{-4}; *C* and *D*, 20 and 40 min, respectively, after washing out the atropine.

Effect of hexamethonium and of large doses of nicotine. Ambache (1951) and Ambache & Edwards (1951) found that the inhibitory action of nicotine was abolished by hexamethonium or by ganglion-paralysing doses of nicotine. In our experiments too, hexamethonium bromide added to the bath in concentrations similar to those required to block the response to stimulation of the pelvic nerve, also blocked the inhibitory effect of nicotine (Text-fig. 3*A*). Although high concentrations of hexamethonium were required, its action could be shown to be specifically ganglion-blocking in that the response to stimulation of the lumbar sympathetic nerves (Text-fig. 6) and the responses to administered acetylcholine or noradrenaline were unaltered.

If large ganglion-paralysing doses of nicotine were added to the bath

and left in contact with the preparation until the tone and activity had returned to their previous level, then the addition of further small doses of nicotine, which had previously caused inhibition, was now ineffective (Text-fig. 3*B*).

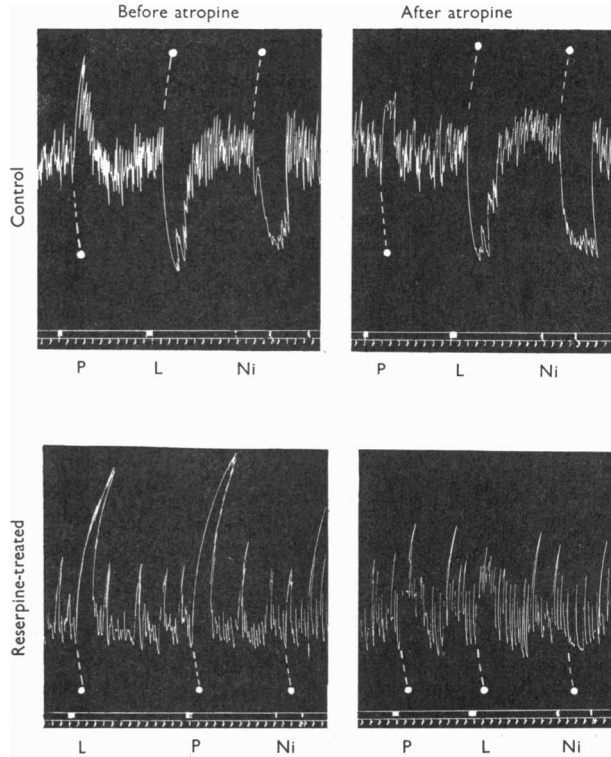
Effect of reserpine. The similarity between the inhibition produced by nicotine and the inhibition which follows stimulation of the sympathetic



Text-fig. 3. *A*. Extinction by 2×10^{-4} hexamethonium bromide (C_6) of the inhibition produced by nicotine 10^{-5} (Ni) in the rabbit colon. *B*. A high concentration of nicotine (8×10^{-5}) left in contact with the preparation abolishes both the inhibitory effect of a small dose of nicotine and the motor effect of a second, larger, dose of nicotine.

lumbar nerves suggested that the nicotine response might be mediated by the release of a catechol amine at some site in the gut wall. It is known that reserpine can produce a depletion of these amines at peripheral adrenergic nerve endings and, less readily, in chromaffin cells (Muscholl & Vogt, 1958); according to these authors' results it would seem that reserpine is more effective in repeated, small, doses, particularly on chromaffin cells. We have accordingly given rabbits single daily intravenous injections

of reserpine 0·2 mg/kg for 10 days. A group of eight animals was used, four treated with reserpine in a suitable vehicle and the other four, serving as controls, receiving daily injections of an equal volume of the vehicle by itself. In the four preparations from the reserpine-treated animals the



Text-fig. 4. Effect of reserpine on the response of the rabbit colon preparation to nicotine 10^{-5} (Ni) and to parasympathetic (P) and sympathetic (L) stimulation. Above, preparation from a control animal injected with the reserpine vehicle; below, from an animal given reserpine 0·2 mg/kg I.V. daily for 10 days. In the control, nicotine causes inhibition and this is enhanced after atropine. In the preparation from the reserpine-treated animal nicotine has little or no inhibitory action either before or after atropine, and the response to sympathetic nerve stimulation is reversed to motor.

inhibitory effect of nicotine was almost completely abolished, whereas in each of the four control animals powerful inhibitory responses were obtained (Text-fig. 4). An unusual finding in these experiments was that after reserpine depletion of the adrenergic nerve endings the response to sympathetic nerve stimulation not only ceased to be inhibitory but was, in fact, replaced by a motor response. This sympathetic reversal has been further investigated and will be reported separately.

Effect of choline 2:6 *xylyl ether* (*TM* 10). The results so far described have shown that the inhibition produced by nicotine in the rabbit colon was a true nicotinic effect of the drug, being blocked by hexamethonium and by large paralysing doses of nicotine, and that the action was mediated by the release of a catechol amine. There remained the problem of the site of liberation of this amine. The two obvious possibilities were (1) adrenergic neurones in Auerbach's plexus and (2) chromaffin cells. The following experiments were designed to distinguish between these two possibilities.

TM 10, although possessing numerous other actions, is known to block the effects of adrenergic nerve stimulation, apparently by interference with the synthesis or release of the transmitter (Exley, 1957). Bain & Fielden (1956) found that it abolished the effect of sympathetic nerve stimulation in the small intestine. Chromaffin cells, as for example in the adrenal medulla, are relatively resistant to the action of TM 10 and no effect on their release of catechols could be shown in acute experiments (Exley, 1957). This drug might therefore be expected to discriminate between an action of nicotine on the adrenergic neurones and on chromaffin cells.

In the present experiments on colon preparations, the response to stimulation of the adrenergic supply in the extrinsic sympathetic nerves was abolished after about 70 min exposure to TM 10. On the other hand, the inhibition produced by nicotine was only slightly reduced (Text-fig. 5) and the motor response to parasympathetic stimulation was also unaffected.

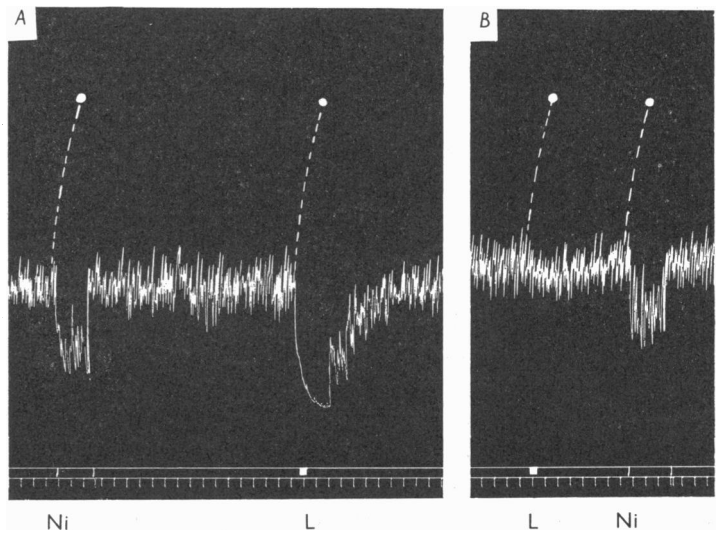
Histological findings. The above results suggested that the action of the nicotine was perhaps on chromaffin cells. We have therefore examined serial sections of lengths of colon for the presence of chromaffin cells or chromaffin tissue. As controls, lengths of colon from rabbits treated with reserpine were similarly examined after the reserpine had discharged the contents of the chromaffin cells. Other tissues examined were: stomach, duodenum, ileum, inferior mesenteric ganglion, and solar ganglion.

In the normal colon the only cells giving the chromaffin reaction were the enterochromaffin cells in the mucosa. The region of Auerbach's plexus was examined particularly carefully in a large number of sections without discovering any chromaffin-staining cells. The enterochromaffin cells also stained well with Sevki's method, assuming a dark reddish-brown or buff colour. In animals reserpine-treated for 5–10 days enterochromaffin cells disappeared completely from the colon, which was examined by both methods of staining.

With Sevki staining one other type of cell was found in the mucosa and submucosa of all parts of the alimentary tract examined (Pl. 1, *g*). They were much commoner in the small intestine than in the colon. These cells contained bright red, discrete granules: the cell outline was ill-defined, the granules often appearing to lie almost free; the cytoplasm was unstained and the disposition of the granules was very variable. The nucleus was commonly single, occasionally bilobed. These cells resembled the chromaffin cells in the skin illustrated

by Adams-Ray & Nordenstam (1956), but were not in fact chromaffin cells, since, in animals treated with reserpine, their staining properties remained unaltered, whereas the known enterochromaffin cells became invisible. From their bright red granules and occasional bilobed 'spectacle' nucleus it may be inferred that they are almost certainly eosinophils, a cell commonly found free in the gut wall. These cells together with typical enterochromaffin cells are illustrated in Pl. 1.

In the inferior mesenteric and solar ganglia, groups of typical chromaffin cells were found, chiefly on the outside of the ganglia, but occasionally single cells or small groups were found buried among the ganglion cells, as described by previous authors (Kohn, 1903; Muschol & Vogt, 1958).

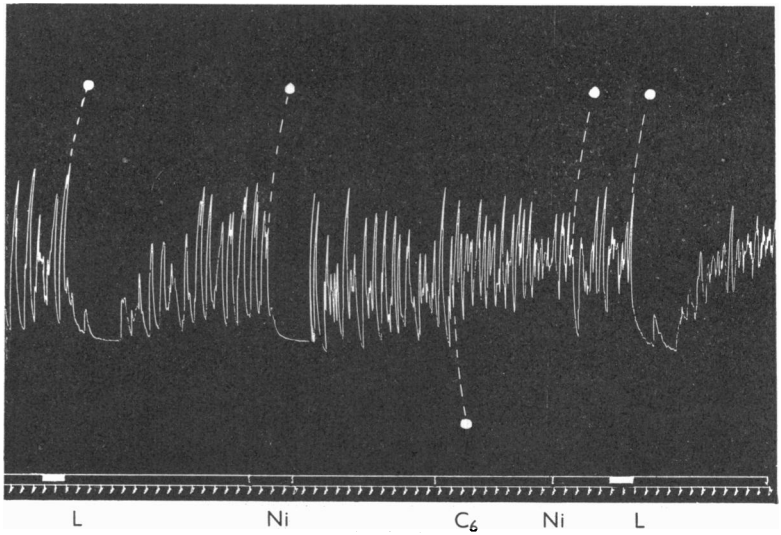


Text-fig. 5. Effect of TM 10 (choline 2:6 xylyl ether) on the response of the rabbit colon preparation to stimulation of the extrinsic sympathetic nerves (L) and to nicotine 10^{-5} (Ni). *A*, inhibitory responses produced initially by nerve stimulation and by nicotine. Between *A* and *B*, TM 10 (2×10^{-5}), abolishing inhibitory effect of sympathetic stimulation but not of nicotine, which is slightly reduced.

Chromaffin cells were not found in the stellate ganglion. It should be mentioned here that in the innervated colon preparation which we used for our nicotine experiments the inferior mesenteric ganglion is retained. Nicotine, however, could not have produced inhibition by liberating adrenaline or noradrenaline from this ganglion into the bath fluid, since even when all the mesentery and external nerves had been removed inhibition was still produced by nicotine. Thus Text-fig. 3*B* is from such a preparation.

Effect of mucosal stripping. The only chromaffin cells demonstrable in the gut wall were the enterochromaffin cells in the mucosa. It is almost certain that these cells contain 5-hydroxytryptamine (5-HT), a substance which

has a motor rather than an inhibitory effect on the smooth muscle of the colon. It was, however, conceivable that these cells might also produce catechol amines, either together with 5-HT in the same cell, or if the cell population were not uniform, in a few cells producing only catechol amines. If such catechol-amine-liberating cells were present, then removal of the mucosa ought to abolish the inhibitory effect of nicotine. After stripping the mucosa, the smooth muscle appeared undamaged since it continued to respond normally to stimulation of both sympathetic and



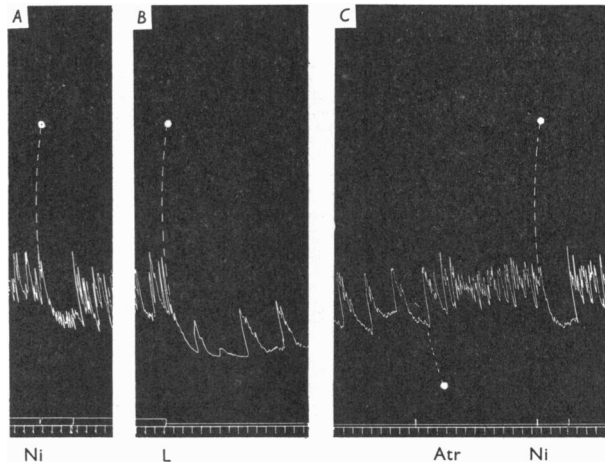
Text-fig. 6. Mucosa-free rabbit colon preparation, showing normal responses to sympathetic nerve stimulation (L) and to nicotine 10^{-5} (Ni). Hexamethonium (C_6) 10^{-4} abolishes the response to nicotine without affecting the response to sympathetic nerve stimulation.

parasympathetic nerves; moreover, the inhibitory response to nicotine was also unaffected, as shown in Text-fig. 6. In this experiment the inhibitory responses to lumbar nerve stimulation and to 10^{-5} nicotine were similar; but the nicotine response was completely blocked by hexamethonium, as before, whereas the response to stimulation of the post-ganglionic sympathetic nerves was unaffected.

Nerve fatigue and degeneration. The histological evidence that the only chromaffin cells present were those found in the mucosa, reinforced by the clear evidence that removal of these cells did not abolish the inhibitory effect of nicotine, redirected attention to the sympathetic nerves as the source of the catechol amines. The possibility that nicotine might release sympathin directly from sympathetic nerve endings was therefore considered. To test this, an attempt was made to reduce or to eliminate this sympathin in the gut wall by two methods. First, the nerves were

stimulated for long periods at high frequency (50/sec) until the preparation had 'escaped' from the initial inhibition; nicotine was then added while the stimulation was continued. The result is shown in Text-fig. 7. Even when the preparation had 'escaped' and recovered much of its original tone and rhythmic activity, i.e. when the amount of sympathin liberated from the nerve endings was presumably reduced, nicotine added to the bath still produced inhibition as before.

Secondly, the sympathetic nerves were cut and allowed to degenerate for 14 days. In these animals the cut peripheral ends of the nerves were stimulated during the subsequent experiment *in vitro*, to demonstrate the completeness of the denervation and degeneration. It was surprising how



Text-fig. 7. Effect of exhaustive stimulation of the sympathetic nerves on the inhibitory action of nicotine. *A*, response to nicotine 10^{-6} before nerve stimulation; *B*, the beginning of sympathetic stimulation; *C*, 100 min later there is some escape from the inhibitory effect; nicotine (after atropine) still produces inhibition of nerve stimulation, as before.

often some residual inhibitory effect was obtained in preparations in which the inferior mesenteric ganglion had been completely removed and both the inferior mesenteric artery and the colonic vein apparently stripped clean. This may be due to the survival of a proportion of intact post-ganglionic fibres, originating in groups of ganglion cells situated distal to the main ganglionic mass but along the course of the artery. In those preparations in which there was a residual response from stimulation of the extrinsic sympathetic nerves nicotine still elicited an inhibitory response. Our impression was that this was weaker than would be expected in normal preparations. In those preparations in which stimulation of the extrinsic sympathetic nerves was ineffective, nicotine when added to the bath produced either no inhibition or, more often, only a small inhibitory response.

DISCUSSION

We have demonstrated that nicotine on the colon of the rabbit causes inhibition of the smooth muscle. This effect is not due to paralysis of intrinsic motor neurones, since it appears at concentrations lower than are required for stimulation of these neurones. The inhibition is abolished by hexamethonium in the same concentration as is required to block post-ganglionic parasympathetic neurones; and the time course of the onset of block and the recovery after washing is similar. The inhibition is therefore a true 'nicotinic' effect. It is apparently due to the release of catechol amines from some site in the gut wall, since reserpine, which produces depletion of these amines, also abolishes the inhibitory effect of nicotine.

Two possible sources of these catechol amines would fit the experimental results, either adrenergic neurones in Auerbach's plexus or chromaffin cells in the gut. In further experiments objections have arisen to both. TM 10 could be shown to block the inhibitory effect of known adrenergic neurones (the extrinsic sympathetic nerves), without substantially affecting nicotine inhibition. Histologically chromaffin cells were present only in the mucosa, yet removal of the mucosa did not abolish the inhibitory action of nicotine. Finally, section and degeneration of the extrinsic sympathetic nerves abolished or reduced the inhibitory response, indicating that the site of liberation of the catechol amines was probably the post-ganglionic extrinsic sympathetic nerve endings or some structure associated with them.

Coon & Rothman (1940) reported fleeting pilo-erection after intradermal injection of acetylcholine or nicotine in man and in the cat. This response was unaffected by acute section of the nerve supplying the area, but was abolished after degeneration of the sympathetic nerves. These authors attributed the effect to an axon reflex in the terminals of peripheral sympathetic pilomotor nerves. Hilton (1954) described a similar effect in the terminals of sympathetic vasoconstrictor fibres. The inhibition of the colonic musculature produced by nicotine appears to be a similar phenomenon in that it is unaffected by acute sympathetic nerve section but is reduced or abolished by degenerative sympathetic section. It could therefore be attributed to an axon reflex in the sympathetic nerve endings. It is, however, difficult to visualize exactly how nicotine initiates this reflex in efferent fibres. Neither nicotine nor acetylcholine even in large concentrations appears able to stimulate nerve fibres (Lorente de Nó, 1944; Hodgkin, 1947), which would suggest that the drug must act on the nerve endings. However, such evidence as there is suggests that nicotine or acetylcholine is as ineffective at efferent nerve endings as on nerve fibres. Acetylcholine added to the fluid perfusing sympathetic ganglia, even in

high concentrations, failed to stimulate the preganglionic sympathetic nerve endings and to initiate action potentials in the preganglionic fibres (Brown & MacIntosh, 1939; Bronk, 1939). Because of these difficulties we would like to suggest the possibility that nicotine acts, not on the adrenergic nerves proper, but on some structure related to these nerves and intervening in some way between them and the effector cells. This structure would then be responsible for the spread of the response, producing its effects by the liberation of a catechol amine.

The histological picture of the final innervation of smooth muscle by the autonomic nervous system is still obscure and the subject of considerable difference of opinion. This mainly centres round the nature of those small cells first described by Cajal (1909) as 'neurones sympathiques interstitiels', which form a network or ground plexus throughout all the tissues innervated by the autonomic nervous system. If these interstitial cells are some form of small, modified ganglion cells, then they would provide a much likelier site of action for nicotine than either nerve fibres or efferent nerve endings. To postulate such a nerve network brings its own difficulties. For example, in the present experiments after section and degeneration of the extrinsic sympathetic nerves the inhibitory effect of nicotine is reduced or abolished. Either the sympathetic ground plexus must also have degenerated, which seems unlikely, or else the catechol amines in the ground plexus are depleted in the absence of the extrinsic sympathetic nerves. This would make the sympathetic ground plexus a structure in which noradrenaline is stored; such stores have been postulated by Burn & Rand (1958) in the neighbourhood of adrenergic nerve endings in the walls of blood vessels and are believed to derive their noradrenaline from these nerve endings.

The existence of such a plexus might also help to explain one remaining inconsistency in our experiments. If nicotine produces inhibition by releasing catechol amines from the extrinsic sympathetic nerves, why is this inhibition not abolished by TM 10 when the effect of nerve stimulation is abolished? If an intervening step between the sympathetic nerves and the effector muscle exists, then it is possible that TM 10 acts on the sympathetic nerves, either to block the synthesis or the release of transmitter, whereas nicotine acts directly on the sympathetic ground plexus to release its stored noradrenaline. An alternative explanation not involving a ground plexus is that TM 10 blocks the release of transmitter from the nerve endings by nerve action potentials but not by nicotine.

Section and subsequent degeneration of the sympathetic nerves to the colon for 14 days produced a variable reduction in the inhibitory effect of nicotine. In one experiment inhibition was completely absent, but in others it was present though reduced. The reason for this is not known.

The post-ganglionic neurones are not confined to the inferior mesenteric ganglion. The few situated more peripherally would remain intact with our denervation technique and may be responsible for the residual inhibitory effect of nicotine. Other possibilities are that the stores from which nicotine releases the transmitter can be replenished by circulating adrenaline, as suggested by Burn & Rand (1959), or that after denervation the smooth muscle acquires a sensitivity to nicotine, as suggested by Winbury (1959). The possibility that in some sites nicotine may act otherwise than through the extrinsic nerves is suggested by the findings of Ginzel & Kottegoda (1953). These workers found that nicotine caused stimulation of rabbit auricles and vasoconstriction of the blood vessels of the ear even after sympathetic denervation and, in the ear, after sensory denervation.

We have found no evidence that the inhibitory effect of nicotine can be mimicked by stimulation of the pelvic parasympathetic nerves. Ambache (1951) suggested that the adrenergic neurones to which he attributed the inhibitory effect in the ileum were inhibitory neurones within the peristaltic reflex arc and might also be innervated by vagal preganglionic fibres. The experimental basis for this vagal innervation was the report by McSwiney & Robson (1929) on the variable character of the response of the stomach to stimulation of the vagus. It is probable that these variations in response are due to a mixture of cholinergic and adrenergic fibres in the vagus (Harrison & McSwiney, 1936; Garry & Gillespie, 1955). Certainly in our experiments after washing out the atropine and waiting for its anti-nicotinic effect to wear off (as shown by the recovery of the inhibitory effect of nicotine) there was no inhibitory response on stimulating the pelvic nerve.

SUMMARY

1. Nicotine in low concentration (10^{-6}–10^{-5}) causes inhibition of preparations of the rabbit colon *in vitro*. In high concentrations ($> 10^{-5}$) it causes contraction. The mechanism of the inhibitory response has been studied.

2. The inhibitory effect of nicotine was abolished by hexamethonium in concentrations similar to those required for blocking the ganglion cells in the parasympathetic pathway. Large, paralysing doses of nicotine likewise blocked the response.

3. The inhibitory action of nicotine was enhanced after atropine. The concentrations of atropine used were high and had some anti-nicotinic action as well as their more usual anti-muscarinic effect. A method is described of overcoming this side effect.

4. After daily intravenous injections of reserpine for 10 days the inhibitory effect of nicotine virtually disappeared, presumably because of

the depletion of catechol amines by the reserpine. Nicotine therefore appears to produce inhibition by liberating a catechol amine.

5. Choline 2:6 xylyl ether bromide (TM 10) suppressed the inhibition produced by sympathetic nerve stimulation but did not abolish the inhibition produced by nicotine.

6. After degenerative section of the extrinsic sympathetic outflow the inhibitory effect of nicotine was reduced or lost.

7. Histologically the only chromaffin cells found in the colon were the enterochromaffin cells in the mucosa. Removal of the mucosa did not abolish the inhibition produced by nicotine. Thus the participation of mucosal chromaffin cells in this effect is excluded.

8. The inhibitory effect of nicotine, therefore, seems to be due to release of catechol amines either (1) from the extrinsic sympathetic nerves or (2) from some structure associated with them. It corresponds to the pilomotor response in the skin described by Coon & Rothman (1940) and attributed by them to an axon reflex in efferent adrenergic fibres. The possibility that nicotine acts on some form of terminal sympathetic nerve network intervening between the sympathetic nerves and the smooth muscle is tentatively suggested.

We are indebted to Professor W. A. Bain for the supply of TM 10; to Messrs May and Baker and Ciba for hexamethonium bromide and reserpine respectively; and to Miss G. Docherty, Miss A. Gilroy and Miss A. McCaffery for skilful technical assistance. The Rankin Medical Research Fund helped to provide apparatus.

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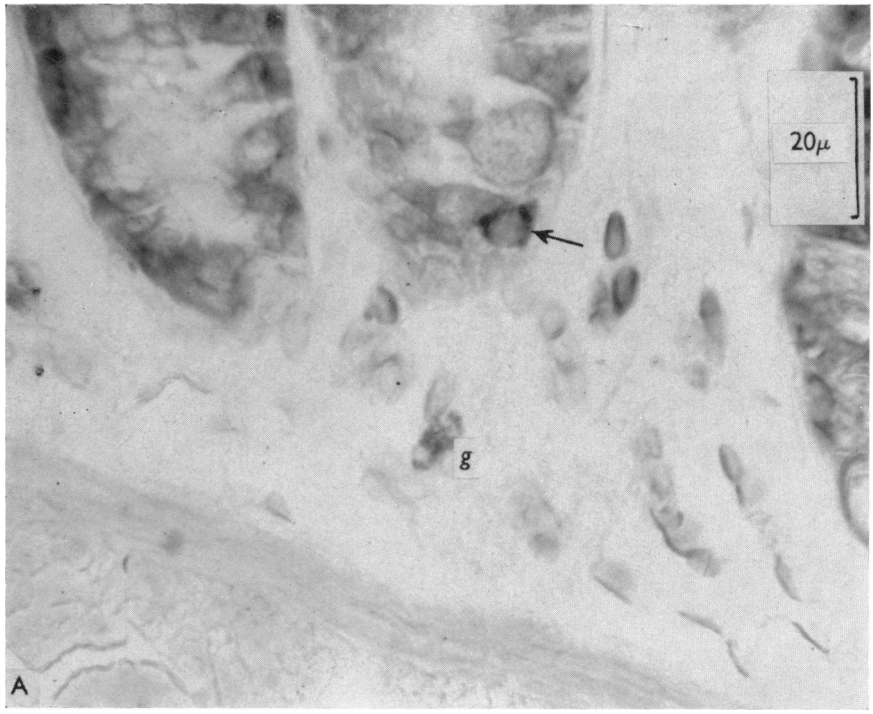
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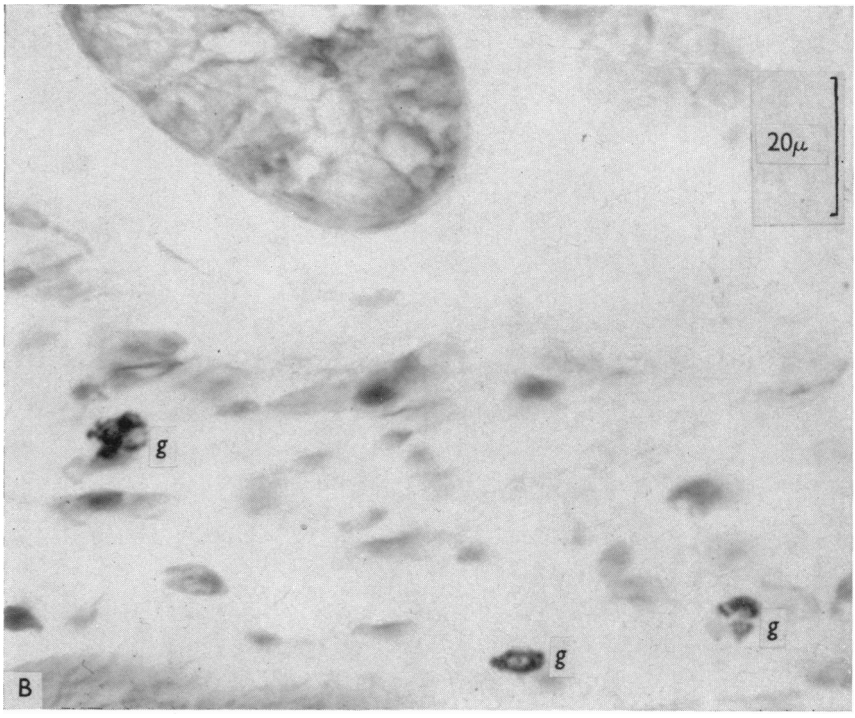
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EXPLANATION OF PLATE

PLATE 1

A. Section of normal rabbit colon stained by Sevki's method and showing an enterochromaffin cell (arrowed) in the mucosal glands and a granular cell (*g*) lying free in the stroma of the mucosa.

B. Section of the colon from a rabbit treated for 5 days with reserpine 0·2 mg/kg daily for 3 days and 1 mg/kg daily for a further 2 days. Enterochromaffin cells are absent, but the granular cells are unaltered.