Br. J. Pharmac. Chemother. (1968), 33, 342–356.

PHARMACOLOGICAL RESPONSES OF THE ISOLATED INNERVATED INTESTINE AND RECTAL CAECUM OF THE CHICK

BY

SALLY D. EVERETT*

From the Department of Pharmacology, School of Pharmacy, University of London

(*Received January* 17, 1968)

The extrinsic nerve supply of the fowl intestine consists of parasympathetic fibres from the vagus and sympathetic fibres which reach the intestine via the coeliac plexus and then, in part, via Remak's nerve and the mesenteric plexus (Nolf, 1934a, b, c). The plexuses which distribute nerves to the viscera and vessels in the abdominal and pelvic cavities are continuous with each other. They originate from the splanchnic nerves from the sympathetic trunks of the thoracic and lumbosacral parts and from the vagus and pelvic splanchnic (parasympathetic) nerves (Hsieh, 1951). The myenteric nervous system, including Auerbach's (myenteric) and Meissner's (submucosal) plexuses, is well developed in the avian intestine (Ábrahám, 1936; Kolossow, Sabussow & Iwanow, 1932). Its function is modified by the extrinsic nerves.

A limited study of the effects of drugs on the isolated fowl intestine was carried out by De Souza (1961). The responses to drugs of the intestinal muscle suspended in Locke solution at 40° C resembled that of corresponding preparations from mammalian species.

The innervation of the rectal caeca is continuous with that of the ileum (Hsieh, 1951). The rectal caecum is one of the few isolated organs of the fowl which have gained any useful pharmacological interest. Barsoum & Gaddum (1935) first reported the actions of a number of drugs on the isolated fowl rectal caecum. Since then, the caecum of the adult hen has been widely used as a sensitive method for assaying adrenaline and noradrenaline in biological extracts (von Euler, 1948a, b, c; 1949; von Euler & Hellner, 1951) and more recently for the assay of substance P (Cleugh, Gaddum, Holton & Leach, 1961). In these procedures only the part of the caecum adjacent to the rectum is used.

Very little work has been done on the effects of stimulating the extrinsic and intrinsic nerves of these organs, and the experiments described in this paper were done in an attempt to identify the neuro-transmitters, and also to evaluate the usefulness of these organs, taken from very young chicks, as general pharmacological tools.

METHODS

Segments of duodenum or ileum 2–3 cm long or whole rectal caeca were removed and cleared of excess mesentery. The preparations were suspended in Krebs-Henseleit solution (containing (g/l.) NaCl 6.95, KCl 0.34, $CaCl_2$ 0.28, KH_2PO_4 0.162, $MgSO_4$ 0.294, $NaHCO_3$ 2.1, dextrose 2) at 35° C

* Present address: Department of Physiology, University of Bristol, University Walk, Bristol 8.

and continuously gassed with 95% oxygen and 5% carbon dioxide. The body temperature of the chick is about 42° C and in preliminary experiments the temperature of the bath solution was varied over the range 32°–42° C. Similar responses to nerve stimulation occurred throughout this range but at 35° C optimal responses were obtained with a minimum of spontaneous activity.

The responses were recorded on smoked paper with an isotonic frontal writing lever loaded with 2 g and amplifying the contraction 10 times. The perivascular nerves were stimulated at various frequencies with rectangular pulses of 0.5 or 1 msec duration and of a strength such that the response for a given frequency was maximal. The electrodes used were of the type described by Burn & Rand (1960). The preparations were also stimulated coaxially according to the method of Paton (1955), between an electrode (the anode) in the lumen of the organs and a second electrode (the cathode) in the external fluid. Stimuli of 0.5 msec duration and supramaximal strength were used. In these conditions it was assumed that post-ganglionic nerve elements were stimulated because hexamethonium (20 μg/ml.) was without effect on responses. Strips of circular muscle from the rectal caecum were prepared and set up according to the method described by Harry (1963), who used the guinea-pig ileum.

Histochemical demonstration of cholinesterases

Both true and non-specific cholinesterases were demonstrated in the intestine by the method described by Hebb, Mann & Perkins (1966). The presence of acetylcholinesterase (I.E.C. 3.1.1.7.) was demonstrated by incubating sections with acetylthiocholine (6.3 mM) in the presence of pseudocholinesterase inhibitor, ethyl propazine (final concentration 5×10^{-4}M). The presence of butyrylcholinesterase (I.E.C. 3.1.1.8.) was demonstrated by incubating with butyrylthiocholine (6.3 mM) alone, because the use of specific acetylcholinesterase inhibitors seemed to make no difference in the staining. Blaber & Cuthbert (1962) also noted differences between fowl and mammalian cholinesterases in the specificity of inhibitors. Control sections were treated similarly but without inhibitor.

The drugs used were acetylcholine chloride (Roche), physostigmine sulphate, atropine sulphate, hyoscine hydrobromide, cocaine hydrochloride, choline chloride, tetramethylammonium bromide (TMA), nicotine hydrogen tartrate and (−)-adrenaline (British Drug Houses), dimethylphenylpiperazinium iodide and (−)-noradrenaline bitartrate (Koch-Light), hexamethonium bromide, pempidine tartrate and mepyramine maleate (May & Baker), phentolamine methanesulphonate, guanethidine sulphate and reserpine (CIBA), pronethalol hydrochloride and propranolol hydrochloride (Imperial Chemical Industries), bromolysergic acid diethylamide (Sandoz), laevisoprenaline bitartrate (Wyeth), *N*,*N*-diisopropyl-*N'*-isamyl-*N'*-diethylamino-ethylurea (P-286, Pitman-Moore), hemicholinium dibromide (HC-3, Aldrich) and mecamylamine (Merck, Sharpe & Dohme). The doses of nicotine, (−)-adrenaline, (−)-noradrenaline, laevisoprenaline, reserpine, P-286, hemicholinium-3 and mecamylamine refer to the base or to the cation; the remaining doses refer to the salts.

RESULTS

Action of drugs on non-stimulated preparations

The duodenum, ileum and rectal caecum contracted in response to acetylcholine (0.01 μg/ml. and above). The contractions were blocked by atropine (0.01 μg/ml.) or hyoscine (0.01 μg/ml.) and were potentiated by physostigmine (0.05 μg/ml.). In the presence of atropine (0.1 μg/ml.), larger concentrations of acetylcholine (0.2 μg/ml. and above) produced small relaxations.

No appreciable differences in the responses to ganglion stimulants were found in any particular region of the duodenum or ileum. Nicotine (0.04 μg/ml.), TMA (0.04 μg/ml.) or DMPP (0.04 μg/ml.) produced contraction of the duodenum and ileum, and occasionally this response was preceded by a small relaxation. In all cases, contraction was the predominant response while the relaxation remained small and apparently maximal at

all effective dose levels. Relaxations of the duodenum and ileum in response to ganglion stimulants and acetylcholine were inconsistently produced and often not reproducible even in the presence of large concentrations of atropine or hyoscine (up to 10 μg/ml.) which were sufficient to abolish contractions elicited by acetylcholine. These blocking drugs were often ineffective in abolishing the contractions produced by ganglion stimulants and it seemed that tolerance to them developed, because the antagonism was usually only transient. Atropine and hyoscine decreased the tone of the preparations and this may have masked any potentiation of the relaxations. The responses were regularly abolished by the ganglion blocking drugs hexamethonium (10 μg/ml.), mecamylamine (1 μg/ml.) and pempidine (1 μg/ml.). Responses to nicotine were also abolished by cocaine (5–10 μg/ml.) and no potentiation of the relaxations, if present, was obtained with smaller concentrations.

In low concentrations (0.05–1 μg/ml.), nicotine produced relaxation of caeca (see Fig. 3), but higher concentrations (1 to 10 μg/ml.) elicited biphasic responses in which the relaxations were preceded by contractions. Tachyphylaxis to both phases was usually evident with concentrations of 10 μg/ml. and above, but on a few occasions relaxations were observed with concentrations as high as 25 μg/ml. Relaxations induced by small concentrations of nicotine or of acetylcholine in the presence of atropine (1 μg/ml.) have been reported by Mattila (1963), but their nature is uncertain because neither dichloroisoprenaline (2 μg/ml.) nor phentolamine (2 μg/ml.), added alone or together, blocked them. In the present investigation, similar experiments with pronethalol (2 μg/ml.) and phentolamine (2 μg/ml.) confirmed the inability of these drugs to block the relaxations. In addition, it was found that the relaxations were unaffected by guanethidine (up to 10 μg/ml.) as illustrated in Fig. 3.

The effects of acetylcholine and nicotine were also studied on strips of circular muscle taken from the rectal caeca. Strips were set up and left for about an hour, during which time sporadic bursts of spontaneous activity developed to a constant level. The circular muscle was less sensitive than the longitudinal muscle to acetylcholine, 0.5 μg/ml. usually being required to evoke contraction of the circular muscle. Nicotine, even in concentrations up to 50 μg/ml., was completely without effect on circular muscle preparations.

(−)-Adrenaline (0.04 μg/ml.), (−)-noradrenaline (0.08 μg/ml.), dopamine (5.0 μg/ml.) and laevisoprenaline (0.04 μg/ml.) all relaxed the duodenum and ileum to an equivalent degree. Concentrations 40 times lower (1 ng/ml. adrenaline, etc.) produced similar effects in the rectal caecum. Frequently adrenaline, noradrenaline and dopamine, but never laevisoprenaline, caused a small contraction before relaxation. The contractile component was abolished by phentolamine (1.0 μg/ml.) and potentiated by atropine (0.02 μg/ml.) which lowered the tone of the intestine. This concentration of phentolamine also reduced the relaxation responses to noradrenaline and adrenaline but did not affect those to laevisoprenaline. Pronethalol (1.0 μg/ml.) also partially blocked relaxations in response to noradrenaline and adrenaline, but abolished those to laevisoprenaline. Relaxations elicited by adrenaline and noradrenaline were abolished by a combination of both blocking drugs. Relaxation of caecal circular muscle strips produced by noradrenaline (0.5 μg/ml.) and adrenaline (0.5 μg/ml.) was occasionally preceded by contraction, but in many preparations the effect was one of relaxation only. The type of response elicited appeared to depend on the background tone of the preparation.

Nerve stimulation

Stimulation of the perivascular nerves supplying all parts of the duodenum, ileum and rectal caeca produced biphasic responses consisting of contraction followed by relaxation in varying proportions. The amplitude of each component depended partly on the frequency of stimulation but was chiefly influenced by the tone of the preparation, low tone favouring contraction and high tone favouring relaxation. The contractile component was of short duration in comparison with the relaxation component, the latter commencing during the 10 sec stimulation period. In most preparations, the size of the contractile phase exceeded or equalled that of the relaxation phase, but a few preparations exhibited exceptionally high tone and with these the inhibition was greater than the contraction.

The lowest effective frequencies of stimulation (0.5–2/sec) produced contraction only in most preparations, but higher frequencies yielded both contraction and relaxation, both of which increased in size with increasing frequency of stimulation until one or both phases became maximal.

Coaxial stimulation gave responses identical to those produced by stimulation of the perivascular nerves. The duodenum, the ileum and the rectal caecum also responded to drugs in a qualitatively similar way. The rectal caecum, however, was less prone to spontaneous and drug-induced changes in tone, and to spontaneous pendular or rhythmic activity than was the intestine. In the intestine, blocking drugs lowered the tone and anticholinesterase drugs raised it, and these effects complicated the more specific effects of the drugs on responses to nerve stimulation. For this reason, some of the effects of blocking drugs and anticholinesterases, which occurred in all three stimulated preparations, have been illustrated for the rectal caecum only.

Atropine and hyoscine. The contractions produced by periarterial nerve stimulation were selectively abolished by atropine (0.02μg/ml.) or hyoscine (0.02 μg/ml.). Both drugs tended to relax the longitudinal muscle of the intestinal preparations with a resultant masking of the relaxations induced by nerve stimulation. These concentrations of atropine and hyoscine did not usually affect the tone of the caecal preparations (Fig. 1). Figure 4 includes an example of an experiment in which hyoscine (0.04 μg/ml.) had comparatively little effect on tone while selectively blocking the contractions elicited by periarterial nerve stimulations at 8/sec. Relaxations of the caeca were frequently potentiated by atropine in concentrations which abolished contractions.

Phentolamine and pronethalol. Any residual contraction remaining after atropine blockade was abolished by phentolamine (1 or 2 μg/ml.). In the experiment of Fig. 1, this concentration had little effect on the relaxations. On the other hand, the relaxations were completely abolished by pronethalol (2 μg/ml.), both in the untreated and in the atropinized preparation. The contractions evoked by nerve stimulation were potentiated in the presence of pronethalol, which also revealed small after-contractions in the absence of relaxations. These after contractions commenced after cessation of the stimulus (see also the section on reserpine). Figure 1 illustrates these effects in an experiment in which the contractions of the caecum produced by coaxial stimulation had been blocked by atropine (0.04 μg/ml.) and the relaxations by pronethalol (2 μg/ml.); physostigmine (see later) was added in the continued presence of these drugs. In this experiment, the after-contractions

were almost indistinguishable from the first contractions, but in fact most of the response occurred after cessation of the stimulus.

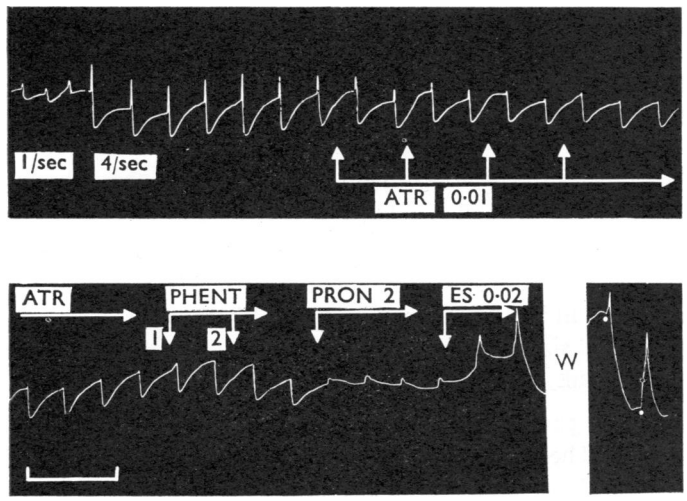

Fig. 1. Rectal caecum. Effect of atropine and physostigmine. Responses to coaxial stimulation. During the periods indicated, atropine (ATR), phentolamine (PHENT), pronethalol (PRON) and physostigmine (ES) were present. At W the bath fluid was changed. The numerals denote the bath concentration in μg/ml. Time marker, 10 min.

Phentolamine (1–2 μg/ml.) generally abolished relaxations of the intestine elicited by nerve stimulation, at the same time depressing tone and spontaneous rhythm. Occasionally, phentolamine in the above doses also reduced contractions of the intestine when the tone was unaffected.

In the intestinal preparations, the β-receptor blocking drugs pronethalol (2 μg/ml.) and propranolol (0.5 μg/ml.) abolished the relaxation component of the response to periarterial nerve stimulation and, very rarely, pronethalol partially depressed the contractions in addition. This latter effect was probably related to the sympathomimetic action of the drug itself (Barrett, 1965; Black, Duncan & Shanks, 1965) which also progressively lowered the tone and therefore interfered with the effects of nerve stimulation.

Guanethidine and dexamphetamine. In the intestine, guanethidine (1–5 μg/ml.) caused a transient relaxation, and reduced spontaneous activity. Relaxations in response to nerve stimulation were blocked by the lower doses (1 or 2 μg/ml.) with only slight depression of the contractions. Higher doses (2–5 μg/ml.) blocked contractions and relaxations, but it is probable that doses of this order also possessed ganglion-blocking activity (Gertner & Romano, 1961) in addition to their adrenergic neurone-blocking activity. This would account for the abolition of the contractile responses.

Low concentrations of guanethidine (0.5 μg/ml.) selectively blocked the relaxation component of the caecal response to either periarterial or coaxial stimulation. Higher concentrations (5 μg/ml. and above) usually depressed the contractions to some extent, probably as a result of ganglion blockade. With low concentrations of guanethidine, just

sufficient to block the relaxations, however, the block was immediately reversed by dexamphetamine (0.25 μg/ml.–0.5 μg/ml.). Figure 2 shows the results of two such reversals in the same preparation. The upper tracing illustrates complete blockade of the relaxations in response to coaxial stimulation at 8/sec, and potentiation of the contractions by guanethidine 0.5 μg/ml. Dexamphetamine (0.5 μg/ml.) reversed the block almost completely. When the concentration of guanethidine was reduced to 0.25 μg/ml., a rise in tone and partial block of the relaxations ensued, although in normal circumstances an increase in tone would enhance the relaxations. In the presence of this dose of guanethidine, dexamphetamine (0.25 μg/ml.) again reversed the block and in addition potentiated the relaxation.

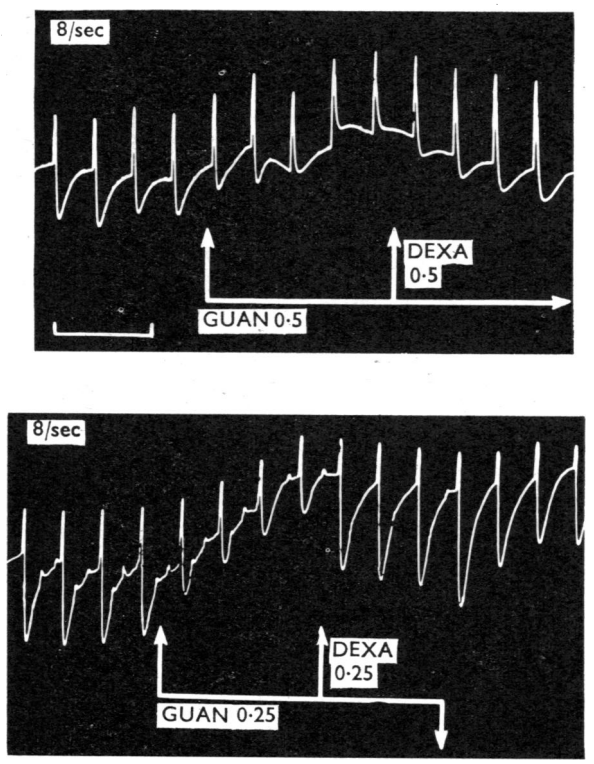

Fig. 2. Rectal caecum. Effects of guanethidine and dexamphetamine. Responses to coaxial stimulation. During the periods indicated, guanethidine (GUAN) and dexamphetamine (DEXA) were present in the bath. The numerals denote the bath concentrations in μg/ml. Time marker, 10 min.

Relaxations produced by nicotine were also investigated in the coaxially stimulated caecum, in order to compare the effect of guanethidine on these responses with its effect on relaxations produced by nerve stimulation. Care had to be taken in this procedure on account of the depressant effect of nicotine on responses to coaxial stimulation. A valid comparison was obtained, however, by restricting the number of responses to nicotine to a minimum and allowing an interval of at least 20 min between them. In these conditions,

it was necessary, after nicotine had been added, to increase the frequency of stimulation from 2 to 4/sec in order to maintain relaxations comparable with those produced before nicotine, but block of the relaxations evoked by coaxial stimulation and their reversal to small contractions by guanethidine (0.5 μg/ml.) was still clearly demonstrable. In contrast, comparable relaxations produced by nicotine (1 μg/ml.) were unaffected by this concentration of guanethidine added at the height of the block of the coaxially stimulated relaxations (Fig. 3). This result confirmed those described for the non-stimulated caecum.

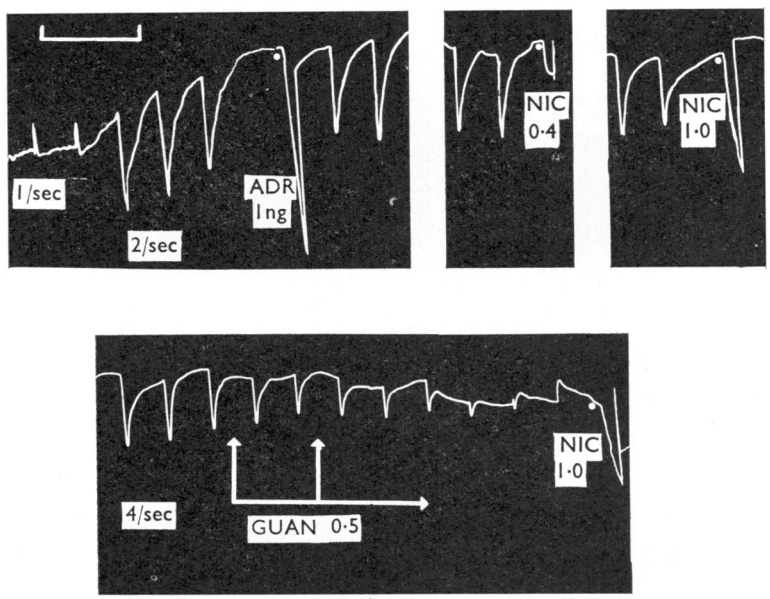

Fig. 3. Rectal caecum. Effect of guanethidine. Responses to coaxial stimulation (unmarked at the frequencies indicated) and nicotine (NIC). The upper left-hand panel includes a response to adrenaline (ADR, 1 ng/ml.) for comparison. At the points indicated, guanethidine (GUAN) was added to the bath and was present until the end of the experiment. The numerals denote the bath concentration in μg/ml. Time marker, 10 min.

P-286 and hexamethonium. The compound *N,N*-diisopropyl-*N'*-isoamyl-*N'*-diethylaminoethylurea (P-286) has been shown to possess selective blocking action in vagal ganglia and an antagonistic action to acetylcholine in the adrenal medulla (Gardier, Abreu, Richards & Herrlich, 1960; Levy & Ahlquist, 1962). P-286 (5 μg/ml.) was found to block the contractions of the intestine produced by periarterial nerve stimulation (8/sec), at the same time producing a submaximal decrease in tone and thereby masking part of the relaxation response. These actions are illustrated in the upper tracing of Fig. 4 and may be compared with a similar action exhibited by hyoscine in the same figure. The blocking activity of P-286 was easily reversible on washing, as was the fall in tone. It has been reported (Pitman-Moore data) that this compound in doses of 1–5 mg/kg intravenously causes a transient relaxation of the duodenum of anaesthetized dogs ; a similar effect was always found with chick intestinal preparations and could not be separated from its blocking activity.

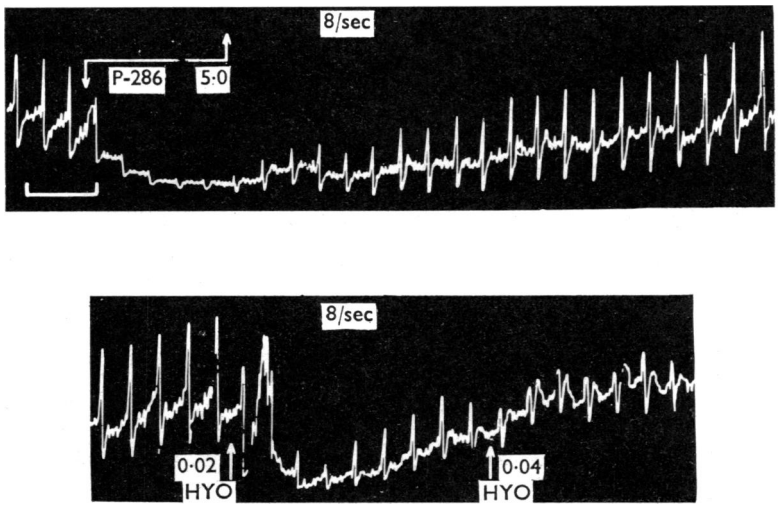

Fig. 4. Ileum. Periarterial nerve stimulation. Upper tracing: During the period indicated, the ganglion blocking drug P-286 was present in the bath. Lower tracing: At the arrows, hyoscine (HYO) was added to the bath and was present until the end of the experiment. The numerals denote the bath concentration in μg/ml. Time marker, 10 min.

Results with hexamethonium were less conclusive than those with P-286. Concentrations up to 50 μg/ml. usually caused a gradual increase in the tone of the ileum (which interfered with the interpretation of the results) and often did not affect the responses of the caecum. These effects may have been related to the anticholinesterase activity of hexamethonium (Schneider, 1966) for other ganglion blocking drugs, mecamylamine (1 μg/ml.) and pempidine (1 μg/ml.), usually abolished the contractile component of the response to periarterial nerve stimulation, leaving the relaxation component unaffected or potentiated. Occasionally the contractions were not entirely blocked by mecamylamine or pempidine, especially in chicks more than 3 weeks old.

Physostigmine. The contractions of all preparations evoked by nerve or coaxial stimulation were potentiated by physostigmine (0.05 μg/ml.). Physostigmine (0.02 μg/ml.) also restored contractions to nerve stimulation when these had been abolished by atropine (0.02 μg/ml.) or hyoscine (0.02 μg/ml.) (Fig. 6).

The contractile component of the response of the rectal caecum to coaxial or perivascular stimulation was potentiated by physostigmine (0.1 μg/ml.), but the relaxation component remained unaffected. In conditions where after-contractions were present—that is, after blockade of the relaxation by pronethalol (2 μg/ml.)—these also were potentiated by physostigmine (0.02 μg/ml.) as shown in Fig. 1.

Hemicholinium and choline. A slowly developing block of the contractions of the caecum evoked by perivascular or coaxial stimulation resulted when small concentrations of hemicholinium-3 (5 to 10 μg/ml.) were added. The block was maximal 45 min after addition of hemicholinium to the bath, while the relaxations remained unaffected throughout. Figure 5 illustrates such a blockade and shows reversal of the block by choline (40 μg/ml.) added in the continued presence of hemicholinium (10 μg/ml.). Further

increase in the concentration of hemicholinium (up to 20 μg/ml.), at the height of the block of the contractile component, still did not reduce the relaxations, which instead were potentiated.

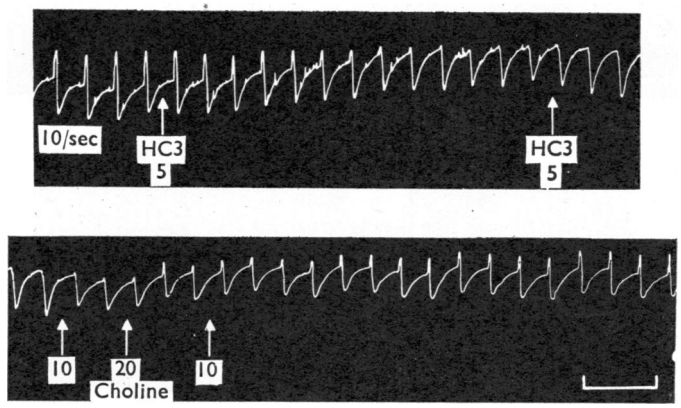

Fig. 5. Rectal caecum. Effect of hemicholinium and choline. Responses to coaxial stimulation. At the points indicated, hemicholinium (HC3) and choline were added to the bath and were present until the end of the experiment. The numerals denote the bath concentration in μg/ml. Time marker, 10 min.

Similar effects with hemicholinium were not usually demonstrable in the intestine because the responses to periarterial and coaxial stimulation were less reproducible for the necessarily long periods of time.

Mepyramine. Mepyramine in concentrations of 0.01–0.5 μg/ml. did not affect the responses to nerve stimulation although the latter concentration was 50 times that required to block the effect of histamine (0.1–1 μg/ml.). In larger concentrations (1–10 μg/ml.), however, mepyramine progressively blocked the responses to nerve stimulation, and caused relaxations in tone when this was high. These effects were probably caused by the local anaesthetic action of mepyramine (Halpern, 1942).

Bromo-lysergic acid diethylamide. Like mepyramine, bromo-lysergic acid diethylamide (BOL), in concentrations of 1 μg/ml. and higher, produced relaxation of the intestine and thus interfered with the effects of nerve stimulation; apart from this action BOL did not seem to have a more specific effect on the responses to nerve stimulation.

Reserpine. Responses to perivascular stimulation of the ileum of chicks which had been pretreated with reserpine differed from those in untreated animals. Two types of response were observed in the preparations taken from reserpinized chicks, and examples are shown in Fig. 6. In chicks which had received one dose only (5 mg/kg) of reserpine 20 hr previously, stimulation of the nerves to the ileum resulted in comparatively large contractions, part of which occurred after the cessation of the stimulation, without accompanying relaxations. These contractions were depressed but not abolished by hyoscine (0.02 μg/ml.) and restored and potentiated by physostigmine (0.2 μg/ml.) in the continued presence of hyoscine. However, in most cases in which chicks received a dose of reserpine (5 mg/kg) on each of 2 days and were used 12 hr after the second dose, the response consisted of a small relaxation during the period of stimulation (15 sec)

followed by a large after-contraction. The after-contractions commenced after the cessation of the stimulus and were slow to subside (Fig. 6, lower panel). They resembled those described by Campbell (1966) following stimulation of the intramural nerves of guinea-pig taenia coli. The after-contractions were abolished by hyoscine (0.02 μg/ml.), but they quickly returned on washing out the bath, which suggests that this effect of hyoscine may not have been due to its persistent anti-acetylcholine action.

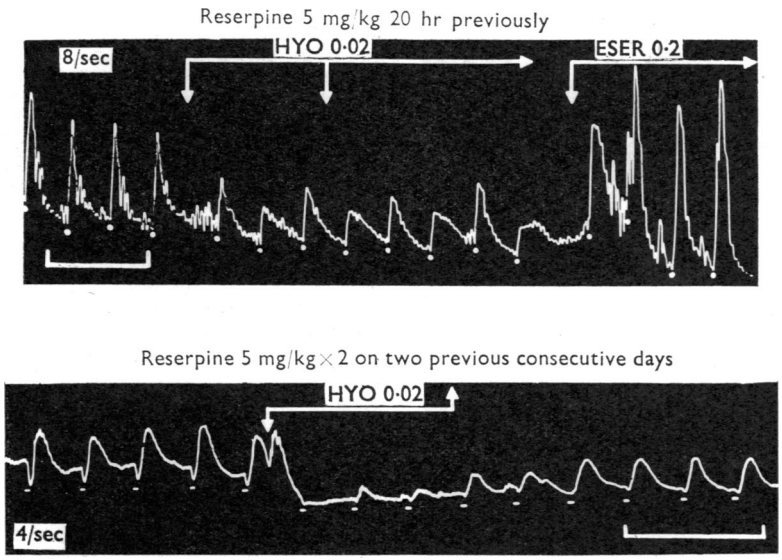

Fig. 6. Ileum from reserpinized chicks. Effect of hyoscine and physostigmine. Chicks pretreated as indicated above each tracing. Responses to periarterial nerve stimulation at the frequencies denoted. During the periods indicated, hyoscine (HYO) and physostigmine (ESER) were present in the bath. The numerals denote the bath concentration in μg/ml. Time marker, 10 min.

The relaxations which occurred after treatment with reserpine were unaffected by guanethidine (1–5 μg/ml.). Guanethidine-resistant relaxations following stimulation of the intramural nerves of the guinea-pig taenia have also been described by Bennett, Burnstock & Holman (1966a, b) and Burnstock, Campbell & Rand (1966).

Histochemical demonstration of cholinesterases. Nerve fibres containing acetylcholinesterase were found in the plexuses and muscle layers of the duodenum and ileum (the rectal caecum was not examined). It was interesting to note that in the duodenum, which has a relatively dense adrenergic innervation (Everett & Mann, 1967), cholinergic fibres were comparatively sparse, while they were more numerous in the ileum, which was shown to contain fewer adrenergic fibres. Non-specific (butyryl) cholinesterase also seemed to be present to a small extent in some of the sections, particularly in the villi. The significance of its presence in the villi is obscure, but it is possible that the intestinal lipases or esterases (other than cholinesterases) may also have taken up the substrate. Sections stained for true and non-specific cholinesterases are shown in Fig. 7.

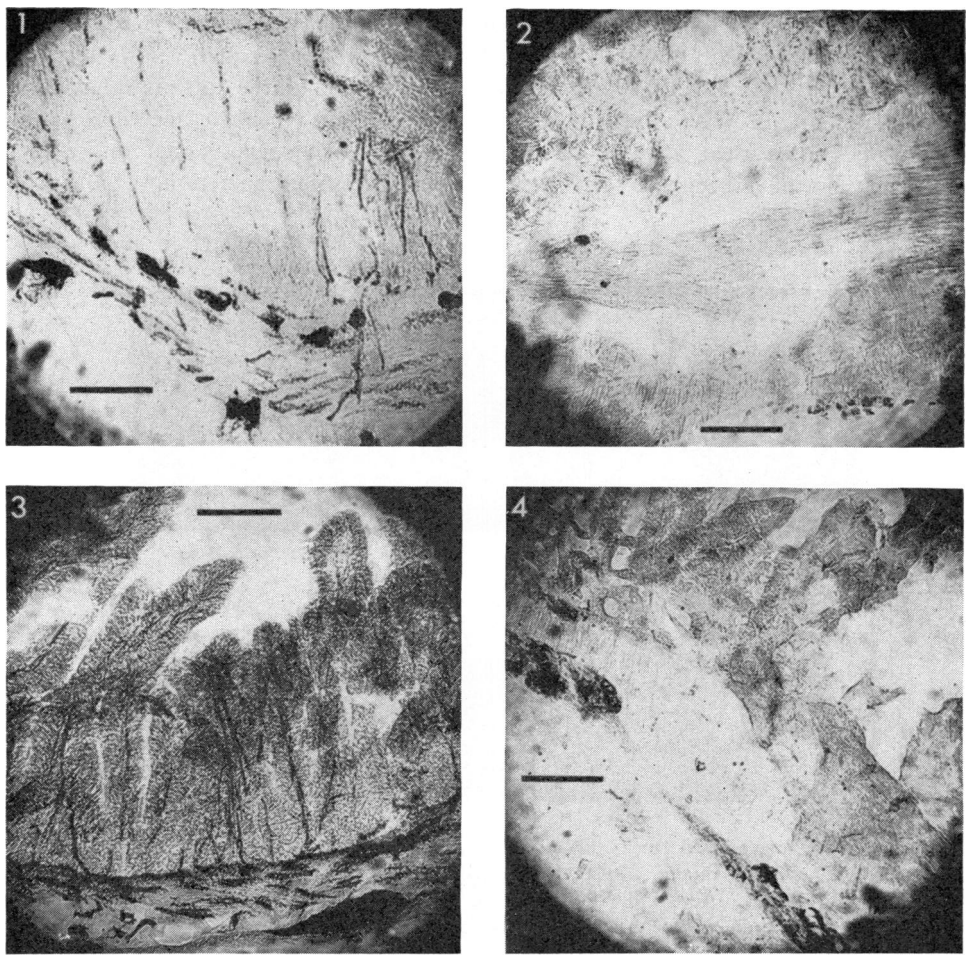

Fig. 7. Duodenum and ileum. Cholinesterases. Ileum (1) and duodenum (2) stained for true (acetyl) cholinesterase. Ileum (3) and duodenum (4) stained for non-specific (butyryl) cholinesterase. Scales, 100 μ.

DISCUSSION

Stimulation of the perivascular nerves supplying the intestine and the rectal caecum usually produced biphasic responses consisting of an initial contraction followed by a relaxation. Acetylcholine produced contraction whereas adrenaline, noradrenaline and laevisoprenaline produced relaxation.

Contractions produced by acetylcholine and the contractile response to nerve stimulation were blocked by atropine or hyoscine, and restored and augmented by physostigmine. The contractile response to stimulation was also blocked by ganglion blocking drugs and by hemicholinium, and the latter effect was reversed by choline. Relaxation produced by adrenaline or by perivascular stimulation was partially blocked by α or β-adrenoreceptor blocking drugs and was completely blocked by a mixture of both. Relaxations

in response to stimulation were also abolished by guanethidine and then restored by dexamphetamine. The presence of nerve fibres staining for cholinesterase was demonstrated histochemically. The presence and distribution of adrenergic nerve fibres in the chick intestine has been described elsewhere (Everett & Mann, 1967). These results show that a mixture of motor cholinergic fibres and inhibitory adrenergic fibres is present in the perivascular nerves to the intestine and caeca. Nolf (1934a, b) also demonstrated vagal enhancement and sympathetic inhibition of intestinal motility in the fowl. The observation that phentolamine occasionally depressed contractile responses of the organs in the absence of an alteration in tone, together with the finding that small contractions were often elicited by adrenaline and noradrenaline before relaxation, suggests that a small number of motor fibres may be adrenergic. The principal response of the intestine to ganglion stimulant drugs was contraction, but small relaxations often preceded the contractile responses. In the rectal caecum relaxations produced by nicotine were much more marked. Relaxations produced by ganglion stimulants in intestinal preparations are well known (Kuroda, 1917 ; Ambache & Edwards, 1951 ; Ambache, 1951 ; Evans & Schild, 1953 ; Jarrett, 1962 ; Greeff, Kasperat & Osswald, 1962 ; Weiss, 1962 ; Burn & Gibbons, 1964 ; Bucknell & Whitney, 1964 ; Fishlock & Parks, 1966 ; Burnstock, Campbell & Rand, 1966). Such relaxations produced by nicotine in preparations from other species are usually revealed or potentiated in the presence of atropine, but in the chick gut the contractile action of nicotine, TMA and DMPP were often resistant to the blocking action of atropine and hyoscine, and little or no potentiation of the relaxations occurred. The relaxations of the intestine or of the caecum produced by ganglion stimulants were not reduced by guanethidine or by α and β-adrenoreceptor blocking drugs in concentrations which abolished relaxations induced by perivascular stimulation. Mattila (1963) also found that relaxations of the caecum induced by nicotine were unaffected by α and β-adrenoreceptor blocking drugs. Other workers, using mammalian preparations, have also described nicotine-induced relaxations which were not blocked by guanethidine or bretylium (Jarrett, 1962 ; Burnstock, Campbell & Rand, 1966) and which have therefore been considered to be non-adrenergic in nature. Nevertheless, some authors do consider them to be adrenergic responses (Burn & Gibbons, 1964 ; Bucknell & Whitney, 1964 ; Fishlock & Parks, 1966).

An inhibitory, non-adrenergic response to perivascular stimulation of the intestine was also revealed by pretreatment with reserpine in doses sufficient to deplete the tissue of catecholamines as demonstrated by histochemical examination (Everett & Mann, 1967). After reserpine treatment, the response to perivascular stimulation was a small inhibition occurring during the period of stimulation and this was followed by a large after-contraction which commenced when stimulation stopped. The inhibitory component, like that induced by ganglion stimulants, was not affected by guanethidine or by α and β-adrenoreceptor blocking drugs. Similar, non-adrenergic relaxations have been observed in the chick gizzard (Everett, unpublished observations). It is therefore concluded that the innervation of chick intestine includes a small non-adrenergic, non-cholinergic inhibitory component. Burnstock, Campbell & Rand (1966) have suggested that similar apparently non-adrenergic, inhibitory fibres in the taenia of the guinea-pig caecum may be involved in the descending inhibition of peristalsis.

The initial contractile response to perivascular stimulation was absent in chicks which had received two doses of reserpine. All the evidence pointed to much of this initial

response being cholinergic in nature and no explanation of its disappearance after reserpine can be offered. The large after-contraction produced by perivascular stimulation in reserpine-treated chicks was blocked by hyoscine. The block by hyoscine was rapidly reversed on washing, however, in contrast to the persistent blockade of responses to acetylcholine. This suggests that the after-contraction was probably not cholinergic in origin, and a more likely explanation may be that it reflected the rebound membrane depolarization which often occurs after inhibitory (hyperpolarization) responses (Bennett, 1966a, b ; Campbell, 1966).

The selective block by hemicholinium of the cholinergic contractile response to stimulation, which left unaffected or augmented the inhibitory adrenergic response, constitutes evidence against the Burn & Rand (1959) hypothesis of a cholinergic step in adrenergic transmission.

In general, the interpretation of the effects of nerve stimulation on the intestine was complicated by the marked relaxing action of the antagonists used (atropine, phentolamine, pronethalol, guanethidine and BOL). The rectal caecum possesses a similar innervation and its responses were more easily studied because it maintains a stable tone in the presence of antagonists. In addition, the caecum of the young chick (from hatching to 6 weeks old) proved to be equally if not more sensitive to the relaxing action of catecholamines than that of the adult caecum preparation described by Barsoum & Gaddum (1935). It therefore seems unnecessary to use preparations from adult birds in the assay of catecholamines both from the point of view of economy and ease of preparation. Preparations taken from young chicks also respond more quickly, and drug induced responses may be washed out more easily, than those from adult birds. As an isolated organ for general pharmacological purposes, including teaching, the rectal caecum of the chick has much to offer, for most preparations have stable tone and contract and relax about a comparatively constant baseline in response to drugs and nerve stimulation. In this respect, the duodenum and ileum are less suitable.

SUMMARY

1. Responses of the duodenum, ileum and rectal caecum of the very young chick to acetylcholine, physostigmine, ganglion stimulant drugs, sympathomimetic amines and the appropriate blocking drugs mainly resembled those in comparable mammalian preparations.

2. Nicotine in low concentrations and acetylcholine in the presence of atropine or hyoscine produced relaxation of the rectal caeca and to a small extent of the duodenum and ileum. The relaxations were unaffected by guanethidine and by α and β-adrenoreceptor blocking drugs and were not potentiated by small doses of cocaine.

3. Stimulation of the perivascular nerves supplying all three regions or coaxial stimulation elicited biphasic responses.

4. The contractile component of the responses to nerve stimulation was abolished by atropine and hyoscine and restored by physostigmine, abolished by *N,N*-diisopropyl-*N'*-isoamyl-*N'*-diethylaminoethylurea (P-286) and usually by ganglion blocking drugs, and slowly blocked by hemicholinium and restored by choline.

5. The relaxation component of the responses to nerve stimulation was abolished by pronethalol and by guanethidine; the latter effect was reversed by dexamphetamine. Small non-adrenergic relaxations which preceded large contractions were sometimes revealed in ileum taken from reserpine-pretreated chicks.

6. The presence in the duodenum and ileum of fibres containing cholinesterase was demonstrated histochemically.

7. It is concluded that the perivascular nerves supplying the duodenum, ileum and rectal caecum contain both cholinergic and adrenergic fibres. In addition there is evidence of a non-adrenergic mechanism in the ileum and rectal caecum.

8. The rectal caecum from the very young chick is recommended as a useful isolated preparation for the assay of catecholamines and for the study of drugs.

I am most grateful to Professor W. C. Bowman for his help and advice during this work, which was financially supported by a grant made to him by the British Egg Marketing Board.

REFERENCES

ÁBRAHÁM, A. (1936). Beiträge zur Kenntnis der Innervation des Vogeldarmes. *Z. Zellforsch. mikrosk. Anat.*, **23**, 737–745.

AMBACHE, N. (1951). Unmasking after cholinergic paralysis by botulinum toxin, of a reversed action of nicotine on the mammalian intestine, revealing the probable presence of local inhibitory ganglion cells in the enteric plexus. *Br. J. Pharmac. Chemother.*, **6**, 51–67.

AMBACHE, N. & EDWARDS, J. (1951). Reversal of nicotine action on the intestine by atropine. *Br. J. Pharmac. Chemother.*, **6**, 311–317.

BARRETT, A. M. (1965). The effects of some autonomic blocking agents on the heart rates of anaesthetized and pithed rats. *Br. J. Pharmac. Chemother.*, **24**, 302 (title only).

BARSOUM, G. S. & GADDUM, J. H. (1935). The pharmacological estimation of adenosine and histamine in the blood. *J. Physiol., Lond.*, **85**, 1–14.

BENNETT, M. R. (1966a). Rebound excitation of the smooth muscle cells of the guinea-pig taenia coli after stimulation of intramural inhibitory nerves. *J. Physiol., Lond.*, **185**, 124–131.

BENNETT, M. R. (1966b). Transmission from intramural excitatory nerves to the smooth muscle cells of the guinea-pig taenia coli. *J. Physiol., Lond.*, **185**, 132–147.

BENNETT, M. R., BURNSTOCK, G. & HOLMAN, M. E. (1966a). Transmission from perivascular inhibitory nerves to the smooth muscle of the guinea-pig taenia coli. *J. Physiol., Lond.*, **182**, 527–540.

BENNETT, M. R., BURNSTOCK, G. & HOLMAN, M. E. (1966b). Transmission from intramural inhibitory nerves to the smooth muscle of the guinea-pig taenia coli. *J. Physiol., Lond.*, **182**, 541–558.

BLABER, L. C. & CUTHBERT, A. W. (1962). Cholinesterases in the domestic fowl and the specificity of some reversible inhibitors. *Biochem. Pharmac.*, **11**, 113–123.

BLACK, J. W., DUNCAN, W. A. M. & SHANKS, R. G. (1965). Comparison of some properties of pronethalol and propranalol. *Br. J. Pharmac. Chemother.*, **25**, 577–591.

BUCKNELL, A. & WHITNEY, B. (1964). A preliminary investigation of the pharmacology of the human isolated taenia coli preparation. *Br. J. Pharmac. Chemother.*, **23**, 164–175.

BURN, J. H. & GIBBONS, W. R. (1964). The sympathetic postganglionic fibre and the block by bretylium; the block prevented by hexamethonium and imitated by mecamylamine. *Br. J. Pharmac. Chemother.*, **22**, 549–557.

BURN, J. H. & RAND, M. J. (1959). Sympathetic postganglionic mechanism. *Nature, Lond.*, **184**, 163–165.

BURN, J. H. & RAND, M. J. (1960). The relation of circulating noradrenaline to the effect of sympathetic stimulation. *J. Physiol., Lond.*, **150**, 295–305.

BURNSTOCK, G., CAMPBELL, G. & RAND, M. J. (1966). The inhibitory innervation of the taenia of the guinea-pig caecum. *J. Physiol., Lond.*, **182**, 504–526.

CAMPBELL, G. (1966). Nerve-mediated excitation of the taenia of the guinea-pig caecum. *J. Physiol., Lond.*, **185**, 148–159.

CLEUGH, J., GADDUM, J. H., HOLTON, P. & LEACH, E. (1961). Assay of substance P on the fowl rectal caecum. *Br. J. Pharmac. Chemother.*, **17**, 144–158.

VON EULER, U. S. (1948a). Identification of the sympathomimetic ergone in adrenergic nerves of cattle (Sympathin N) with laevonoradrenaline. *Acta physiol. scand.*, **16**, 63–74.

VON EULER, U. S. (1948b). Assay of noradrenaline and adrenaline in extracts of tissues and nerves. *Nature, Lond.*, **162**, 570–571.

VON EULER, U. S. (1948c). Preparation, purification and evaluation of noradrenaline and adrenaline in organ extracts. *Archs int. Pharmacodyn. Thér.*, **77**, 477–483.

VON EULER, U. S. (1949). Noradrenalin und Histamin als Wirkstoffe vegetwer Nerven. *Z. Vitam.-, Horm.- u. Fermentforsch.*, **2**, 596–608.

VON EULER, U. S. & HELLNER, S. (1951). Excretion of noradrenaline, adrenaline and hydroxytryptamine in the urine. *Acta physiol. scand.*, **22**, 161–167.

EVANS, D. H. L. & SCHILD, H. O. (1953). The reactions of plexus-free circular muscle of cat jejunum to drugs. *J. Physiol., Lond.*, **119**, 376–399.

EVERETT, S. D. & MANN, S. P. (1967). Catecholamine release by histamine from the isolated intestine of the chick. *Eur. J. Pharmac.*, **1**, 310–320.

FISHLOCK, D. J. & PARKS, A. G. (1966). The action of nicotine on the circular muscle of the human ileum and colon *in vitro*. *Br. J. Pharmac. Chemother.*, **26**, 79–86.

GARDIER, R. W., ABREU, B. E., RICHARDS, A. B. & HERRLICH, H. C. (1960). Specific blockade of the adrenal medulla. *J. Pharmac. exp. Ther.*, **130**, 340–345.

GERTNER, S. B. & ROMANO, A. (1961). Action of guanethidine and bretylium on ganglionic transmission. *Fedn Proc.*, **20**, 319.

GREEFF, K., KASPERAT, H. & OSSWALD, W. (1962). Paradoxical effects of electrical stimulation of the vagus nerve on the isolated stomach and atrial preparation of the guinea-pig and its alteration by ganglionic blocking agents, sympatholytics, reserpine and cocaine. *Arch. exp. Path. Pharmak.*, **243**, 528–545.

HALPERN, B. N. (1942). Les antihistamineques de synthèse. Essais de chimiothérapie des états allergiques. *Archs int. Pharmacodyn. Thér.*, **68**, 339–408.

HARRY, J. (1963). Action of drugs on the circular muscle strip from the guinea-pig isolated ileum. *Br. J. Pharmac. Chemother.*, **20**, 399–417.

HEBB, C., MANN, S. & PERKINS, D. (1966). Histochemical demonstration of catecholamines and of cholinesterases on adjacent sections of tissue. *J. Physiol., Lond.*, **184**, 12–13*P*.

HSIEH, T. M. (1951). The sympathetic and parasympathetic nervous systems of the fowl. Ph.D. thesis, Univ. Edinburgh.

JARRETT, R. J. (1962). Action of nicotine on the rabbit muscular organ (ileo-colic sphincter). *Br. J. Pharmac. Chemother.*, **18**, 397–404.

KOLOSSOW, N. G., SABUSSOW, G. H. & IWANOW, J. F. (1932). Zur innervation des Verdauungskanals des Vögel. *Z. mikrosk.-anat. Forsch.*, **30**, 257–294.

KURODA, M. (1917). Observations of the effects of drugs on the ileo-colic sphincter. *J. Pharmac. exp. Ther.*, **9**, 187–195.

LEVY, B. & AHLQUIST, R. P. (1962). A study of sympathetic ganglion stimulants. *J. Pharmac. exp. Ther.*, **137**, 219–228.

MATTILA, M. (1963). Pharmacological properties of some pyrrolidine N-substituted nornicotine derivatives. *Academic dissert., Dept. of Pharmacology, Univ. Helsinki.*

NOLF, P. (1934a). Les nerfs extrinsiques de l'intestin chez l'oiseau. Première partie—Les nerfs vagues. *Archs int. Physiol.*, **39**, 113–164.

NOLF, P. (1934b). Les nerfs extrinsiques de l'intestin chez l'oiseau. Deuxième partie—Les nerfs coeliaques et mesenteriques. *Archs int. Physiol.*, **39**, 165–226.

NOLF, P. (1934c). Les nerfs extrinsiques de l'intestin chez l'oiseau. Troisième partie—Les nerfs de Remak. *Archs int. Physiol.*, **39**, 227–256.

PATON, W. D. M. (1955). The response of the guinea-pig ileum to electrical stimulation by coaxial electrodes. *J. Physiol., Lond.*, **127**, 40–41*P*.

SCHNEIDER, R. (1966). The longitudinal muscle component of the peristaltic reflex in the guinea-pig isolated ileum. *Br. J. Pharmac. Chemother.*, **27**, 387–397.

DE SOUZA, R. (1961). Farmacologia do Aparello digestivo de Aves. *Archos. Esc. sup. Vet. Est. Minas Gerais*, **13**, 21–30.

WEISS, J. (1962). Biphasic responses of guinea-pig's taenia coli induced by 1,1-dimethylphenylpiperazinium (DMPP). *Acta pharmac. tox.*, **19**, 121–128.