PHARMACOLOGICAL ANALYSIS OF INTRINSIC INTESTINAL REFLEXES[1]

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I. INTRODUCTION

The problem of the mode of action of drugs on intestinal motility was reviewed ten years ago by Vaughan Williams (218), who divided his attention about equally between the results obtained from experiments on isolated tissues and those from observations on whole animals and man. As the most important

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recent advances in our knowledge are the result of experimental work on isolated segments of small or large intestine, the present review will deal mainly with this method of approach. However, when experiments on the whole animal or human being can be clearly shown to make important contributions to the analysis of the intrinsic reflexes of the gut, their results are included.

Although the intestine is perhaps the organ in which pharmacological methods have been most frequently used to elucidate function, the organization of its tissue is so complex that the success of the experimental work reported in the literature does not seem to be commensurate with the effort expended. While the research of recent years has answered some questions, it has posed new, and more difficult, problems.

The review will begin with the more important advances resulting from morphological research, followed by an account of the results of recent physiological and pharmacological experimental work. The problems of the electrophysiology of smooth muscle and of impulse transmission from nerve to muscle will be dealt with only briefly as they have recently been reviewed by Burnstock *et al.* (42) and Burnstock and Holman (41).

II. MORPHOLOGICAL AND PHYSIOLOGICAL CONSIDERATIONS

Movements of the gut are almost always due to active contraction of one of the layers of smooth muscle cells present in the wall. The longitudinal and circular coats proper are in this respect more effective than the muscularis mucosae. Contractions of the muscle coats may be due to the inherent rhythmic property of the muscle cells themselves or to stimuli acting on them, either directly or indirectly. Since drugs may act, therefore, on the muscle cells or on some points of the neurone or neurones innervating them, it is necessary to consider the morphological and functional relationships between the individual muscle cells, their nerve supply, the sensory receptors and, finally, the intramural nerve plexuses.

A. Relationships between smooth muscle cells

The question of the morphological relationship between individual smooth muscle cells is of great importance for the understanding of the process of transmission of excitation from one cell to another and also of the mode of action of drugs having an effect directly on the muscle cells. Unfortunately, even the results obtained with the electron microscope have not given unequivocal answers.

Most investigators, *e.g.*, Thaemert (206), Prosser *et al.* (170), and Dewey and Barr (59), expressed the view that bridges of some kind exist between cells. There is, however, disagreement on the problem of whether or not there is complete protoplasmic continuity across these processes. Bridges have been seen not only in intestinal smooth muscle cells, but also in those in other organs, *e.g.*, in the ureter by Bergman (20). On the other hand, Caesar *et al.* (45) found no bridges in mouse urinary bladder or uterus, but Barr (12) drew attention to the difficulties of demonstrating bridges even in electron micrographs and pointed out that these bridges are very labile and easily destroyed by techniques used in preparing sections.

The physiological importance of intercellular bridges lies in the possibility that electrical excitation is transmitted along them. However, Burnstock *et al.* (43) argued that whether or not there is protoplasmic continuity, such bridges could not be of great significance in the conduction process, since the resistance across them would be too high. They suggested that the bridges may serve a mechanical function and that an area of close contact of adjacent cell membranes may be of greater importance for the conduction of excitation. Such areas of close proximity (50 Å) have been found in mouse intestine by Taxi (205). The evidence that conduction takes place from muscle cell to cell is very strong and has been reviewed recently by Prosser (169). In this context it is only right to recall the experiments of Magnus (154, 155), who showed as early as 1904 that in the circular muscle of the cat conduction of the excitation wave can occur in the absence of the plexuses of Auerbach and Meissner.

B. Nerve supply of intestinal smooth muscle cells

The controversial subject of the structure of autonomic neuroeffector junctions has been reviewed by Hillarp (109). The most significant progress is due to the recent careful and critical investigations by Richardson (175, 176), who obtained evidence from electron micrographs that in the intestine of the rabbit the interstitial cells of Cajal are not nervous structures and therefore cannot play a role in the transmission of the excitatory impulse from nerve fibres to smooth muscle cells. The autonomic ground plexus, which issues from Auerbach's plexus, consists of Schwann-sheathed nerve fibres, forming miniature plexiform bundles. The autonomic ground plexus is distributed within the muscle coats but the relation between its terminations and the muscle cells is still not clear. Some of the nerve fibres in the two muscle coats are filled with vesicles similar to those found at synaptic ganglionic terminals. However, nerve endings are rarely seen in intestinal smooth muscle; there is apparently no Schwann membrane interposed between nerve and muscle, the distance between their surface membranes being about 200 Å, a separation comparable with that found at ganglionic synapses. Similar observations have been made by Taxi (205) on the circular muscle of mouse intestine. He considered that the neuroeffector junction is not of a truly synaptic nature since the space between neurite and muscle cell shows great variation and the vesicles have widely differing sizes, but he assumed that numerous muscle cells are in more or less close contact with a neurite and that transmitter is secreted by the vesicles. It is possible that axons passing close to the muscle fibres may not possess discrete endings but may release transmitter substance along their length. In contrast to the findings on the intestine, Richardson (177) found that in the vas deferens of the rat numerous nerve terminals form synaptic contacts with all, or almost all, muscle fibres.

There are only a few electrophysiological investigations in which the effects of stimulation of autonomic nerves on the membrane potential of single smooth muscle cells have been studied. Gillespie (86, 87) examined this problem on the isolated colon of the rabbit, a preparation which is spontaneously active. The membrane potential shows rhythmic changes lasting 5 to 6 sec; each depolarizing

phase culminates in the discharge of a short burst of spike potentials, associated with a contraction of the muscle. Single stimuli applied to the parasympathetic nerve cause a depolarization after a delay of 400 msec, which is too long to be accounted for by only one synapse in the intramural plexuses. The depolarization lasts 600 msec and, if large enough, may give rise to a spike. Single stimuli to the inhibitory sympathetic fibres are ineffective but a stimulation rate of 2/sec reduces the rate of depolarization of the slow waves and a rate of about 10/sec causes suppression of slow waves and spikes as well as complete mechanical inhibition. In the guinea-pig vas deferens, Burnstock and Holman (40) showed that junction potentials are obtained in every cell impaled; this finding indicates that sympathetic nerve endings are widely distributed amongst the smooth muscle cells. There is also a marked degree of convergence of each of the postganglionic axons on each individual or small group of muscle cells. However, in a later paper Merrilees *et al.* (161) correlated their electron microscopic findings with their electrophysiological observations and concluded that it is unlikely that every muscle fibre is in close contact with an axon and that it is not possible for every fibre to have such contacts. It is assumed that muscle fibres are activated both by diffusion of transmitter from naked portions of axons a fraction of a micron distant and by electrotonic spread of activity from the neighbouring cells.

It is of interest to note that, while Bülbring and Burnstock (30) showed that the contraction of the taenia coli of the guinea-pig is normally associated with depolarization of the membrane and spike potentials, Evans *et al.* (68) found that smooth muscle preparations of chick amnion, rat uterus, guinea-pig ileum and cat intestine can still respond to the same stimulant drugs after the membrane of the muscle cells has been depolarized by potassium chloride or sulphate; however, responses to electrical stimulation are greatly reduced and conducted responses to mechanical stimulation are abolished.

C. Sensory receptors in the intestine

The presence in the wall of the small and large intestine of sensory receptors sensitive to distension has been postulated from experiments by Kosterlitz *et al.* (128) and Kosterlitz and Robinson (132) on the isolated ileum of the guinea-pig.

In a paper which also reviews the literature, Schofield (192) reports on an experimental morphological study on cats and rats and arrives at the following conclusions. Large nerve fibres (2 to 4 μ) in the gut are extrinsic in origin; some of the fibres arise in spinal ganglia and terminate in the mucous membrane of the stomach and intestines. Enteric neurones, some of which are situated in the submucous plexus, innervate the mucous membrane with medium-sized (1 to 2 μ) and small (<1 μ) fibres. Nerve fasciculi in the mesentery of the small intestine contain a number of nerve fibres which are centripetal processes from enteric neurones. However, no sensory terminals are discernible by the light microscope.

Bülbring *et al.* (36) showed that nerve fibres penetrate into the mucous membrane from ganglion cells situated in the submucous plexus and that removal of the mucous membrane abolishes the emptying phase of the peristaltic reflex in the isolated ileum of the guinea-pig. However, the findings of Ginzel (92, 93) do

not seem to be in agreement with these results; he showed that coagulation of the mucous membrane with silver nitrate does not abolish the peristaltic reflex. Diament *et al.* (60) found that the isolated ileum of the guinea-pig sheds most of its mucous membrane during the first two hours after setting up the preparation in the organ bath and concluded that the tension receptors are in either the deep layers of the mucosa or the submucous and muscular layers.

A different approach was used by Iggo (114), who showed that stimulation of tension-sensitive receptors in the cat intestine evokes action potentials in the vagus nerve. The responses of these receptors are unaffected by removal of the mucosa or submucosa. Contraction of smooth muscle at the site of the receptor sets up discharges of impulses in the afferent fibre and this response is not dependent on changes in intraluminal pressure. Iggo concluded from these findings that the receptors are tension-signalling devices "in series" with the contractile elements in the smooth muscle.

D. Intramural nerve cell plexuses

Present knowledge of the finer architecture of the submucous and myenteric plexuses is still very incomplete. It is certain that many more nerve cells are present in the plexuses than there are preganglionic parasympathetic fibres and the arrangement between nerve cells and preganglionic fibres is still uncertain (6, 65, 98, 118, 138, 139, 202). The ratio seems to vary from species to species; Ambache (6) calculated it to be 6000 to 1 in the cat.

There is also uncertainty as to the possible connexion between sympathetic fibres and the nerve cells of the intramural plexuses. A finding which may be of importance in this respect is that not all nerve cells in the myenteric plexus of the cat contain cholinesterase (126, 139).

E. Intrinsic reflexes of the small intestine

There are different types of intestinal movement; some depend on the integrity of the intramural nervous elements while others are due to the intrinsic rhythmic activity of the smooth muscle cells. We shall not concern ourselves with the latter movements as these have been reviewed recently by Burnstock *et al.* (42).

The intrinsic or peristaltic reflexes of the intestine were first demonstrated in 1899 by Bayliss and Starling (13, 14). In 1917, Trendelenburg (214) systematically examined the conditions in which these reflexes can be elicited in isolated segments of the small intestine of various species, particularly the guinea-pig. He showed that when the lumen is distended by pressures of 1 to 2 cm H_2O, the longitudinal muscle contracts first, causing a shortening of the piece of gut; this is followed by a contraction of the circular muscle, as a contraction wave travelling in an aboral direction and expelling the contents, while the longitudinal muscle relaxes. Trendelenburg accordingly distinguished the first, or preparatory phase, from the second, or emptying, phase. A number of workers re-examined Trendelenburg's findings without adding significant factual or theoretical knowledge, until Feldberg and Lin (70), and shortly afterwards Paton and Zaimis (168), showed that the emptying phase is inhibited by ganglion-blocking agents such as

tubocurarine or hexamethonium. Feldberg and Lin (70) found also that the emptying phase, but not the contraction of the longitudinal muscle during the preparatory phase, is depressed or abolished by local anaesthetic agents, *e.g.*, cocaine, procaine, or cinchocaine (dibucaine). For this reason, the authors thought that the contraction of the longitudinal muscle was not neurogenic but myogenic.

A few years later, Kosterlitz *et al.* (128) examined the peristaltic reflex again, using isometric recording for the longitudinal muscle, monitoring changes in intraintestinal pressure, and measuring the amount of fluid expelled during the emptying phase. With a stimulus of 0.5 to 1.5 cm H_2O intraintestinal pressure, the only change is a rise in the longitudinal muscle tension. When the intraintestinal pressure is raised to 1.5 to 3 cm H_2O, there is a further increase in the longitudinal muscle tension, followed by the emptying phase. While the contraction wave of the circular muscle travels in an aboral direction, there is a spike-like rise and fall in intraintestinal pressure. In repetitive reflex activity the longitudinal muscle tension increases early during the filling of the lumen, while the circular muscle contracts when the distension of the lumen is maximal. Although the contractions of the longitudinal and circular muscle layers appear to follow each other in a fixed pattern, the contraction of one muscle layer does not trigger the contraction of the other, since ganglion-blocking agents block the contraction of the circular muscle only and high concentrations of acetylcholine, carbamylcholine (carbachol), or histamine cause a nonspecific block of the longitudinal muscle without affecting the circular muscle. The time lapse between the contractions of the two layers is due to the fact that a greater distension of the lumen is required for the contraction of the circular muscle than for that of the longitudinal muscle.

The stimulus which, in the presence of hexamethonium, elicits the reflex contraction of the longitudinal muscle coat, is the deformation of the sensory receptors by radial stretching, not by increased transmural pressure; if radial distension is prevented by slipping a glass or perspex tube over the gut, increased pressure in the lumen does not lead to a reflex contraction of the longitudinal muscle (132, 225). A similar technique shows that the same kind of stimulus is responsible for the triggering of the emptying phase (92). Further evidence for the view that radial stretch is the adequate stimulus can be obtained by raising the luminal and external pressures to the same extent; thus the lumen cannot distend and the longitudinal muscle coat does not contract (132). Finally, Schaumann *et al.* (183) and Kosterlitz and Robinson (132) have shown that neither a slow nor a sudden stretch applied to the longitudinal muscle coat, in its long axis, causes a shortening of this muscle.

There is strong evidence that the relaxation of the longitudinal muscle which occurs as the circular muscle contracts during the emptying phase is an active process. Schaumann *et al.* (183) showed that when the longitudinal muscle is first contracted by acetylcholine or histamine, and then the reflex is elicited by distension of the lumen, the longitudinal muscle relaxes during distension of the lumen and contracts again when the pressure in the lumen is reduced to zero. These findings have been confirmed and extended recently by Thouvenot and

Harichaux (209–211) and by Kosterlitz and Watt (136). The identity of the inhibitory transmitter has not yet been established.

The fact that the reflex contraction wave of the circular muscle is blocked by hexamethonium indicates that the ganglion cells of the intramural plexus are indispensable. Klinge (125) obtained direct evidence for this view by showing, in experiments on tubular segments of the mid-ileum of the cat, that the reflex can be elicited only when the myenteric plexus of Auerbach is intact, although acetylcholine can induce propagated contractions in the absence of the plexus.

Although Trendelenburg (214) distinguished two phases of the peristaltic reflex, there is no evidence that the shortening of the gut during the first, or preparatory, phase is necessary for, or even facilitates, the evacuation of the intestinal contents during the second, or emptying, phase. Kosterlitz *et al.* (128) showed that prevention of shortening by isometric recording of the longitudinal contraction or by blocking the contraction altogether in no way interfered with the efficacy of the emptying phase.

When the first, or preparatory, phase is elicited alone and the emptying phase is suppressed by hexamethonium, the response of the longitudinal muscle is graded, *i.e.*, the contraction increases with the degree of distension. This holds for both isotonic and isometric recording (99, 132). This graded response can also be observed in the absence of hexamethonium when the degree of intestinal filling is below the threshold of the emptying phase. Kosterlitz and Robinson (132) called the graded response type I contractions; type II contractions of the longitudinal muscle are observed during the emptying phase and are inhibited by hexamethonium. When the degree of intestinal filling reaches the threshold for the emptying phase, type II contractions are superimposed on the type I contractions as a sudden, additional rise in the longitudinal muscle tension.

The two phases of the peristaltic reflex would appear to have little in common, except that they are evoked by the same stimulus. We therefore propose to call the first phase the graded reflex of the longitudinal muscle and the second phase the peristaltic reflex proper. The complete peristaltic reflex, as observed in the isolated ileum preparation without addition of drugs, would thus be made up of the graded reflex of the longitudinal muscle and the peristaltic reflex proper.

With regard to the reflex arcs involved, it has already been stated that the arc of the peristaltic reflex proper has at least one cholinergic synapse. Since the graded reflex of the longitudinal muscle is not inhibited by hexamethonium and drugs with similar action, its reflex pathway contains either no synapses or only synapses in which transmission is not cholinergic. In both reflex arcs neuromuscular transmission, however, appears to be mainly cholinergic (36, 130, 132).

III. EFFECTS OF VARIOUS AGENTS ON INTRINSIC REFLEXES OF THE SMALL INTESTINE

A. Anoxia

Job *et al.* (119) found that anoxia increases the excitability of the guinea-pig jejunum before causing complete paralysis. During the early period of anoxia, the peristaltic reflex proper and the reflex contraction of the longitudinal muscle

are elicited by lower degrees of distension than in the absence of anoxia. At a later stage, rapid rhythmic contractions of both muscle coats supervene and are apparently myogenic since they are not inhibited by morphine or nicotine, in contrast to the coordinated contractions of the earlier phase of anoxia.

The reflex contraction of the longitudinal muscle is more readily inhibited by anoxia (183) or by sodium cyanide (132) than the contractions produced by agonists acting directly on the muscle, as, for instance, acetylcholine.

B. Cooling

Kosterlitz and Robinson (130), Beleslin and Varagić (15), and Thouvenot and Harichaux (208) showed that gradual lowering of the temperature of the bath fluid in which the guinea-pig ileum is suspended affects first the peristaltic reflex proper and then the graded reflex of the longitudinal muscle. With cooling to 25°C, the rate of contraction of the circular muscle is slowed, the ejection velocity diminished, and the emptying period prolonged. At about 21°C, coordinated contractions of the circular muscle and type II contractions of the longitudinal muscle cease. The type I contractions become slower and smaller as the temperature of the bath fluid is lowered further to 16°C. These effects of cooling are readily reversible. The depression of the reflexes by cooling is not due to inability of the muscle to contract since Innes *et al.* (117) and Day and Vane (57) have shown that the responses of the longitudinal muscle to acetylcholine, carbamylcholine, and histamine are either unaltered or potentiated at these temperatures. Thus cooling exerts its effect somewhere on the nervous elements involved in the two reflexes, the peristaltic reflex proper being the more sensitive. Attention is drawn to this particular difference in sensitivity of the two reflexes, which will be observed repeatedly with other inhibitory agents.

It is important to differentiate between the effect of lowering the temperature of the organ bath and that of storing the tissue at low temperature. The inhibition of the reflex when a segment of guinea-pig ileum is kept at 5°C with adequate O_2 supply is reversible for as long as 8 hours (15). On the other hand, when the ileum is stored at 4°C for 24 or 48 hours without ensuring the supply of oxygen, the peristaltic reflex proper is lost irreversibly; this is assumed to be due to damage to nervous structures. The graded reflex of the longitudinal muscle survives longer, usually up to 48 hours, and can be elicited at all degrees of filling (2, 117, 185).

In this context it is of interest that, in the gut of the trout, Burnstock (39) observed inhibition of the peristaltic reflex, which requires intact nervous structures, by a rapid rise in temperature from 12 to 18°C. On the other hand, myogenic pendular contractions increase in both frequency and amplitude with temperature changes from 4 to 30°C.

C. Ganglion-blocking drugs

It has already been mentioned that ganglion-blocking drugs, added to the fluid of the organ bath, inhibit the peristaltic reflex proper but leave the graded reflex of the longitudinal muscle unaffected. Drugs having this effect are tubocurarine,

nicotine, pentamethonium, hexamethonium, dimethylphenylpiperazinium, and bretylium, among others (70, 127, 130, 148, 168).

When hexamethonium is present in concentrations insufficient to inhibit the peristaltic reflex proper, the rate at which the intraintestinal pressure rises during the contraction of the circular muscle is reduced and the ejection of the intestinal contents is incomplete.

The inhibitory effect of nicotine is more complex in that, with large doses, there is also a transient depression of the graded reflex of the longitudinal muscle (70, 106). However, when this occurs, the responses of the longitudinal muscle to acetylcholine and histamine are also reduced (70, 130); this result indicates a nonspecific effect of the type described by Cantoni and Eastman (48).

Busse *et al.* (44) have shown that when ganglion-blocking drugs are used in threshold concentrations, their action on the peristaltic reflex proper in the guinea-pig ileum can be antagonized by drugs which apparently have only a direct action on smooth muscle. Thus the block produced by tubocurarine or hexamethonium can be overcome by histamine and the block produced by nicotine is antagonized by carbamylcholine. There are several possible interpretations of these observations and further analysis is required.

D. Local anaesthetics

Feldberg and Lin (70) have shown that local anaesthetics such as cocaine, procaine, and cinchocaine (dibucaine), applied to the serosal surface, have an effect similar to that of ganglion-blocking drugs; they inhibit the peristaltic reflex proper but do not interfere with the graded reflex of the longitudinal muscle unless the concentration of the drug also inhibits the contraction of the longitudinal muscle caused by acetylcholine added to the bath fluid. The potency of local anaesthetics in inhibiting the peristaltic reflex proper is correlated with their anaesthetic potency; thus, a lower concentration of cinchocaine is required than of cocaine.

Procaine applied to the mucosal surface has an inhibitory effect similar to that observed after serosal application (32).

E. Catecholamines

It has been shown repeatedly, *e.g.*, by Leo (142), Bozler (26), McDougal and West (153), and Kosterlitz and Robinson (130), that in the rabbit and guinea-pig the peristaltic reflex is inhibited by adrenaline and other catecholamines.

The peristaltic reflex proper is particularly sensitive to the inhibitory action of these compounds; adrenaline (12 μg/l) inhibits the emptying phase without affecting the graded reflex of the longitudinal muscle but the latter is very much reduced by a higher adrenaline concentration (50 to 150 μg/l). The contractions of the longitudinal muscle induced by acetylcholine are not affected by such concentrations of adrenaline, while those due to histamine are depressed by the higher concentrations (130). Since cholinoceptive synapses are present in the reflex arc of the peristaltic reflex proper, but not in that of the graded reflex contraction of the longitudinal muscle, it is possible that these synapses may be

the site of the inhibitory action of adrenaline and noradrenaline on the peristaltic reflex proper.

McDougal and West (153) examined the effects of a large number of phenylalkylamines on the peristaltic reflex in the isolated ileum of the guinea-pig. They found that all dihydroxyphenylalkylamines are inhibitors, adrenaline being the most potent and isoprenaline (isoproterenol) the least. Their inhibitory action is antagonized by adrenergic blocking agents of the benzodioxan, imidazoline, and β-haloalkylamine classes. Thus it would appear that the inhibitory action of catecholamines on the peristaltic reflex is on adrenergic receptors of the α- type.

Monohydroxyphenylalkylamines and phenylalkylamines, also, exert an inhibitory action on the peristaltic reflex but only in much higher concentrations than the dihydroxyphenylalkylamines, and this action is not blocked by adrenergic blocking agents. It is of interest that *m*-monohydroxyphenylalkylamines, which are much more potent than the corresponding *p*-monohydroxy compounds in exciting the rabbit uterus or inhibiting spontaneous movements of the rabbit ileum, are about as ineffective on the peristaltic reflex as the *p*- substituted compounds (153).

F. 5-Hydroxytryptamine

The analysis of the action of 5-hydroxytryptamine by Bülbring and Crema (31–33) and Bülbring and Lin (35) demonstrated for the first time that the effects of drugs applied to the mucosal surface of the isolated intestine may be different from those obtained after application to the serosal surface. From observations of this kind conclusions may be drawn with regard to the site of action of a drug. Bülbring and her collaborators examined also the conditions under which 5-hydroxytryptamine is released into the lumen from the mucous membrane.

Serosal application. The observations of Kosterlitz and Robinson (130), Ginzel (91), Bülbring and Lin (35), Bülbring and Crema (31), and Lembeck (141) have shown that application of 5-hydroxytryptamine to the serosal surface causes only a transient stimulation and then abolishes the peristaltic reflex proper in the isolated ileum of the guinea-pig, but not of the rabbit. The inhibitory action is very similar to that of ganglion-blocking drugs, since it does not affect the graded reflex contraction of the longitudinal muscle (132); this action is possibly due to specific desensitization of tryptamine receptors. The transient stimulation is particularly marked when the reflex is depressed by cooling (15) or partly inhibited by hexamethonium or atropine (32), and may be explained by a sensitization of the longitudinal muscle and ganglion cells to acetylcholine; it is antagonized by lysergic acid diethylamide or 2-bromo-lysergic acid diethylamide (31, 91).

Mucosal application. The application of 5-hydroxytryptamine to the mucosal surface lowers the intraluminal pressure which is required to trigger the peristaltic reflex proper. When the peristaltic reflex is elicited in the experimental arrangement of Bülbring *et al.* (34), the filling of the lumen proceeds continuously at a slow and constant rate, the amount of fluid expelled can be measured, and drugs

can be added to fluid passing through the lumen. After application of 5-hydroxytryptamine to the mucosal surface the peristaltic waves occur more frequently and more fluid is transported through the intestinal lumen but prolonged presence of the drug leads to paralysis (33). This effect has been shown to occur in the small and large intestine of all species investigated, namely, guinea-pig, rabbit, rat, and monkey (29). The 5-hydroxytryptamine antagonists, lysergic acid diethylamide and 2-bromo-lysergic acid diethylamide, when applied to the mucosal surface, have an effect opposite to that of 5-hydroxytryptamine. They raise the intraluminal pressure required to trigger peristalsis, reduce the frequency of peristaltic waves, and diminish fluid transport but they do not block peristalsis completely (35).

In vivo experiments. It is of interest that, when in the guinea-pig ileum *in vivo* the intraluminal pressure in an isolated loop is low and the ileum is quiescent, the stimulating effect of 5-hydroxytryptamine is more marked after arterial (0.1 to 0.2 μg) than after intravenous (4 to 10 μg) injection or after intraluminal (100 to 300 μg) application (33). When, however, the fluid passing through the lumen has a constant concentration of 5-hydroxytryptamine, there is no stimulating effect in quiescent or in active loops of intestine. Moreover, spontaneous peristalsis is inhibited more readily *in vivo* than *in vitro* by the prolonged presence of 5-hydroxytryptamine in the lumen. It is probable that *in vitro* the local production of 5-hydroxytryptamine gradually declines in the course of an experiment and therefore the introduction of 5-hydroxytryptamine into the lumen stimulates peristaltic activity, while *in vivo* the local production is normal and therefore introduction of 5-hydroxytryptamine into the lumen or intravenously causes the local concentration to become too high and thus leads to inhibition of peristalsis. Moreover, a great part of the mucous membrane is shed during the course of an *in vitro* experiment (60) and the resultant change in histological structure may alter the sensitivity of the sensory receptors to 5-hydroxytryptamine.

The administration of 5-hydroxytryptamine by any of these routes necessarily produces effects other than stimulation of sensory receptors, as for instance ganglionic block. For this reason, slow intraarterial injection of 5-hydroxytryptophane, which is converted to 5-hydroxytryptamine and selectively stored in the enterochromaffin cells, is a more powerful and persistent stimulant of peristalsis than 5-hydroxytryptamine, provided it is not given in very large doses (33).

Analysis of action. The stimulatory effects of serosal and mucosal applications of 5-hydroxytryptamine were used by Bülbring and Crema (31) to determine the sites at which 5-hydroxytryptamine acts. During block by procaine introduced into the lumen, mucosal, but not serosal, application of 5-hydroxytryptamine reestablishes peristalsis. During partial inhibition of ganglionic transmission by hexamethonium or of neuroeffector transmission by atropine, both acting from the serosal surface, 5-hydroxytryptamine restores peristalsis both by serosal and by mucosal application; when the block is complete, serosal but not mucosal application is effective. During block by serosal application of 5-hydroxytryptamine itself or of morphine, phenoxybenzamine, or dihydroergotamine, mucosal, but not serosal, application of 5-hydroxytryptamine restores the peristaltic reflex.

When lysergic acid diethylamide or 2-bromo-lysergic acid diethylamide is applied to the serosal surface, 5-hydroxytryptamine is without effect from either surface. Beleslin and Varagić (15) showed that serosal or intraluminal application of 5-hydroxytryptamine regularly restores peristaltic activity in the isolated ileum in which peristalsis has been abolished by cooling to 20 to 25°C.

It may be concluded that 5-hydroxytryptamine facilitates the peristaltic reflex at two sites: when introduced into the lumen it stimulates sensory receptors situated near the mucosal surface (36, 60, 92, 93); when acting from the serosal surface it sensitizes the muscle to the transmitter, acetylcholine. The action on the intramural ganglia is inhibitory after transient stimulation.

Physiological significance. It is not certain whether 5-hydroxytryptamine plays an indispensable role in the initiation of the peristaltic reflex. Bülbring and Crema (32) found that peristalsis in reserpine-treated guinea-pigs is abnormally active and occurs at low threshold, although the 5-hydroxytryptamine content may be as low as 1 to 2% of normal when the isolated ileum is set up. Even addition of 2-bromo-lysergic acid diethylamide does not stop peristalsis in the ileum obtained from reserpine-treated guinea-pigs. However, no definite statement on the indispensability of 5-hydroxytryptamine for the initiation of peristalsis can be made until a means is found whereby synthesis of 5-hydroxytryptamine can be completely blocked, especially since Bülbring and Crema (33) have found that intraarterial or intraluminal administration of 5-hydroxytryptophane causes an increase in peristaltic activity, in the mucosal content of 5-hydroxytryptamine, and in its release. Such an increase in the release of 5-hydroxytryptamine from the ileum is observed even in reserpine-treated guinea-pigs although there is no increase in the 5-hydroxytryptamine content of the mucous membrane.

In this context, the observations of Hukuhara *et al.* (113) may be of importance. They found that in the denervated jejunal loop of the dog, stroking of the mucosal surface or application of hydrochloric acid elicits peristaltic responses after specific desensitization of the mucosal receptors to 5-hydroxytryptamine. These observations, if confirmed for other species, would indicate that 5-hydroxytryptamine is not necessary for the excitation of mucosal sensory receptors.

Using a technique slightly different from that of Bülbring and her collaborators, Lembeck (141) arrived independently at the conclusion that 5-hydroxytryptamine applied to the lumen of the isolated ileum of the guinea-pig stimulates peristalsis, while it has an inhibitory action from the serosal side. He, too, holds the view that the facilitation of the reflex is due to a sensitization of the sensory structures to the stimulus of stretch.

Stimulus for release. The adequate stimulus for the release of 5-hydroxytryptamine from the intestinal mucosa into the lumen is thought by Bülbring and Lin (35) and Bülbring and Crema (32) to be pressure on the mucous membrane. When an intestinal segment is inverted and slipped on to a perspex rod, the effects of movements of the muscles on the mucous membrane are excluded and the lumen cannot distend; application of different hydrostatic pressures to the exposed mucous membrane results in larger amounts of 5-hydroxytryptamine being released with higher pressures. However, since the absolute amounts re-

eased are smaller than those appearing in the lumen during normal peristalsis, the inversion experiments do not exclude the importance of luminal distension and peristaltic movements. Indeed, Bülbring and Crema (32) state that the enterochromaffin cells may be particularly exposed to mechanical effects resulting from the distension of the gut wall normally associated with increased filling pressure. That such mechanical deformation may be the most important factor determining release of 5-hydroxytryptamine follows from the observation that, at a constant filling pressure, the release is very much greater during bursts of peristalsis than during the intervening periods of rest.

However, stimuli other than pressure or distension of the lumen can cause release of 5-hydroxytryptamine. For instance, Resnick and Gray (174) found that perfusion of the upper small intestine of the rat with 3 ml of 0.1 N hydrochloric acid over a period of 15 minutes causes reduction of the 5-hydroxytryptamine content and degranulation of the argentaffin cells; only a small fraction of the 5-hydroxytryptamine lost from the gut wall was found in the lumen.

Several investigations indicate that 5-hydroxytryptamine facilitates peristalsis in man *in vivo* (52, 58, 105, 107, 173).

Movements of villi. It is of interest that 5-hydroxytryptamine facilitates not only peristalsis but also the movements of the duodenal villi in the dog. Ludány *et al.* (151) showed that the movements of the villi are enhanced after intra-arterial or intravenous injection or mucosal application of 5-hydroxytryptamine. The increase in the movements is preceded by capillary constriction and an increase in the tone of the smooth muscle of the villi and is accompanied by increased peristaltic movements of the duodenum. Chlorpromazine and lysergic acid diethylamide, but not atropine, prevent the action of 5-hydroxytryptamine.

G. Substance P

The results of the experiments with substance P cannot be considered as final since all the investigators used impure preparations.

Beleslin and Varagić (17) found that application of substance P to the serosal surface in the isolated ileum of the guinea-pig blocks the peristaltic reflex. However, the sample they used contained only 1 unit/mg, whereas the pure substance contains about 120,000 units/mg (100). Moreover, Kosterlitz and Robinson (132) showed that high concentrations of a slightly purer sample of substance P (13 units/mg) do not inhibit the graded reflex contraction of the longitudinal muscle.

Beleslin and Varagić (16) showed that application of a crude sample of substance P to the mucosal surface of the isolated ileum increases the number and amplitude of peristaltic waves. The same sample of substance P produces a similar effect in preparations in which peristalsis has been depressed by fatigue, by lowering the temperature of the bath, or by saturation of the tryptamine receptors after serosal or mucosal application of 5-hydroxytryptamine. This effect is abolished by removal of the mucous membrane or application of hexamethonium from the serosal side. Medaković and Radmanović (160) confirmed these findings and showed that mucosal or serosal application of atropine or morphine inhibits the effect of substance P.

Liljedahl *et al.* (147) showed that, in man, intravenous injection of 600 to 1000 units of substance P, containing 600 to 800 units/mg, causes an increase of segmental and of peristaltic movements, lasting 20 minutes.

Intravenous injection of substance P increases movements of the villi of the duodenum of dogs anaesthetized with chloralose. Hexamethonium, which blocks the excitatory action of villikinin on the villi, does not depress the action of substance P (149, 150).

H. Atropine and hyoscine (scopolamine)

In the isolated ileum of the guinea-pig, atropine and hyoscine inhibit both the peristaltic reflex proper and the graded reflex contraction of the longitudinal muscle (99, 130, 185, 214). The graded reflex contraction of the longitudinal muscle is inhibited about equally well at all degrees of distension (99). Kosterlitz and Watt (135) showed that the responses to distension cannot be inhibited beyond a certain extent by increasing the concentration of atropine or hyoscine. However, lowering the bath temperature to 15 to 19°C abolishes almost completely the contraction which persists in the presence of these drugs. Since the contraction of the longitudinal muscle induced by agonists acting on the muscle directly, *e.g.*, bradykinin, is not depressed at these temperatures, the possibility of a hyoscine-resistant reflex pathway has to be considered.

Schaumann (185) found that, in the rabbit jejunum, the reflex contraction of the longitudinal muscle is only slightly inhibited by atropine and slightly potentiated by morphine, even when large concentrations (1 μg/ml and 10 μg/ml, respectively) are used.

I. Morphine-like drugs and their antagonists

The inhibitory action of morphine on the peristaltic reflex proper and on the graded reflex of the longitudinal muscle of the isolated ileum of the guinea-pig has been known since the work of Trendelenburg (214) and has been confirmed many times (99, 129, 130, 181, 182, 185).

Gyang *et al.* (99) showed that in contrast to the inhibitory action of atropine or hyoscine, the depressant action of morphine on the graded reflex contraction of the longitudinal muscle tends to be greater at low than at high degrees of distension of the lumen. The graded reflex is never completely abolished by morphine, even when its concentration is increased tenfold. When hyoscine or atropine is added to the bath after a maximal inhibitory concentration of morphine has been present for some time, no further inhibition is observed, except at the highest degrees of distension. Similarly, morphine does not cause any further inhibition when added to the bath containing a concentration of atropine or hyoscine that produces a maximal effect. However, the morphine- and hyoscine-resistant reflex contractions are reversibly depressed by cooling the preparation to 15 to 19°C. These observations seem to suggest that, apart from the reflex pathway which is inhibited by morphine and hyoscine, there is another reflex arc on which morphine and hyoscine have no effect (135).

It should be stressed that the inhibitory action of morphine, methadone, or

levorphanol on the graded reflex of the longitudinal muscle is not due to a depression of the response of the muscle to the transmitter, acetylcholine (99, 130, 131, 182), although the actions of acetylcholine, histamine, and bradykinin on the longitudinal muscle may be slightly depressed by morphine (131, 146).

Schaumann (186, 188) showed that morphine (10 μg/ml) reduces the output of acetylcholine from the distended isolated ileum incubated in Tyrode solution; later (189), he found that adrenaline and noradrenaline (5 μg/ml) also reduce the output of acetylcholine and proposed that morphine-like compounds may act by liberating catecholamines or by occupying the same receptors as catecholamines. However, the evidence presented in favour of this view rests on the use of insufficiently specific adrenergic blocking drugs and cannot be considered to be definite.

All drugs with a morphine-like action depress the peristaltic reflex proper and the graded reflex of the longitudinal muscle, their efficacy being closely correlated with their analgesic potencies (95, 99, 181, 182).

Straub and Viaud (199) examined the effect of morphine on the peristaltic reflex in the guinea-pig by modifying the method of Trendelenburg (214) in such a manner that it could be used *in vivo* on the ileum with intact blood supply. They found that morphine (0 05 mg/kg) increases the level to which the intraintestinal pressure has to be raised in order to elicit the reflex. These findings were confirmed and extended by Schaumann (184), who showed that pethidine (meperidine), *laevo*-methadone, or levorphanol inhibits the peristaltic reflex and that their inhibitory potency is closely correlated with their analgesic potency. The *dextro*-isomers, *dextro*-methadone and dextrorphan, have little or no effect. Pethidine or, in small doses, morphine, but not methadone or levorphanol, decreases the resistance of the ileum to filling; this result confirms the findings of Hildebrandt and Matthäy (108). Therefore the increase in distensibility cannot be the cause of the inhibitory action of morphine, as had been suggested by Straub and Leo (196).

This view finds further support in the observations of Thorp (207) that in the isolated ileum of the rabbit both *dextro*- and *laevo*-methadone have a similar antiacetylcholine action, while the *laevo*- isomer is 15 times more potent in inhibiting the peristaltic reflex in the isolated ileum of the guinea-pig (181). While neither levorphanol nor dextrorphan has a significant antiacetylcholine action (171), only the *laevo*- isomer inhibits the peristaltic reflex (99, 171, 181).

Schaumann (185) found that, in preparations of isolated ileum of the guinea-pig stored at 4 to 5°C for 24 hours in order to abolish the peristaltic reflex proper, the graded reflex contraction of the longitudinal muscle which is depressed by morphine or atropine can recover despite the drugs being left in the bath. This does not occur in all experiments; in some there is only partial recovery, in others none at all. Using the technique of Straub and Viaud (199), he found also that refractoriness to morphine-like compounds can develop in the ileum of the guinea-pig *in vivo* and that under these conditions atropine (0.1 mg/kg) still has an inhibitory effect (184). The peristaltic reflex in isolated segments of ileum obtained from morphine-tolerant guinea-pigs is less sensitive to the depressant action of

morphine than in ileum of normal animals (172); no such tolerance can be produced with pethidine (123).

Medaković (159) showed that morphine inhibits the peristaltic reflex also when applied to the mucous membrane. This action is antagonized by 5-hydroxytryptamine but not by substance P, acting from the lumen (160).

The inhibitory action of morphine and morphine-like drugs is antagonized *in vitro* and *in vivo* by the allyl- analogues, nalorphine and levallorphan, although these compounds may have a transient morphine-like action themselves (78, 99, 121, 131).

It is noteworthy that morphine in concentrations of up to 150 μg/ml does not cause contractions of the longitudinal or circular muscle of the isolated ilea of rats, rabbits, guinea-pigs, dogs, and human beings (53).

J. Other inhibitory substances

In the isolated ileum of the guinea-pig, Hobbiger (110) has shown that γ-aminobutyric acid depresses the peristaltic reflex proper and the graded reflex of the longitudinal muscle in low concentrations (0.1 to 1 μg/ml), but only when the intraluminal pressure is low, *i.e.*, near threshold level; if the pressure is high enough to produce regular peristaltic responses, a concentration of 100 μg/ml is ineffective. γ-Aminobutyric acid acts to a limited extent as an antagonist of acetylcholine, nicotine, and histamine; the antinicotine effect, which is more effective at low than at high concentrations of nicotine, is more marked than the antiacetylcholine effect. In the rabbit ileum, γ-aminobutyric acid has no inhibitory effect on the peristaltic reflex.

Many quaternary phenothiazine derivatives depress the peristaltic reflex proper in the rabbit ileum, with or without altering the resistance of the gut wall to distension (61). Chlorpromazine-like compounds inhibit the peristaltic reflex proper and the graded reflex contraction of the longitudinal muscle of the guinea-pig only in concentrations which also exert strong antiacetylcholine effects. Antipyretic analgesic drugs, such as phenazone (antipyrine) or amidopyrine, have no inhibitory action on the peristaltic reflex unless their concentration is raised to a level at which there is a strong antiacetylcholine effect (99).

Schneider and his colleagues (21, 190, 191) have shown that wheat gluten contains substances which inhibit the peristaltic reflex in the isolated jejunum of the rat and the twitch response of the isolated jejunum of the guinea-pig on coaxial electrical stimulation. These substances reduce the output of acetylcholine during rest as well as during electrical stimulation. The active material is water-soluble, ultrafiltrable, and resistant to peptic-tryptic digestion and may owe its inhibitory effect to its adenosine content, as both the ultrafiltrate of gluten extract and adenosine inhibit the peristaltic reflex and are inactivated by incubation with mammalian small intestinal mucosa and with purified adenosine deaminase.

Sodium diphenylylbutylacetate inhibits release of acetylcholine from the isolated ileum of the guinea-pig, probably by depressing acetylcholine synthesis (82, 90), and abolishes the reflex response of the longitudinal muscle but not the peristaltic reflex proper.

K. Adrenal cortical hormones

Streeten *et al.* (200) have shown that adrenal cortical extract in low concentrations (0.7 to 4 μl/ml) enhances peristaltic activity of isolated segments of the small intestine of rabbits, dogs, and human subjects, while in high concentrations (6.5 to 40 μl/ml) it reversibly inhibits peristalsis. From experiments *in vivo* the authors concluded that the electrolyte-controlling corticoids are responsible for this effect. Vogt (219) found that in rabbits adrenalectomy depresses the response of the circular muscle of isolated segments of small intestine to substances acting on the myenteric plexus, but this effect is not reversed by cortical extract and is therefore due to changes secondary to adrenal deficiency. The response of the longitudinal muscle to directly acting drugs is normal. This agrees with observations that the responses of the longitudinal muscle of the adrenalectomized rat to acetylcholine or adrenaline are normal (51).

IV. EFFECTS OF DRUGS ACTING ON THE INTRAMURAL NERVOUS STRUCTURES AND MUSCLE FIBRES OF THE SMALL INTESTINE

Drugs acting on the intramural ganglia or their axons may have excitatory or inhibitory actions. The analysis of the site of action of excitatory drugs depends largely on the antagonism by inhibitory agents known to act at certain sites, *e.g.*, the ganglia, nerve endings, neuroeffector junction or the muscle fibres. The effects of inhibitory drugs cannot be demonstrated in an intestine lacking tone and spontaneous movements without first causing it to contract. This can be achieved by stimulating sensory receptors by distension of the lumen, by electrical stimulation of the nervous or muscular structures in the gut wall, by stimulation with excitatory drugs, or by electrical stimulation of extrinsic nerves. The first of the four experimental approaches leads to quite complex responses and has already been dealt with in section III; it is now proposed to consider the other three possibilities.

A. Effects of drugs on the responses of the longitudinal muscle to electrical stimulation

1. *Stimulation with coaxial electrodes.* The technique of coaxial electrical stimulation, which was devised by Paton (165), consists of passing current pulses from one electrode in the bath fluid, in which a piece of guinea-pig ileum is suspended, to another within the lumen of the gut. Twitches of the longitudinal muscle in response to single stimuli of 50 μsec duration can be obtained; such twitches are augmented and prolonged by eserine, are insensitive to hexamethonium and nicotine, are reduced by procaine, and are abolished by small doses of atropine. Paton concluded from the character of the strength-duration curve and from these and other pharmacological responses that the nerves supplying the muscles are stimulated. If the intestine is distended by raising the intestinal pressure, an emptying reaction can be seen in response to single shocks; it resembles the peristaltic reflex and is inhibited by ganglion-blocking agents. After blocking the neuroeffector transmission with hyoscine or atropine, the smooth muscle can be stimulated directly but a pulse of much longer duration is required.

In an analysis of the action of morphine, Paton (166) found that it depresses the twitch caused by a single stimulus and also the prolonged contraction due to

stimuli applied at frequencies of 1 to 5/sec. Other morphine-like drugs, phenadoxone, dihydromorphinone, metopon (methyldihydromorphinone), methadone, heroin, and codeine, also reduce the size of the twitch, their relative potencies being related to their analgesic potencies. It is interesting that nalorphine, also, has a marked depressant effect and Paton concluded from his qualitative observations that nalorphine acts on the guinea-pig ileum as an analogue of morphine rather than as an antagonist. The analysis of the action of morphine is complicated by the fact that the ileum rapidly develops tachyphylaxis or tolerance to morphine and morphine-like drugs, and also to nalorphine. The inhibitory action of morphine is not due to a depression of the response of the longitudinal muscle to acetylcholine; of the morphine-like drugs tested by Paton, only pethidine has a mild atropinic effect.

Schaumann (189) showed that the contractions of the longitudinal muscle caused by coaxial stimulation are inhibited not only by morphine-like drugs but also by adrenaline and noradrenaline (0.03 to 0.06 μg/ml); the effects of the morphine-like drugs and the catecholamines are antagonized by phentolamine and tolazoline.

Release of acetylcholine. When the release of acetylcholine into the bath fluid is studied, it can be shown that morphine reduces the output of acetylcholine at rest and during coaxial stimulation (166). There seems to be a fundamental difference between the effects caused by stimulation at low frequencies (less than 1/sec) and those at higher frequencies. First, the output/shock is higher at low frequencies (0.16 to 0.4 ng/shock) than at higher frequencies (0.05 to 0.11 ng/shock) and, secondly, morphine reduces the output at low frequencies to 0.03 to 0.06 ng/shock while at higher frequencies the values after morphine are 0.04 to 0.1 ng/shock, that is, there is no reduction.

Recently Paton (167) extended these findings. He showed that there are two mechanisms concerned with the output of acetylcholine; one is important at low rates of stimulation, yields a constant output of transmitter per unit of time and is sensitive to morphine, the other is brought in at higher frequencies of stimulation, yields a more constant output per volley and is resistant to morphine. In this context he drew attention to the findings of Kosterlitz and Taylor (133) and Cairnie *et al.* (47), who showed that neuroeffector transmission at the sino-auricular node of the rabbit and rat heart and at the cat nictitating membrane is depressed by morphine and that morphine is more effective at low than at high frequencies of stimulation.

There is no evidence that morphine affects impulse transmission in the axons (46, 134). Since morphine has little or no effect on the response of the smooth muscle to acetylcholine, the conclusion appears to be justified that morphine interferes with the release of acetylcholine from the nerve endings.

Harry (101) examined the effects of cooling to 13°C, of local anaesthetics, and of botulinum toxin on the response of the slightly distended guinea-pig ileum to coaxial electrical stimulation. The responses, which consist of contractions of the longitudinal and circular muscle coats, are correlated with the amount of acetylcholine found in the bath fluid in which the ileum is suspended. Cooling

to 13°C reversibly abolishes the contractions caused by electrical stimulation, with a concurrent fall in the output of acetylcholine to 16 % of that of the control period. Similar reductions in responses and acetylcholine output are observed in the presence of procaine (100 μg/ml), which does not reduce the contractions due to histamine, and also after exposure of the ileum to botulinum toxin A for one hour. In contrast to these findings, papaverine, in concentrations which abolish the responses of the longitudinal and circular muscle layers to coaxial stimulation, does not reduce the output of acetylcholine. Harry points out that there are several possible sites of release of acetylcholine after coaxial stimulation, namely, cholinergic nerve endings in the longitudinal muscle, similar endings in the circular muscle, and the cholinergic ganglionic synapses of the intrinsic nerve plexuses.

Origin of acetylcholine. The problem of the origin of the acetylcholine released from the gut has been investigated by Feldberg and Lin (71, 72), who found that the spontaneous output of acetylcholine of the resting guinea-pig or rabbit small intestine is not inhibited by nicotine, tubocurarine, or cocaine. They concluded that, if the nervous structures in the intestinal wall were the site of acetylcholine metabolism, the spontaneous release would be independent of any excitation of the nerve fibres of the plexuses; alternatively, not all of the released acetylcholine originates in the nervous structures of the wall. This view is supported by the finding that choline-acetylase is present in non-nervous tissue, particularly in the glandular part of the mucous membrane. Johnson (120) reinvestigated this problem and found that cooling the preparation to 25°C or the addition of cocaine (5 μg/ml) or procaine (10 μg/ml) greatly reduces the resting output of acetylcholine; this is in contrast to the findings of Feldberg and Lin (71), who used a higher concentration of cocaine (30 μg/ml). Moreover, reduction of the calcium content of the Krebs solution to 5 % of its normal value or a fourfold increase of the magnesium content inhibits the release of acetylcholine. These findings indicate that at least part of the spontaneously released acetylcholine is derived from nervous structures, a conclusion which is supported by the observation of Paton (166) that morphine depresses the resting output. There is also evidence that adrenaline has a similar depressant effect (212).

Chujyo and his collaborators (49, 50, 115) found that radial, but not longitudinal stretching of the wall of the segments of the eserinized isolated small intestine of the guinea-pig causes a release of acetylcholine into the fluid in which the intestine is suspended; at the same time, there is an increase in the acetylcholine content of the stretched segments. In the noneserinized intestine there is a considerable decrease in the acetylcholine content of the wall immediately after stretching, but this loss is no longer to be found when the wall has been stretched for 30 minutes. There is insufficient experimental evidence to explain these interesting observations.

2. *Stimulation with external surface electrodes.* Härtfelder *et al.* (103, 104) used field stimulation of the isolated ileum of the guinea-pig by means of sinusoid alternating current (50 cyc/sec); they confirmed Paton's (166) findings that morphine and atropine have inhibitory effects. They found, further, that in their

experimental arrangement hexamethonium inhibits the responses of the longitudinal muscle to submaximal stimulation; therefore this response results in part from stimulation of preganglionic elements. The depressant action of adrenaline or noradrenaline is blocked by Dibenamine, phentolamine, or tolazoline.

Kern and Lembeck (122) re-examined the effect of hexamethonium on the isolated ileum of the guinea-pig, stimulated with rectangular pulses through external electrodes. Hexamethonium has no inhibitory effect on contractions caused by single stimuli but depresses the responses to trains of 50 impulses at a frequency of 100/sec. The authors concluded that the responses to repetitive stimulation are due to stimulation not only of the final motor fibres but also of preganglionic elements. Their observation that tubocurarine (6 to 10 μg/ml) depresses the responses to single stimuli is not readily explained since this drug does not depress the response of the longitudinal muscle to acetylcholine (70).

Bozler (26) showed that electrical stimuli of submaximal intensity applied by external electrodes to the small intestine or colon of the rabbit produce polar responses in which there are contractions in an ascending, oral direction but never in a descending, rectal direction. In the guinea-pig ileum, Schaumann (187) found that there is never a strictly polar response but, with submaximal stimuli, the ascending contractions are always stronger than the descending contractions. Hexamethonium (1 to 100 μg/ml) and cocaine (1 μg/ml) depress the ascending contractions but have little effect on the descending contractions. Schaumann assumed, therefore, that at least a component of the ascending contractions is mediated by ganglion cells. The descending, as well as the ascending, contractions are depressed by morphine (0.1 to 1 μg/ml) or atropine (0.01 to 0.1 μg/ml), the responses to submaximal stimuli being completely inhibited, those to maximal stimuli by 70%.

The effect of electrical stimulation applied at the two ends of the lumen of an isolated piece of the terminal ileum of the guinea-pig was studied by Munro (164). The terminal segment differs from a more oral segment in that adrenaline causes contraction of the longitudinal muscle instead of relaxation. The motor response produced by direct electrical stimulation is first abolished by atropine but later replaced by an atropine-resistant sluggish contraction which is inhibited by cocaine but not by Dibenamine, ergotoxine, diphenhydramine, or antazoline. It is considered that these may be noncholinergically mediated responses; the arguments are similar to those used for the morphine- and atropine-resistant component of the graded reflex of the longitudinal muscle in response to distension of the lumen (135).

B. Effects of drugs stimulating intramural nervous structures and effects of their antagonists

1. *General considerations.* The excitatory action of drugs on the intestine may be due to an action on the motor neurones, *i.e.*, ganglion cells or nerve terminals, or on the smooth muscle fibres, or to a mixture of both actions. It is often difficult to determine the site of action of drugs since, at present, knowledge of the architecture of the intramural neurones is very limited. One difficulty is, for instance,

the possibility that, apart from cholinergic neurones, there are adrenergic neurones and also neurones which release unidentified transmitters or which are excited or inhibited by unknown transmitters. Furthermore, it would be dangerous to draw conclusions as to the nature of the transmitter from experiments with apparently specific antagonists, unless this evidence can be corroborated by other means of identification of the transmitter. Boyd *et al.* (25) have given strong support to this view because of the difficulties they encountered in attempts to distinguish between adrenergic and cholinergic nerves with apparently specific antagonists.

The difficulties associated with this problem have been presented clearly by Ambache (4, 6). For instance, he defined the criteria of an ideal drug with which to excite intramural ganglion cells as follows: 1) ability to stimulate all known autonomic ganglion cells; 2) susceptibility to both varieties of ganglion-blocking agents, *i.e.*, the depolarizing and the nondepolarizing competitive types, and 3) restriction of the action to the ganglion cell-body to the exclusion of all other tissue components. However, substances with nicotine-like activity on ganglion cells are also capable of stimulating certain types of nerve-endings, or even the muscle cells. Further, ganglion cells which do not respond to nicotine-like substances are not likely to be blocked by anticholinergic ganglion-blocking drugs of either the depolarizing or the competitive type.

Anoxia and cooling. Anoxia and lowering the temperature of the bath fluid in which isolated tissues are suspended, affect all nervous structures, but transmission across ganglionic synapses is more susceptible than transmission across neuroeffector junctions (57, 96, 117). The size of the responses of the smooth muscle to directly acting drugs is little affected by these procedures but the character of the contractions is usually altered (83, 117, 223).

Botulinum toxin. Ambache and Lessin (9) have used botulinum toxin D, which prevents the release of acetylcholine from nerve endings, to distinguish between drugs acting directly on the muscle fibres and those acting on nervous structures. Thus, after exposure of the isolated ileum of the guinea-pig to the toxin, the responses to nicotine and dimethylphenylpiperazinium are abolished or reversed while those to acetylcholine, muscarine, and histamine are unaltered, or even potentiated. Barium and potassium chloride have a mixed action, in small doses their effect is mainly neuronal. In the rabbit ileum, the histamine responses are reduced after exposure to botulinum toxin and therefore appear to be predominantly neuronal.

Morphine-like drugs and their antagonists. Morphine interferes with release of acetylcholine from nerve endings (166, 188) and thus reduces the response of the longitudinal muscle of the isolated ileum of the guinea-pig to drugs acting on nervous structures, *e.g.*, nicotine (131, 185), 5-hydroxytryptamine (57, 81, 129, 131), and barium chloride (131). While most authors state that morphine has only a small, if any, depressant effect on agonists stimulating smooth muscle directly, Lewis (146) found that morphine did definitely depress the action of such drugs although less than the action of drugs acting on nervous structures.

The allyl- analogues of morphine and levorphan, nalorphine and levallorphan,

have varying effects on the contractions of the longitudinal muscle of the guinea-pig ileum caused by nicotine, 5-hydroxytryptamine and barium chloride. For instance, as far as 5-hydroxytryptamine is concerned, they depress the responses about as much as morphine and do not act as antagonists of morphine. On the other hand, they only rarely depress the responses to nicotine and, in this case, behave as antagonists of morphine (131). In the isolated ileum of the rat, the depressant action of nalorphine is more pronounced than that of morphine; moreover, nalorphine does not antagonize the action of morphine on the responses of the longitudinal muscle to nicotine (157).

The ileum from a morphine-tolerant guinea-pig is much less sensitive to morphine than the ileum from a nontolerant animal; the concentrations which depress the responses to nicotine and 5-hydroxytryptamine are 10 to 50 times higher than in the nontolerant animal. In the ileum from a morphine-tolerant rat, morphine and nalorphine depress the responses to barium chloride and nicotine although higher concentrations are required than in the nontolerant rat (157).

Atropine and hyoscine. If most, and perhaps all, neuroeffector junctions in the longitudinal and circular muscle layers are cholinergic, it would follow that the effects of coaxial electrical stimulation and of drugs acting on the nerves innervating these muscles are inhibited by atropine or hyoscine. If responses are only partly blocked by these drugs, mediation by noncholinergic fibres has to be considered; this has been stressed for the action of 5-hydroxytryptamine by Day and Vane (57) and for the graded reflex response of the longitudinal muscle to distension by Kosterlitz and Watt (135). However, the absence of an inhibitory action of atropine is not easily interpreted and does not necessarily mean that acetylcholine is not the transmitter. This is particularly true in the rabbit, in certain strains of which atropinase is present in the tissues. This problem has been fully discussed by Ambache (6). For instance, the actions of nicotine and Darmstoff are inhibited by atropine in the guinea-pig ileum but not in the rabbit intestine, although botulinum toxin blocks the action of nicotine and Darmstoff in the rabbit. It would seem that the presence of atropinase cannot explain this species difference fully since morphine, too, depresses the actions of these substances in the guinea-pig but not in the rabbit (5, 8, 63, 131, 185, 220, 221).

2. *Responses of the longitudinal muscle.* The available evidence shows that, as far as the longitudinal muscle of the guinea-pig ileum is concerned, acetylcholine, histamine, and bradykinin, in the concentrations normally used, do not have an action on nervous elements. In the rabbit ileum, however, the action of histamine is to a great extent indirect, *i.e.*, on nervous structures (9). Most other drugs have a dual action, *viz.*, on nervous structures and, in higher concentrations, on the muscle cells.

Nicotine. In the guinea-pig ileum, nicotine causes contraction of the longitudinal muscle. This response is due mainly to stimulation of intramural neurones and their axons since it is depressed or abolished by cocaine, hexamethonium, morphine, atropine, or botulinum toxin (9, 69, 131, 185). As has already been mentioned, in the small intestine of the rabbit atropine and morphine are very much less effective as inhibitory agents, or even without effect at all (8, 63, 185).

An important observation was made by Ambache and Edwards (3, 8) when they showed that, after blocking neuroeffector transmission by atropine in the kitten or by botulinum toxin in the mouse and rabbit, nicotine causes not a contraction but a relaxation of the longitudinal muscle. This reversal of nicotine action may be interpreted as being due to the unmasking of an action on inhibitory, possibly adrenergic, neurones.

The evidence as to a direct excitatory action of nicotine on the longitudinal muscle is still somewhat uncertain. While Ambache and Lessin (9) found that botulinum toxin D abolishes the responses of the longitudinal muscle of the rabbit and guinea-pig ileum to nicotine, Kosterlitz and Robinson (131) showed that atropine does not block completely the response of the longitudinal muscle of the guinea-pig to nicotine, and Day and Vane (57) found that in anoxia nicotine still causes a contraction. Day and Vane thought that the observations of Ambache and Lessin could be explained best by the fact that nicotine also stimulates a relaxing mechanism.

The ganglionic stimulants, dimethylphenylpiperazinium and tetramethylammonium, have also a direct action on the longitudinal muscle of the guinea-pig ileum, the drugs acting not only on hyoscine- but also on phenoxybenzamine-sensitive receptors. The action on phenoxybenzamine-sensitive receptors is small with dimethylphenylpiperazinium and nicotine. This direct action of these drugs is mainly on receptors for acetylcholine; the phenoxybenzamine-sensitive receptors may possibly be receptors for histamine or 5-hydroxytryptamine (57).

Barium. Barium chloride acts on at least three different structures. First, it excites neurones which subserve the peristaltic reflex proper and cause emptying movements of the circular muscle. This action is suppressed in the guinea-pig and rabbit ileum, but not in the rat ileum, by hexamethonium (62, 69, 131, 213); this finding suggests that neurones other than the final motor neurones are involved. The contraction of the longitudinal muscle induced by barium chloride in the presence of hexamethonium is depressed by exposure to botulinum toxin (4) and by morphine and atropine (131), observations which indicate that barium chloride stimulates the nerve fibres innervating the muscle. When in the presence of a blocking dose of morphine or hyoscine the concentration of barium chloride is increased, the longitudinal muscle shows a powerful contraction due to direct stimulation of the smooth muscle fibres.

5-Hydroxytryptamine. Present evidence indicates that 5-hydroxytryptamine acts on both nervous and muscle receptors. Rocha e Silva *et al.* (179) thought that the site of action is at the postganglionic cholinergic fibres of the intramural nervous system of the guinea-pig ileum. Gaddum and Hameed (80) showed that this action of 5-hydroxytryptamine is inhibited by atropine or cocaine and specifically blocked by large doses of 5-hydroxytryptamine but not by hexamethonium; they concluded that in the nervous tissue of the ileum of the guinea-pig, possibly in the ganglion cells, there are two types of receptors, one of which is stimulated by nicotine and the other by 5-hydroxytryptamine; this view is shared by Levy and Michel-Ber (145). Kosterlitz and Robinson (129, 131) found that the effect of 5-hydroxytryptamine is partly blocked by morphine; they

thought that the morphine- and atropine-resistant residual action is directly on the muscle. Gaddum and Picarelli (81) showed that some of the 5-hydroxytryptamine receptors in the guinea-pig ileum are blocked by morphine and others by phenoxybenzamine (Dibenzyline), the M and D receptors. However, phenoxybenzamine also antagonizes the action of acetylcholine on the guinea-pig ileum (18, 25). Day and Vane (57) adduced evidence that the dose-ratio for 5-hydroxytryptamine obtainable with morphine is smaller than that with anoxia and concluded that most of the normal response to 5-hydroxytryptamine is due to its action on nervous receptors and that phenoxybenzamine antagonizes also nervous receptors. Kosterlitz and Robinson (131) and Day and Vane (57) showed that similar dose-ratios are obtained with morphine on the one hand and atropine or hyoscine on the other. Brownlee and Johnson (28), however, found that hyoscine blocks the action of very high concentrations of 5-hydroxytryptamine almost completely. In other respects, the latter authors confirmed and extended the earlier observations; their most important finding was that the depolarizing ganglion-blocking drugs, nicotine and dimethylphenylpiperazinium, inhibit the response to 5-hydroxytryptamine. Agreeing with Gaddum and Hameed (80), they concluded that 5-hydroxytryptamine activates specific receptors situated at the intramural parasympathetic ganglion cells. It must be stressed, however, that the pharmacological evidence does not necessarily differentiate between an action at the soma and one at other reactive sites on the neurone, for instance, the nerve endings.

The depressant actions of morphine-like drugs on the response of the longitudinal muscle of the guinea-pig ileum to 5-hydroxytryptamine acting indirectly are closely correlated with the analgesic potencies of these drugs (158). The activity decreases in the following order: dihydromorphinone, methadone, morphine, pethidine, codeine, and ethylmorphine.

The direct action of 5-hydroxytryptamine on the longitudinal muscle of the guinea-pig and rabbit is antagonized by 2-bromo-lysergic acid diethylamide but not by lysergic acid diethylamide (80, 194).

Darmstoff and irin. Vogt (220, 221) showed that, in the guinea-pig ileum, the action of Darmstoff, which is a mixture of acid phosphatides, is partly suppressed by morphine and atropine. Its action on the rabbit ileum is blocked by botulinum toxin (5). The response to Darmstoff is therefore due partly to excitation of nervous structures and partly to a direct action on the muscle. On the other hand, the action of irin, a lipid-soluble acid described by Ambache (7), is not blocked by atropine.

Angiotensin and substance A. The actions of these two polypeptides on the longitudinal muscle of the guinea-pig ileum are also partly inhibited by morphine and atropine (124, 180, 222). The action of angiotensin on the longitudinal muscle of the rabbit ileum is blocked by botulinum toxin A (178).

γ-Aminobutyric acid. Hobbiger (111) found that γ-aminobutyric acid, in concentrations of 10 to 100 μg/ml, depresses the response of the longitudinal muscle of the isolated guinea-pig ileum to 5-hydroxytryptamine and, to a somewhat lesser extent, to nicotine. When the responses to 5-hydroxytryptamine are reduced by addition of morphine to the bath fluid, the efficacy of γ-aminobutyric

acid is very much reduced; a similar, but much less pronounced, interaction is found when γ-aminobutyric acid is added first to the bath fluid and then followed by morphine. It appears that the antagonism produced by γ-aminobutyric acid has many characteristics in common with that caused by morphine. Similar results were obtained by Inouye *et al.* (116). Neither morphine nor γ-aminobutyric acid antagonizes the action of 5-hydroxytryptamine on the rabbit ileum, the rat duodenum or the rat or guinea-pig uterus (111, 157). In the guinea-pig ileum γ-aminobutyric acid has also some antiacetylcholine and antihistamine effect (110). Florey and McLennan (77) showed that the inhibitory Factor I does not antagonize the action of 5-hydroxytryptamine on the longitudinal muscle of the guinea-pig ileum but can cause complete inhibition of acetylcholine- and nicotine-induced contractions, which are only partly blocked by γ-aminobutyric acid. The relative anti-5-hydroxytryptamine, antinicotine, and antiacetylcholine activities of γ-aminobutyric acid are altered by N-methylation, phenylation, acetylation, or methyl esterification (203, 204). γ-Aminobutyrylcholine has an anti-5-hydroxytryptamine action similar to γ-aminobutyric acid on the guinea-pig ileum but is much less potent (97).

Catecholamines. The analysis of the action of catecholamines in the intestine is made difficult by the presence of several receptors sensitive to their action. In the guinea-pig, the peristaltic reflex, the graded reflex response of the longitudinal muscle, and the contractions of the longitudinal muscle induced by nicotine are more sensitive to catecholamines than the contractions induced by acetylcholine or histamine; furthermore, the nervous structures are much less sensitive to isoprenaline than to adrenaline or noradrenaline, while this difference is less marked for acetylcholine-induced contractions (130, 153). Similar observations have been made on rabbit intestine (152).

Experiments by Ahlquist and Levy (1, 143, 144) and Furchgott (79) with the α-adrenergic blocking drugs, phenoxybenzamine and dibozane [1,4-bis(benzodioxane-2 yl-methyl)piperazine], and the β-adrenergic blocking drug, dichloroisopropylnoradrenaline, have shown that there are α- and β-adrenergic inhibitory receptors in the rabbit and dog intestine. Phenylephrine, methoxamine, metaraminol, hydroxyamphetamine, and ephedrine act on α- receptors, isoprenaline and buphenine (nylidrin) on β- receptors, and adrenaline, noradrenaline, and ethylnoradrenaline on both α- and β- receptors. In this context, mention should be made of Munro's work (162–164) showing that, in the terminal segment of guinea-pig ileum, adrenaline causes not relaxation but contraction of the longitudinal muscle; this effect is not inhibited by atropine. If the muscle is contracted by the action of nicotine prior to the addition of adrenaline, adrenaline causes relaxation, pointing to a dual action of adrenaline. When the motor response to adrenaline is antagonized by ergotoxine or Dibenamine, the inhibitory response is unmasked.

Miscellaneous. The mode of action of substance P on the longitudinal muscle is not clear; it is not affected by morphine or atropine but depressed by lowering the bath temperature and anoxia (22, 117, 129). The undecapeptide, eledoisin, appears to behave in a similar fashion (64).

An important observation seems to be the fact that the longitudinal muscle of

the isolated ileum of the guinea-pig can still respond to nicotine, barium chloride, or 5-hydroxytryptamine after the ileum has been stored at 4°C for 24 hours. In such ilea the peristaltic reflex proper is no longer present but the graded reflex of the longitudinal muscle can still be elicited (2, 129, 131, 185). It is tentatively suggested that in such preparations transmission across cholinergic synapses is no longer possible but that the nerve cells, or at least their axons or nerve endings, are still excitable.

An interesting finding is the potentiation by lysergic acid diethylamide of the actions of acetylcholine, bradykinin, substance P, and substance A on the longitudinal muscle of the guinea-pig ileum; the action of histamine is not affected. The mechanism of this potentiation is not clear (137, 193).

3. *Responses of the circular muscle.* The responses of a strip of circular muscle to drugs are very different from those of the longitudinal muscle. Sperelakis and Prosser (195) showed that circular muscle strips of the cat ileum are insensitive to 5-hydroxytryptamine and much less sensitive to acetylcholine than the longitudinal muscle, and recently Harry (102) and Brownlee and Harry (27) subjected the responses of the circular muscle of the isolated ileum of the guinea-pig to a full pharmacological analysis. Incubation of the preparation with the anticholinesterase mipafox (N, N′-di-isopropylphosphorodiamidic fluoride) sensitizes both the longitudinal and the circular muscles to acetylcholine, the former 16 times and the latter 4000 times. Even after treatment with mipafox, however, the longitudinal muscle is more sensitive to acetylcholine than the circular muscle. Bradykinin and substance P do not stimulate the circular muscle. After, but not before, mipafox treatment, the circular muscle responds to 5-hydroxytryptamine, histamine, and nicotine. Morphine, atropine, and hyoscine antagonize the stimulant actions of nicotine, 5-hydroxytryptamine, and histamine on the circular strip, but only those of nicotine and 5-hydroxytryptamine on the longitudinal muscle. The contractions of the circular strip caused by 5-hydroxytryptamine, nicotine, and histamine are also abolished by procaine and by botulinum toxin A. Hexamethonium has no effect on the responses of the circular muscle strip to acetylcholine, 5-hydroxytryptamine, or histamine. It would appear that the circular muscle strip is less sensitive to all drug action than the longitudinal muscle and that, in contrast to the longitudinal muscle, the action of histamine is indirect by stimulation of nervous structures. In the circular muscle strip of the cat, the action of nicotine is abolished or reversed by botulinum toxin D (9).

Fishlock (74) showed that circular strips of human ileum contract in response to 5-hydroxytryptamine (0.005 μg/ml). Lysergic acid diethylamide (0.05 to 0.1 μg/ml) completely blocks this action while morphine (10 μg/ml) and hexamethonium (50 μg/ml) cause a partial block. It is assumed that, as in the guinea-pig, 5-hydroxytryptamine acts on nervous elements and also directly on the smooth muscle.

Evans and Schild (66, 67) found that ganglion-free rings of circular muscle of the cat jejunum, stripped of the myenteric and mucous plexuses, are stimulated by acetylcholine and nicotine. Barium chloride causes rhythmic contractions while in ganglion-containing circular muscle powerful contractions are produced

which culminate in spasm. Ganglion-free strips which contain nerve fibres are less sensitive to acetylcholine than those containing ganglion cells, which in turn are less sensitive than longitudinal muscle containing myenteric plexus. Nicotine causes contraction or, when the muscle has been contracted by acetylcholine or eserine, relaxation. As serial sections showed that there were no ganglion cells, this could be due only to stimulation of the muscle cells or intramural nerve fibres and endings. When cat circular muscle has been freed of the myenteric plexus *in vivo* 13 to 27 days before the experiment, nicotine either stimulates or relaxes the muscle. This indicates that these effects are not subserved by ganglion cells of the myenteric plexus and their axons; however, the submucous plexus is intact in such preparations. These results, which do not exclude nerve endings as the site of action of nicotine, are in agreement with the earlier findings of van Esveld (215), but not with those of Magnus (156) and Gasser (85).

C. Effects of drugs on the responses to stimulation of the extrinsic nerves

Van Harn (216) found that, in the cat, stimulation of the cervical vagus nerve causes an increase in mechanical activity of the previously inactive jejunum. Stimulation of the thoracic sympathetic chain is inhibitory when the jejunum is spontaneously active but may cause an increase in activity in a previously inactive gut. When the catecholamine stores are depleted by administration of reserpine, 2.5 mg/kg daily for two days, both vagal and sympathetic stimulation are always excitatory.

In the guinea-pig *in vivo*, Straub and Stefánsson (198) found that stimulation of the vagus just above the diaphragm with an alternating current of 40 cyc/sec evokes a short burst of peristaltic waves in the small intestine preparation of Straub and Viaud (199). The duration of this burst cannot be prolonged by increasing the duration of stimulation; each burst is followed by a refractory period during which vagal stimulation is ineffective. Atropine blocks the effect of vagal stimulation.

When the vagus of the isolated vagus stomach-duodenum preparation of the guinea-pig is stimulated by rectangular pulses at 10 impulses/sec, the duodenum exhibits contractions which are blocked by cocaine or hexamethonium. Atropine (0.01 μg/ml) and morphine (0.1 μg/ml) reduce the tone of the preparation and also depress the contractions caused by electrical stimulation. Schaumann (187) concluded that hexamethonium blocks intramural ganglia, and that morphine and atropine block neuroeffector transmission.

Finkleman (73) showed that the effect of stimulation (20 to 40/sec) of the periarterial nerves of an isolated segment of rabbit duodenum results in an inhibition of the spontaneous rhythmic contractions. Stimulation at a low frequency (2 to 4/sec) occasionally causes a contraction soon after setting up the preparation. Ephedrine blocks the inhibition caused by adrenaline or electrical stimulation. Using a superfusion technique, Finkleman was able to show that on electrical stimulation of the periarterial nerves at high frequency, the intestine releases a substance which relaxes a second piece of gut. Day and Rand (55) found that stimulation at a frequency of 10 to 20/sec causes a contraction in young, but

not in adult, rabbits. In the cat small intestine, too, motor responses are more readily elicited by low than by high frequency of stimulation of the periarterial nerves (216). In the terminal ileum of the guinea-pig, stimulation of the periarterial nerves with high frequencies has varying results; it should be remembered that catecholamines produce a motor response in this segment (164). Contraction on stimulation occurs most frequently at the most distal parts of the ileum; this response is sometimes abolished and sometimes potentiated by atropine. It would appear that this motor response may be due to either cholinergic or adrenergic excitatory fibres.

The inhibitory effect obtained by stimulating the periarterial nerves of the rabbit and cat small intestine with high frequencies is blocked by drugs interfering with the release of noradrenaline from nerve endings, as, for instance, 2,6-choline xylyl ether or xylocholine (10, 11, 54), bretylium (19, 23, 24, 38, 54, 201), guanethidine (19, 24, 54–56), dimethylphenylpiperazinium (19, 224) and phenyltrimethylammonium (37). In the presence of these drugs the inhibitory responses to adrenaline or noradrenaline added to the bath fluid are unaltered or potentiated. Day (54) found that in the rabbit the action of the blocking drugs persists for a considerable time after washing out the drugs. It is readily reversed by addition of the following compounds to the bath fluid: dopamine, tyramine, dexamphetamine, hydroxyamphetamine, or ephedrine. If these amines are given first, adrenergic blocking agents are ineffective. Noradrenaline, adrenaline, isoprenaline, and phenylephrine were found to have no protective action, but Boyd *et al.* (24) obtained reversal of bretylium and guanethidine blockade with noradrenaline. Eserine, atropine, or hexamethonium has no effect on the inhibition caused by stimulation of the periarterial nerves (19).

Isolated segments of ileum from rabbits injected with guanethidine (12.5 mg/kg) 1 or 4 hours before the experiment show block of the effect of periarterial stimulation; this block is reversed by adding dopamine to the bath fluid. Cocaine, when given together with bretylium or guanethidine, prevents the blocking action of the two drugs; this effect is not due to the inhibitory action of cocaine on amine oxidase since amine oxidase inhibitors, which have no sympathomimetic activity, do not antagonize the action of bretylium or guanethidine. Day (54) put forward the hypothesis that xylocholine, bretylium, and guanethidine act on the site of stored catecholamines and prevent their release, and that the sympathomimetic substances which antagonize the blocking drugs act probably by releasing stored noradrenaline and may thus displace the blocking drugs from these sites. This concept has been studied in more detail for dexamphetamine by Day and Rand (56), who produced evidence suggesting that the antagonism between guanethidine and dexamphetamine may be competitive.

Bretylium and guanethidine not only block the inhibition caused by stimulation of the periarterial nerves of the rabbit intestine but can also change the inhibition to a contraction. Day and Rand (55) thought that this reversal is due to unmasking of postganglionic cholinergic sympathetic fibres because the motor response is not blocked by hexamethonium nor abolished by degenerative section of the cervical vagus nerve. This interpretation supports the hypothesis of Burn

and Rand (38). Bentley (19), also, found reversal of the inhibition with guanethidine but showed that hexamethonium blocks this motor response. He therefore favoured the possibility that the motor response is due to unmasking of the effects of parasympathetic fibres present in the periarterial nerves. Boyd *et al.* (24) showed that reversal of the response after bretylium and guanethidine is obtained more readily at low than at high stimulus frequencies, a finding which they consider supports the view that the motor response is due to an admixture of parasympathetic fibres. Pretreatment of rabbits *in vivo* with bretylium or guanethidine also converts the response of isolated segments of the mid ileum to periarterial stimulation from an inhibitory to a motor response.

Gillespie and Mackenna (89) showed that, in the rabbit mid ileum, pretreatment with reserpine for 1 to 10 days converts the response to periarterial nerve stimulation from inhibition to contraction without affecting the responses to adrenaline or noradrenaline. The motor response can be elicited best with low stimulus frequencies (2.5 to 10/sec). The inhibitory response is restored by soaking the preparation in solutions of adrenaline or noradrenaline but the restored inhibitory effect is easily fatigued by stimulation. Bentley (19) found that, with stimulus frequencies of 30 to 50/sec, a motor response is obtained only occasionally in ilea of reserpinized rabbits. However, the addition of bretylium, guanethidine, or dimethylphenylpiperazinium to the bath fluid usually converts the reduced inhibitory response to a motor response which is unaffected by atropine, potentiated by eserine, and reversibly blocked by hexamethonium or pempidine. Gillespie and Mackenna, as well as Bentley, hold the view that the motor responses are the result of stimulating parasympathetic fibres in the periarterial nerves, the stimulation of the sympathetic fibres having been made ineffective by the depletion of the noradrenaline stores.

Day and Rand (56a) have shown recently that α-methyldopa, α-methyldopamine, and α-methylnoradrenaline are about as effective in restoring the inhibitory response of the isolated ileum obtained in reserpinized rabbits as the nonmethylated compounds. Stimulation of the cervical vagus or the thoracic sympathetic chain in the reserpinized cat also gives rise to a motor response in the jejunum (216).

Gillespie and Mackenna (89) examined the effects of tolazoline and ergotamine on the inhibitory response to stimulation of the periarterial nerves in the rabbit ileum. The results were variable; at frequencies of 50 stimuli/sec or above, these drugs enhanced the inhibitory effect, while at lower frequencies motor or biphasic responses were obtained.

In the guinea-pig ileum which has little or no tone, Szerb (201) examined the action of drugs on the inhibition of the histamine-induced contraction of the longitudinal muscle by stimulation of the periarterial nerves. The inhibitory responses are depressed by morphine (0.1 μg/ml), an effect partly antagonized by nalorphine (1 μg/ml). Hexamethonium (10 μg/ml) is without effect and cocaine (10 μg/ml) enhances the inhibitory response to stimulation. Surprisingly, atropine (0.01 μg/ml) prevents the inhibitory response; no explanation can be offered for this observation. Dopamine inhibits the response to histamine more at 37°

than at 28°C. Morphine, but not the other drugs, prevents the inhibitory action of dopamine at 37°C but not its reduced effect at 28°C. It is suggested that dopamine, at 37°C, may act by releasing noradrenaline from adrenergic nerve endings.

When interpreting the results of experiments in which apparently specific adrenergic blocking agents are used, great caution has to be used. Kosterlitz and Lees (127) have shown that bretylium has a weak atropine-like action and also blocks transmission across cholinergic synapses. Boyd *et al.* (25) have pointed out that phenoxybenzamine often blocks responses to acetylcholine and to cholinergic nerve stimulation in concentrations lower than those required to block adrenergic nerves. Guanethidine, bretylium, tolazoline, and phentolamine may exhibit considerable antiacetylcholine activity.

V. EFFECTS OF DRUGS ON THE COLON

Bayliss and Starling (14) observed that in the anaesthetized dog and rabbit the peristaltic contractions of the colon were larger, less frequent, and more prolonged than in the small intestine. In the guinea-pig and cat, Straub and Schild (197) showed that, *in vivo*, the main characteristics of the large intestine are that the peristaltic contractions are sluggish and the tone is greater than in the small intestine. These observations were confirmed for isolated preparations of the guinea-pig and rabbit by Lembeck (141) and Lee (140). The oral part of the distal colon shows more tonic contraction and a greater nonpropulsive activity than the aboral part. The pressure required to elicit the peristaltic reflex is higher than in the small intestine *in vitro* (140) and *in vivo* (197).

A. Effects of drugs on peristalsis

Lee (140) examined the pharmacological responses of the isolated colon and found that in the guinea-pig colon hexamethonium, nicotine, and atropine block peristalsis as they do in the ileum. However, the propulsive activity of the rabbit colon elicited by distension of the lumen is not affected by hexamethonium, nicotine, or atropine although these drugs produce a block in a piece of ileum obtained from the same animal. This is the more remarkable because ganglion-blocking drugs and atropine abolish the response of the rabbit colon to pelvic nerve stimulation. Peristalsis evoked by mechanical stimulation of the mucosa of the denervated colon of dogs under urethane-morphine anaesthesia is blocked by hexamethonium (112).

Lembeck (141) found that, when 5-hydroxytryptamine (1 to 10 μg/ml) is added to the bath fluid, the peristaltic reflex evoked by distension of the lumen of the guinea-pig colon is facilitated. On the other hand, Lee (140) showed that 5-hydroxytryptamine depresses peristaltic activity when applied from the serosal surface (1 to 10 μg/ml) but enhances it when applied from the mucosal surface (0.01 to 1 μg/ml). In the rabbit colon, intraluminal or serosal application enhances propulsive activity. The response to pelvic nerve stimulation, however, is facilitated by serosal application of 5-hydroxytryptamine in both species.

Lee (140) showed further that the amount of 5-hydroxytryptamine released into the lumen during peristalsis of the oral portion of guinea-pig distal colon is

of about the same order as in the small intestine but is much smaller in the caudal portion. The amounts of 5-hydroxytryptamine released are related directly to peristaltic activity and not to distension of or pressure in the lumen, since there is no increase in the release when the intraluminal pressure is raised and the peristaltic movements prevented by asphyxia or adrenaline. Sympathetic nerve stimulation inhibits propulsive activity in the rabbit colon and reduces 5-hydroxytryptamine release by only 30%; pelvic nerve stimulation has no effect on 5-hydroxytryptamine release. Therefore release of 5-hydroxytryptamine is controlled by an intrinsic mechanism associated with peristaltic activity but is not controlled by the extrinsic nerves. The observations also suggest that the mechanical changes associated with propulsive activity may be a contributory factor in the release of 5-hydroxytryptamine.

Using the technique of Straub and Viaud (199), Straub and Leo (196) showed that in guinea-pigs morphine (1 mg/kg) depresses the peristaltic reflex; this effect is antagonized by eserine (8 μg/kg). Tolerance to morphine seems to develop more readily in the colon than in the small intestine (197).

B. Effects of drugs acting on the intramural nervous structures and muscle fibres

In isolated segments of rabbit colon, Gillespie and Mackenna (88) showed that nicotine causes inhibition in low concentrations (1 to 10 μg/ml) but contraction in higher concentrations. The inhibitory effect of nicotine is blocked by hexamethonium in concentrations similar to those required for blocking ganglion cells in the parasympathetic pathway. Larger, paralyzing concentrations of nicotine also block the inhibitory response of small doses of nicotine. Atropine in high concentrations (100 μg/ml) converts the contraction induced by a high concentration of nicotine to a relaxation. In colon preparations from reserpinized rabbits, nicotine has no inhibitory effect, probably because of depletion of the catecholamine stores. After degenerative section of the extrinsic sympathetic nerves, the inhibitory effect of nicotine is abolished or at least very much reduced; on the other hand, xylocholine does not affect the inhibitory response of nicotine but abolishes the inhibition induced by stimulation of the extrinsic sympathetic nerves. Removal of the mucosal chromaffin cells does not block the inhibitory response to nicotine. The actual site of the inhibitory effect of nicotine is not certain but it is very likely that it is due to release of catecholamines from the extrinsic sympathetic nerves or some structure associated with them.

Fishlock and Parks (76) and Fishlock (75) made the interesting observations that nicotine (2 to 10 μg/ml) causes relaxation of isolated preparations of the circular muscle strips of the human colon. This inhibition is blocked by procaine, hexamethonium, bretylium, guanethidine, and the β- adrenergic blocking drugs, dichloroisoprenaline and pronethalol (nethalide). 5-Hydroxytryptamine (0.5 to 10 μg/ml), too, causes a relaxation which is not prevented by lysergic acid diethylamide, morphine, hexamethonium, or mepyramine. Quite recently, Bucknell and Whitney (28a) have shown that nicotine and dimethylphenylpiperazinium (0.5 to 40 μg/ml) cause a relaxation also of a longitudinal muscle strip, namely, the isolated taenia coli of the sigmoid region of the human colon. These

effects are blocked by hexamethonium, procaine, or pronethalol, as in the experiments on the circular muscle strip. In the presence of a high concentration of hyoscine (1 μg/ml), acetylcholine (8 μg/ml) causes a relaxation although in the absence of hyoscine a low concentration of acetylcholine (0.2 μg/ml) induces a contraction. These observations are explained best by assuming that the ganglionic stimulants excite adrenergic intramural nerve cells or the axons arising from them, or both.

C. Effects of drugs on the responses to stimulation of the extrinsic nerves

Garry and Gillespie (84), who reviewed the earlier literature, found that in the isolated preparation of the rabbit colon stimulation of the pelvic nerves causes contraction and stimulation of the sympathetic lumbar outflow causes inhibition. The initial tone of the preparation does not influence the results. Maximum contraction is obtained when the pelvic nerves are stimulated with 10 stimuli/sec; stimulation at any frequency between 1 and 1000 stimuli/sec causes contraction. Maximum inhibition is obtained when the lumbar outflow is stimulated at 100 stimuli/sec or over; stimulation at frequencies below 5 stimuli/sec is rarely effective. These results seem to exclude the possibility that the pelvic nerves carry a large proportion of sympathetic fibres or the lumbar outflow a large proportion of parasympathetic fibres. The response to pelvic nerve stimulation is rapid but not well sustained; it is abolished by large doses of hexamethonium (200 μg/ml) or atropine (100 μg/ml). The response to stimulation of the lumbar outflow is not affected by hexamethonium or atropine. Simultaneous stimulation of both outflows causes contraction at low frequencies and inhibition at high frequencies.

Varagić (217) confirmed the findings of Garry and Gillespie (84) and observed that, in the presence of tolazoline (16 μg/ml), stimulation of the lumbar outflow to the colon at 5 stimuli/sec causes a biphasic response consisting of an initial contraction followed by relaxation. This phenomenon is much less marked at stimulus frequencies of 50 to 100/sec. The initial contraction is potentiated by eserine and abolished by atropine and hexamethonium. Varagić concluded that tolazoline unmasks the presence of cholinergic motor fibres in the lumbar sympathetic outflow to the colon.

Gillespie and Mackenna (89) found that tolazoline, ergotamine, or xylocholine fails to unmask a motor response after stimulation of the sympathetic lumbar outflow at 50 stimuli/sec, xylocholine being the only one of the three drugs which can be relied upon to give a selective sympathetic block. However, in reserpinized rabbits, the response to sympathetic stimulation is converted from inhibition to contraction and the motor responses to parasympathetic and sympathetic nerve stimulation (5 to 50/sec) are now similar in appearance. Both motor responses are blocked by hexamethonium and atropine; the inhibitory effect of the sympathetic nerves is restored by treating the preparation with adrenaline, noradrenaline, dopamine, or dopa. The motor response to sympathetic nerve stimulation has a frequency sensitivity similar to that of normal parasympathetic fibres; it is abolished by fatigue of the pelvic nerves and by degenerative section of the

pelvic nerves. These observations led Gillespie and Mackenna (89) to the conclusion that the motor response of sympathetic nerve stimulation after reserpine cannot be due to the presence of parasympathetic fibres or of cholinergic sympathetic fibres in the sympathetic nerve; in the absence of a ready explanation, the authors raised the possibility that in the rabbit colon, after treatment with reserpine, sympathetic nerve stimulation can, in some way unknown, activate the parasympathetic fibres.

These conclusions were modified later to some extent by observations of Boyd *et al.* (24). When, in isolated nerve-colon preparations of the rabbit, the sympathetic fibres are blocked by prolonged exposure to bretylium or guanethidine, stimulation of the sympathetic nerve only occasionally leads to a small motor response. Again, this motor response is more readily demonstrated at low (10/sec) than at high (50/sec) frequencies of stimulation. It is suggested that the motor response is due to the presence of a few parasympathetic motor fibres running with the sympathetic nerves. It is difficult to interpret the differences between small motor responses after drugs blocking adrenergic transmitter release, such as bretylium and guanethidine, and the much larger motor responses readily observed in the colon obtained from rabbits pretreated with reserpine. The authors assumed that the action of reserpine is due not only to its ability to deplete the noradrenaline stores at the nerve endings, but also to an action peculiar to the drug itself. It would appear that the final interpretation of these interesting observations will have to await further analysis.

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