THE EXTRINSIC AND INTRINSIC INNERVATION OF THE COLON

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I N T R O D U C T I O N.

Interest in the physiology of smooth muscle and its innervation by the autonomic nervous system is increasing, but there are still numerous and disconcerting gaps in our knowledge. Most smooth muscle is innervated by both the sympathetic and the parasympathetic divisions of the autonomic nervous system, and these are generally considered to be antagonistic in function, one producing contraction, the other inhibition.

The experimental evidence for this antagonism shows certain anomalies. Such inconsistencies are most prominent in the alimentary canal. In this region the parasympathetic is usually considered to be motor and the sympathetic inhibitor to the general musculature. If the tonus of the smooth muscle is high, however, the motor effect of parasympathetic stimulation is reversed to inhibition. If tone is low then the inhibitory effect of sympathetic stimulation may be reversed to a motor one. From these observations it is postulated that the type of response is at least partly determined at the periphery by the state of activity. This modification of the older view has not proved fertile in promoting any better understanding of smooth muscle innervation. Indeed all surgical experience runs directly counter to this later addition. If hyperactive smooth muscle is relaxed by impulses in either division of the autonomic nervous system then it is

illogical to cut either sympathetic or parasympathetic outflows, or to block their action with drugs, in an attempt to relieve this spasm. In practice, at least temporary relief of smooth muscle spasm follows sympathectomy in vasospasm (Raynaud's disease); in pylorospasm, and in achalasia of the cardia. Sympathectomy or spinal anaesthesia may relieve megacolon (Hirschsprung's disease). Vagotomy has been found useful in the treatment of peptic ulcer where the stomach is often hyperactive, and blocking the action of this nerve with atropine has long been a recognised feature of medical treatment. In all of these conditions smooth muscle spasm is relieved by cutting the conventional motor nerve, which presumably therefore still exerts a motor influence in spite of the high tonus.

Evidence that the state of activity determines the type of response has been found in certain regions only. The most important of these has been the stomach when the vagus and splanchnic nerves were stimulated. Smooth muscle in many other sites responds consistently to stimulation of sympathetic or parasympathetic nerves. The extrinsic innervation of the stomach and also the arrangement of the muscle in the walls of this organ is complex. These local peculiarities may be responsible for the vagaries in the response to nerve stimulation. This thesis describes investigations into another region of the gut, the colon, and its responses to stimulation of the sympathetic and parasympathetic nerves using the sacral component of the

parasympathetic outflow (the pelvic nerve) in place of the cranial (the vagus nerve). Here it is possible to study in detail the separate influences of the parasympathetic and sympathetic outflows. The results have been in themselves rewarding and may, moreover, provide a clue to the source of the erratic responses obtained on stimulation of the outflows to other regions of the gut.

PART I.

REVIEW OF THE LITERATURE.

The descriptive anatomy of the autonomic nervous system, in common with the rest of the nervous system, has always been more advanced than the understanding of its mode of functioning. Galen recognised the existence of the vagus nerve and also the sympathetic chain which he misinterpreted as a costal branch of the vagus. He described the distribution of these nerves to the viscera and believed their function was to produce "consent" or "sympathy" between the visceral organs whereby they could work in harmony with one another. This concept of function was held almost without change for the next sixteen centuries. Even in the 18th century when Winslow abandoned the term "intercostal nerve" in favour of the "great sympathetic nerve" the function of this nerve was still to promote "sympathy" among the viscera. The introduction in the 19th century of the technique of animal experiment was followed by rapid progress in understanding how these nerves influenced the viscera. The inhibitory action of the vagus on the heart (Weber) and the motor effect of sympathetic nerves on blood vessels (Cl. Bernard; Brown-Sequard) were quickly demonstrated and in the second half of that century several observations on the effect of vagal and splanchnic nerve stimulation on the alimentary canal were made. From these experiments it was clear that stimulation of the cranio-sacral (parasympathetic) and of the thoracico-lumbar (sympathetic) outflows, in general produced opposite responses from smooth muscle cells. The particular effect, contraction or inhibition, differed according to the site of the smooth muscle.

In the alimentary canal the sympathetic was inhibitory to the smooth muscle while the parasympathetic was motor. About the turn of the century, work, chiefly by Elliot and by Langley & Anderson, showed that in the region of the sphincters the effects of extrinsic nerve stimulation were reversed. The sympathetic outflow was motor to the smooth muscle of the sphincters and the parasympathetic was inhibitory. From these observations a workmanlike theory of the extrinsic innervation of the alimentary tract was produced and is still largely accepted to-day. The parasympathetic outflow is motor to the gut promoting activity of the propulsive musculature and relaxing those regions specialised in restraining forward movement of the gut contents. The sympathetic outflow is inhibitory to the gut producing quiescence of the propulsive musculature and increasing activity of the sphincters.

Almost from the first experiments, however, unaccountable vagaries were reported. Stimulation of the parasympathetic outflow would sometimes produce inhibition, stimulation of the sympathetic outflow would sometimes produce contraction and, most common of all, stimulation of either could produce various forms of biphasic response in which both contraction and inhibition appeared. The factors promoting these variations in the response were at first not recognised. Many of the earlier investigators simply record them as one of the possible responses. Bayliss & Storling (1899), for example, reported both motor and inhibitor responses from the small intestine of the dog on stimulating the

vagus nerve but do not report what circumstances favoured each response. Information on the experimental conditions is often inadequate in these older reports and the possibility of reflex responses, or responses due to current escape to other nerves cannot always be excluded. For these reasons some of these reports are excluded from this review. McCrea, McSwiney & Stopford (1925) reviewed most of them. Gradually, however, certain circumstances were recognised as being likely to alter the response of the alimentary canal to stimulation of the extrinsic nerves. Of these the first to be recognised was the state of maintained contraction, the tonus, of the muscle. Later the frequency of nerve stimulation was also found to alter the response and these two factors have remained in all subsequent work the most fruitful source of variation both in the gut and elsewhere. Other less frequently reported factors which may alter the response are the strength of the stimulating current, the duration of stimulation and certain drugs.

These anomalous responses were intensively studied during the '20's and '30's of this century particularly by the school led by McSwiney. These workers determined many of the conditions under which reversal of the accustomed response appeared. Explanatory theories were produced but none has gained general acceptance or disturbed appreciably the generally held concept that the sympathetic and parasympathetic are antagonistic in action. Since that time this subject does not seem to have been

specifically reinvestigated although sporadic references to biphasic responses and responses the reverse of that classically attributed to the nerve stimulated, still appear in the literature.

It is useful to consider separately each of the factors which have been reported to reverse the normal response to nerve stimulation.

Tonus. Courtade & Guyon (1899), stimulating the vagus and splanchnic nerves and studying the response of various regions of the stomach, observed motor, inhibitor and biphasic responses from either nerve. This variability was particularly prominent in the cardia of the stomach and the type of response was determined by the tonus of the muscle at the time of stimulation. If tone were high the response was inhibition, if tone were low the response was contraction. These authors quoted an earlier statement by Cl. Bernard, "that the same muscle responds in a different fashion to excitation of its motor nerve according to whether it is in a state of contraction or relaxation when excited" in support of their observations. Confirmation of these findings was provided by Carlson, Boyd & Pearcy (1922) who used rabbits and cats in addition to dogs. A species difference was found in the response of the cardia of the stomach to splanchnic stimulation. In the dog this was always motor, in the cat motor or inhibitor depending on tonus, high tone favouring inhibition, and in the rabbit only inhibition was observed. The influence of muscle activity on the response was intensively investigated in the

in the researches of McSwiney and his group (McCrea, McSwiney & Stopford 1925; McCrea & McSwiney 1926; McCrea & McSwiney 1928; McSwiney & Wadge 1928). They found that the response of the stomach to stimulation of the vagus and splanchnic nerves varied according to the state of activity of the muscle at the moment of stimulation (cat, rabbit, dog). On the basis of these results they elaborated the theory of the "peripheral mechanism" according to which the effect of extrinsic nerve stimulation was dependent on the state of the muscle and the activity in the local plexuses. Only one of these references (McCrea & McSwiney 1928) refers to the splanchnic nerve. In later experimental work variations in the frequency of stimulation appear to have been more effective in reversing the response to sympathetic stimulation. There are no subsequent references from these workers of variations in tonus altering the response of the stomach to sympathetic nerve stimulation.

About this time Veach in America was also studying these inhibitory responses of the stomach on stimulating the vagus nerve. He brought forward evidence that this was a form of Wedensky inhibition and appeared only with high frequencies of stimulation (Veach 1925). McSwiney & Wadge (1928) were unable to confirm this; they re-affirmed the previous claim that the response was determined solely by the "peripheral mechanism". Veach, Schwartz & Weinstein (1929) repeated the original work

of Veach after modifying the experimental methods to correspond with that of McSwiney & Wadge. They in turn were unable to repeat the work of McSwiney & Wadge and confirmed the previous findings that the frequency of stimulation was the determining factor. The solution to this controversy is probably that both frequency of stimulation and the state of activity can alter the response to stimulation. Variations in frequency will only do so if the organ is already moderately active. If it is very active or quite inactive the response is little affected by the frequency of stimulation. Brown & Garry (1932) confirmed that in general the response of the stomach (cat) to stimulation of the vagus nerve was dependent on the tonicity of the muscle but when this was intermediate between high and low tone then variations in the strength, frequency and duration of nerve stimulation could alter the response.

Rabinovich (1928) and McSwiney & Robson (1929a, b; 1931a, b) described in vitro preparations of stomach muscle from the cat, dog and rabbit and from the small intestine of the cat in which either the extrinsic parasympathetic or sympathetic nerves, or both, were retained. The parasympathetic nerves were small branches of the vagus, the sympathetic the periarterial nerves from the coeliac ganglion. The responses to stimulation of these nerves could be altered either by altering the frequency of stimulation or by drugs. No mention is made of the effect of

muscle tonus on the response but in a later article in which similar preparations were used (Harrison & McSwiney 1936) spontaneous reversal was observed which may have been related to tonus. Such reversal was said to be very rare. This may have been due to the absence of sufficient variation in tone of these isolated preparations. It may be significant that the motor response to stimulation of the periarterial (sympathetic) nerves at low frequency could be reversed to inhibition if the tone of the preparation was first raised by stimulation of the vagus nerve (McSwiney & Robson 1931b).

Laughton (1929) obtained motor, inhibitor and biphasic responses from the stomach of the cat on stimulating the vagal nuclei in the medulla. The type of response depended on the tonus of the stomach, high tone favouring inhibition. Paterson & Rubright (1934), working with monkeys, found the response of the stomach to vary with its tone from inhibition (high tone) through various forms of biphasic response to contraction (low tone). Their work is particularly interesting in that the same variation was observed in the reflex responses of the stomach produced by central stimulation of the sciatic nerve or by pressing on the eyeball. More recently Hsu (1945) has reported motor, inhibitor or biphasic responses of the small intestine of the dog on stimulating the vagus either in the neck, inside the skull or over the nuclei of origin in the medulla.

Frequency and intensity of stimulation. These two factors are taken together because it is probable that altering the intensity of stimulation acts through altering the frequency of impulses set up in the nerve. At high frequencies of stimulation successive stimuli fall in the relatively refractory period following the preceeding nerve impulse. If a strong stimulating current is used it will be above threshold and so stimulate the nerve; if a weak current then it will be below threshold for the nerve at that moment and therefore ineffective. The next stimulus, when recovery is more complete, may be above threshold and so give rise to an impulse in the nerve. Kato, Hayoshi, Ota, Nakayoma, Tamura, Takeuchi, Kanai & Matsuyama (1929) described this phenomenon in its relationship to Wedensky inhibition. It is further supported by the fact that those investigators who describe variations in the response of the alimentary canal according to the intensity of stimulation almost always describe simultaneously variations according to the frequency of stimulation. Lowering the frequency or reducing the intensity have similar effects. Another possible explanation of the intensity effect is that two groups of fibres in a nerve might have different thresholds and one might be activated alone by stimulation at a suitable strength. There is little evidence that in autonomic nerves (even pre and post--ganglionic nerves) there is any great difference in excitability.

While variations in tone are most often reported as varying

the response to vagal stimulation, variation in the frequency or intensity of stimulation seem more often to be successful in reversing the response from stimulation of sympathetic nerves. Thus McSwiney & Robson (1929a, 1931a), stimulating in vitro the periarterial nerves to muscle strips from the stomach, found that low frequencies of stimulation or weak currents were motor while high frequencies were inhibitor. Brown, McSwiney & Wadge (1930) stimulated the thoracic sympathetic trunk in cats and reported motor responses with low frequencies or weak currents and inhibitor responses with high frequencies. Finkleman (1930), again working with innervated in vitro preparations, found that occassionally at the beginning of an experiment stimulation of the periarterial nerves to a length of small intestine from the rabbit at low frequencies or weak currents produced motor responses. Variations have been reported, though less frequently, in the response from stimulation of the vagus nerve. Veach (1925a) found low frequencies caused contraction and high frequencies inhibition of the stomach of cats. Veach et al (1929) re-investigated and confirmed this observation. Veach (1925b) also investigated what effect altering the frequency of stimulation of the splanchnic nerve had on the response of the stomach in the cat. He obtained motor, inhibitor and biphasic responses but these he believed were secondary to the vaso-constrictor action of the nerve. The response was not

related to the frequency or intensity of stimulation. Examination of his results however suggest that low frequencies tended to produce motor responses, high frequencies inhibitor, and intermediate frequencies biphasic responses. For example one experiment shows the following responses at the frequencies indicated:-

3·3	5·5	11·5	185 (Stimuli/sec)
+	+	+ -	-

Autonomic nerves to smooth muscle are sometimes considered to be iterative nerves i.e., they require a train of impulses to produce an effect. This does not apply to all smooth muscle, the nictitating membrane is an obvious exception. In the alimentary tract Rogers & Bercovitz (1921) were unable ever to elicit a response from the stomach of the dog from a single stimulus to the vagus nerve. Veach (1925a), on the other hand, found a single make-break stimulus from an induction coil applied to the vagus nerve of the cat produced contraction of the stomach. Rabinovich (1928), stimulating the vagus nerve to <u>in vitro</u> preparations of the stomach of the cat, rabbit or dog, found single pulses from a battery source ineffective but single pulses from an induction coil occassionally effective. Using similar preparations he describes in a later article the characteristics of the response to single pulse stimulation suggesting that these were then more frequently obtained

(McSwiney & Robson 1929b). McSwiney & Wadge (1928), studying the response of the stomach of the cat in vivo to stimulation of the vagus nerve, found mechanical stimulation effective, presumably a single stimulus. Bain & McSwiney (1936) reported single stimuli of the vagus nerve effective for both the striated and smooth muscle of the terminal oesophagus. Iggo (1954) found that a single pulse was ineffective in producing a response of the rumen of the sheep unless the stomach was sensitised by previous section of the other vagus nerve. Von Euler (1948) has shown that the efferent autonomic fibres in the vagus do not respond to continuous electrical or thermal stimulation by repeated discharge in contrast to somatic C fibres (pain fibres). In the experiments quoted, therefore, the single stimulus applied to the nerve probably did represent a single impulse set up in the nerve. The evidence suggests that single impulses in the vagus nerve are able to produce a response. Bishop & Heinebecker (1932) say that a nerve impulse is probably always effective at the periphery. The apparent threshold is determined by the minimum effect which can be measured rather than by the effectiveness of each single impulse. I have, however, been unable to find any instance in the literature of a response from a single stimulus applied to sympathetic nerves to the gut. Fischer (1944) declares that an inhibitor mechanism has never been demonstrated to a single nerve volley.

The duration of stimulation. Under this heading those references to difference in the temporal characteristics of the response from stimulation of the sympathetic and parasympathetic outflows, differences which might explain biphasic responses, will also be reviewed. McSwiney & Robson (1931a) reported that short periods of periarterial stimulation caused contraction of the *in vitro* muscle strips from the stomach, while long periods of stimulation produced inhibition. Courtade & Guyon (1899) noted that the response of the stomach to parasympathetic stimulation had a short latent period, developed quickly but was short lived. The effects of sympathetic stimulation on the other hand were slow to appear and slow to develop. Bayliss & Starling (1900a), stimulating the nervous outflows to the colon, found the pelvic motor responses easily fatigued but the lumbar inhibitor nerves very resistant to fatigue. Rogers & Bercovitz (1921) reported a similar relationship between motor and inhibitor responses of the stomach but this time from stimulation of a single nerve - the vagus. The easy fatigue of the vagus nerve *in vitro* was commented on by Rabinovich (1928). Iggo (1954) has reported that the inhibitory effect of splanchnic stimulation continues into the post-stimulatory period. The evidence suggests that the parasympathetic motor effects are rapid in onset and development but easily fatigued; the sympathetic inhibitor responses by contrast are slow in onset, persistent in action and

slow to fatigue. As was previously pointed out inhibitor responses have never been reported to single stimuli. The longer latent period of sympathetic responses compared with parasympathetic may be related to the number of impulses required to produce a detectable response. If this is true then obviously short periods of stimulation of a mixed nerve containing both groups of fibres would elicit motor (parasympathetic) responses.

Drug reversal. Drugs which have been used to reverse the normal response of the smooth muscle of the alimentary canal to stimulation of its extrinsic nerves fall into two groups.

1) Those which block specifically one or other of the nerve outflows; atropine, nicotine and ergot derivatives.
2) The barbiturates whose effect is less well understood but which does not seem to depend on specific block of one or other group of fibres.

Langley (1898-99) suggested the presence of inhibitor fibres for the stomach in the vagus nerve because of the appearance of inhibition on stimulation of this nerve after atropine or curare (rabbit, cat). McSwiney & Robson (1929a, b) revealed with atropine an inhibitor effect of vagal stimulation on preparations of the stomach in vitro. Kuré, Ryaji, Ichiko & Yasuhiko (1931), Kuré, Ichiko & Ishikawa (1931) demonstrated a motor effect on the stomach and small intestine from stimulation of the splanchnic nerves after painting the coeliac ganglion with nicotine 1 - 2%

(dog, guinea pig, toad, frog). Ergotoxine has been used in the hope that it would abolish those responses due to stimulation of true sympathetic fibres. Unfortunately neither ergot derivatives nor any other sympathetic blocking agent is effective in blocking the inhibitory effects of sympathetic nerve stimulation. The concentrations of ergotoxine used were often very large and themselves acted on smooth muscle causing contraction. McSwiney, who in earlier work used ergotoxine to demonstrate that certain motor effects of sympathetic stimulation were due to true sympathetic fibres and not to an admixture of cholinergic fibres, eventually withdrew this evidence (Harrison & McSwiney 1936) and demonstrated that, in the concentrations which they had earlier used, ergotoxine also abolished the motor effect of acetylcholine.

The experiments with the barbiturates showed that amytal (sod. ethyl isoamyl barbiturate) reversed inhibitor responses of the stomach from vagal nerve stimulation (Brown & Garry 1932) and that luminal (sod. ethyl phenyl barbiturate) converted into contraction the inhibitor effects of splanchnic stimulation on the stomach (Brown & McSwiney 1932). In both reports inhibitor responses were still obtained, although with difficulty, from the other nerve. Reversal with luminal was confined to the stomach, the effect on the small intestine of splanchnic nerve stimulation remained inhibitor. Wright, Florey & Jennings (1938) carried out similar experiments in the large bowel of the cat. Luminal antagonised

peripherally the action of the pelvic nerve but reversed neither this motor effect nor the inhibitor effect of sympathetic stimulation. The reversal described by Brown & Garry and by Brown & McSwiney may be due to some local peculiarity of the stomach.

A certain number of references deal with the reversal by atropine or botulinum toxin of the action of nicotine and similar drugs. These drugs are intended to represent post-ganglionic nerve stimulation. To apply these results to the action of the extrinsic nerves is dangerous considering the multiple action of nicotine, its toxic effects and our ignorance of the composition and function of Auerbach's plexus.

Variations in the response of smooth muscle to indirect stimulation are not confined to the alimentary tract. Furthermore the same factors determine the type of response in other regions. De Zilwa (1901), stimulating directly the retractor penis of the dog, observed both motor and inhibitor responses depending on tonus. High tone favoured inhibition. Cushny (1910) pointed out that the response of the uterus of the pregnant or recently pregnant cat to stimulation of the hypogastric nerve was contraction, whereas in the non-pregnant animal it was inhibition. Greene (1935) reported constrictor, dilator and biphasic responses of the coronary arteries to stimulation of the vagus nerve in the neck. The response was dependent on the tone of the vessels at the moment of stimulation. High tone favoured dilatation. The ultimate cause

of these variations, according to this last author, was the presence of sympathetic (dilator) and parasympathetic (constrictor) fibres in the vagus nerve in the neck. Bülbring & Burn (1935) reported constrictor and dilator responses of the blood vessels of the hind leg of the dog. Short periods of stimulation favoured dilatation, long periods constriction. Folkow & Uvnas (1948), in a somewhat similar investigation, found the same variations in response but they related it to the frequency of stimulation. Low frequencies favoured dilatation, high frequencies constriction. Both they and Bülbring & Burn demonstrated that the constrictor response is due to adrenergic fibres, the dilator to cholinergic fibres. Mellanby & Pratt (1939) report that the bladder of the cat may respond by inhibition or contraction followed by inhibition to stimulation of the hypogastric nerve. Low frequencies and intensities of stimulation augment the initial motor component.

All smooth muscle, however, does not respond in this inconsistent manner to extrinsic nerve stimulation. Bishop & Heinebecker (1932), for example, never observed inhibition of the nictitating membrane whatever the frequency of stimulation. Nor are all blood vessels equally prone to vary in their response as are those of the limb muscles. Daly, Eggleton, Hobb, Linzell & Trowell (1954), studying the effects of prolonged perfusion of the dog, found that, while stimulation of the intact or central end of the vagus could produce dilator, constrictor

or biphasic responses of the systemic blood vessels, the response of the pulmonary vessels remained constrictor. Weak stimuli favoured dilator responses of the systemic blood vessels.

Several theories have been produced to explain these anomalous responses. These can be divided into two groups according to whether the nerves stimulated are believed to contain only one type of fibre or not. Those who believe that they are all of one type consider the type of response to be determined at the periphery. Those who consider that two functionally different fibre groups account for the vagaries presumably imply that these fibre groups can be selectively stimulated under certain conditions. These theories will be briefly reviewed.

GROUP I. THE NERVE OUTFLOWS ARE "PURE".

1) The "peripheral mechanism". This term was introduced by McCrea et al (1925) to explain the variations in the effects of nerve stimulation with the tonus of the muscle. The "peripheral mechanism" was composed of the smooth muscle and the intrinsic nerve apparatus. The relative part played by each could not be determined. Variations both in the length and tension of the muscle fibres and in the activity of the intrinsic nerves altered this "peripheral mechanism". If the "peripheral mechanism" was active extrinsic nerve impulses induced inhibition, if inactive contraction was produced. The function of this mechanism was therefore homeostatic, opposing equally, excess activity or inactivity. The

sympathetic and parasympathetic outflows were no longer antagonistic to one another although possibly in the middle range of peripheral activity they would appear to be. The elimination of the antagonistic function of the two divisions of the autonomic nervous system has commended this theory to those (chiefly histologists) who believe in a terminal syncytium mediating all afferent and efferent nervous activity. In such a nerve net direct antagonistic systems are not possible; the nerve net can mediate one effect only. It has been suggested that the dual response of smooth muscle is due to variations in the physico-chemical environment of the muscle cells altering their response to the same stimulus. Alternatively the stimulus may influence the environment of the cells, by vasoconstriction for instance, and thereby produce a response opposite to that which the nerve would produce by its direct action on the muscle itself (Schäefer 1952). The concept of the "peripheral mechanism" as introduced by McSwiney was abandoned following the demonstration of cholinergic and adrenergic fibres in both the vagus and sympathetic nerves to the stomach (Harrison & McSwiney 1936).

2) Wedensky inhibition or fatigue. Since high frequencies of stimulation favour inhibition whether stimulating sympathetic or parasympathetic nerves it is possible that this is due to Wedensky inhibition i.e., a block of the nerve impulses somewhere in the pathway to the effector muscle without diminution in the ability

of that muscle to respond. By itself this is an insufficient explanation. If the effect is one of Wedensky inhibition then the responses should always be biphasic and inhibition when it appears should be a disappearance only of the previous motor effect imposed by nerve stimulation. The normal tonus and rhythmic activity, if myogenic, should be unaltered. Veach (1925) countered this by supposing that the spontaneous activity and tonus of the gut was maintained by a low basal level of activity in the intrinsic nerve apparatus. If this activity was increased by extrinsic nerve stimulation the motor effect increased up to a certain frequency. Above this frequency Wedensky inhibition appeared and all activity in the intrinsic nerves was suppressed including the basal activity. As a result a true inhibition of tone and activity below the base line appeared. On stopping stimulation the activity in the intrinsic nerves was supposed to pass once more through a phase of heightened activity. This accounted for the appearance of after-contraction. This explanation of after--contraction is difficult to understand. If, during stimulation, Wedensky inhibition is induced then there will be no activity whatever distal to the region of block. On stopping stimulation one would expect only a gradual reappearance of basal activity in these nerves. There can be no region in which nerve impulses are stored up as it were; impulses which, when extrinsic stimulation stops, discharge themselves at a diminishing frequency.

Cannon, Raule & Schäefer (1954) have attempted to explain the dilator and constrictor responses of blood vessels to stimulation of the sympathetic chain at different frequencies on the basis of Wedensky inhibition. They demonstrate, by means of a reduction in the compound action potential, that the sympathetic fibres to the heart will not conduct at frequencies above 20 pulses/sec ($^{P}/_{S}$). The spontaneous activity in these nerves is at a very low frequency, about $3^{P}/_{S}$. If artificial stimulation at frequencies above $20^{P}/_{S}$ is superimposed on this natural activity then both are said to be supressed by Wedensky inhibition with the loss of the previous tonic state. Their interpretation of these results is open to several objections. A reduction in the compound action potential is not Wedensky inhibition in which all nerve impulses should be supressed. It does not even imply that the fibres are no longer transmitting each impulse unless temporal dispersion of the individual fibre responses and a reduced amplitude of these responses from travelling in the relative refractory period of their predecessors can be excluded. The biggest stumbling block however is in applying their theory to the results from stimulation of the sympathetic chain. According to the theory high frequencies of artificial stimulation should supress both themselves and the natural tonic impulses in the nerve fibres. They should surely therefore produce dilatation of the blood vessels whereas in fact high frequencies produce constriction and low frequencies

dilatation.

3) Variable effect of a single chemical transmitter. This group is of less importance. None of the suggestions have been more than a tentative explanation of a few of the variations which have been reported.

Cannon & Rosenblueth (1933) found that when sympathetic nerves were stimulated a substance was liberated into the blood stream whose presence could be detected by its action on smooth muscle suitably sensitised. Two substances were liberated, one from smooth muscle contracted by sympathetic stimulation and which itself caused contraction of other smooth muscle which it reached via the blood stream (Sympathin E) and one liberated from muscle inhibited by sympathetic stimulation and itself causing inhibition of any appropriate smooth muscle it reached through the circulation (Sympathin I). Sympathin I was never obtained alone as all tissues liberating it also contained blood vessels which were a source of Sympathin E. Cannon & Rosenblueth suggested that a single mediator substance M was liberated from the nerve endings and that this then combined with some receptor in the muscle cell. This receptor could be of two types E or I. The combination M E caused excitation of the muscle locally and via the blood stream of other similar muscle. The combination M I produced inhibitor phenomena. The authors suggested that the varying response of the uterus to sympathetic stimulation or to adrenaline

might be due to an alteration during pregnancy of the I receptor to E. It is possible that these receptor groupings might alter not only over long periods as in pregnancy but from minute to minute with the state of the muscle and so explain many inconsistencies. The discovery that Sympathin E is probably non-adrenaline and Sympathin I adrenaline however suggests that many of Cannon & Rosenblueth's observations were due to the liberation of two slightly different transmitter substances.

Bozler (1940) showed that hypogastric nerve stimulation or adrenaline will reduce the excitability of uterine muscle to electrical or mechanical stimulation. He states that a similar result can be shown in the intestine. He also confirmed the variation in the response of the uterus to nerve stimulation or adrenaline and reported biphasic responses, the motor component first. Bozler postulates that the action of the transmitter substance is primarily to produce contraction. Subsequently it depresses excitability which leads eventually to conduction block in the muscle and inhibition. There is little experimental support for this theory. The changes in excitability he obtained were slight and variable. All responses would have to be biphasic which is certainly not always so in smooth muscle in other regions.

Burn & Robinson (1951) studied the response of the blood vessels of the isolated rabbit's ear perfused for up to 3 days. Such long continued perfusion could reverse the normal dilator

effect of acetylcholine to constrictor if large concentrations were used. The constrictor response to adrenaline could be reversed to dilator by ephedrine. These findings they believe are due to an abnormal combination of the drug molecules with the receptors when the drug is in excess of the available number of receptors. Such conditions were produced in their experiments by ephedrine occupying impotently the same receptors as adrenaline, and by cholinesterase becoming ineffective in destroying acetylcholine. It is not easy to see any application of this theory to the rapid variations in response of other smooth muscle to nerve stimulation under more physiological conditions.

GROUP 2. ADRENERGIC AND CHOLINERGIC FIBRES

1) Sympathetic and parasympathetic fibres. These are the obvious origin of adrenergic and cholinergic fibres although fibres anatomically belonging to the sympathetic outflow may liberate acetylcholine as transmitter. Certain of the macroscopic anatomical nerves whose stimulation produces mixed responses are known to contain both sympathetic and parasympathetic fibres. The periarterial nerves to the small intestine (Finkleman 1930) or to the stomach (McSwiney & Robson 1929a; 1931a, b) are in this group, a fact recognised by McSwiney & Robson who used differences in latent period between the motor response from periarterial stimulation and that from vagal stimulation, as well as the blocking effect of ergotoxine, to argue that the effects they

observed were all due to sympathetic fibres. The differences in latent period were hardly significant and the conclusions drawn from the action of ergotoxine were withdrawn in a later paper. Sympathetic fibres are known to exist in the branches of the vagus nerve below the diaphragm. The anterior and posterior vagal trunks, which enter the abdomen along the oesophagus, divide into numerous branches several of which anastomose with sympathetic nerves from coeliac ganglion. This is particularly true of the vagal fibres for the pylorus of the stomach which pass to the hepatic fissure to join with sympathetic fibres for the same region. These connections are illustrated in the beautiful plates of Swann (1834) and later confirmed by McCrea (1924-25). It is not surprising then that Rabinovich (1928) and McSwiney & Robson (1929a, b) were able to reveal an inhibitor response after atropine when stimulating small terminal branches of the vagus nerve.

In the nerves so far considered there can be no reasonable doubt that both sympathetic and parasympathetic nerve fibres are, or may be, present. It is more difficult to explain the presence of sympathetic fibres in the vagus in the neck or parasympathetic fibres in the splanchnic nerves or thoracic sympathetic cord. The vagus however is known to have numerous interconnections with the sympathetic chain in the neck (Duncan, 1928; Greene, 1935). At the level of the diaphragm the vagus is as thick as at its exit from the cranium in spite of losing both cardiac and

pulmonery fibres. This is due to the acquisition of sympathetic fibres (Mitchell, 1953). Kuré, Ichiko & Ishikawa (1931) point out that a section of the vagus at the level of the diaphragm shows two groups of fibres, a myelinated group and a large number of unmyelinated fibres. In their experiments all but four of the myelinated fibres degenerated on sectioning the vagus high in the neck but the unmyelinated fibres were unaltered. Extirpation of the cervical sympathetic from above the superior cervical ganglion to below the stellate ganglion was followed by complete degeneration of the unmyelinated fibres, the myelinated group remaining intact. Kuré et al were convinced that the vagus was a combined parasympathetic-sympathetic nerve and that both types of fibre were distributed to the stomach.

There is also some physiological evidence that fibres leave the sympathetic chain in the neck region, join the vagus and are distributed to the stomach. Carlson et al (1922) pithed the spinal cord, cut both splanchnic nerves and sometimes both vagi. They then stimulated the thoracic sympathetic chain at varying levels. At the level of the stellate ganglion they occassionally observed a response from the cardia of the stomach. Kuré, Ichiko & Ishikawa (1931) noted that if the vagus was cut low in the neck there was little delay in emptying of the stomach; if the section was high in the neck there was loss of tone and delayed emptying. This they attributed to intact inhibitor

sympathetic fibres. If now the cervical sympathetic was extirpated the delay in emptying was reduced. Much of this evidence for sympathetic fibres in the vagus nerve has been reviewed by Mitchell (1953) in his book. He concludes that this nerve should be looked on as a mixture of sympathetic and parasympathetic fibres.

The evidence for vagal fibres in the splanchnic nerves is much less convincing. Duncan (1928) investigated whether some of the numerous connections between the cervical sympathetic and the vagus were not in fact conveying vagal fibres to the sympathetic chain which later left in the splanchnic nerves. He cut one vagus nerve and after allowing a sufficient time for degeneration, examined the thoracic sympathetic chain and splanchnic nerve (rabbit, cat, dog). In a few animals he found degenerated fibres presumably of vagal origin but he considered them too inconstant and too infrequent to be of physiological importance. The other possible source of parasympathetic fibres is the spinal cord via the dorsal roots; the spinal parasympathetic fibres. These were first described by Kuré and his co-workers and their relationship with the alimentary canal elaborated in a series of articles in 1931. Some of this work has been questioned, particularly the experimental evidence but there still remains certain observations which are not otherwise easily explained. The dorsal roots they found to contain a large proportion of small myelinated fibres

which on sectioning the root between the spinal cord and the dorsal root ganglion did not degenerate in the central stump i.e., their cells of origin were on the spinal cord side (Kuré, Ichiko & Ishikawa 1931). Further histological evidence suggested that the cells of origin were in the spinal cord between the anterior horn and the substantia gelatinosa (Kuré, Ikida, Ichiko & Yasuhiko 1931). These fibres amounted to between 25% and 55% of the fibres in the dorsal roots and were maximal in the lower cervical and lower lumbar regions. They formed with the cranio-sacral fibres a continuous parasympathetic outflow from the brain stem to the sacral cord. These fibres were motor to the gut muscle and dilator for its blood vessels. Their function could be displayed by stimulating the splanchnic nerve after applying nicotine to the coeliac ganglion. The activity of the stomach and small intestine was increased. Stimulating of the lumbar sympathetic chain, even without nicotine, produced segmental activity of the small intestine and the first part of the large intestine. Nicotine applied to the lumbar or inferior mesenteric ganglia reinforced this motor effect. In some of these experiments the vagi had been cut one month previously. Two objections to these experiments are, first, that few animals were used and, secondly, the methods were such that strong reflex (sympathetic) inhibition of the gut was almost certainly present. Under these circumstances interrupting transmission at the coeliac ganglion would be expected

to lead to increased activity without implying the presence of motor fibres. They report however (Kuré, Ichiko & Ishikawa 1931) that sectioning the rami communicantes or splanchnic nerves caused relaxation of the stomach and small intestine. This suggests that these nerves were maintaining a tonic motor influence. Unfortunately none of their experimental work seems to have been successfully repeated.

The histological part of Kuré's investigations has more support. Barron & Matthews, from electroneurographic studies, concluded that about 40% of the fibres in the dorsal roots carried centrifugal impulses. These they believed to be recurrent fibres of sensory afferents entering other roots. Kuré & Kajiyama (1936) investigated this by cutting a large number of successive dorsal roots on one side leaving one root in the middle of the series intact. After allowing time for degeneration this intact root was examined for degenerating fibres. None was found. They also cut one or two nerve roots and then examined all of the other intact roots on the same side for degenerating fibres. Again none was found. The presence of unmyelinated fibres in somatic nerves unconnected with the sympathetic outflow has been reported. Sheehan (1932-33) showed that these fibres were present in large numbers in both muscular and cutaneous nerves and that they were unaffected by bilateral sympathectomy. He thought that they corresponded to the fibres described by Kuré.

The evidence for the presence of sympathetic and parasympathetic fibres in nerves producing anomalous responses can be summarised as follows. Periarterial nerves and branches of the vagus below the diaphragm are certainly mixed nerves. There is good evidence that the vagus, even in the neck, contains sympathetic fibres for the stomach. The splanchnic nerve and thoracic sympathetic chain are unlikely to contain a sufficient number of parasympathetic fibres of vagal origin to produce a detectable response; they may contain fibres of spinal origin homologous with the vagal parasympathetic fibres.

2) <u>The fibres are all of similar origin</u>. Dale & Feldberg (1934) have demonstrated the cholinergic nature of the sympathetic fibres to sweat glands in the cat. It is therefore possible that both the sympathetic and parasympathetic outflows contain adrenergic and cholinergic fibres, both groups in each outflow corresponding to the pattern of organisation of its appropriate division. The findings of Folkow & Üvnas (1948) that both the cholinergic and adrenergic fibres of the lumbar sympathetic chain which supply muscle blood vessels come from the ventral nerve roots, support this. The observations that inhibitor responses of the stomach can still be obtained by stimulating the vagus high in the neck (Carlson et al 1922; Veach 1925); intracranially (Hsu 1945) or over the nucleus of origin (Laughton 1929; Hsu 1945) suggest a medullary origin for the corresponding fibres. If these inhibitor fibres in the vagus nerve

correspond to parasympathetic fibres there ought to be post--ganglionic inhibitor neurones in Auerbach's plexus. Langley (1922) postulated the presence of such neurones connected to extrinsic vagal fibres. Ambache (1951) and Ambache & Edwards (1951) have produced pharmacological evidence for the presence of inhibitor neurones in the gut wall. Hsu (1945) demonstrated a synapse in the gut wall on the pathway of inhibitor fibres in the vagus to the small intestine. He recorded from two regions of the gut; if one of these was painted with 1 - 2% nicotine then vagal stimulation, previously producing inhibition of this site, was rendered ineffective. The untreated region still responded with inhibition. Youmans (1948), in a diagram representing the innervation of the gut, includes adrenergic and cholinergic post-ganglionic fibres in both outflows. Both adrenergic and cholinergic post-ganglionic neurones of the anatomical parasympathetic outflow are in Auerbach's plexus.

If, instead of nerve stimulation, the response to adrenaline and acetylcholine were studied one would imagine that it would be possible to decide whether the anomalies of nerve stimulation were due to a pure outflow with peripheral selection or a mixed outflow containing two types of fibre. This has been done on several occasions with results which favour peripheral selection. Brown & McSwiney (1926) and McSwiney & Brown (1926-27) found that muscle strips from the fundus and body of the stomach of the cat,

dog and rabbit responded to adrenaline by contraction, inhibition or contraction followed by inhibition depending on the tone of the muscle. Muscle tone was altered in these experiments by drug action. Certain regions however did not vary. The pylorus responded only with inhibition, the subcardiac region only with contraction whatever the tonus. Furthermore the motor effect of pilacorpine was never reversed in any region. McCrea & McDonald (1928), recording from the entire stomach in vivo, observed a similar variability in the response to intravenous injection of adrenaline. They also reported acetylcholine as producing both motor and inhibitor responses depending again on the tonus of the organ. They remarked, however, that the action of adrenaline did not correspond with that of splanchnic nerve stimulation. A reversed response to one was not necessarily accompanied by reversal of the other. Carlson, Smith & Gibbins (1927), recording with balloons the responses of the stomach, small intestine and colon to intravenous injection of choline, also report contraction, inhibition and inhibition followed by contraction. Hsu (1945) found that close-arterial injection of acetylcholine produced a biphasic response, contraction followed by inhibition, of the small intestine of the dog. After atropine only inhibition appeared. Munro (1951, 53) reported motor and inhibitor responses of the terminal ileum of the guinea pig to adrenaline. The type of response depended on the concentration

of adrenaline. Ergotoxine abolished the motor component. In the guinea pig the type of response seems to be dependent on the region of muscle studied. Newman & Thienes (1933) found the response of the small intestine either to sympathetic nerve stimulation or to adrenaline to vary from contraction, with an occassional secondary inhibition (duodenum), to inhibition with sometimes a small preliminary contraction (terminal ileum).

Variations in the response of smooth muscle to adrenaline and acetylcholine are not confined to the alimentary canal. Cannon & Rosenblueth (1933) and Bozler (1940) reported inhibition or contraction of the uterus of the cat to adrenaline, the determining factor being hormonal. Edge (1933), recording the response of the entire bladder to adrenaline or non-adrenaline, observed both inhibition and inhibition preceeded by contraction. It is well known too that adrenaline in small concentrations may dilate but high concentrations constrict muscle blood vessels.

In spite of the variable effect of drug action the evidence from nerve stimulation is overwhelmingly in favour of two nerve fibre groups as being chiefly responsible for the inconsistencies in the response. Although the action of adrenaline and acetylcholine is not uniform in all regions it is so in some regions. This is just as difficult to explain on the basis of the "peripheral mechanism" as are the variations on the basis of two fibre groups in the nerves. Furthermore, the variations in

drug action have been reported only under circumstances in which other factors may be operating. For example, muscle strips, whose response varies with the tonus of the muscle, can only be obtained from certain regions of the stomach. In other sites the response is uniform. It is possible that two types of muscle are present in the former. Recording from the whole organ (stomach or bladder) also involves this risk since in these organs there are specialised regions whose muscle responds in the opposite direction to the general musculature, e.g., the trigone of the bladder.

It is more difficult to come to a decision on the origin of the two types of nerve fibre. The evidence for both opinions seems fairly good. A closer investigation, however, shows certain weaknesses in the evidence that the outflows are pure sympathetic or parasympathetic in origin and organisation but each containing cholinergic and adrenergic fibres. There is no evidence that I can find to link the organisation of the cholinergic fibres in the splanchnic nerves with that of the sympathetic outflow. I can find no report in which the motor effects corresponding to these cholinergic fibres were blocked by nicotine to the presumed cell station, the coeliac ganglion. Those reports suggesting inhibitor neurones in Auerbach's plexus connected to extrinsic vagal fibres are not completely convincing. Langley (1899, 1922) appears to have based his views on the presence of inhibitor effects of vagal stimulation especially after atropine. This does not of itself

prove a cell station in the bowel wall. The experiments of Ambache (1951) were purely phramacological, to interpolate his inhibitor neurones on the vagal pathway is speculation only. The illustrations of Hsu (1945), showing an inhibitor response to vagal stimulation abolished in the nicotinised sector of bowel, also shows that the activity of the gut is reduced and the inhibitor effect of splanchnic stimulation much reduced. Since splanchnic inhibition was initially greater than the inhibitor response from vagal stimulation, the abolition of the latter may be a non-specific effect. Nor is the evidence for the intracranial origin of the inhibitor fibres free from criticism. Current escape can so easily occur in this limited space that unless the spinal cord is severed effects may be mediated through the splanchnic nerves. This was not done in the experiments of Hsu. The records illustrated by Laughton (1929) showing inhibition following stimulation of the vagal nuclei are not convincing although he did take the precaution of cutting the spinal cord.

All inconsistencies in the response of the alimentary canal to stimulation are probably not due to the same thing. One important cause of these may be the presence of two types of fibre in many of the nerves stimulated. Other possibilities are:-

1) The presence of two types of smooth muscle, one sphincteric in function, the other belonging to the general propulsive musculature.

2) The integrative action of the intrinsic nerve mechanism, particularly when stimulation initiates peristalsis. Below the region of activity inhibition will be recorded, succeeded by contraction if the peristaltic wave travels far enough. At the site of activity the record will show pure contraction.

3) The interpretation of inhibition is not always comparable between investigators. To some the disappearance of a previous motor response is inhibition (Wedensky inhibition often), to others a fall in tonus and activity after stimulation has ceased is inhibition, while yet others interpret a prolonged latent period as inhibition. I do not consider these to be true inhibitory responses to stimulation. Inhibition implies a lowering of tone or activity below the base line which existed before stimulation began, and occurring while stimulation is still continuing.

Most of the work on the effect of nerve stimulation on the gut has been carried out on the stomach. The colon has been little studied. The few observations which have been made suggest that inconsistencies are fewer. Langley & Anderson (1895) reported motor responses from stimulation of the pelvic nerve in the rabbit. "Brief" inhibition was occassionally seen but such results were a rarity. The same workers found that stimulation of the caudal pair of inferior mesenteric ganglia or of any of the peripheral branches

to the colon (lumbar colonic nerves, ascending mesenteric nerves, hypogastric nerves) could produce motor, inhibitor or biphasic responses. Langley & Anderson were of the opinion that these motor responses were due to stimulation of ascending parasympathetic fibres of sacral origin. Stimulation of the cranial pair of ganglia or pre-ganglionic fibres produced only inhibition. Courtade & Guyon (1897) found the sympathetic purely inhibitor and the parasympathetic purely motor to the colon in the dog. Bayliss & Starling (1900a) found the same thing in the rabbit but in the dog they reported biphasic responses from parasympathetic stimulation. Munro (1953), using the colon of the guinea pig and stimulating periarterial mesenteric nerves, observed both motor and inhibitor responses. The presence of tone favoured inhibition.

In none of these reports has a deliberate attempt to reverse the usual response been made. Those factors predisposing to reversal which have been so intensively investigated in the stomach have never, so far as I can discover, been studied in the colon. This is probably due to the ease with which the extrinsic nerves to the stomach, both sympathetic and parasympathetic, can be isolated and stimulated <u>in vivo</u> or <u>in vitro</u>. The parasympathetic outflow to the colon is contained in the pelvic nerves which are delicate and not easily accessible for stimulation. This thesis describes investigations on the response of the colon to stimulation of the extrinsic sympathetic and parasympathetic nerves. An <u>in vitro</u>

preparation has been used so as to eliminate, or at least reduce, the danger that the integrative action of the gut wall would conceal the primary effect of nerve stimulation. The conditions altering the type of response of the stomach to stimulation have been reproduced and their effect on the response of the colon studied.

PART II.

ANATOMY & HISTOLOGY.

A N A T O M Y.

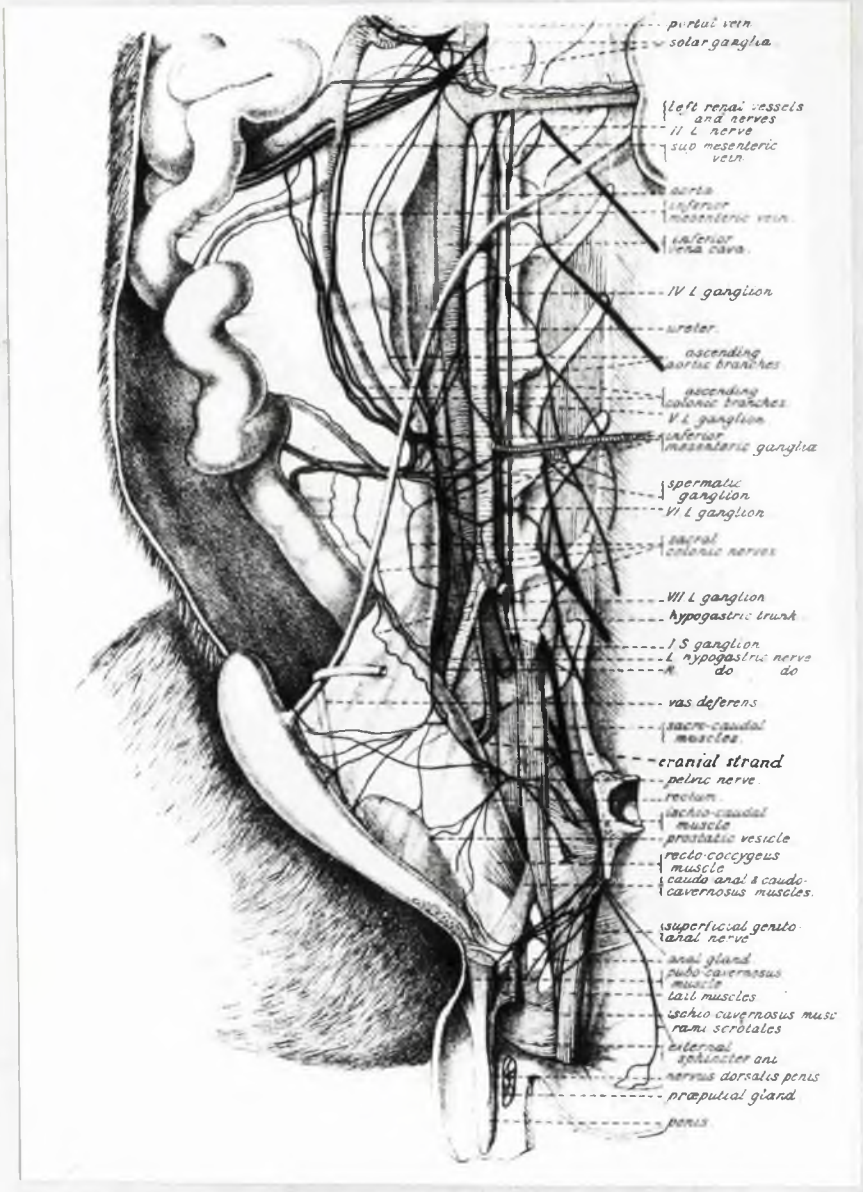


Fig. 1 A copy of Langley's drawing of the inferior mesenteric and pelvic plexuses. The cranial strand, to which reference is made in the text, has been arrowed.

The anatomical relations and the function of the nerves to the pelvic viscera were brilliantly described in a series of articles by Langley in the years 1895 and 1896. His description of the nerve supply to the colon of the rabbit in three articles (Langley 1895, 1896a, b) differs in only one important respect from the description to be given here. It now seems unlikely that the efferent parasympathetic fibres to the smooth muscle of the colon synapse in the ganglia of the pelvic plexus rather than in the ganglion cells in the plexus of Auerbach. His terminology has been used except that the nerve bundle he called the anterior strand I have called the cranial strand since there is some possibility of confusing anterior with ventral. The inferior mesenteric vein I call the lumbar colonic vein. This vein, as can be seen clearly in the photomacrograph of Fig. 3 does not identify itself closely with the inferior mesenteric artery. At the point where the artery swings through the mesocolon to reach the aorta the vein, instead of accompanying the artery and ending in the inferior vena cava, takes a sharp right angled bend and continues cranially to end in the portal venous system. Since it drains the whole of the colon but does not accompany the inferior mesenteric artery for much of its course, the lumbar colonic vein seems a more fitting description. Many authors, including Langley, recognise the colon and rectum as distinct regions in the rabbit. I have not been able to see any sharp distinction in the

terminal part of the large intestine which would permit one part to be regarded separately as the rectum. The colon lies in the midline of the abdominal and pelvic cavities suspended by its mesocolon. It is a narrow tube whose circular muscle coat contracts down firmly on the faecal pellets within the lumen, giving it a beaded appearance. This is continued uniformly right to the anus with no evidence of a region specialised for storage or evacuation. Consequently I refer to the whole of this region as the colon.

The mesocolon is a continuation of the parietal peritoneum of the dorsal abdominal wall. This has a "fold" in it produced, I believe, by the retroperitoneal fat on either side of the midline which raises the peritoneum from the abdominal wall, the bulge so formed overlapping the dorsal part of the mesocolon. This is illustrated diagrammatically in Fig. 2.

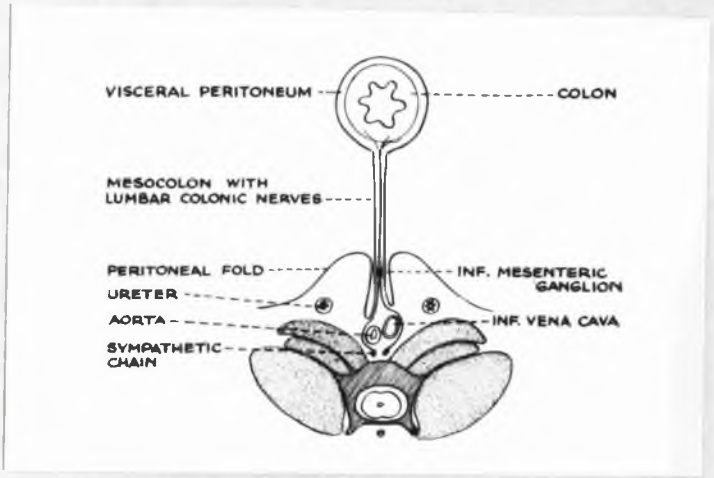


Fig. 2 Diagrammatic representation of a cross section through the lower lumbar region in the rabbit. The fold of parietal peritoneum which overlaps the mesocolon is shown.

This fat-containing fold of peritoneum may obscure the inferior mesenteric ganglia and hypogastric nerve which otherwise are lying in a transparent mesentery. Fortunately it is easy, if the correct cleavage plane is found, to reflect this peritoneal fold off the mesocolon proper. The entire mesocolon can be removed from the colon so as to retain within the mesocolon the inferior mesenteric and pelvic plexuses. Such a preparation stained with gold chloride is a useful adjunct in studying these nerve plexuses (Fig. 3).

Fig. 3 Photomacrograph and line drawing of a preparation of the inferior mesenteric and pelvic plexuses. Gold chloride staining.

The Parasympathetic Outflow. These fibres originate in the sacral region of the spinal cord and emerge in the ventral roots of the 2nd, 3rd and 4th sacral nerves and the 1st coccygeal nerve. The sacral nerves as they lie on the dorsal wall of the pelvis in the triangular space bounded by the sacrocaudal, ischio-caudal and pyriformis muscles give rise to five or six nerve filaments which form the pelvic nerve. The relative size of the contributions from individual nerve roots varies according to whether the lumbosacral plexus is of the anterior or posterior type. If anterior, there is no coccygeal contribution; if posterior, no fibres from S2. The filaments may unite to form a single nerve or, instead, form a loose plexus. Whichever form is present the fibres, after a short course, separate into two divisions, an anterior (cranial) and a posterior (caudal). There are numerous connections between these two divisions. The parasympathetic fibres for the colon pass first into the posterior division but almost immediately leave it as the Cranial Strand of the posterior division. This nerve passes craniad towards the pelvic brim and after a course of about 2 cm unites with the dorsal division of the hypogastric nerve. In Langley's drawing (Fig. 1) this cranial strand is shown lying alongside the colon well away from the pelvic wall. This can be misleading. When the dissection is completed and the restraining fascia which binds the peritoneum to the pelvic wall removed this is certainly the appearance seen

on pulling the bladder forward. Before dissection, however, the cranial strand pursues a backward and upward course as shown in the diagram of Fig. 4. This brings it close to the pelvic wall where it may easily be damaged during dissection.

At the junction of the cranial strand with the hypogastric nerve there is a collection of ganglion cells just visible to the naked eye and called by Langley (after Frankenhäuser) the "ganglion uterinum", although, as Langley pointed out, it is present in both sexes. From the combined cranial strand-hypogastric nerve complex a series of nerve fibres are given off which pass ventrally through the mesocolon to the inferior mesenteric artery. These are the sacral colonic nerves composed almost entirely of the parasympathetic fibres to the colon. A particularly conspicuous branch is given off from the ganglion uterinum (Fig. 3 photomacrograph). These parasympathetic fibres on reaching the inferior mesenteric artery join with the sympathetic fibres accompanying this vessel and both fibre groups are then distributed to the colon with the terminal branches of the inferior mesenteric artery and lumbar colonic vein. The most cranial of the sacral colonic fibres passes into and through the inferior mesenteric plexus and reaches the colon either alongside the upper branches of the inferior mesenteric artery or with the ascending mesenteric branches of the inferior mesenteric plexus. The latter group of nerve filaments are more closely related to the lumbar

colonic vein than to the inferior mesenteric artery (Fig. 5).

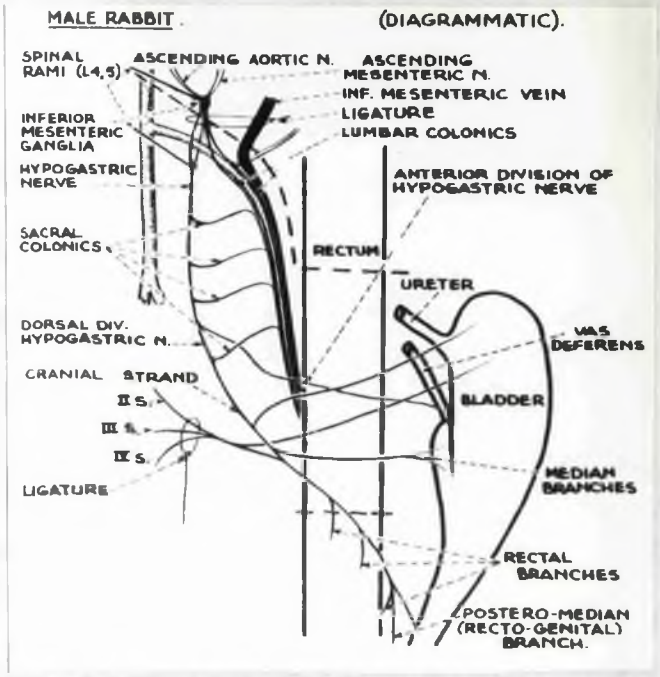


Fig. 4 A simplified diagrammatic representation of the pelvic and inferior mesenteric plexuses. The dotted lines represent the line of section of the colon and mesocolon when removing the preparation.

The sacral parasympathetic outflow gives rise to numerous other groups of fibres innervating the other pelvic viscera. These can be dismissed briefly. They are represented diagrammatically in Fig. 4.

1) Antero-median fibres: Arise from the anterior division of the pelvic nerve to innervate the bladder.

2) Median fibres: Arise from the anterior division and cranial strand to innervate the prostate, seminal vesicles, vas deferens, urethra and the vagina in the female.

3) Postero-median fibres: These are the fibres remaining after the cranial strand has left the posterior division. They curve

across the rectum and run eventually in the mesentery between the urethra and the colon to end in anastomoses with the pudic nerves through which they supply the genitalia. Rectal fibres are given off. This group, sometimes known as the recto-genital fibres, innervates the rectum, urethra and external genitalia.

4) Posterior fibres: A few very delicate inconspicuous fibres innervating the recto-coccygeus smooth muscle.

The Sympathetic Outflow. This arises as three delicate rami from the lumbar sympathetic chain on each side. The ganglia of origin are L4, L5 and the third ramus usually from the trunk between the 5th and 6th lumbar ganglia. (The origin of these fibres from the spinal cord will be at least one segment craniad). These three rami, called the lumbar splanchnic nerves, enter the collection of ganglionic tissue around the origin of the inferior mesenteric artery. This consists of two main ganglionic masses, a large elongated one cranial to the artery and a smaller collection of cells caudal to the artery. These two ganglia are connected by numerous nerve strands, some containing ganglion cells, which form a ring round the inferior mesenteric artery (Fig. 3). The inferior mesenteric ganglia are macroscopically single structures but according to Langley are really paired but lying within a single connective tissue sheath. The pre-ganglionic fibres synapse in this ganglion and the post-ganglionic fibres for the colon leave by two routes:-

1) The lumbar colonic nerves. These lie between the inferior mesenteric artery and the lumbar colonic vein and accompany these vessels to the colon. They are joined at intervals by the parasympathetic fibres of the sacral colonic nerves with which they are distributed. The lumbar colonic nerves as a consequence contain, except at their origin from the ganglion, both sympathetic and parasympathetic fibres. This is the route taken by the sympathetic fibres in the innervated preparation of the colon to be described later.

2) The ascending mesenteric nerves. These form a large bundle of nerve filaments which pass cranially through the mesocolon from their origin in the inferior mesenteric ganglion. Their branches to the colon pass straight through the mesocolon. The ascending mesenteric nerves end by anastomosis with the superior mesenteric and coeliac plexuses.

Another important branch of the inferior mesenteric plexus is the hypogastric nerve. This starts as an apparently single structure from the small ganglion mass caudal to the inferior mesenteric artery. It passes caudally in the midline and divides a variable distance above the pelvic inlet into the right and left hypogastric nerves. These enter the pelvic cavity and almost immediately divide into a ventral and dorsal division. The ventral division swings forward to end in the bladder mainly, while the dorsal continues caudally to unite with the cranial strand of the

pelvic nerve at the ganglion uterinum. The most important anatomical branches of this nerve before it joins the pelvic plexus are the sacral colonic nerves: physiologically these are para-sympathetic in nature. It also supplies a few fibres to the ureters and the aortic plexus. In the rabbit it receives no additional direct contribution from the lumbar sympathetic ganglia. Langley (1896b) describes one collection of ganglion cells on this nerve just before it divides into the right and left hypogastric nerves. Examination of serial sections of this region (Fig. 5) or, a less tedious method, examination of the whole nerve stained by gold chloride, showed numerous collections of ganglion cells along its whole length but particularly in the first cm or so below its origin where they could easily be made out with a hand lens. Similar collections have been described in the hypogastric nerve of the cat (Kuntz & Moseley 1935).

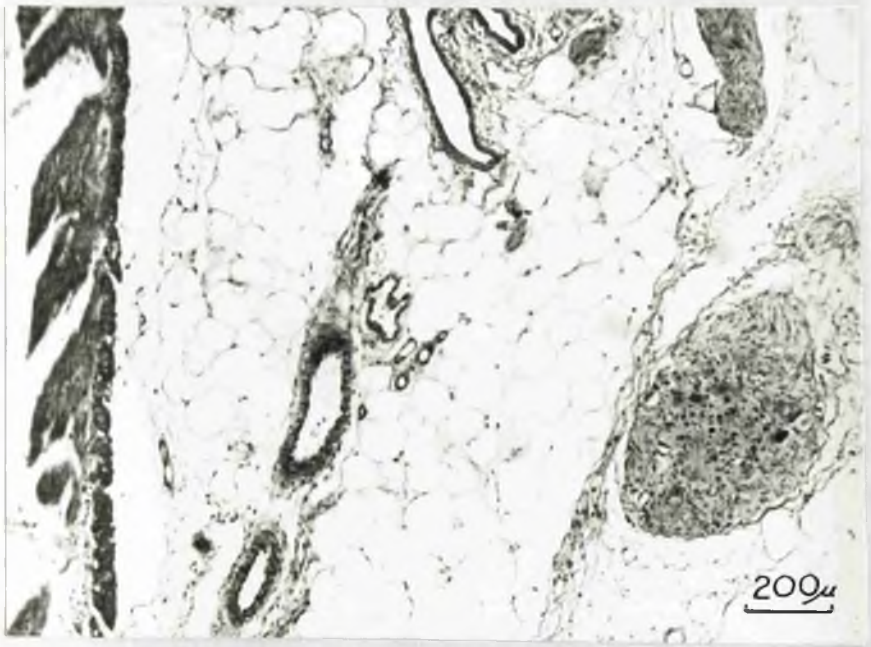


Fig. 5. The colon and mesocolon of the rabbit in transverse section, the mucosa has been stripped from the muscularis. The large nerve trunk lying in the mesocolon is the hypogastric nerve, numerous ganglion cells are visible. Auerbach's plexus can just be seen lying between the narrow longitudinal muscle layer and the thick circular muscle layer. Masson's stain.

Other branches of the inferior mesenteric plexus of less interest are:-

1) Ascending aortic filaments: These cranially directed fibres start from the upper ganglionic mass and pass through the mesocolon close to the aorta to end in the renal and superior mesenteric plexuses.

2) Branches to the testicular/ovarian artery: These often arise from a small separate collection of ganglion cells close to the inferior mesenteric ganglia.

The ramifications of the numerous branches of the pelvic and hypogastric nerves constitute the pelvic plexus. The fibre constitution of any peripheral branch of this plexus can only be determined by histological examination after degeneration of one or other of the outflows. In this way it has been shown that the sacral colonic nerves, branches apparently of the hypogastric, a sympathetic nerve, are derived from the pelvic nerve; certain of the median branches of the pelvic nerve in fact are derived from the hypogastric nerve (Langley 1896a, b). The connections of the numerous small groups of ganglion cells found throughout the plexus are probably, as in the cat (Kuntz & Moseley 1935), both sympathetic and parasympathetic. The cell station of the efferent parasympathetic fibres for the colon however are in the plexus of Auerbach.

H I S T O L O G Y.

Several staining methods were used to demonstrate the intra--mural nervous elements. The best of these was the modified Bielschowsky-Gros silver staining by the method described by Garven & Gairns (1952). Material from three animals was stained by this method. Two other animals were tried with gold chloride staining (Gairns 1930); this gave a good picture of the general arrangement of the plexuses but the detail of individual cells was insufficient to identify them or to study their relations with one another. Intra-vital staining by methylene blue infusion after the method of Schabadasch was carried out in three animals. The interpretation of the results of this method I found difficult, partly no doubt because of the small number of experiments. This is the method above all, however, for demonstrating the presence of those much debated cells, the sympathetic interstitial neurones of Cajal. Their presence in the stomach, small intestine and in the colon was confirmed. Transverse sections of the gut wall from one animal were stained with Masson's triple stain to demonstrate the position of the nerve plexus between the muscle layers and the relative thickness of the two muscle coats.

In preparing the tissue for the Bielschowsky-Gros staining lengths of colon and small intestine were removed from the animal as quickly as possible after its death. These were then distended with the fixative, 12% neutral formol, and immersed in a large

bath of the same solution. The material was left in the fixative for three days and then suitably sized areas of the wall cut out and the mucosa stripped off with forceps. In a few preparations the two muscle coats were separated. This is a simple procedure in the small intestine where the longitudinal muscle can be removed, if need be, from the whole diameter of the bowel. In the colon the longitudinal muscle is a very thin layer, incomplete at places and difficult to strip from the underlying thick circular coat. When such muscle stripping was successful on the colon Auerbach's plexus was found to adhere mainly to the circular coat in contrast to the small intestine where almost the entire plexus remained on the longitudinal layer. The preparations were therefore usually of the complete muscularis and serosa. They were further trimmed into small rectangular pieces, the long axis of the rectangle parallel to the circular muscle fibres. This reduced the tendency of the preparations to "curl" on being put into the silver nitrate solution.

As Meyling (1953) has said, "A clear and indisputable description of the organisation of the nervous apparatus of the intestines, based on histological investigations, does not yet exist". This, one would expect, would be a severe handicap in interpreting the results of physiological and particularly of pharmacological experiments. But this is not so, just because a convenient histological organisation, based on the classical neurone theory, is assumed! It is very doubtful if this classical theory can be made to fit, without drastic modification, the

microscopical appearances. What then becomes of our conventional interpretations?

The core of this problem of innervation is whether or not the nerve fibres retain their individuality from their origin in the prevertebral ganglia or Auerbach's plexus, right to the periphery, or whether they lose their identity in some form of terminal nerve net which is the final common pathway between post-ganglionic nerve fibres on the one hand and the effector cells on the other. This problem in turn depends on the interpretation placed on those cells called by Cajal the "neurones sympathicque interstitielles". These are small cells and are found throughout all the tissues of the body which are innervated by the autonomic nervous system. They have numerous processes which are in protoplasmic continuity with one another forming a syncytium. The protoplasm is fibrillar in structure. Histologists differ as to whether or not these cells are nervous in nature; they are uncertain about their relations with the post-ganglionic autonomic nerves. Some of these views can be summarised as follows:-

1) These cells are non-nervous, probably connective tissue in nature and have no direct connection with the autonomic nervous system (De Castro, 1929; Johnson & Palmer, 1931; Nonidez, 1944).

2) They are Schwann cells or lemmoblasts. They form a syncytium within which the neurofibrillae of the post-ganglionic fibres are conducted to the effector cells. The post-ganglionic fibres do not lose their identity within this

syncytium (Lawrentjew, 1934; Hillarp, 1946).

3) They are true nerve cells forming a syncytium in which post--ganglionic sympathetic and parasympathetic as well as sensory afferent fibres lose their identity. This syncytium has been variously named the sympathetic ground plexus (Boeke, 1940), the Terminalreticulum (Stöhr Jr. 1955), the autonomic interstitial cell nerve net (Meyling, 1953). Although these various workers are grouped together because of this common conviction that there is a terminal nervous syncytium interposed between the individual autonomic nerve fibres and the innervated tissue, their conception of the organisation of the intrinsic nerves of the gut differs widely in other ways.

Objections to the nervous nature of these cells which have been put forward are:-

1) Those stains used to demonstrate them (methylene blue, silver) are non specific and will eventually stain almost any tissue so that a subjective element is introduced in determining at what stage specificity is lost.

2) Certain silver salts which do not stain nervous tissue will stain these cells.

3) Reticular cells are argyrophilic and naturally would show processes forming a reticulum.

4) The cells and processes may be stained with connective tissue stain.

The weight of evidence, particularly the work of Leeuwe (1937)

favour their nervous nature. This can be summarised as follows:-

1) Many experienced histologists from Cajal onwards have thought that morphologically these cells resembled most closely small ganglion cells.

2) They stain with those stains which are most selective for nervous tissue.

3) They contain neurofibrils and these surround the cell nucleus unlike Schwann cells where the nucleus is applied to the outside of the neurofibrillar bundle (Leeuwe, 1937; Meyling, 1953; Stöhr Jr. 1941).

4) They contain Nissl granules which not only stain specifically but show the chemical reactions of this substance (not dissolved by fat solvents, acetic acid or gastric juice, easily dissolved by 1% KOH or pancreatine solution) (Leeuwe, 1937; Meyling, 1953).

5) Peroxidase and oxidase ferments are present as in nerve cells (Leeuwe, 1937).

6) The cells and processes stain positively for di-phenols, presumably adrenaline (Leeuwe, 1937).

7) These cells develop from neuroblasts and their neurofibrillae develop independent of connections with the more centrally placed ganglion cells.

Dogiel (1896) described two types of nerve cell in Auerbach's and Meissner's plexus and later in 1899 three types. The type I cell was smaller than the others. It had a large number (up to 20)

of short thick branching dendrites which anastomosed in the ganglion of origin with the processes of similar cells and a single long axone which he thought he could trace to the smooth muscle layer. The vagal fibres formed pericellular apparatuses around these cells and they were motor in function. The type II cells were in general larger and had several (up to 10) long processes. It was difficult to distinguish an axone among these. The dendrites, although showing none of the stubby processes of the type I cell, did divide in a dichotomous fashion which the axone never did, the axone was also more slender than the dendrites. All of these processes left the ganglion of origin, the dendrites to the submucous layer, the axones to pass through numerous other ganglia and fibre tracts giving off delicate collaterals to ganglion cells as it passed. Dogiel believed these cells to be sensory in function and provide the afferent side of local reflexes in these plexuses. The type III cell in form and size resemble the type II according to Dogiel (1899) although he has been quoted as comparing them with type I (Hill 1927). They are found anywhere in the ganglion, often in the short thick bundle connecting adjacent ganglia. Their dendrites (up to 10), like type I cells, give off large numbers of short branches but the dendrites themselves are many times longer than those of type I. Like type I cells, however, they are limited to their ganglion of origin within which they form a network unconnected to the other ganglion cells. Dogiel never successfully followed the axone to its termination. He made no suggestion of the function of these cells and subsequent authors have tended to

ignore this type altogether. His illustrations of type III cells do look like type I cells so that Hill's interpretation of his views may have some justification. Of interest is the appearance of a definite claw like appearance of the terminations of the dendrites.

Most investigators are agreed that cells corresponding to Dogiel's types I and II can be identified in the gut wall but their relationship with the extrinsic nerves and their function is disputed. A summary of some of these views is given below.

Author.	Functions.		Relationship to
	Type I.	Type II.	Extrinsic Nerves.
Dogiel (1899)	motor	sensory	vagus ends round type I
Cajal (1911)	motor	motor	-
Hill (1927)	intercallary	motor	vagus ends round type II
Müller E (1921) } Leeuwe (1937) }	motor	inhibitor	vagus ends round type I sympathetic ends round type II
Lawrentjew (1931)	motor	spontaneous activity	vagus & pelvic nerve end round type I
Stöhr Jr. (1941)	not possible to decide function		-
Kuntz (1922) }	not possible to divide		-
Johnson & Palmer (1931) }	the cells into two types		-

The majority of investigators, particularly those on the Continent, attribute a motor function and a parasympathetic connection to the type I cell. An interesting correlation is possible between Irwin's (1932) observations that the density of

ganglion cells is at its maximum in the pylorus of the stomach, falls markedly in the small intestine to rise again in the large intestine and rectum, and Lawrentjew's (1931) findings that type I cells predominate in the stomach while type II increases steadily in the small intestine and amounts to 50% of the cells in the ileum. The proportion of type I cells increases once more in the large intestine and in the rectum these are almost the only type of cell found. Lawrentjew relates this varying distribution of the two types of cell along the length of the alimentary tract, the cytoarchitectonics of Auerbach's plexus as he calls it, to the influence of the vagus and pelvic nerves. These nerves are distributed mainly to the stomach and terminal colon; section of either is followed by degeneration of pericellular endings around type I cells. These type I cells he suggests have migrated along the parasympathetic pathways and superimposed themselves on the type II cells derived from the neural crest region. Further evidence for this is that type I cells can be found in the vagus trunk itself alongside the oesophagus. These he believes are cells which failed to complete their migration (Lawrentjew, 1929). That the stomach and large bowel are more dependent on the extrinsic parasympathetic nerves than the small bowel would agree well with everyday experience. The visceral regions affected by alterations in emotional states are typically stomach, bladder and rectum. It would also explain the heavy incidence in the stomach

and colon of those diseases of the alimentary tract believed to be due to an abnormal pattern of activity in the extrinsic nerves secondary to some psychological disturbance (ulcerative and mucous colitis, pylorospasm, peptic ulcer). Another feature in the distribution of these cells is the predominance of type II cells in Meissner's plexus (Hill, 1927; Kuntz, 1922; Lawrentjew, 1931).

Personal observations. In the rabbit colon most of the structures described by other investigators were found. The limited amount of material examined probably explains the inability to identify certain features such as the subserous plexus. For the same reason only tentative conclusions can be drawn on this complex problem of the intrinsic nervous organisation within the bowel wall.

The plexuses of the colon. The familiar Auerbach's and Meissner's plexuses were present in their accustomed positions, Auerbach's between the muscle layers, Meissner's in the submucosa (Fig. 6). From Auerbach's plexus fibres were given off which, running among and parallel with the muscle fibres, formed the intramuscular plexus. This was well developed in the circular muscle and poorly developed in the longitudinal, a reflection of the amount of muscle in the two layers. The general plan of Auerbach's plexus in the colon differs little from that in the small intestine as can be seen on comparing the two (Fig. 7). The difference between these regions lies in their cell content.

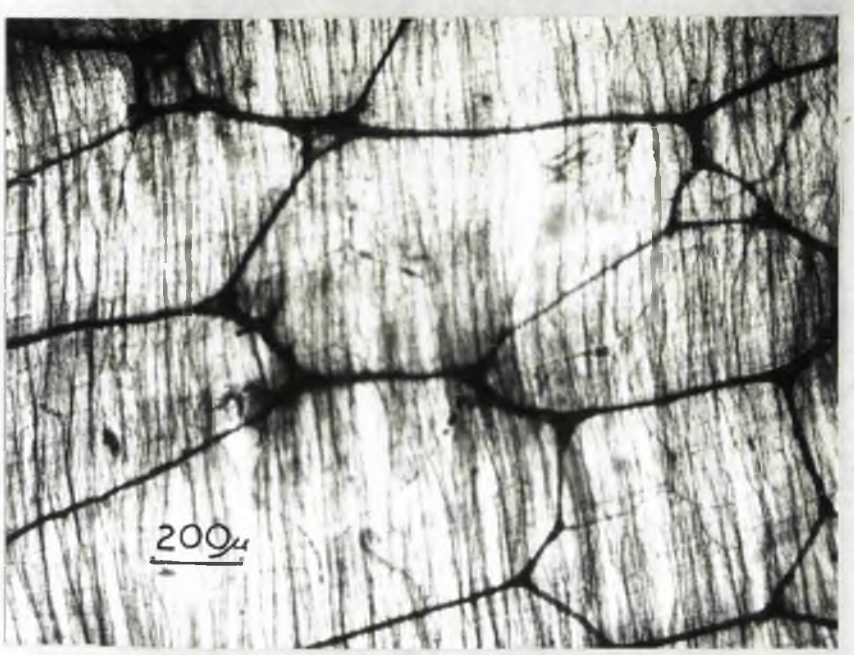
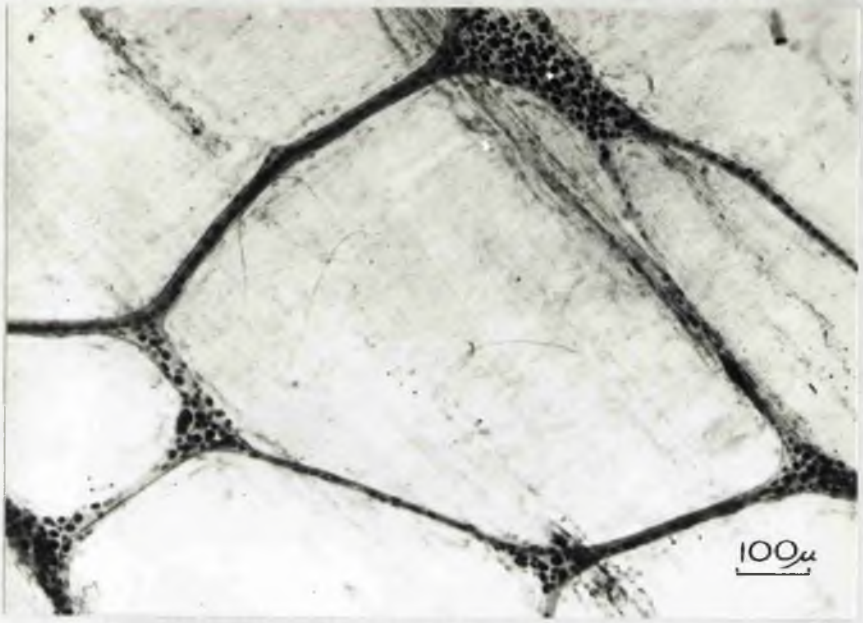
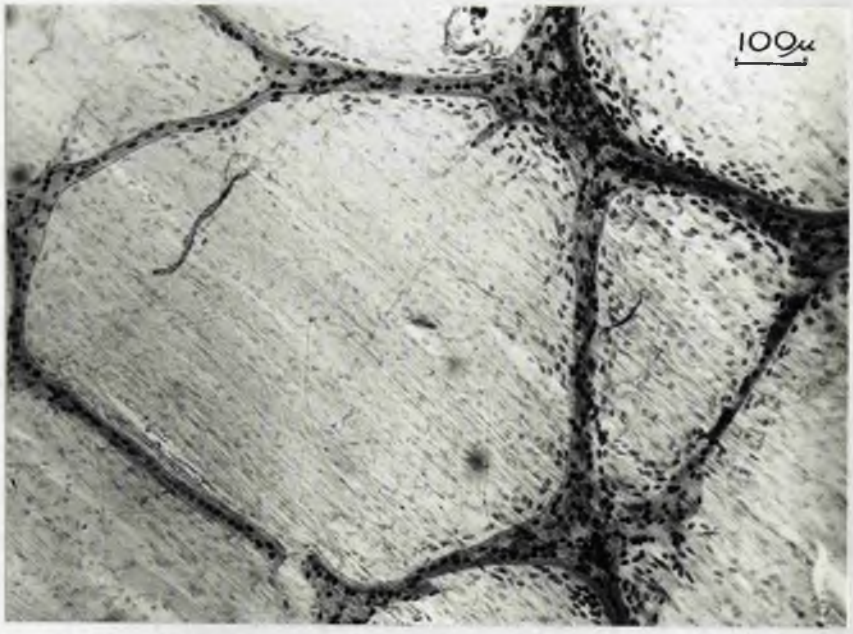


Fig. 6 Colon of the rabbit, gold chloride staining. The thick heavily stained strands of Auerbach's plexus are shown and between them another more delicate plexus also containing ganglion cells can be seen. This is the submucous plexus of Meissner.

In the material examined, although the other plexuses were well stained, no clear evidence was found of the subserous plexus described by Hill (1927). The syncytial terminal reticulum formed by the autonomic interstitial cells was seen in the specimens stained with methylene blue.



Colon



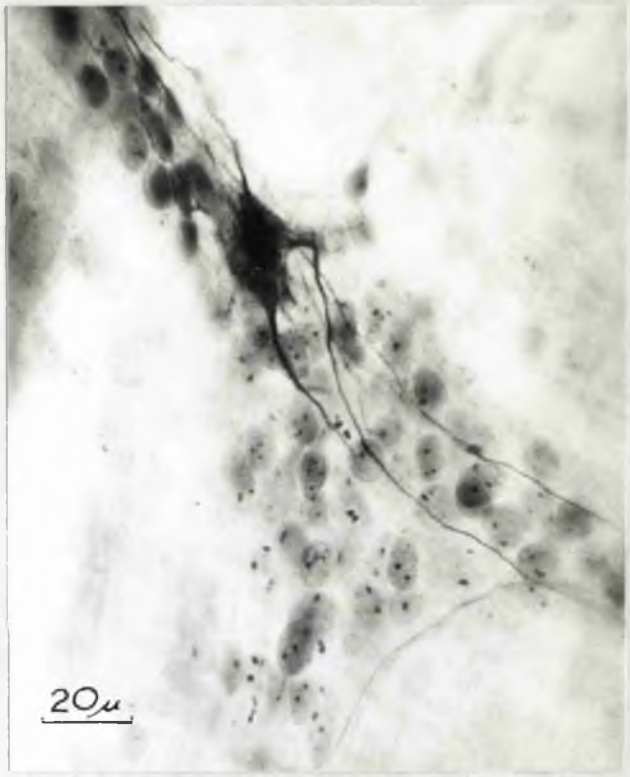
Small intestine

Fig. 7 Auerbach's plexus in the colon and small intestine of the rabbit. Bielschowsky-Gros staining.

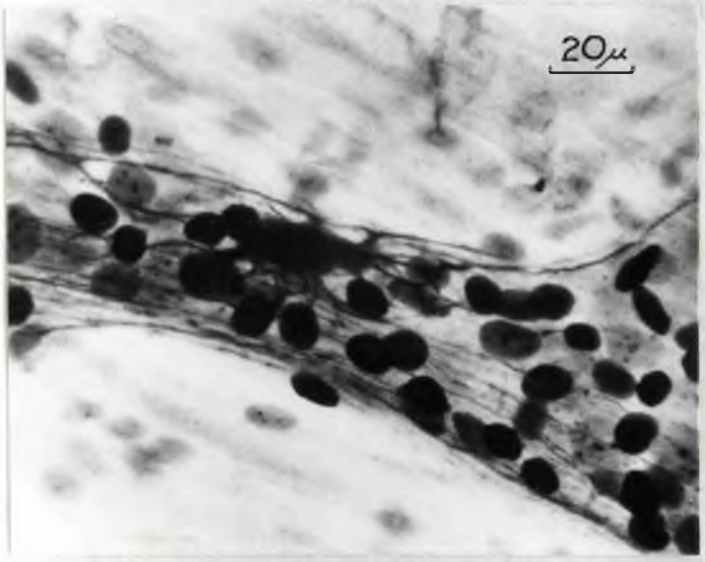
Ganglion Cells. These were very clearly impregnated by the silver method, their processes could be followed for long distances. Every ganglion cell did not stain however, some remaining as pale areas. The ganglion cells could be divided into type I and type II cells according to the criteria of Dogiel. The same difficulty which probably led him to recognise a type III cell arose in interpreting the present material. Some of the cells were typical type I, crab-like in appearance with very short branching dendrites appearing from the entire circumference of the cell and ending in the immediate vicinity, and one long axonal process which could be followed for long distances through the plexus. Typical type II cells were also found, though less frequent in the colon than type I. These had several (up to 6) long processes any of which might have been the axone. These processes showed none of the short stubby branching or "claw" formation of the other cell types but presented as smooth "clean" nerve processes which, if they did divide, did so in a dichotomous manner. Examples of these two types of cell from the colon and ileum are illustrated in Fig. 8.

Type I Colon

Type II Colon



Type I Ileum



Type II Ileum

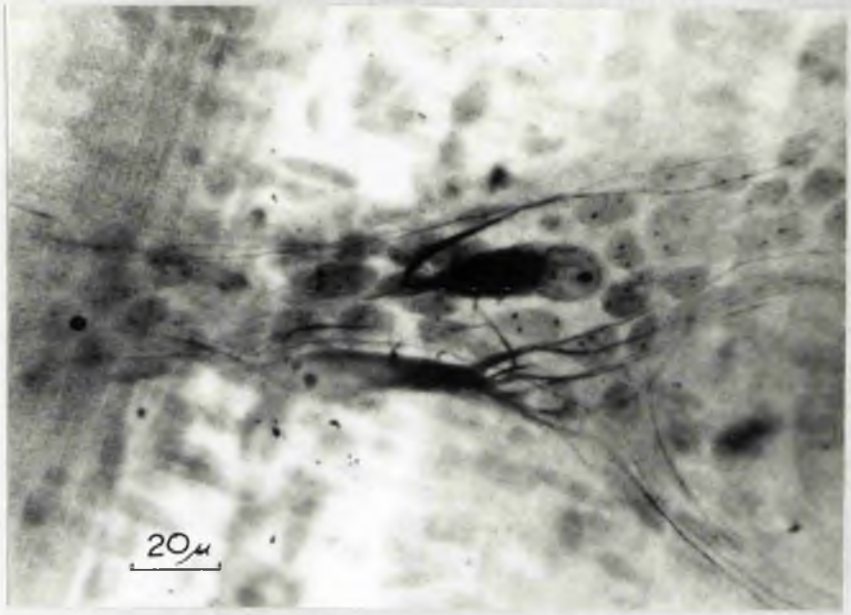
Fig. 8 Dogiel's type I and type II cells in the small intestine and colon of the rabbit. Bielschowsky-Gros staining.

In addition to these two types there was a third, the most common of all in the colon, which corresponds closely to Dogiel's type III. This cell was large. It had numerous processes some of them quite long but if traced under the 2 mm oil immersion objective they were limited to the ganglion in which the cell body lay or to limited extensions into the fibre tracts in the immediate neighbourhood. These cells had usually one process, the axone, which could be followed for long distances through the plexus. The dendritic processes typically showed multiple branching which terminally formed large "claws". Examination of these under the oil immersion lens showed that they ended in brush like arborisations usually in relation to the numerous non--ganglionic "sheath" and "neighbouring" cells. The dendrites often seemed to pass to the surface of the ganglion and then to bend round the edge giving an appearance of binding the structure together. An excellent example of this type of cell is illustrated in Fig. 9 and shows most of the features described. Because of the multiple branching of its dendrites, their limitation to the region of the ganglion and the presence of a single long axone these cells have been included as type I. As Dogiel observed, the large size and multiple long dendrites of these cells can on occasion give difficulty in distinguishing them from type II cells. Nevertheless,I feel that they should be regarded as variants of type I cells which are the most characteristic and

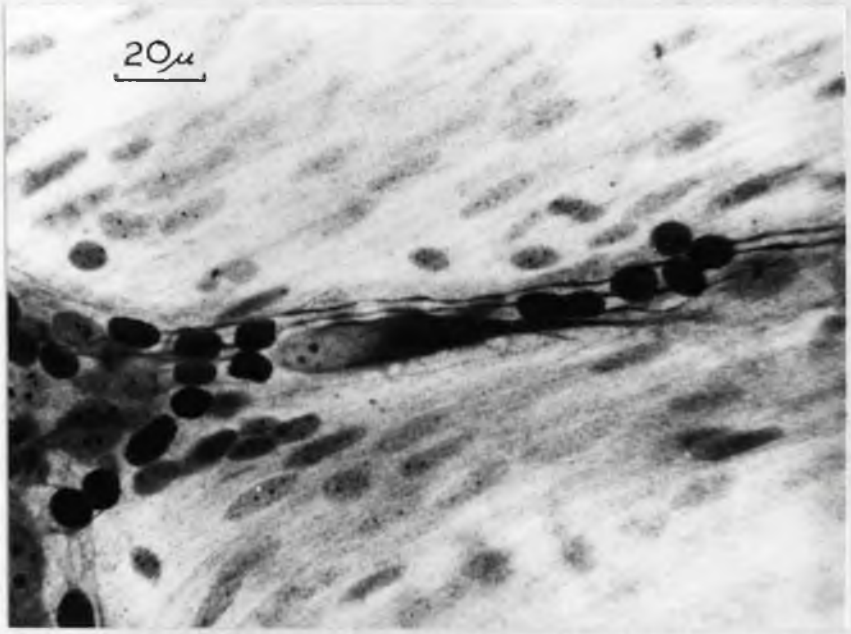
easily recognised of all the cell types in this region.

Fig. 9. A type I cell corresponding to that described by Dogiel as type III from the rabbit colon showing the claw shaped terminations of the dendrites and also dendrites ending in close relationship with sheath cells (arrowed). Bielschowsky-Gros staining.

Elongated pear shaped unipolar and bipolar cells were also seen although rare compared to the multipolar types. These, according to the criteria adopted, were grouped as type I cells to which group Dogiel (1899) also assigned them. They have numerous very short processes from the cell soma and one long well defined axone. C. Hill (1927) grouped these with the type II cells and did not illustrate any of the very short processes leaving the cell soma. An example of a unipolar cell is shown in Fig. 10 in which these short processes can be seen.



Colon



Ileum

Fig. 10 Unipolar ganglion cells in the small intestine and colon of the rabbit. Bielschowsky-Gros silver staining.

Lawrentjew's observation that, in the dog, type I cells predominate in the rectum while, in the ileum, type I and II cells are about equally common was confirmed in the rabbit. A majority of the cells in the region of the colon examined (equivalent to the rectum) were found to be type I.

Ganglion cells were not the only cell type found in the ganglia of Auerbach's plexus. In silver stained preparations large numbers of round or oval vesicular nuclei with well marked nucleoli were visible (Fig. 11). At first these were thought to be the nuclei of ganglion cells which were incompletely stained. Their number however made it unlikely that, if this were so, there would be sufficient space in the ganglion for all of the cell bodies. Convincing evidence that these cells were not ganglion cells was obtained from one preparation in which the ganglion cells were unstained but their presence revealed by a corona of their processes which were stained. The appearance was of a large pale area with a very pale nucleus recognisable by its nucleoli only. Around this area were the nuclei of these other cells and penetrating between them and appearing to end in relation to them the short processes of the nerve cell (Fig. 11). This was confirmed in numerous other preparations and there is no doubt that the short dendritic processes of type I cells seemed to end in relation to these sheath cells (see Fig. 11).

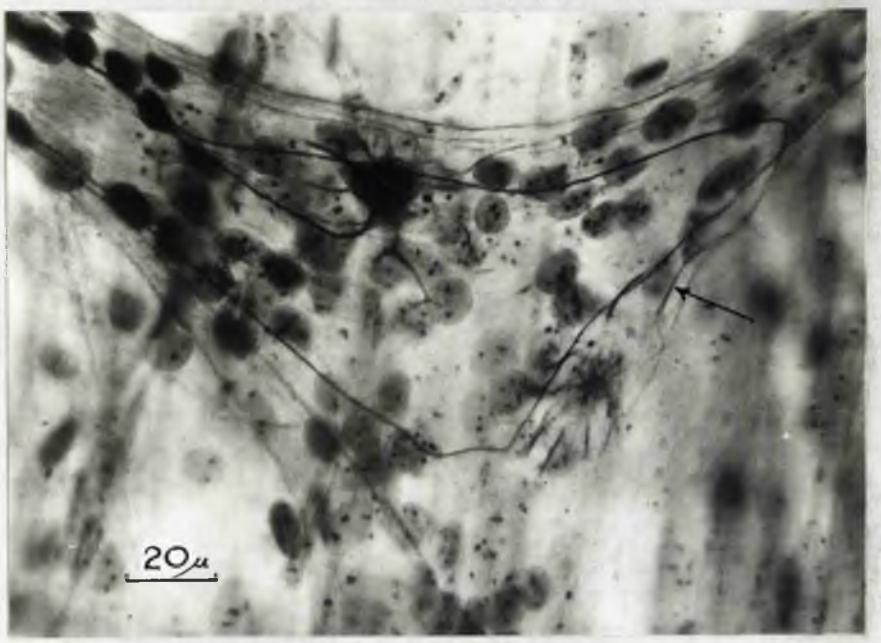


Fig. 11 Colon of the rabbit. Bielschowsky-Gros silver staining. The processes of a type I ganglion cell (right centre) have stained but the cell soma can be seen as a pale area with some light central staining. The cell axone is arrowed.

The nuclei of these cells were smaller and stained more strongly than those of the ganglion cell. They were of two types, some small, oval and dark staining, others larger, round and pale staining. They were present in Auerbach's plexus in both the large and small intestine. Their cytoplasm was not stained. They were not Schwann cells whose elongated, rather granular and intensely staining nuclei could be seen elsewhere in the preparations. (Fig.15). According to Stöhr Jr. (1941) Schwann cells stop short at the periphery of the ganglia. These cells are identical with those described and

illustrated by Stöhr Jr. (1941) and which he called "Nebenzellen". These he divided into those forming a sheath around the ganglion cells, the "Hüllplasmodium" or sheath syncytium and those groups of cells lying between neighbouring ganglion cells, the Nebenzellen proper. There was no histological difference between the two. These cells formed a continuous syncytium with one another and with the ganglion cells. The processes of the ganglion cells ended in protoplasmic continuity with these cells. Stöhr Jr. (1955) suggests that they may have a secretory function. This revolutionary conception of the peripheral autonomic system as a neuro-secretory apparatus will be discussed later. It is sufficient for the moment to say that cells identical with those illustrated by Stöhr Jr. were seen in these preparations both in the colon and in the small intestine, and that, as he describes, the short processes of the type I cell appear to end in relation to them. The nuclei of these cells were all observed to be in the same plane in these preparations of the rabbit gut, and none overlapped the ganglion cell. It appears therefore that if there is a continuous syncytial sheath around the ganglion cell the nuclei of this syncytium must form a ring around the equator of the cell. Similar cells have been recognised by other histologists and variously named. Capsule cells, neurilemmal cells, mantle cells, Scheidenzellen, amphicytes, satellite cells, Belegkerne and Nebenzellen all occur in the literature. Hill (1927) was unable to

see these cells and none is illustrated in her drawings. She does however mention and illustrate processes of ganglion cells which end in the spaces between adjacent cells.

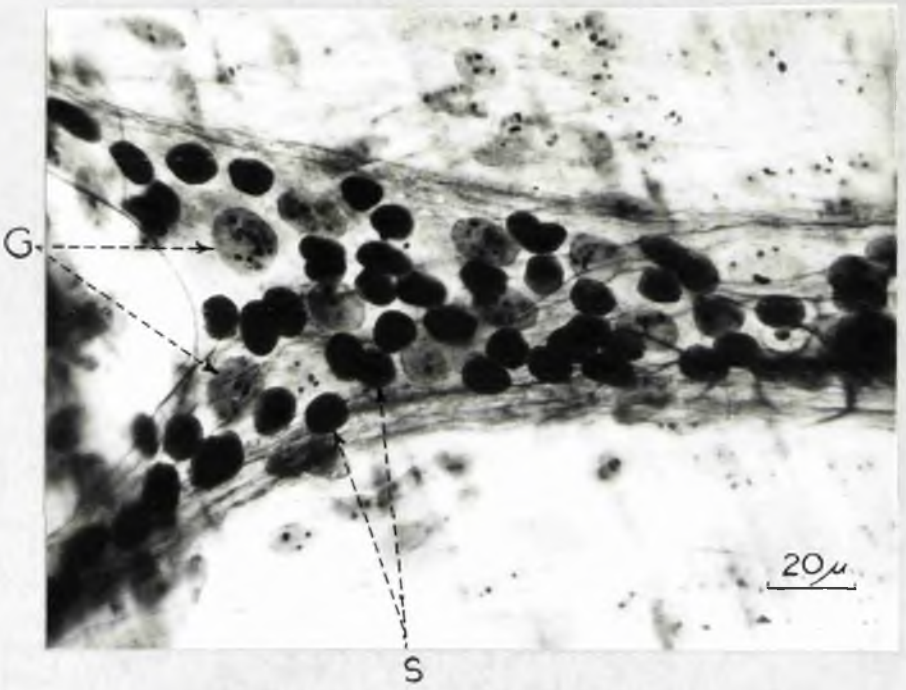
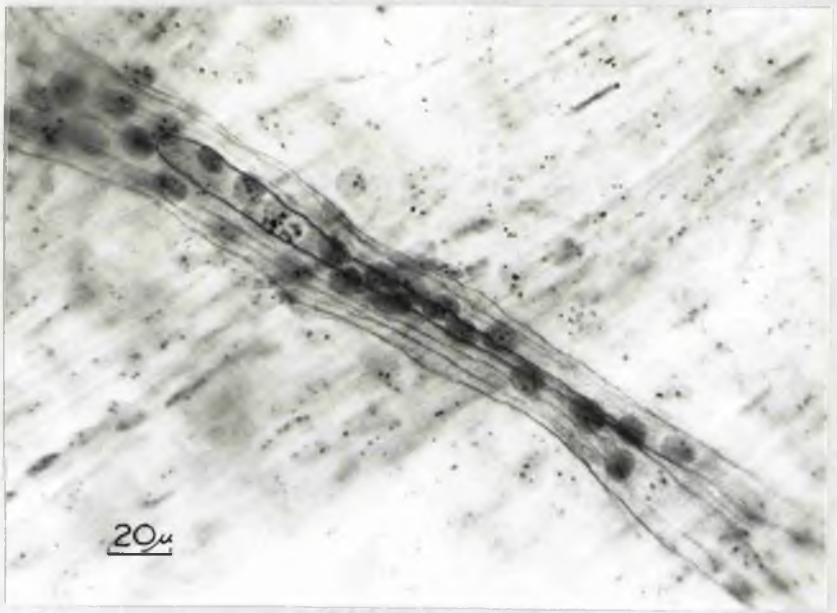


Fig. 12 Colon of the rabbit. Bielschowsky-Gros silver staining. The nuclei of the sheath cells, S have stained particularly densely. Their distribution at the periphery of ganglion cells which themselves are unstained except for their large pale nucleus, G is well shown. The ganglion cell nuclei are larger and fewer than those of the sheath cells.

An attempt was made to follow the axonal processes of the ganglion cells. This was unsuccessful. No single axone was

was followed from its parent ganglion cell to its ending on another ganglion cell or in the muscle coats. This does not mean that these long processes were badly stained or difficult to follow. On the contrary they were often boldly stained and easily followed.



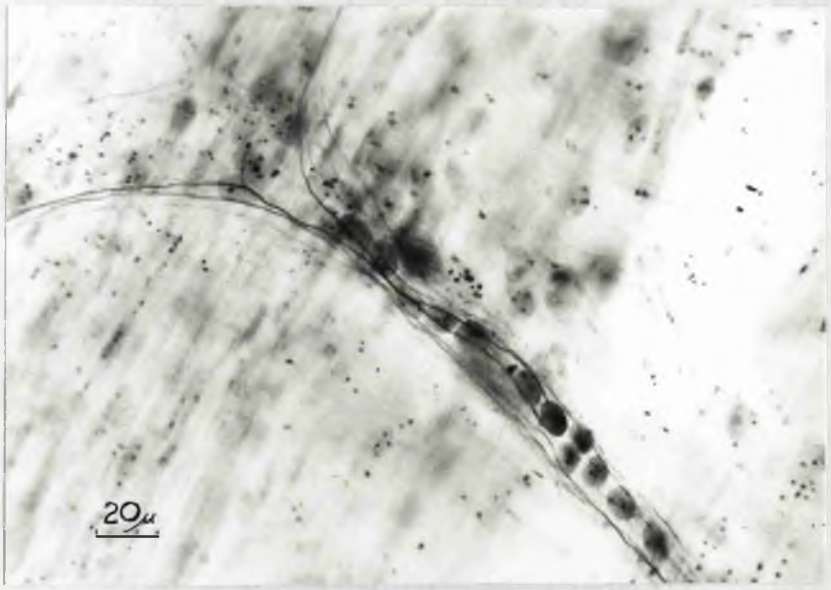


Fig. 13 Colon of the rabbit; Bielschowsky-Gros silver staining. Twice in the course of the same fibre it enters a fibre tract but after a variable distance doubles back to enter some other part of the plexus.

The difficulty was the distance they travelled through the ganglionic plexus. Axones of type I cells were followed for long distances through fibre tracts and ganglia. Often they passed near other ganglion cells but never ended. Always they were finally lost by coming to the cut surface of the preparation or by becoming associated with several other fibres so closely that it was impossible to say which was which when the fibres separated. In their course these fibres would sometimes enter a fibre tract, run along it for a considerable distance, and then double back to enter some other tract. On occasions more than one such loop was observed in following one axone (Fig. 13). The fibres always tended to travel in the periphery of the fibre tracts. Dogiel (1899) confessed himself unable to follow the axone of his type III cell (included here as type I) and Stöhr Jr. (1941) states that no-one has been able to bring indisputable evidence of the mode of ending of type I cells.

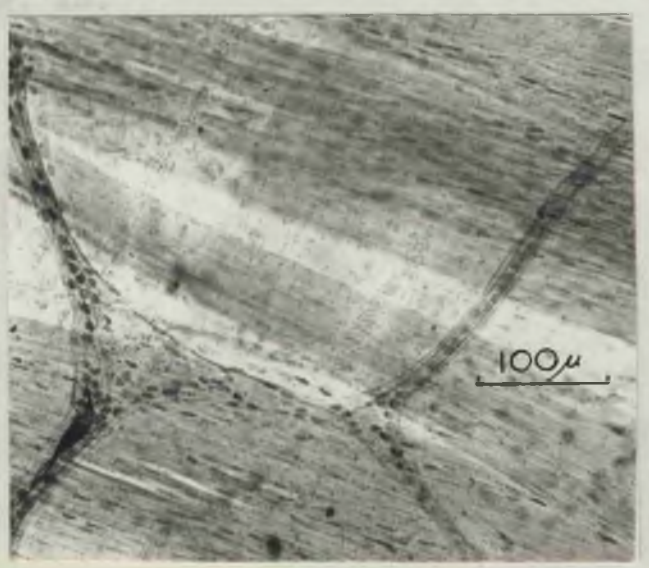


Fig. 14. The colon of the rabbit; Bielschowsky-Gros staining. The nerve fibres both in the fibre tracts and in the ganglion tend to occupy the periphery.

The inability in the present investigation to follow these axones to their termination may be related to the predominance of type I cells in the colon. In following these axones within the plexus it was essential to use the 2 mm oil immersion lens. Although the fibres were often quite large and easily seen under a dry lens the limited plane of focus of the 2 mm objective allowed fibres which ran across or close to one another to be identified correctly. On several occasions a fibre which under the dry high power lens appeared to end on a ganglion cell was shown in fact to be continued, though possibly staining more or less intensely, beyond the cell.

Fibres were seen to leave the plexus and enter the smooth muscle coats but these never showed discrete nerve endings. Often they could be followed as single nerve fibrils, enclosed in the cytoplasm of Schwann cells, through the muscle coat to re-enter another part of the plexus. Such a fibre is illustrated in Fig. 15.

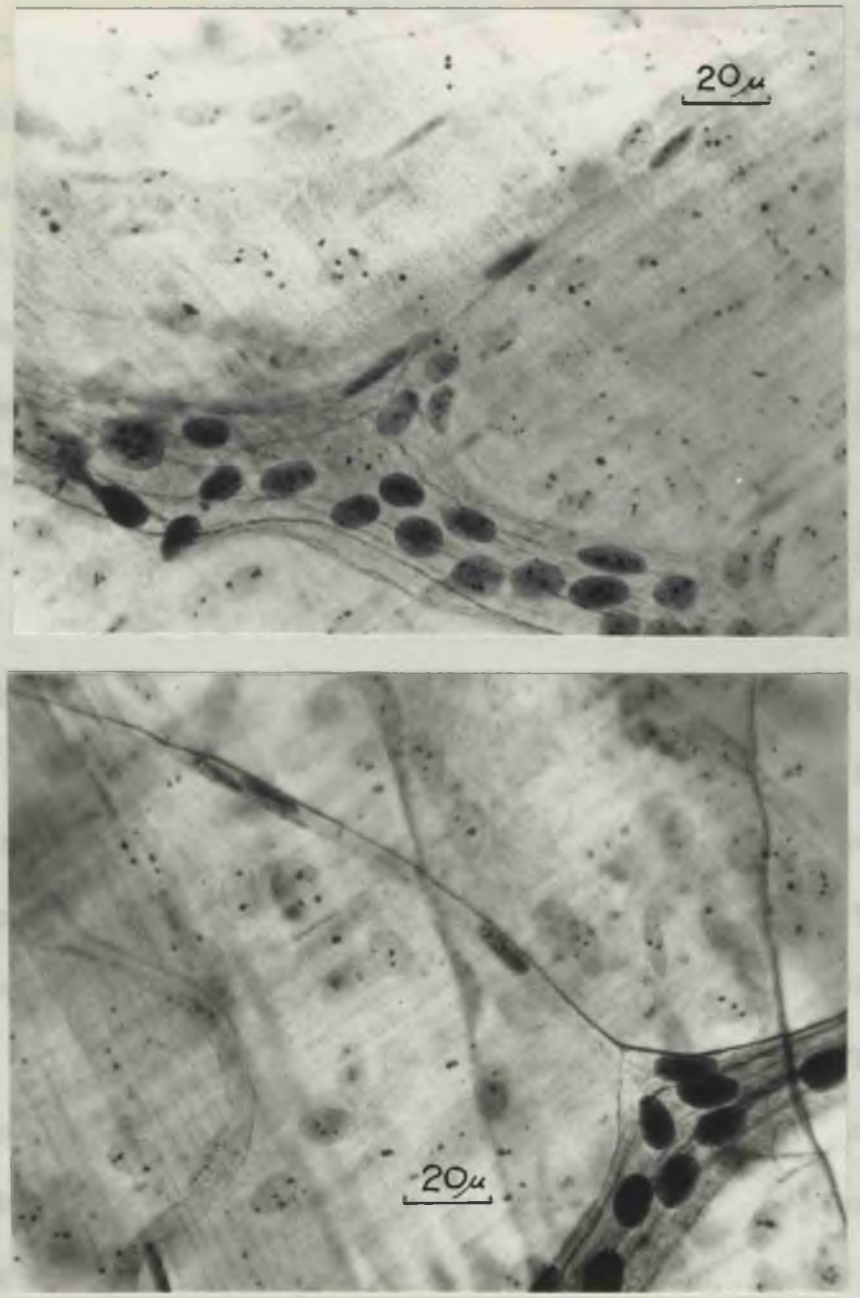


Fig. 15 Colon of the rabbit. Bielschowsky-Gros silver staining. Single nerve fibres can be seen leaving Auerbach's plexus and entering the muscle coat. The elongated nuclei of Schwann cells can be seen accompanying them and in the lower illustration the cytoplasm of these cells can just be made out, forming a sheath around the fibre. The nuclei of the Schwann cells are quite different from the nuclei of the cells lying within the plexus.

Langley (1922) has pointed out that there is a great disparity between the small number of vagal fibres to the small intestine and the large number of ganglion cells in the plexuses of this region. He argued that it was impossible for all of these ganglion cells to be innervated by separate nerve fibres. He postulated the presence of a few "mother" cells directly innervated and these in turn innervating a large number of "local" cells from which the fibres to the effector cells arose. Lawrentjew explained this same discrepancy by assuming that each extrinsic vagal fibre divided several times to innervate a group of cells. This he called the multiplication phenomenon (Lawrentjew, 1931). It is not easy to imagine sufficient branching of the extrinsic fibres to innervate the "hundreds of thousands" of nerve cells referred to by Langley. If Langley's explanation is correct one would expect to see bifurcation of nerve axones in the intrinsic plexuses. This was specifically looked for in the present preparations and found beyond any doubt. This of course does not prove in any way Langley's theory but it is a pre-requisite for that theory.

Relation of extrinsic nerves to Auerbach's plexus. The extrinsic nerves, containing both sympathetic and parasympathetic fibres, could easily be followed into the plexus but it was not possible to trace their further connections. These extrinsic nerves were easily recognised by their solid, rounded, intensely staining appearance; the fibres within the nerve were wavy and

accompanied by dark staining Schwann cells. These Schwann cells continued for some distance into the plexus but eventually disappeared or at least lost their close connection with the nerve fibres. The entry of an extrinsic nerve is illustrated in Fig. 16.

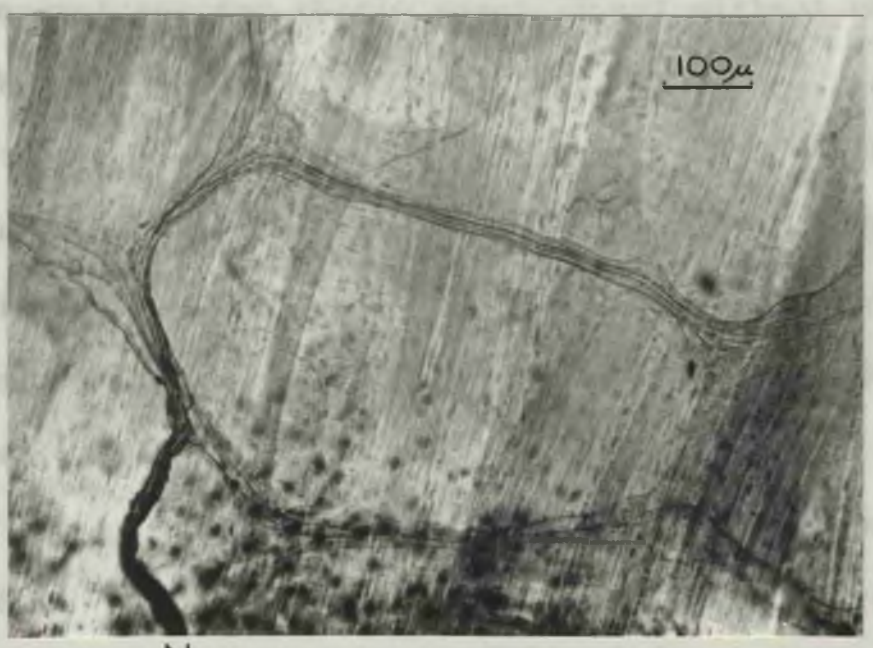


Fig. 16 Rabbit colon; Bielschowsky-Gros silver staining. A large extrinsic nerve N can be seen entering Auerbach's plexus and sending fibres into several fibre tracts.

D I S C U S S I O N.

The description of the innervation of the gut most commonly taught in this country is that of C. Hill (1927). It is rather difficult to understand at first sight why this scheme has remained in general use for thirty years almost without modification in spite of the fact that much subsequent observation by experienced microscopists was inimical to it. The explanation may be twofold. First, the scheme is built up on the classical neuronal theory with nerve cells linked to one another synaptically, post-ganglionic fibres innervating effector cells and sensory fibres derived from the two surfaces, luminal (mucosa) and serosal, at which environmental changes might be expected. This type of organisation fits well with that of the somatic and central nervous system and so has the attraction of all large generalisations. Secondly, and perhaps more important, this scheme explains best the experimental findings of physiologists and particularly of pharmacologists. Drugs which block nerve conduction specifically at the synapse or at the periphery or which stimulate sensory nerve endings are referred to regularly in the literature. The great disadvantage of Hill's theory is the increasing gap between it and the histological findings. Her illustrations show nerve cells which have less profuse branching than in fact exists, there is no illustration of these sheath cells which form such a conspicuous

part of the ganglion and it is hard to avoid the conclusion that impregnation was incomplete.

Those theories which invoke some form of terminal reticulum as the final common pathway for all of the post-ganglionic fibres going to the smooth muscle and glands, as well as the sensory fibres arising in the bowel wall (Leeuwe, 1937; Boeke, 1940; Meyling, 1953; Stöhr Jr. 1955) have rarely taken account of the functional problems of such a synctium. This network has been compared to the primitive nerve net in invertebrate animals (Meyling, 1953). This ignores the increase in complexity of the responses mediated by the nerve net in the gut wall. In the invertebrate stimulation at any one point is followed by a centrifugal spread of the impulse throughout the whole nerve net and a response of the entire organism. The impulse is required to spread uniformly through the unpolarised nerve net. There is no need for localisation. In the gut there is good experimental evidence that certain responses are produced by some form of reflex mechanism with its centre in the spinal cord or in Auerbach's plexus. There is some form of sensory receptor providing the afferent side of these responses and post-ganglionic nerve fibres providing the efferent link. If both efferent and afferent fibres are simultaneously active and both traversing a common unpolarised nerve net at the periphery it would imply that this nerve net was conducting impulses in opposite directions in the same neurofibrillae.

The action of those drugs which specifically inhibit or antagonise the action of one or other division of the autonomic nervous system would also require revision if a synctium common to both divisions was accepted as the true mode of innervation. Such substances as atropine and ergot are believed to act by antagonising the appropriate transmitter substance at the periphery and thereby block the action of the corresponding nerve. If in fact the "periphery" is common to both divisions then another site of action for these drugs would have to be found which would allow discrimination between sympathetic and parasympathetic outflows.

Both Meyling (1953) and Stöhr Jr. (1955) assume that the transmitter substance of this terminal reticulum is adrenaline or sympathin, yet both suggest that this nerve net is responsible in some way for the spontaneous rhythmic activity of the gut. It is unlikely that the continual release of adrenaline would promote activity of any kind in the gut wall.

The histological findings in the present, very superficial, investigations resemble most closely those of Stöhr Jr. This histologist believes that the entire intrinsic nervous system of the gut wall forms one giant synctium, but more than that, this syncytium contains cells, the Nebenzellen, which histologically are not nerve cells. The neurofibrillae within the syncytium are continuous from one ganglion to the next and also with the autonomic interstitial nerve network (Stöhr's Terminalreticulum)

where they are in close contact with the effector cells. This Terminalreticulum is the site of neuro-effector transmission by means of Sympathin. It includes sympathetic, parasympathetic, efferent and afferent fibres (Stöhr Jr., 1955). The extrinsic nerves end not in intimate relation with the ganglion cells but with the Nebenzellen. These cells he suggests may be secretory in function, producing the transmitter substance acetylcholine. In his view the "synapse" is transferred from the ganglion cell surface to these sheath cells and "ganglion paralysis" he suggests may in reality be an inhibition of these sheath cells. This revolutionary conception although producing many problems would fit certain peculiarities of the innervation of the alimentary canal and is not without precedent. For example:-

1) There are already in the adrenal medulla and neuro-hypophysis of the pituitary examples of pre-ganglionic autonomic fibres innervating cells homologous with post-ganglionic neurones of the sympathetic (adrenal) and parasympathetic (neuro--hypophysis) divisions of the autonomic nervous system whose nervous function has been replaced by a secretory one.
2) There is a continual production of acetylcholine by the ganglion cell layer in the isolated bowel wall (Dikshit, 1938). This could be explained more easily by a low basal activity in neurosecretory cells than by continuing nervous activity.
3) If the ganglion cells are surrounded by a sheath of cells it is rather surprising that drugs and other substances gain

access to them so easily in the isolated tissue. The number of ganglion cells remaining unstained in the silver preparations and the appearance of staining proceeding inwards along the nerve cell processes in partially stained cells (Fig. 11) suggests that at least for this staining method access is not easy and that penetration through the plasmodium may be along the dendritic processes. If, however, the site of action of drugs active at ganglion cells was really the sheath cells their rapid action and the sensitivity of the tissue could be explained.

4) It will be shown later in this thesis that atropine in low concentration may reduce the response from stimulation of the pelvic nerve but that very large concentrations about 1000 times greater than this threshold concentration are needed to abolish the response completely. The response to acetylcholine added to the bath is abolished by low concentrations of atropine similar to those producing the first reduction in the response to nerve stimulation. A similar phenomenon has been reported by Henderson & Roepke (1934) in the bladder. If the effect of pre-ganglionic nerve stimulation were to liberate from the sheath cells acetylcholine part of which initiated an impulse in the neurofibrillae of the syncytium while the other diffusing outwards stimulated the smooth muscle cells directly, then this wide range of effectiveness of atropine could be explained. That effect of the transmitter which initiated

post-ganglionic nervous activity would not readily be blocked by atropine but that effect due to transmitter diffusing to the muscle cells would be as open to block as acetylcholine added to the bath.

This brief discussion contains much that is speculative but it may illustrate some of the difficulties of the present theories of autonomic innervation. Hill's hypothesis is useful, indeed throughout much of this thesis it is used, but it rests on shaky histological foundations. The newer concept, in one of its many forms, of a terminal reticulum is attractive but does not so far provide a basis for the physiological facts derived from experiment.

S U M M A R Y.

1) The anatomy of the extrinsic nerves supplying the colon is described. The sympathetic outflow originates in the lumbar region of the spinal cord. The pre-ganglionic fibres pass through the sympathetic chain to synapse in the inferior mesenteric ganglion. The post-ganglionic fibres accompany the branches of the inferior mesenteric artery as the lumbar colonic nerves. A few fibres for upper regions of the colon run free of this artery in a group of nerve bundles known as the ascending mesenteric nerves. The parasympathetic outflow arises in the sacral region of the spinal cord. The fibres leave the cord in the 2nd, 3rd and 4th sacral and sometimes the 1st coccygeal nerves. These pre-ganglionic fibres unite to form the pelvic nerves. The fibres for the colon leave this nerve as the cranial strand and unite with a branch of the hypogastric nerve. They leave this cranial strand - - hypogastric nerve complex at intervals as the sacral colonic nerves which run freely through the mesocolon to join the lumbar colonic nerves around the inferior mesenteric artery which they then accompany to the colon. The pre-ganglionic parasympathetic fibres synapse with post-ganglionic neurones in Auerbach's plexus.

2) Material from the stomach, small intestine and colon of seven

rabbits was stained to demonstrate the intrinsic nerve elements. Gold chloride staining gave a good picture of the general arrangement of the intra-mural plexuses but little detail of their constituent elements. The results of supra-vital staining with methylene blue after the manner of Schabadasch were found difficult to interpret. A modified Bielschowsky--Gros silver staining technique gave excellent results and this method was chiefly used.

3) Auerbach's plexus between the muscle layers and Meissner's plexus in the submucosa were identified. The intra-muscular nerve plexus was best seen in specimens stained with gold chloride. A subserous plexus was not seen. Autonomic interstitial cells were identified in specimens stained with methylene blue.

4) Cells corresponding to the type I and type II cells of Dogiel were seen in both colon and ileum. Other cells corresponding to Dogiel's type III were also found; reasons are given for including them in the same group as type I cells. Cells of type I pre-dominate in the colon, in fact they were almost the only type seen in this region.

5) The axones of type I cells travelled long distances through the plexus but were never followed to their termination. Fibres leaving Auerbach's plexus for the muscle coat were followed as single delicate fibrils enclosed in a Schwann cell syncytium.

These fibrils never showed discrete endings in the muscle and eventually re-entered Auerbach's plexus.

6) The majority of the cells in the ganglia of Auerbach's plexus are not ganglion cells. These non-ganglionic cells form a sheath around the ganglion cells. The short processes of type I cells appear to end in close relation to these sheath cells. They are not Schwann cells. Their possible function as neurosecretory cells is considered.

7) These findings are discussed in relation to the various theories of the organisation of the intrinsic nervous elements of the gut wall.

PART III.

THE IN VITRO INNERVATED PREPARATION OF THE COLON OF THE RABBIT AND THE EFFECT ON IT OF STIMULATION OF THE EXTRINSIC NERVES

METHODS

All experiments have all been made on *in vitro* preparations of the gut subjected to various forms of electrical and of pharmacological stimulation. To avoid tiresome repetition methods common to the remaining two parts of this Thesis will be described here; thereafter only variations peculiar to the experiments forming Part IV will be described separately.

For the doubly innervated preparation of the colon rabbits of either sex were used in a wide weight range of from 0·8 kg to 2·8 kg. The most convenient weight, avoiding on one hand the disadvantage of the small nerves of the young animal and on the other the excess of fat in the old animal, was 1·7 - 2 kg and most of the animals were in this range. The animals were stunned by a blow on the head and bled by opening the carotid arteries. The abdominal skin was incised in the midline and reflected from the underlying muscle. The inferior arch of the pubis was cleared by cutting the cartilaginous bar crossing it and also the ischio-cavernosus and pubo-cavernosus muscles. The abdomen was now opened in the midline, the symphysis pubis split and the pelvic ring forcibly opened with dislocation of the sacro-iliac joints. From this stage in the dissection onwards chilled Ringer's solution was frequently and liberally applied to the tissues to keep them moist and cold. This lowering of the temperature has a double advantage, it reduces tissue

metabolism and thereby avoids the danger of anoxia - nerve cells are reputed to be very sensitive to oxygen lack. Moreover a low temperature produces an inactive and usually flaccid colon from which it is relatively easy to remove faecal pellets. During the subsequent dissection direct handling of the bowel was avoided as far as possible by taking advantage of the mesenteric attachments between the prostate, seminal vesicles and pelvic colon in the male or the uterus and pelvic colon in the female. If either the seminal vesicles or the uterus were grasped by forceps it was possible to draw the colon forward putting the dorsal mesocolon on the stretch and facilitating dissection. The filaments of origin of the pelvic nerve from the 2nd, 3rd and 4th sacral nerve roots were identified on either side. The inferior haemorrhoidal artery was found to be a useful index of the position of the pelvic nerve. This artery lies immediately above and lateral to the nerve as it is approached from the ventral surface. The artery and its accompanying vein were severed; this could usually be done simply by pulling them away with forceps if care was first taken to send the vessels into spasm. This spasm was induced by compressing the vessels with forceps and applying gentle tension, insufficient to rupture the vessels until the column of blood within them had disappeared. If the vessels were torn when still patent there was slight but annoying haemorrhage obscuring the precise site of dissection. The two pelvic nerves were cleared from the gelatinous mesenchymal

tissue in which they lie at their origin until a length of about 1 cm was obtained free from fascia. The two nerves were then ligatured separately as close to their origin from the sacral roots as possible using fine nylon thread. One end of each ligature was left long. The fat-containing fold of parietal peritoneum which overlaps the mesocolon was removed. This was a simple matter if the correct cleavage plane was found when the whole length of the fold could be peeled off. The inferior mesenteric ganglion with its various branches was now easily identified in the mesocolon cranial to the inferior mesenteric artery (Fig. 17). The hypogastric nerve which arises from the accessory ganglion tissue immediately caudal to the inferior mesenteric artery was followed to its junction with the cranial strand of the pelvic nerve. This hypogastric - cranial strand nerve complex contains most of the parasympathetic fibres for the colon and since it runs close to the pelvic and dorsal abdominal wall it is easy at some stage in the dissection inadvertently to damage it. This dorsal position of these nerves is not quite clear in the illustrations of Langley & Anderson (1896) in which the length of this nerve complex lying within the pelvis is shown quite far forward of the dorsal pelvic wall. This position is only seen when the dissection is completed; the removal of restraining fascia allows the cranial strand to come forward, away from the pelvic wall.

The lumbar colonic nerves which convey the post-ganglionic sympathetic outflow from the inferior mesenteric ganglion to the

colon were ligatured at their origin from the ganglion, the ligature including the lumbar colonic vein as shown in Fig. 17. One end of the ligature was left long. The mesocolon was separated from the dorsal abdominal wall, taking care to preserve the hypogastric nerve. The bladder, urethra, and, in the female, the uterus, were removed from the ventral aspect of the colon, keeping as far from the bowel as possible when cutting the mesenteric folds. Finally the colon itself was cut through, the lower section about 1 cm caudal to the pelvic nerves, the upper section 4 - 5 cm cranial to this. The upper cut is continued through the mesocolon in an oblique curve above and parallel to the inferior mesenteric artery and following it to its origin from the aorta. The inferior mesenteric artery was cut through and the preparation removed to a Petri dish containing chilled Ringer's solution.

Faecal pellets were removed gently from the anal end of the preparation using fine forceps and taking care not to stretch the gut. This removal of faeces can, on occasions, be very difficult if the gut is irritable and forms tone rings which clamp down in front of the faeces. In a few experiments a small glass cannula, connected to a saline reservoir, was tied into the upper end of the preparation and an attempt made to remove the faeces by inducing peristalsis with Ringer's solution at a pressure of 3 cm of saline. This was fairly successful in removing faeces but the distension of the colon seemed to impair its vitality and the

method was discontinued.

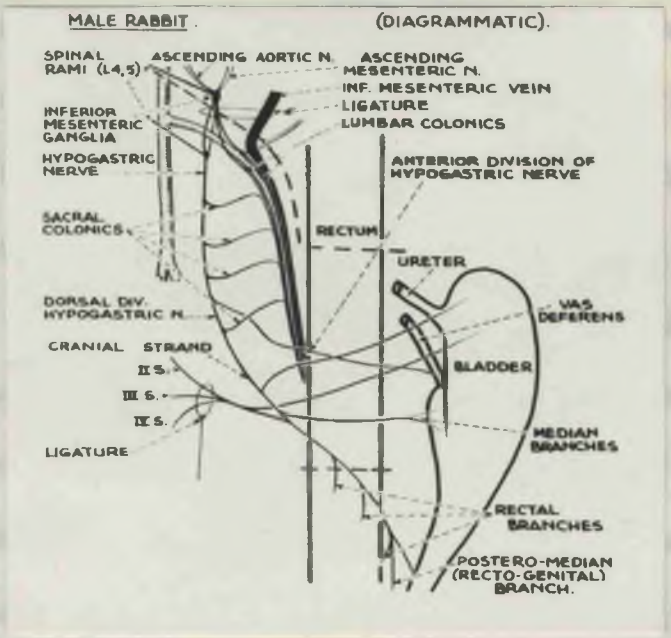


Fig. 17. A diagram of the colon of the rabbit showing the lines of section (dotted) used in removing the preparation and also the position of the ligatures on the nerves.

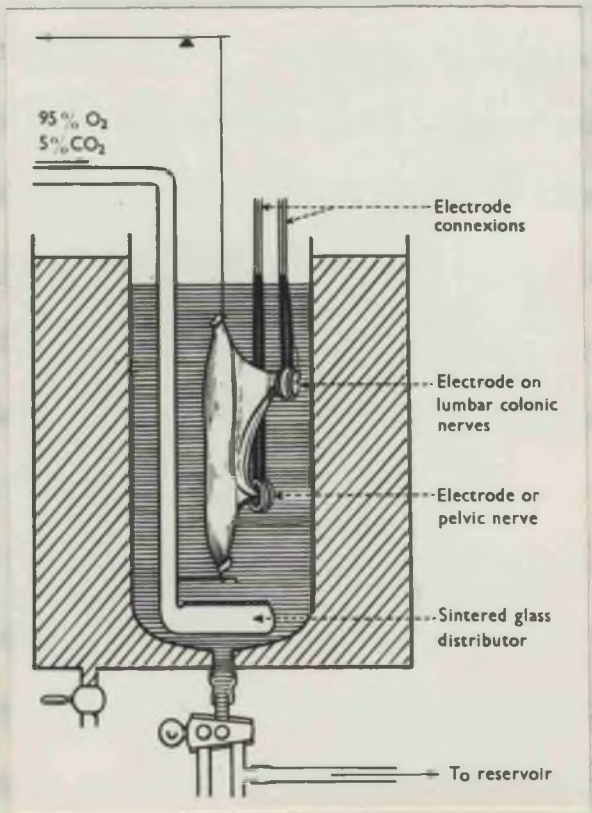


Fig. 18. The arrangement of the preparation and the electrodes in the bath. The upper electrode is on the sympathetic outflow, the lower on the parasympathetic outflow.

The preparation was suspended as a Magnus preparation from a light isotonic gimbal lever exerting a tension of 0·5 g on the preparation and magnifying its movements 3·5 times. The lower end was fixed by a loop of thread to a glass hook above the sintered glass disc of the gas distributor (Fig. 18). The gimbal lever, gas distributor and fluid electrode clamps were all suspended from a common upright which could be raised from or lowered into the saline solution by means of a rack and pinion. While the preparation was suspended above the fluid in the inner vessel of the organ bath the ligatures attached to the nerves were threaded by means of a fine, sharp needle through the condom rubber diaphragms of the two fluid electrodes. One or both pelvic nerves in the lower electrode, the lumbar colonic nerves in the upper electrode. The nerves were pulled into place, the closed half cell of each electrode filled with saline and sealed by a "Perspex" disc. The ligature attached to the nerve was caught between the "Perspex" shell of the electrode and this disc, thereby fixing the nerve in position. The whole assembly was then racked down into the inner vessel of a "Complete isolated organ bath" as supplied by Messrs. C.F. Palmer Ltd.

This dissection, once familiar, can be completed fairly rapidly but the removal of faeces, suspension of the preparation and introduction of the nerves into the electrodes are all time consuming so that the interval between killing the animal and racking the

preparation into the bath was usually about one hour. In spite of this there was no evidence of impaired vitality. Two honours students have used this preparation in their research projects and were naturally somewhat slower in the dissection. In spite of long periods between the animal's death and the preparation's immersion in the oxygenated bath, in one instance over 2½ hours, these preparations were rhythmically active and responded well to nerve stimulation. The dissection is not particularly difficult and a high proportion of successes have been obtained. In the past 100 experiments 81% were completely successful judged on the presence of a response to stimulation of both nerve outflows, 14% were partially successful responding to one or other nerve only, and 5% gave no response to stimulation of either nerve. Most of the unsuccessful experiments were in the first 50. In the last 50 experiments 96% were completely successful, 4% partially successful, and none in which there was no response to stimulation of either outflow.

The fluid electrodes used were a modification of those described by Garry & Wishart (1951). In the earliest experiments it was found that even in those preparations which initially responded well to nerve stimulation, this response quickly diminished and eventually disappeared. Various stratagems were used in an attempt to reverse this decline in the response and the results are shown in Table 1.

Successful	Unsuccessful
Using the other pelvic nerve	Adding eserine
Using the sacral colonic nerves	Cooling to 29°C
Advancing the electrode	...
Increasing voltage	...
Lowering frequency	...

Table 1. Methods used in restoring, or attempting to restore, the response from nerve stimulation when this response declined early in the experiment.

It is obvious from the first column that the peripheral structures including the ganglia within the wall of the bowel were still functioning. The complete restoration of the response in several experiments by advancing the electrode about 2 mm along the nerve suggested that the cause of the failure of nerve stimulation lay in this small region of nerve. It was possible that either the proximity of the silver chloride or some substance in the rubber diaphragm was exercising a toxic effect or that the narrow mouth of the original electrode was damaging the nerve mechanically. The small diameter of the electrode was particularly suspect since the

nerve, following the movements of the gut, moves through an arc of a circle whose centre is the point where the nerve pierces the rubber diaphragm. This movement might be sufficient to kink the nerve over the "perspex" wall of the electrode. This is made even more likely if the nerve is not exactly central in the electrode. In these early experiments an all copper Burn-Dale bath was in use and with this it was not possible to see the true position of the nerve in the electrode. This rapid decline in the response was eventually overcome by modifying the fluid electrode. The external diameter was increased to 16 mm and the internal diameter to 10 mm. This increase in size prevented kinking of the nerve and the increased thickness of the wall allowed the silver electrode loops to be recessed deeply into the "Perspex" shell. A diagram of the original electrode and the modified form is shown in Fig. 19 A & B. This diagram also illustrates a third electrode C with two rubber diaphragms and three leads. This electrode was used in a few experiments when studying the fatigue which appears with long continued stimulation (Part IV). The output of the stimulator was first applied to the lead in the closed cell and the middle lead, the nerve was therefore stimulated somewhere in the neighbourhood of the first diaphragm. Stimulation was continued until fatigue had appeared. The stimulator output was then suddenly switched so that the current now flowed from the middle lead to the lead in the open cell. The point of stimulation of the

nerve was therefore moved several mm peripherally to the neighbourhood of the second diaphragm. If fatigue was due to some alteration in the nerve at the site of stimulation then this should abolish it temporarily.

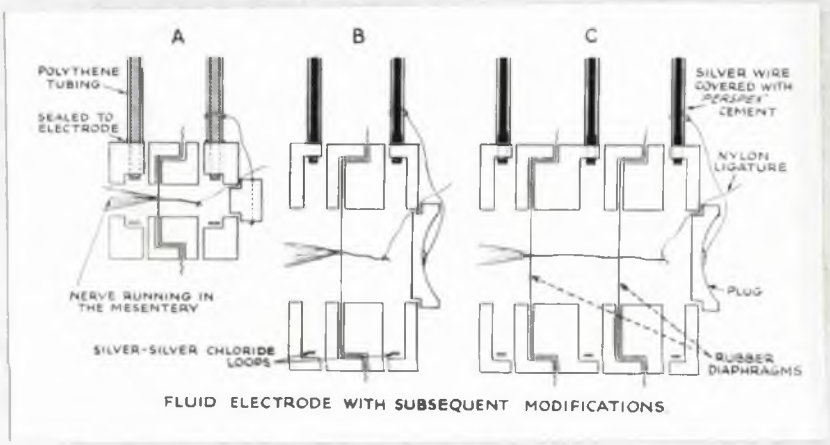


Fig. 19 Fluid electrodes:

A. The electrode as originally described by Garry & Winhart.

B. The modified electrode in which the internal diameter was increased to prevent kinking of the nerve, and the electrode loops of Ag - AgCl were recessed deeply in the perspex wall.

C. A double electrode with two rubber diaphragms and three leads. Switching the current from the 1st and 2nd lead to the 2nd and 3rd advanced the point of stimulation of the nerve.

The original fluid electrodes used multi-strand copper wire leads running in polythene tubing. These were replaced by leads consisting of twelve strands of fine copper wire each strand 0·004" in diameter and the whole covered with polyvinyl chloride (P.V.C.) plastic. The two, or in some cases three such leads from each fluid electrode were sealed together with "perspex" cement diluted with chloroform. Such connections were pliable, easily handled, and much less bulky than the original. The electrodes were held in clamps suspended from a horizontal bar well above the level of the fluid in the inner vessel. These clamps could be moved along the horizontal bar, swivelled about the bar and raised or lowered. In this way the position of the electrode could be so adapted to that of the nerve entering it that there was no tension on the nerve or any possibility of its being dragged against the "Perspex" shell of the fluid electrode. This positioning was done once the preparation had been racked down into the inner vessel. It was made easy by the transparent perspex walls of the organ bath.

The silver loops in each fluid electrode were coated with silver chloride by immersing them in 0·9% Na Cl solution and passing current from a 4 volt battery through the solution using each loop in turn as anode and a large silver plate as cathode. Thirty seconds of current passage was usually sufficient; if chloriding was continued too long, or carried out too rapidly, the silver

chloride film became thick and flaked off easily, exposing bare silver. The electrodes when chlorided were tested for polarisation by measuring their resistance on an Avo 8 testmeter. Before chloriding, measurement of this resistance showed a steady increase from about 500 Ω up to several thousand Ω within one minute as the small current passed by the instrument in making the resistance measurement polarised the electrodes. After chloriding the resistance was between 150 Ω and 200 Ω and remained constant. A refinement of this method was to arrange a reversal switch so that the direction of current flow from the meter could be altered quickly. The resistance was then measured; if there was any polarisation an E.M.F. was built up on the electrodes opposing the passage of current and causing a rise in the resistance proportional to this opposition. If the polarity of the meter was then rapidly reversed this E.M.F. from the electrodes was then augmenting current flow from the meter source and giving a much lower resistance reading. This method was unduly sensitive; almost every fluid electrode showed some short lived reduction in its resistance on reversing the polarity of the meter and the method was discontinued.

In addition to testing for polarisation, resistance measurements were used to detect electrical leakage from one side of the rubber diaphragm to the other. Theoretically with an unperforated rubber diaphragm in position there should have been no

conductive electrical pathway from one half of the fluid electrode to the other even though the electrode was immersed in saline. Measurement of resistance under these circumstances should have been a measurement of the resistance of the rubber composing the diaphragm. In practice however there were always lower resistance pathways than this, probably because the "Perspex" disc closing one half of the electrode was not an exact fit. The magnitude of these leakage pathways was estimated by measuring the resistance with a diaphragm in position. If leakage was slight this resistance was found to be about 20,000 Ω compared with 150 Ω without the diaphragm. The criteria finally adopted for the electrodes were that they should have a resistance of under 200 Ω without a diaphragm, over 10,000 Ω with the diaphragm in position and that the resistance measured without the diaphragm should remain constant for at least one minute.

In a group of eighteen experiments the resistance was measured at the end of the experiment with the nerve threaded through the diaphragm. The nerve was then removed and the resistance measured without it. The resistance with the nerves filling the diaphragm aperture was greater than when the aperture was filled with saline. The difference between these two readings was not, of course, the true resistance of the nerve but only the amount by which this nerve resistance exceeded the resistance of the column of saline which filled the hole in its absence. The "nerve"

resistances obtained in this way for both pelvic and lumbar colonic nerves in these eighteen experiments are shown in Table 2. The usual range for the pelvic nerve was 700 - 900 Ω and for the lumbar colonic nerves 1100 - 1400 Ω. This increase in resistance produced by the presence of the nerve depended on the degree to which the nerve filled the aperture. The results in Table 2 illustrate this.

Exp. No.	Pelvic Nerve	Lumbar Nerve	Remarks
81	800	1100	...
82	790	1135	...
83	850	1400	...
84	650	1050	...
85	800	725	...
86	665	1190	...
87	315	1335	Diaphragm holes burned
88	425	2000	Diaphragm holes burned
89	800	735	...
90	225	75	Both diaphragms ripped
91	715	1275	...
92	875	1325	...
93	890	1100	...
94	1385	1725	Specially sharpened needle
95	950	1225	Specially sharpened needle
96	1000	1410	Specially sharpened needle
97	1075	1300	Specially sharpened needle
98	1075	1310	Specially sharpened needle

Table 2. Measurement of the increase in the resistance brought about by the presence of the nerves in the hole of the rubber diaphragm.

When the holes in the diaphragm were burned with a hot needle before pulling the pelvic nerves through as in experiments 87 and 88, or when the diaphragm was accidently ripped as in experiment 90, the "nerve" resistance was greatly reduced since a large proportion of the current passed from one side of the diaphragm to the other by the electrolyte present in the large perforation incompletely blocked by nerve. A similar explanation would account for the increase in resistance of the pelvic nerve from experiment 94 onwards. In these a very fine needle was specially sharpened so as to make as small a hole as possible, this increased the tightness of the diaphragm around the nerve producing an increase in resistance. These variations were seen best with the pelvic nerve. This was understandable since the ligature round the lumbar colonic nerves included the lumbar colonic vein, fat, and some mesentery. This bulkier tissue was more successful in filling the hole in the diaphragm. These measurements were useful in that they sometimes explained an unusually high threshold for nerve stimulation, especially of the pelvic nerve. Obviously if the diaphragm had ripped in pulling the nerve into place and most of the current was being conducted through the electrolyte, then a higher total current had to be passed before that fraction through the nerve reached the threshold for stimulation.

The nerves were stimulated by means of an electronic rectangular pulse stimultor delivering negative pulses. The

frequency of the pulses, their duration and voltage could all be varied independently. The cathode of the fluid electrode was always peripheral i.e., towards the preparation. In most experiments as a final safeguard against polarisation a 1 MFD. condensor was included in the negative line from the stimulator to the preparation. This capacity was such that with the short pulse durations and small currents involved it did not charge sufficiently to oppose current flow during the pulse, but in the interval between pulses it discharged, the current passing in the opposite direction to that during the original pulse. The amount of this current passed was equal to that passing during the stimulator output pulse but instead of being a high voltage current for a short period it was a very low voltage current for a long period. The stimulator output and the effect on it of introducing such a condensor is shown in Fig. 20 and it is clear that there is very little distortion of the pulse form.

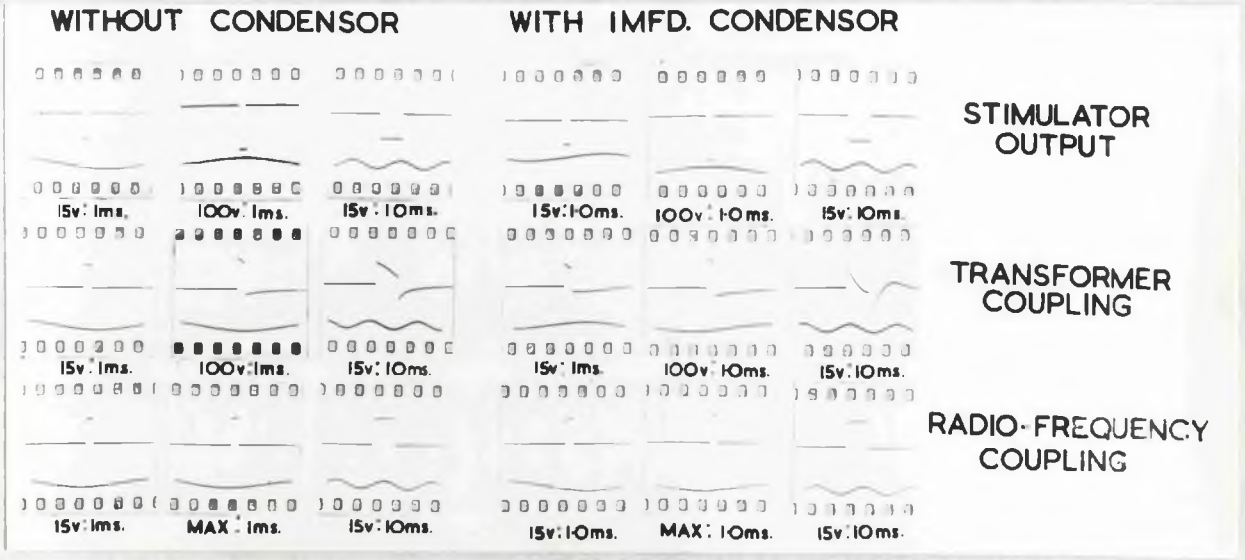


Fig. 20 The pulse form of the stimulator output and the effect on it of transformer and radio-frequency coupling. The effect of a large condensor in series is also shown; distortion is produced with long pulses coupled through a transformer.

When one nerve was being stimulated part of the current flowing in the fluid electrode on that nerve could cross the electrolyte and earth itself via the other fluid electrode if that other electrode were connected to earth. Such leakage of current made interpretation of the results of stimulation very difficult. The fraction of current passing to the other electrode was often sufficient to stimulate the nerve in that electrode thereby reinforcing or reducing the effect of the nerve being studied according to whether the two outflows were similar or antagonistic. Even more confusing, the fraction of current passing to the other electrode was occassionally sufficient to reduce a previously supra-maximal stimulus to a submaximal one. In the experiment of this section only one nerve was stimulated at any one time. Leakage of current from one fluid electrode to the other was therefore easily eliminated by having the electrode not in use "floating", that is without earth or other connections. In addition the two halves of the fluid electrode were short circuited when not in use.

The interval between periods of stimulation was five minutes which allowed complete recovery from the previous stimulus and avoided fatigue. The duration of each period of stimulation was ten seconds except when the effect of the duration of the period of stimulation on the response was investigated.

The volume of the vessel in which the preparation was suspended was either 200 or 300 ml. The large capacity of the inner vessel

allowed considerable freedom to the fluid electrodes which were able to follow any side to side swaying movements the preparation made. It was a disadvantage of this large volume that in studying the action of drugs it was not possible to change the fluid in the inner vessel by the technique of overflow. This inner vessel was both drained and refilled from below, a side limb on the drainage tube bringing in the fresh Ringer's solution. This was first passed through two glass warming coils lying in the bath proper so that alterations in temperature were kept to a minimum.

The formula of the saline solution used has undergone several changes during the course of these experiments. Originally a simple balanced salt solution similar to that described by Burn (1952) but without glucose was used. The formula was as follows: NaCl = 0·9%: KCl = 0·4%: $CaCl_2$= 0·25%: $NaHCO_3$= 0·05%. The response to stimulation of the pelvic nerve, when the preparation was suspended in this solution, was not maintained if stimulation was long continued. Feldberg & Solandt (1942) reported that in the absence of glucose the rhythmic activity of the rabbit small intestine declines and eventually disappears, as does the response to acetylcholine added to the bath: both of these effects were reversed by adding glucose to the bath. In view of these findings the effect of adding 0·1% dextrose to the Ringer's solution was tried. This caused a slight increase in tone in a few experiments but did not prevent the decline in the response when nerve stimulation was

long continued. The colon of the rabbit, in the absence of glucose, does not show the same depression and eventual disappearance of rhythmic activity as Feldberg & Solandt found in the small intestine of the same animal. In spite of the absence of any very definite indication that the presence of glucose was advantageous to this preparation it was felt wise to avert possible criticism on this account and glucose has been added in all experiments from 113 onwards (66 experiments in all). More recently (last 20 experiments) Krebs' saline has been used. This solution was tried following the report by Creese (1954) that the rat diaphragm in such a solution showed only a slight initial fall in the concentration of intra--cellular potassium which subsequently remained constant. It seemed possible that potassium leakage from either the nerve or muscle elements of the colon preparation might be contributing to the inability to maintain a tetanic response. The findings of Cantoni & Eastman (1946) that the post-stimulatory inhibition of the guica pig ileum after maximal contraction induced by acetyl--choline or pilocarpine can be prevented by increasing the potassium content of the saline also suggested that Krebs' saline with its high K^+ content might prevent a similar inhibitory phase which appears in the colon after a maximal contraction induced by stimulation of the pelvic nerve. There was no observable alteration in the rhythmic activity of the preparation or in the response to stimulation of the pelvic nerve when Krebs' saline was

used. The threshold for stimulation of the pelvic nerve, however, was lowered. Using Ringer-Locke solution the threshold voltage usually lay between 5V and 8V; in Krebs' saline the threshold was between 2V and 4V. The formula of Krebs' saline is usually given in terms of each ion rather than as a percentage of the salts used in its preparation. For comparison with the previous formula the percentage composition of the salts is as follows:- NaCl = 0·69%: KCl = 0·035%: $CaCl_2$= 0·029%: KH_2PO_4= 0·0160%: $MgSO_4$= 0·029%: $NaHCO_3$= 0·21%: dextrose = 0·2%. The main differences between this and the Ringer-Locke saline were in the high potassium content and in the presence of the magnesium, phosphate and sulphate ions. The bicarbonate content was also much greater. The PH. of the solution, however, was similar to that of Ringer-Locke (7·2) but differed from that solution in that it could only be maintained at this value by vigorous aeration with the gas mixture which contained 5% CO_2.

The method of preparation was that described by Krebs & Henseleit (1932). The stock solutions were sufficient for five experiments and were then replaced. Krebs' saline very easily precipitated its calcium content if the solution was allowed to become slightly more alkaline by loss of CO_2. This happened quickly if the solution was kept at 36°C in an open vessel. Once the solution had become cloudy from precipitation gassing with CO_2 did not restore completely the calcium to solution as reported by

Krebs & Henseleit. For this reason fresh saline for replacing that in the inner vessel was kept warm in a 2 litre volumetric flask. As the CO_2 was liberated the pressure inside the flask rose until equilibrium was established. This pressure was not high and there was no danger of the flask breaking. If the flask was almost filled with saline the total quantity of CO_2 lost in establishing this pressure was small.

The saline was aerated by a mixture of 95% O_2 + 5% CO_2 delivered through a sintered glass gas distributor. The pore size of the sintered glass disc was 40 - 90 microns. The bath temperature was thermostatically maintained at $36^{\circ}C \pm 0{\cdot}5^{\circ}C$ except in those experiments in which the "peripheral mechanism" was depressed by cooling.

Innervated lengths of the rabbit colon suspended as Magnus preparations in the way that has been described continued to respond to intermittent stimulation of the motor and inhibitor nerves for a long time. Responses have been obtained from preparations 18 hours after the death of the animal, and after leaving the preparation overnight at $10^{\circ}C$.

The effect of stimulation of the extrinsic nerves on the circular muscle was studied using the Trendelenburg preparation. This was set up in the conventional fashion other than the presence of the two stimulating electrodes. The diagram in Fig. 21 illustrates the arrangements without further description.

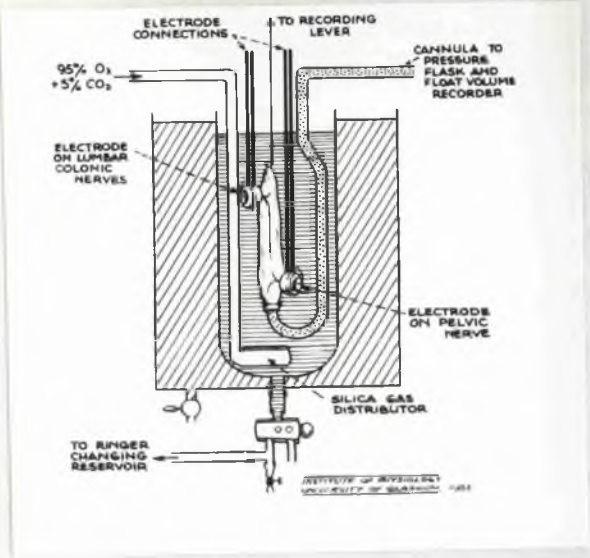


Fig. 21. The colon of the rabbit arranged as a Trendelenburg preparation.

Drugs used in this section were hexamethonium bromide (C6), atropine sulphate, acetylcholine chloride, adrenaline hydrochloride and nor-adrenaline bitartrate. Concentrations of hexamethonium refer to the active base; concentrations of all the other drugs refer to the salt.

The Rhythmic Activity of the Rabbit Colon in vitro and the Response to Stimulation of the Sympathetic and of the Parasympathetic Nerves.

The rabbit colon suspended in the way that has been described has a characteristic rhythmic activity superimposed on a maintained tonic state. This activity consists of a series of contractions often of irregular amplitude and with a frequency of 10 - 11/min. These contractions appear as "spikes" in the kymograph records. Occassionally they are superimposed on slow tone waves. These slow fluctuations in the tone of the muscle fall into two groups according to their period. In one a complete cycle occupies 1 to 2 min; in the other, much slower, the cycle is completed in 5 to 8 min. Both forms of tone waves are easily separated from the rapid "spike" contractions which are superimposed on them without alteration. The possession of both rhythmic activity and tone makes this preparation particularly suitable in studying the actions of the extrinsic nerves on the smooth muscle since the smooth muscle under study can as readily show inhibition as augmentation. No difference in the frequency of the rhythmic contractions or the tone waves was found between the three forms of saline used (plain Ringer's solution; Ringer's solution + 0·1% glucose; Krebs' saline). There was sometimes a slight increase in the tone of the muscle on adding glucose to a bath previously

lacking this substance.

On studying the preparation through the transparent walls of the bath several other forms of activity were apparent which did not appear on the record of longitudinal muscle activity. The most common of these were tone rings. The circular muscle of a limited area, usually near the oral end of the preparation, contracted forming a tight ring of muscle. Commonly only one such tone ring was present at a time, but occassionally two or more were seen. Usually these tone rings were static but waves of contraction moving both anally and orally were seen which started as what appeared to be tone rings. Most of these travelling waves passed in an anal direction. A comparison of the activity of the gut by inspection and the activity of the longitudinal muscle as recorded on the graphic while the experiment was in progress showed that many of the irregularities in the longitudinal muscle contractions were a reflection of these other forms of activity. One other feature of the activity of the preparation was noticed by inspection. The rhythmic contractions sometimes caused not only shortening of the preparation but also a rotation round its long axis. This may be due to the longitudinal muscle being arranged in a very long spiral as has been suggested by Carey (1921). It is interesting, however, that in two preparations showing marked spiral rotation when contracting rhythmically, stimulation of the pelvic nerves produced a strong contraction of the longitudinal muscle without twisting.

The response of both muscle coats to effective stimulation of the pelvic (parasympathetic) nerve was contraction and to stimulation of the lumbar colonic (sympathetic) nerves inhibition. Typical responses in the same preparation from stimulation of these nerves are shown in Fig. 22. That these responses were due to stimulation of the nerves and not just to spread of current through the electrolyte to the preparation was demonstrated in the following way. At the end of an experiment both outflows were stimulated separately and their response recorded. A fine pair of long handled scissors were then lowered into the bath and one nerve cut where it left the electrode. The two outflows were then stimulated again. The nerve which had been cut gave no response even with a sixfold increase in voltage while the response from the other nerve was undiminished.

The response from stimulation of the pelvic nerve appeared after a short latent period averaging 0·8 sec as a smooth tetanic contraction which rapidly reached its maximum. If stimulation was continued there was a gradual decline in the response accompanied by the reappearance of rhythmic contractions. Eventually a steady state was reached in which the amplitude and usually also the frequency of the rhythmic contractions were increased and also the tone. Cessation of stimulation was followed by a rapid relaxation. Tone and rhythmical activity usually passed through a brief phase of depression before returning to the previous base line. The

stronger the preceeding contraction and the longer it lasted the more marked was this phase of depression on stopping stimulation. It resembled the inhibition reported by Cantoni & Eastman (1946) following stimulation of the guinea pig small intestine with pilocarpine, histamine and acetylcholine.

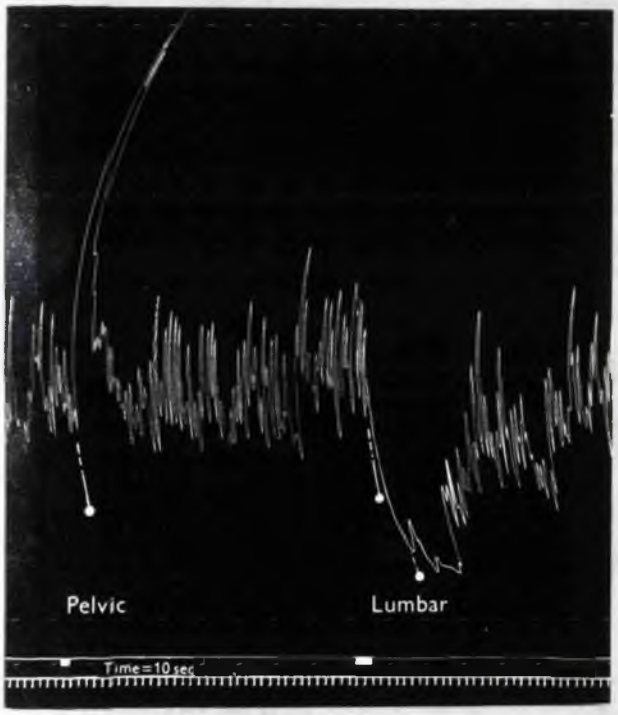


Fig. 22 Colon of the rabbit in vitro. Responses from stimulation of the pelvic nerve at $10^{P/}S$ and the lumbar colonic nerves at $50^{P/}S$. The voltage was 15 and the pulse duration 0·5 msec.

Stimulation of the lumbar colonic nerves produced a very different picture. The latent period between the beginning of stimulation and the onset of the mechanical response was longer, averaging 1·8 sec and the response developed slowly. There was

complete suppression of the rhythmic activity and a gradual fall in tone. If stimulation was continued there was no evidence of "escape" unless long periods of stimulation were used; the preparation remained completely inactive and without tone. Stopping stimulation after the standard period of 10 sec produced a curious sequence of events. There was first a short latent period, then a rapid contraction followed in turn by a restoration, and indeed intensification of the inhibitory state (Fig. 22). This usually lasted about 1 min and was then replaced by a sudden resumption of rhythmic activity and a more gradual return of tone. The nature of this rapid contraction appearing on cessation of stimulation and quickly giving way to further inhibition was investigated a little further. It was most prominent when a short but effective inhibitory stimulus had been applied. If there was little lengthening of the gut during stimulation it was absent or inconspicuous. If, on the other hand, a long period of effective stimulation was given again this feature was slight. It appeared that stretch of the muscles was essential and that the inhibitory effect of stimulation also inhibited this response. With short periods of stimulation maximum inhibition occurs after the "spike" and this slow development of the inhibitory effect of stimulation might have explained why short periods of stimulation favoured this component. It was possible that this was a reflex contraction of the gut from a stretch stimulus and involving local

ganglion cells. It was found, however, that it was still present in the presence of concentrations of hexamethonium and nicotine which were known to block these ganglion cells. It was further arranged in one experiment (Fig. 23) that, during the inhibition produced by stimulation of the lumbar colonic nerves, the lever was supported so that its stretching force of 0·5 gms was removed. Some stretching may still have occurred from the weight of the tissue but, when immersed in saline, this factor must be slight. In spite of this removal of the main source of tension the after contraction was undiminished. The mechanism of this component of the response is quite unknown.

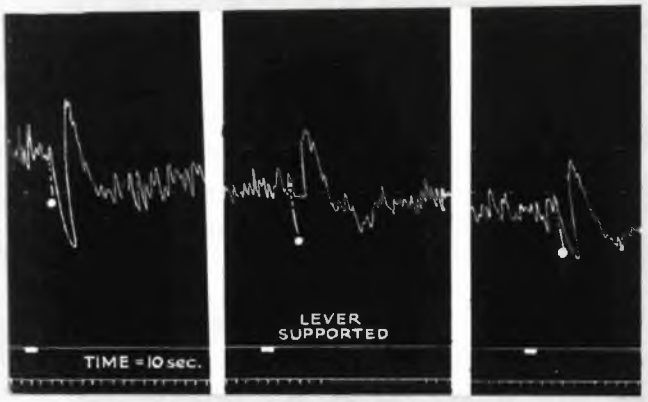


Fig. 23 Colon of the rabbit in vitro. Nicotine 10^{-5} and C6 10^{-4} present in the bath. The spike-like contraction on stopping stimulation of the sympathetic outflow is unaffected by the ganglion blocking agents. The prevention of muscle stretching during inhibition by supporting the recording lever also has no effect on the appearance of the "spike".

Gradually increasing distension of the colon in the way described by Trendelenburg (1917) induced waves of peristalsis which emptied the preparation of the distending fluid. Simultaneous recordings of the activity of the longitudinal and circular muscle demonstrated essentially the same response as was described by Trendelenburg for the guinea pig ileum but certain features made interpretation difficult. The tone of the rabbit colon is high, as a result the peristaltic waves often arose from some point short of the upper (oral) end. The rhythmic activity of that part above the peristaltic wave continued without interruption and was superimposed on the record. According to Trendelenburg increasing distension of the guinea pig ileum causes a rise in longitudinal muscle tone and a fall in circular muscle tone with acceptance of fluid. At the critical distension pressure for initiating peristalsis, usually about 3 cm H_2O, the longitudinal muscle contracts followed almost immediately by contraction of the circular muscle which continues as a wave passing down the gut and emptying the lumen. Subsequent to this first contraction the longitudinal muscle relaxes to its original base line or even below it. Further waves of peristalsis are initiated by contraction of the longitudinal muscle from this relaxed or even inhibited base line. The wave of circular muscle contraction follows the longitudinal muscle contraction usually a ¼ cycle out of phase. Peristalsis in the rabbit colon was similar to this but the initial rise in

longitudinal muscle tone with distension was absent. From the beginning of distension both muscle coats relaxed (Fig. 24).

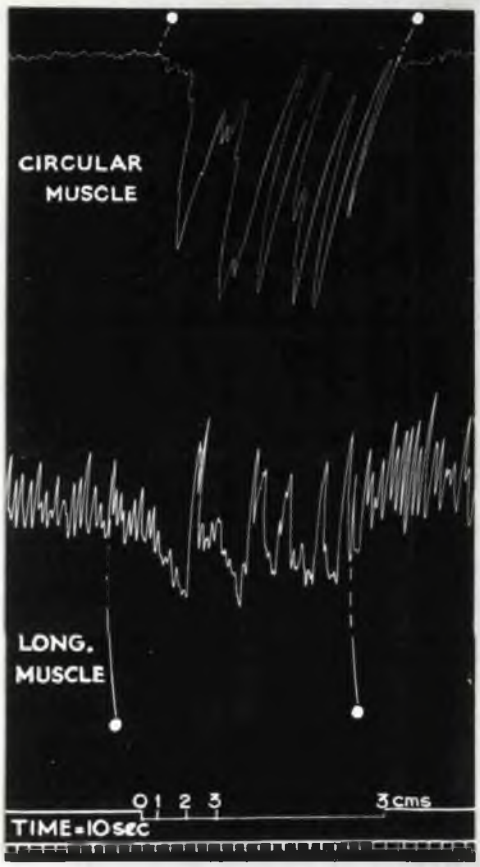


Fig. 24. Rabbit colon *in vitro*, Trendelenburg preparation. Increasing distension produces a fall in tone followed by the appearance of large co-ordinated peristaltic contractions; both muscle coats share in these responses. The normal rhythmic activity of the longitudinal muscle is suppressed during peristalsis.

At the critical distension pressure, usually 2 - 3 cm of saline, the longitudinal muscle contracted, followed by the circular muscle. The wave of circular muscle contraction emptied the colon. The two muscle coats contracted with each succeeding wave of peristalsis but slightly out of phase, the longitudinal muscle leading. (An example of peristalsis in the colon is shown in Fig. 24).

Stimulation of the pelvic nerve caused contraction of both muscle coats, obliteration of the gut lumen, and cessation of peristalsis. Stimulation of the lumbar colonic nerves caused

inhibition of both muscle coats, extreme distension of the preparation and inhibition of peristalsis. The peristaltic waves which re--appeared when sympathetic stimulation was stopped were deeper, more regular and more frequent than before stimulation.

The influence of the frequency of stimulation on the response

In other regions of the alimentary tract, notably the stomach, variations in the frequency of the stimulating pulse have been the most frequently reported method of altering the character of the response. This effect of frequency is in many ways the core of the whole problem. Those investigators who attributed variations produced by altering the frequency to the presence of two types of fibre in the nerve presumably believed that these two fibre groups had a different frequency sensitivity whereby one or other was preferentially stimulated. Unable to get the two groups separate from one another, i.e., to find a region in which stimulation of two separate visceral nerves produced opposite effects on the gut neither showing reversal on altering the frequency, they were unable to put this belief to experimental test.

In the colon of the rabbit, whatever the frequency, the response from effective stimulation of the pelvic nerve was contraction and from stimulation of the lumbar colonic nerve inhibition. Single pulses of varying duration and repetitive pulses ranging in frequency from one pulse every 20 secs up to 1000 pulses per sec ($1000^{P}/_{S}$) were used. The freedom from variation in the character of the response provided the very opportunity, previously lacking, of comparing the frequency sensitivity

of inhibitory and motor fibres to the smooth muscle of the gut. From the earliest experiments it was obvious that there was a difference in the optimum frequency of stimulation of the two outflows. The most effective frequency for the pelvic nerve was $10^{P/S}$ and for the lumbar colonic nerves about $100^{P/S}$. The uniform nature of the responses from stimulation of the two outflows at a wide variety of frequencies is shown in Fig. 25, as well as this difference in the optimum frequency of stimulation.

These differences in frequency sensitivity were investigated more fully and an attempt was made to express them in a mathematical form. In each experiment and on the same graphic the maximum response was selected and given the arbitrary value of 6. The remaining responses were then assessed in terms of this maximum response and expressed as some integral fraction of 6. During the experiment that frequency which was producing the maximum response was repeated at intervals so that any variation in the response of the muscle not associated with frequency was detected. In assessing the magnitude of the response two factors were considered. First the amount of shortening or lengthening produced and secondly the duration of this state. The effectiveness of the different frequencies of stimulation of the pelvic nerve could be assessed almost entirely on the amount of shortening since on cessation of stimulation the response disappears rapidly whatever the frequency. Comparison of the inhibition

produced by various frequencies of stimulation of the lumbar colonic nerves required consideration both of the degree of this inhibition and its duration.

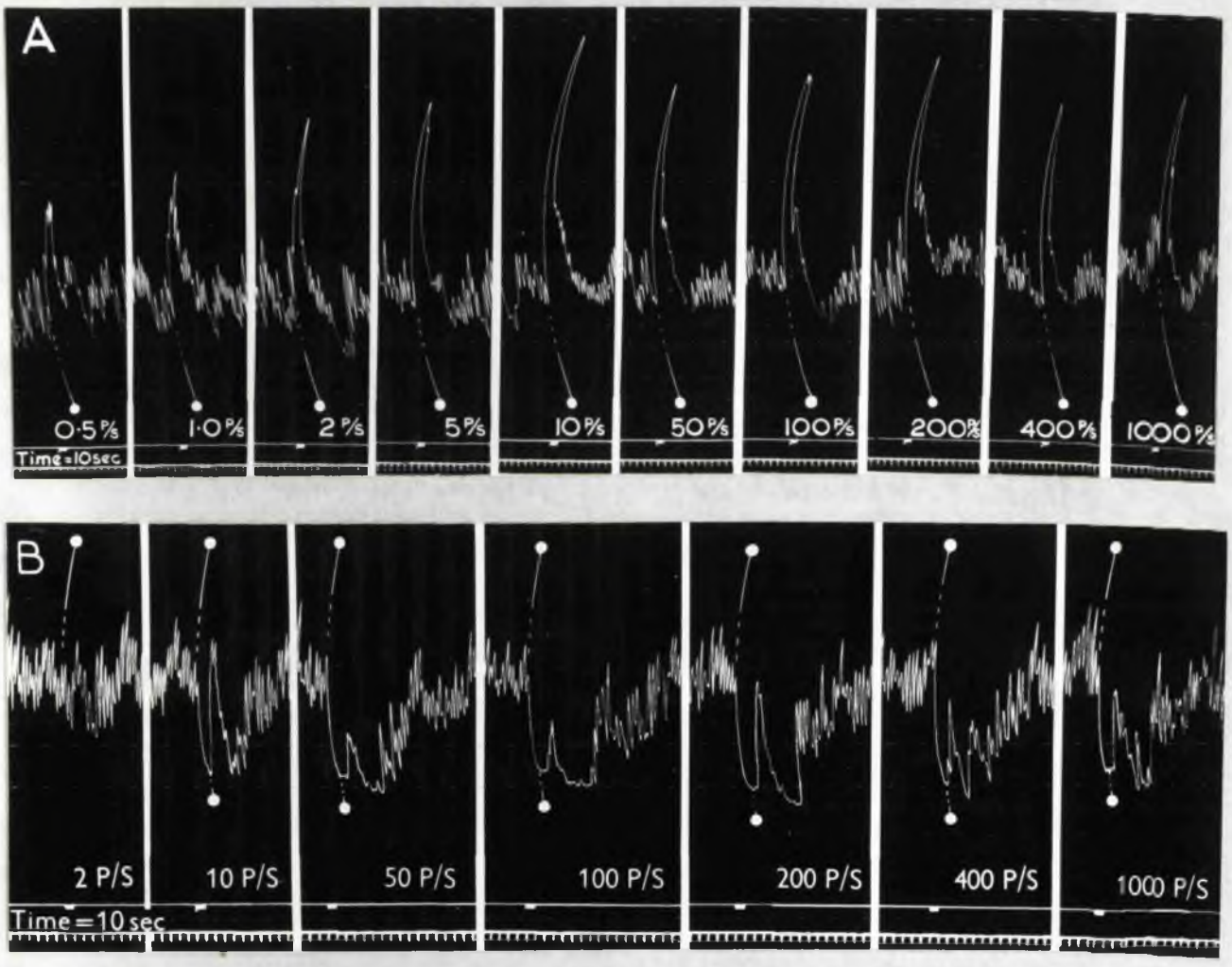


Fig. 25 Colon of the rabbit *in vitro*. The effect of varying the frequency of stimulation. The current voltage was 15 and the pulse duration 1·0 msec except at 1000 P/S when it was 0·5 msec.
A. Pelvic Nerve. The response was always motor. The maximum effect was at 10 P/S and frequencies down to 0.5 P/S were still effective.
B. Lumbar Colonic Nerves. The response was always inhibition. The maximum effect was at about 100 P/S; a frequency of 2 P/S was ineffective.

The results of these experiments are grouped in Table 3.

TABLE 3

The influence of the frequency of stimulation on the magnitude of the response. An arbitrary figure of 6 is given to the maximum response in any one experiment. The frequency is given as pulses per sec at the head of each column. The pulse duration is 1 msec except at a frequency of $1000^{P/S}$ when it is 0·5 msec.

Exp.	0.5	1	2	5	10	20	50	100	200	400	500	$1000^{P/S}$
116	-	-	0	3	5	-	6	4	-	-	-	-
118	-	1	-	4	6	-	2	3	4	5	-	-
120	1	2	3	5	6	-	6	5	5	5	-	-
121	-	-	-	-	6	-	-	5	4	4	-	-
134	2	3	4	5	6	-	5	5	6	5	-	5

Responses from stimulation of the Pelvic Nerve: maximum responses are first obtained at $10^{P/S}$; responses are numerous at frequencies below $5^{P/S}$.

115	-	0	2	-	4	-	5	6	5	-	5	3
116	-	-	0	2	-	4	6	6	6	-	2	-
117	-	-	0	1	-	3	5	6	6	4	-	-
118	0	1	-	3	4	-	6	5	-	-	-	2
119	-	0	-	1	-	4	6	6	6	6	-	-
120	-	-	0	-	2	4	6	6	6	6	-	1
121	-	-	0	1	2	-	5	6	-	-	-	-

Responses from stimulation of the Lumbar Colonic Nerves: maximum responses are first obtained at $50^{P/S}$; responses are few below $5^{P/S}$.

It is obvious from this that the difference in the optimal frequency of stimulation is only one aspect of a difference in the whole "spectrum" of frequencies which are effective in producing responses from the two outflows. This band of effective frequencies is broader for the pelvic nerve because of extension into the lower frequencies, frequencies which are ineffective when applied to the lumbar colonic nerves. Thus below $10^{P}/_{S}$ the pelvic nerve still produced strong contraction but the response from stimulation of the lumbar colonic nerves was greatly reduced. At frequencies below $5^{P}/_{S}$ it was exceptional for stimulation of the lumbar colonic nerves to produce a response whereas the pelvic nerve regularly caused contraction at frequencies as low as 1 pulse every 2 sec. These frequencies do not represent absolute lower limits for effectiveness. These are dependent not only on the frequency but also on the total duration of stimulation. If the standard duration of 10 sec was lengthened to several minutes there was a further displacement of the band of effective frequencies towards the low frequencies. This applied to both outflows but the difference between them remained. Pelvic nerve stimulation at frequencies as low as one pulse every 20 sec in one experiment produced a small but definite increase in activity. Because of the appearance of fatigue when stimulation of the pelvic nerve was prolonged, a standard period of stimulation of 10 sec was adopted in comparing the effectiveness of different frequencies of

stimulation.

Table 3 also shows that the spectrum of effective frequencies continues up to 1000 P/S for both nerves. Autonomic nerves are not capable of transmitting impulses at this frequency so presumably some form of block appeared whereby only a proportion of the stimuli produced nerve impulses conducted to the muscle. For this reason it seemed futile to explore any higher frequency range since whatever effect these might have had on the nerve at the site of stimulation they would not have increased the range of impulses conducted along it.

Single pulses whose duration varied from 1 sec to 1·44 sec were tried in a few experiments. These never produced a response from the lumbar colonic nerves and only once produced a response from stimulation of the pelvic nerves (Fig. 26).

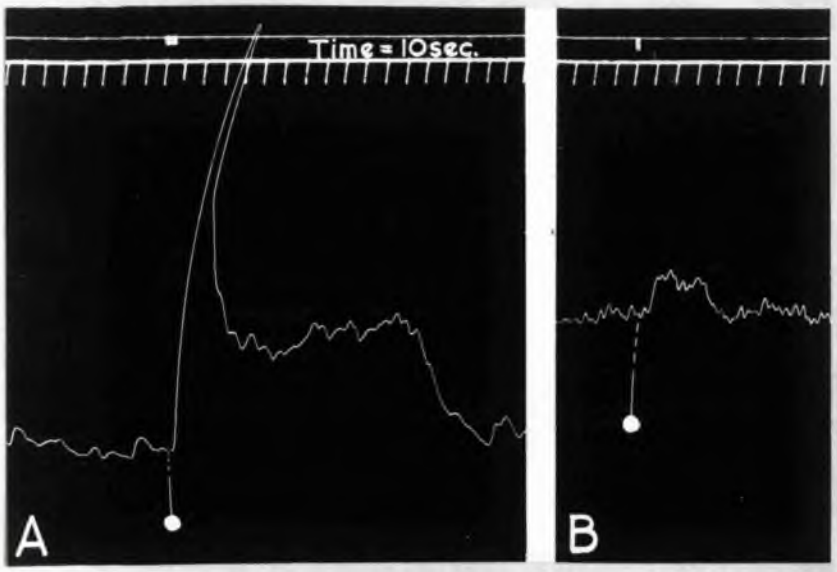


Fig. 26 Rabbit colon <u>in vitro</u>. The activity of the preparation initially was very low.

A. Response to stimulation at 10 P/S, voltage 5, pulse duration 0.5 msec.

B. Response to a single pulse of 1.25 sec duration: Voltage 15.

The threshold duration of this single pulse was between 850 msec which was ineffective and 1·25 sec which produced the response illustrated. Since the pulse duration threshold for repetitive stimulation, as will be shown later, was somewhere in the range 0·01 to 0·05 msec it is likely that these very long single pulses became effective only because they were capable of generating more than one conducted nerve impulse. Probably one single volley, even when every nerve fibre is activated, is never sufficient to cause a response measurable by these methods. Von Euler (1948) however, has reported that mammalian efferent autonomic C fibres in the vagus nerve to the stomach do not respond to a constant current stimulus by repetitive discharge. This contrasts with afferent somatic C fibres (pain) which do respond in this way.

A second method was used to compare the effectiveness of different frequencies of stimulation. Since these experiments were completed a previous description of a similar method by Querido (1924) has been found. He used it to study the influence of the frequency of stimulation on the response of the nictitating membrane. The nerve was stimulated continuously at the frequency believed to be optimal. Without interrupting stimulation this frequency was suddenly increased or reduced. The new rate was maintained for 20 to 30 sec and then restored to the original. If the frequency thus interpolated was less effective than the initial frequency then the response diminished and if more effective the response was increased.

In both instances the original response was restored on restoring the original frequency. Fig. 27 is an example of this method applied to the colon preparation showing the superiority of a frequency of $10^{P/S}$ over $50^{P/S}$ when stimulating the pelvic nerve. This method was applied to the lumbar colonic nerves but alteration of the frequency in the range 50 to $200^{P/S}$ did not affect the response.

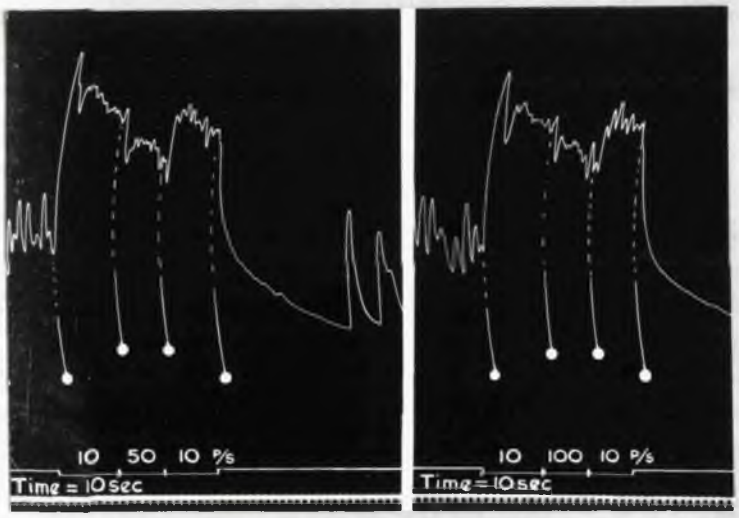


Fig. 27 Colon of the rabbit <u>in vitro</u>. Pelvic nerve stimulation. Increasing the frequency from $10^{P/S}$ to $50^{P/S}$ or $100^{P/S}$ decreases the amplitude of the contraction; the contraction is restored to its old level on reverting to a frequency of $10^{P/S}$.

The method, however, is not a reliable index of the efficacy of a given stimulus frequency under all conditions. The reason for this is that if the initial stimulation frequency ($10^{P/S}$ for the pelvic nerve) is maintained for more than a minute or two it

it "conditions" the preparation in some way so that the change to a higher frequency produces a complete inhibition similar to that described by Wedensky in the frog sciatic-gastrocnemius preparation. This inhibitory effect of high frequencies after conditioning by low frequency stimulation is considered more fully in Part IV. It should be emphasized that all frequencies up to 1000 $^{P}/_{S}$ are effective if applied without such conditioning.

The effect of the frequency of stimulation on the temporal characteristics of the responses were investigated in three experiments. Two measurements were made at each frequency:-

1) The latent period between the beginning of nerve stimulation and the beginning of the mechanical response.
2) The time elapsing from the beginning of stimulation to the moment at which the mechanical response reached its maximum.

The results of these experiments are given in Table 4.

Table 4. The effect of the frequency of stimulation on the latent period of the response and on the time to reach the maximum response. The frequency is indicated as pulses per sec at the head of each column.

	Latent Period (Sec)						Time to reach maximum response (sec)					
	1	2	5	10	50	100 P/S	1	2	5	10	50	100 P/S
Exp.												
131	-	4.1	0.8	0.8	0.7	-	-	9.9	6.5	4.6	4.0	-
132	0.9	0.9	0.7	0.8	0.6	-	10	10.8	6.9	4.8	2.8	-
133	3.0	2.8	0.9	1.0	0.7	-	13	8.5	4.0	3.2	2.9	-
	Responses from stimulation of the Pelvic Nerves: the latent period is short and the responses quickly reach their maximum.											
131	-	-	6	-	2.2	1.0	-	-	60	60	70	85
132	-	5.8	3.8	2.2	1.3	2.4	-	52	51	51	84	64
133	-	-	5.0	3.0	3.0	2.1	-	-	34	53	65	74
	Responses from stimulation of the Lumbar Colonic Nerves: the latent period is long and a considerable time elapses before the maximum is attained.											

The two outflows once more are sharply differentiated. The latent period when stimulating the pelvic nerve is much less than when stimulating the lumbar colonic nerves even comparing them at the optimum frequency for each outflow. This difference is magnified at low frequencies as for example at $5^{P}/_{S}$ where the difference in latent periods is about 4 sec. It is important to note that at this frequency the response from stimulation of the pelvic nerve would almost have reached its maximum before the response from the lumbar colonic nerves had begun if both nerves had been simultaneously stimulated. The time taken for the response to reach its maximum was also different for the two nerves. The contraction from stimulation of the pelvic nerves was rapid even at low frequencies. The maximum in all but a few instances lay within the 10 sec period of stimulation. The inhibition from stimulation of the lumbar colonic nerves developed more slowly but continued to increase in the post-stimulatory period so that the maximum response typically lay outside the period of stimulation. If both nerves were simultaneously stimulated we would expect that the contraction from the pelvic nerve would have disappeared at the time when the inhibition induced by the lumbar colonic nerves was reaching its peak.

The influence of various frequencies of stimulation on the response of the circular muscle was studied using the method of Trendelenburg. Since high frequencies of stimulation are most

commonly reported to reverse the parasympathetic response and low frequencies the sympathetic response corresponding frequencies were particularly studied on the appropriate nerves to the colon. The inability to reverse the response by altering the frequency of stimulation was confirmed. No attempt was made to determine the optimal frequency or the range of effective frequencies for the two nerves.

The influence of the strength of stimulation and of the pulse duration on the response

Altering the strength of the stimulating current has been reported by several workers to change the character of the response from inhibition to contraction or vice versa. Some of these reports were reviewed in the first part of this thesis. In these experiments in which altering the strength of stimulation has altered the response, variations in frequency have often produced a similar change. Lowering the frequency or using a weaker stimulus seem to have had a similar effect in converting inhibitor into motor responses. Kato et al (1929) have shown that reducing the strength of electrical stimulation of a nerve may, especially at high frequencies, reduce the frequency of the nerve impulses set up. The weaker stimulus is ineffective early in the relative refractory period of the nerve so that a certain number of the electrical impulses are not followed by an impulse in the nerve. A form of intermittent block is set up, the number of "dropped pulses" depending on the rate of recovery of the nerve from the last effective stimulus and the strength of the stimulating current. When the nerve excitability has risen sufficiently for the weak stimulus to be at or above threshold another nerve impulse is initiated. It is likely that this mechanism explains at least some of the variations in response

produced by altering the strength of stimulation. In the end it is really variations in the frequency of impulses travelling along the nerve that alter the response.

No variation in the strength of the stimulating current was found to alter the character of the response of the rabbit colon on stimulation of the pelvic or lumbar colonic nerves. Routinely, in each experiment, the current was gradually increased from zero until a response was just obtained. This response was invariably contraction when the pelvic nerve was stimulated and inhibition when the lumbar colonic nerves were stimulated. This threshold voltage was then trebled in subsequent periods of stimulation without altering the type of response. Stimulation in these experiments was, of course, at the optimum frequency for each nerve ($10^{P}/_{S}$ for the pelvic nerve, $100^{P}/_{S}$ for the lumbar colonic nerves). In a few experiments the effects of altering the strength of the stimulating current was investigated at frequencies other than the optimum. Voltages from threshold (usually 5 - 8V in Ringer's solution and 2 - 4V in Krebs') up to 100V (at frequencies from $1^{P}/_{S}$ to $200^{P}/_{S}$) were tried. The response appropriate to the nerve was always obtained.

Pulse durations varying from 0·01 msec to 100 msec for repetitive stimulation and up to 1·44 secs for single pulses were used. At high frequencies the range of possible pulse durations was limited. At a frequency of $100^{P}/_{S}$ it is not possible to use

pulses much longer than 5 msec otherwise the stimulator may intermit and produce a pulse frequency which is some fraction, usually a half, of that selected. As varied combinations of frequency and pulse duration as were possible within this limitation were used for nerve stimulation. None was ever found to alter the character of the response.

Since the sympathetic nerves are post-ganglionic and the parasympathetic pre-ganglionic, presumably with a difference in fibre diameter, it was possible that there was some corresponding difference in excitability. Such a difference would offer another basis, independent of frequency, for preferential stimulation with weak currents of the motor group of fibres (pre-ganglionic, myelinated) in a mixed nerve. An attempt to determine whether a difference in excitability did exist between the two nerve outflows was made.

Bishop & Heinebecker (1932) have shown that the pulse duration threshold of smooth muscle, which responds only to repetitive stimulation of its nerve, can be measured by finding the duration of individual pulses which, when repeated at the necessary frequency, will just produce a response. They found that the threshold of the nerve measured by the appearance of the effector response was "very close" to that measured by stimulating the nerve with a single stimulus and recording the action potential. Scott (1934) and von Euler (1949) have also measured the

excitability of nerves in this way, the latter using it for efferent vagal fibres to the stomach. There is one difficulty inherent in this method. Where the frequency of stimulation is high it is difficult to be sure that the duration of each pulse is sufficiently long for the measurement of the threshold voltage to be a true measure of the rheobase. Scott appreciated this point and for high frequencies he used a different part of the strength duration curve as a measure of excitability. At low frequencies he did use the chronaxie and in measuring the rheobase used pulses of 7·97 msec. Von Euler used a pulse duration of 10 msec in his measurement of rheobase. In the experiments on the rabbit colon it was necessary to compare the pelvic nerve at $10^{P}/_{S}$ with the lumbar colonic nerves at $100^{P}/_{S}$. This range of frequencies has made such long pulses as were used by previous workers impossible. Investigations of the pelvic nerve at low frequencies of stimulation showed that the threshold for voltage was not altered by increasing the duration of the pulses from 1 msec to 10 msec at a frequency of $10^{P}/_{S}$ or even to 100 msec with frequencies lower than $10^{P}/_{S}$. For this nerve at least it appears that the threshold voltage using pulse durations of 1 msec still lies on the flat part of the strength-duration curve. In measuring the rheobase therefore a pulse duration of 1 msec was used but it is appreciated that this is open to objection and that the terms "rheobase" and "chronaxie" may not correspond exactly to these terms as used by Lapicque.

In the present experiments the rheobase was measured by stimulating the nerve at its optimal frequency with a pulse duration of 1 msec and increasing the voltage gradually from zero until the first appearance of a response. The rheobase was doubled for subsequent use in measuring the threshold for pulse duration, the "chronaxie". Progressive reduction of the pulse duration had no effect on the response until a duration of 0·05 msec was reached when there was a slight reduction both with the sympathetic and parasympathetic nerves (Fig. 28). This reduction was present at all frequencies but was a greater proportion of the response at low frequencies. At a pulse duration of 0·01 msec there was no response from stimulation of either outflow with a voltage double the "rheobase". A response with a pulse duration of 0·01 msec could be obtained but only if the voltage was increased to about ten times the "rheobase". The "chronaxie" for both nerves seemed to be between 0·01 and 0·05 msec with a few fibres having a "chronaxie" between 0·05 msec and 0·1 msec. The reduction in the response when the fibres with longer excitation times dropped out was greater at low frequencies than at high. The explanation of this is not known. If, as has been suggested, there is some ubiquitous terminal nerve net in which impulses from the extrinsic nerves may be modified before they are conducted to the effector muscle cells it is possible that, at the optimal frequency of stimulation, every extrinsic fibre is not essential to maintain this

net in maximal activity. Thus fibres which drop out on lowering the pulse duration to 0·05 msec do not cause a corresponding reduction in the response.

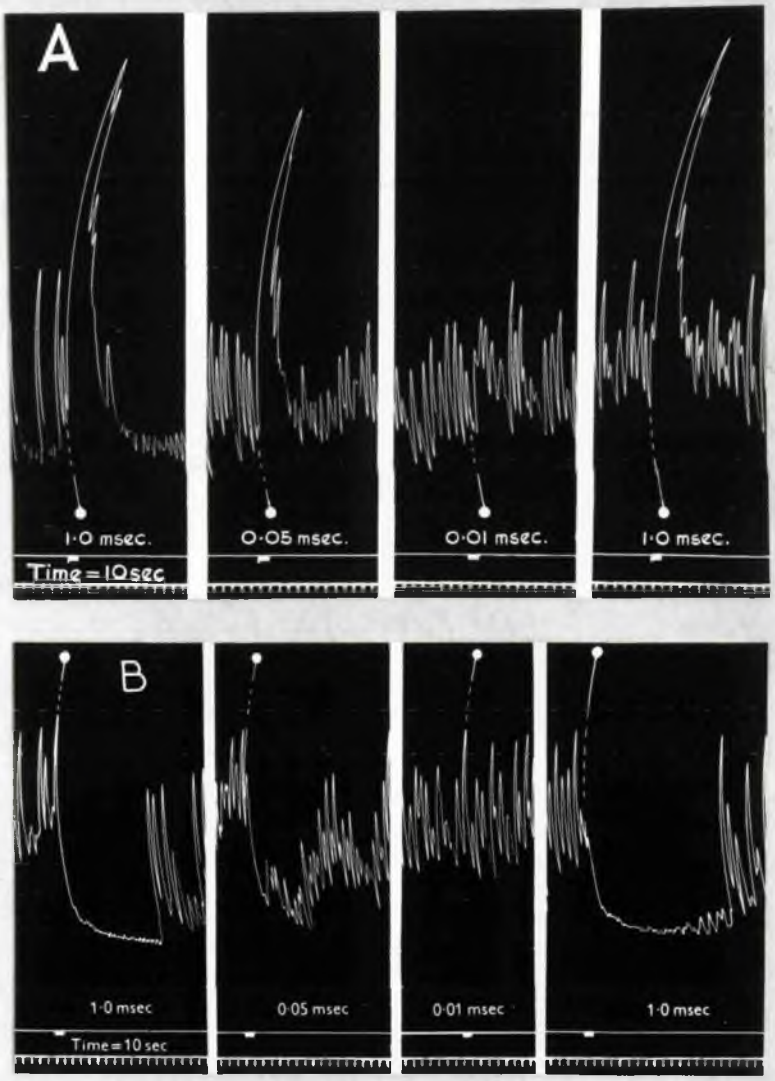


Fig. 28 The colon of the rabbit <u>in vitro</u>. The measurement of the "chronaxie" using repetitive stimulation at a voltage twice the threshold.

A. Stimulation of the pelvic nerves at $10^{P}/S$.

B. Stimulation of the lumbar colonic nerves at $100^{P}/S$.

No evidence was obtained from these experiments to support the belief that the pre-ganglionic parasympathetic and the post--ganglionic sympathetic nerve fibres differ in excitability. This does not agree with the results of Veach & Pereira (1925) who found that the excitability of the post-ganglionic cervical sympathetic fibres is less than the pre-ganglionic. Bishop & Heinebecker (1932) confirmed their findings but believe that they are due to the large number of post-ganglionic fibres which leave the superior cervical ganglion compared with the smaller number of pre--ganglionic fibres entering the ganglion. The increase in the size of the post-ganglionic nerve over the pre-ganglionic requires a larger voltage to produce the same current density. The pre and post-ganglionic fibres are equally excitable.

The influence of the total duration of stimulation on the response

McSwiney & Robson (1931a), stimulating the periarterial nerves of *in vitro* preparations of the stomach in the cat and the rabbit, found that a high frequency ($50^{P}/_{S}$), which normally would cause inhibition, if applied for short periods (0·07 - 2 sec) caused contraction. If these short periods of stimulation were repeated at 15 sec intervals a continued motor effect was produced. These authors did not suggest any explanation for this. In a latter article Harrison & McSwiney (1936) came to the conclusion that the variations in the response of the stomach were due to the presence of adrenergic and cholinergic fibres. This seems to imply that fewer impulses are required to produce a measurable effect when stimulating the cholinergic fibres than when stimulating the adrenergic fibres. Indirect evidence supporting this concept is given by the longer latent period of inhibitor responses compared with motor (McSwiney & Robson,1931a; Garry & Gillespie, 1955) and the complete absence of any report that a single stimulus has ever produced an inhibitor response, while single pulses have, though infrequently, been reported to produce a motor response (Rabinovich, 1928; McSwiney & Robson, 1929b, 1931a; Bain & McSwiney, 1935; Garry & Gillespie, 1955).

Periods ranging from 1 sec to 1 hour for the pelvic nerve and

from 8 sec to 4 min for the lumbar colonic nerves were used in stimulating the nerve outflows to the rabbit colon preparation. The response typical of the nerve stimulated was never reversed. The duration of stimulation did affect the response in another way. Continued stimulation of the pelvic nerve was followed by a decline in the initial tetanic response and by a reappearance of rhythmic contractions. After three or four minutes a steady state was reached in which there was an increase in tone and in rhythmic activity. This remained until stimulation was stopped when it was succeeded by a phase of inhibition followed in turn by a gradual recovery to the state preceeding the period of stimulation. This "fatigue" of the initial tetanic response from stimulation of the pelvic nerve would be paralleled by "escape" from the inhibitor response from lumbar colonic stimulation. No such "escape" was seen with inhibitory stimulation. The lumbar colonic nerves seemed much more resistant to fatigue and continued to exercise an undiminished inhibitory influence to the end of the periods of stimulation used in these experiments. In one experiment, by an honours B.Sc. student, Mr. Kenmure, in which lumbar colonic stimulation was continued for 25 min some evidence of "escape" did appear as a slight rise in tone and an occassional slow contraction towards the end of this period.

The "Peripheral Mechanism"

Variations in the tonus of the gut have, next to variations in the frequency of stimulation, been the most frequently quoted cause of reversal of the response from stimulation of the extrinsic nerves. Many of the references to this reversal were reviewed in Part I of this thesis. McSwiney and his numerous co-workers were particularly impressed by this ability of the state of activity of the organ to determine the nature of the response to stimulation of the extrinsic nerves. They formulated the concept of the "peripheral mechanism" the activity of which determined the state of tonus of the muscle and also the type of response to nerve stimulation. The term "peripheral mechanism" included both myogenic and neurogenic factors. The myogenic was the length and tension of the smooth muscle fibres; the neurogenic the activity of the intrinsic nerve apparatuses. Indications of an active peripheral mechanism were the ability of the stomach to maintain an entogastric pressure (tonus), the presence of rhythmic contractions, a short latent period on indirect stimulation and a propensity for responding to stimulation of the extrinsic nerves with inhibition (McCrea et al, 1925). The experiments in which these effects of tonus were studied were mostly *in vivo*. If an active organ was required the animal was given a meal of lean meat beforehand or the stomach was filled with meat extract three

or four hours before the experiment. To ensure an inactive organ the animal was starved for twelve hours. In this way the activity was more or less determined before the experiment was begun and variations in this activity in any one animal were not easy to produce during the experiment. Comparisons of the effect of extrinsic nerve stimulation on the active and inactive stomach was therefore mostly a comparison between separate animals. In some experiments, because of fortuitous changes in tone or because repeated vagal stimulation had converted an inactive into an active stomach, comparison could be made in the same animal at different times.

In vitro studies of the influence of the peripheral mechanism on the response from stimulation of the extrinsic nerves was disappointing. Work by McSwiney & Robson (1929b, b; 1931a) on the response of muscle strips from the stomach to stimulation of periarterial (sympathetic) nerves or branches of the vagus nerve contained no report of alteration in the response with variations in activity. The reason for this could, of course, be the absence of sufficient variations in activity in vitro. It is interesting that McSwiney & Robson (1931b), using a doubly innervated preparation of the stomach, found the motor response from stimulation of the sympathetic nerve at low frequency to be reversed by first raising the tonus of the preparation by vagal stimulation. More recently Munro (1953) has reported both

inhibitor and motor responses from an *in vitro* preparation of the guinea pig colon. The presence of "tone" favoured inhibition. The importance of peripheral activity in determining the response *in vitro* has been demonstrated more successfully using drugs than nerve stimulation. In some of these experiments the chemical transmitters of nerve action, adrenaline and acetylcholine were used. Increase in peripheral activity by pilocarpine, $BaCl_2$, histamine, choline and pituitrin demonstrated reversal to inhibition of a previously motor response to adrenaline (Brown & McSwiney and McSwiney & Brown 1926).

The innervated rabbit colon preparation shows rhythmic activity *in vitro* and stimulation of the lumbar colonic nerves, or the addition of adrenaline to the bath, cause lengthening of the preparation, presumably due to a lowering of pre-existing tone of the smooth muscle cells. The degree of rhythmic activity and the amount of lengthening of the preparation on stimulating the sympathetic nerves varies from one preparation to another although the length of gut removed from the animal is always approximately the same. It is likely that these variations in activity and tone reflect variations in the "peripheral mechanism". In addition to these spontaneous variations in activity between preparations some method was wanted whereby the activity of any one preparation could be increased or decreased at will and the effects of this on the nerve responses studied. Two methods used in the past to

produce these variations have already been mentioned. The first of these was to use drugs such as pilocarpine and atropine to increase and decrease activity. The second to use stimulation of the antagonist nerve to produce this change of state. Both of these methods are open to objection. Drugs which change the state of the peripheral mechanism by acting on the membrane of the muscle or nerve cell at specific sites may simultaneously interfere with the action of the nerve at this same site. The resulting variation in the response to nerve stimulation may be related more to the mode of action of the drug employed to alter the state of the "peripheral mechanism" than to the alteration produced in this mechanism. This objection can be diminished by using several drugs with different modes of action so that the only thing they have in common is the ability to alter activity. Stimulation of one nervous outflow as a means of altering the "peripheral mechanism" is open to similar objections. The effects produced may not be due to alteration in the "peripheral mechanism" so much as to a direct antagonism (or more complex interaction) between the two nerves. That this can occur is shown by the abolition of the motor response from stimulation of the sympathetic nerves to the stomach if the vagus has previously been stimulated to fatigue (McSwiney & Robson, 1931b). Under these circumstances the tone and activity have returned to, or sometimes below, the previous base line and it would be expected that conditions would, if anything, be improved for demonstrating

a motor response from stimulation of the sympathetic outflow. In fact the motor response of this nerve is abolished. The ideal method of altering activity would be one which acted on the state of the contractile elements within the muscle cell or which increased the excitability of the nerve elements of the gut wall but without acting at any specific receptor site. Small variations in the H^+ content of the Ringer's solution have been shown to alter the tone of smooth muscle in vitro (McSwiney & Newton, 1927) and this might have been a suitable method. Certain technical difficulties however made it less simple than the two methods which were finally selected. These were:-

1) Glucose was added to the previously glucose free bath and the effect on the nerve responses of the rise in tone which follows the addition of this substance noted. This was a disappointing method. Not all preparations showed any rise in tone; when they did it was never very much and the rhythmic activity was little altered.

2) The temperature of the bath was altered. This method produced wide and easily controlled fluctuations in activity. Cooling the preparation diminished activity and lowered tone, eventually producing an arrhythmic and atonic gut. Heating above 36°C increased tone, increased the frequency of the pendular movements and diminished their amplitude.

Whatever variations in activity were observed, either naturally

occurring between preparations, or imposed on one preparation by varying the temperature or adding glucose, the response from stimulation of the pelvic nerve or the addition of acetylcholine to the bath was always contraction, and from stimulation of the lumbar colonic nerves or the addition of adrenaline to the bath, always inhibition. The effect of increasing tone by adding glucose is illustrated in Fig. 29.

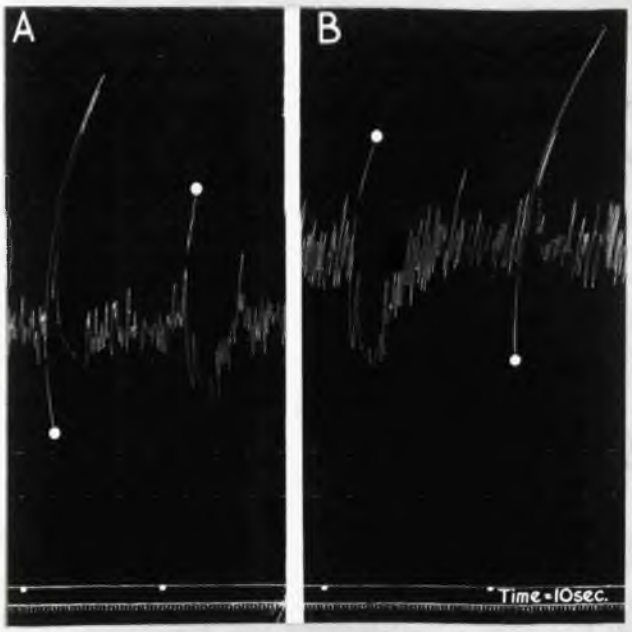


Fig. 29 The colon of the rabbit in vitro. The effect of raising the tone and activity of the preparation on the response to stimulation of the pelvic and lumbar colonic nerves. Between A. & B. sufficient glucose was added to the previously glucose-free Ringer's solution to produce a concentration of 0·1%.

The effect of altering the bath temperature on the movements and tonus of sections of gut in vitro merits fuller consideration. The response differs from that of smooth muscle because the bowel wall contains other important tissues, particularly nerve fibres and ganglion cells, in addition to smooth muscle. The effect of cooling these other structures may be to produce changes opposed to those produced by the change of temperature acting directly on the muscle. This will be clearer if the effects of temperature on the three tissues - smooth muscle, autonomic ganglion cells and nerve fibres are treated separately before considering the response of the organ as a whole.

Smooth muscle. Smooth muscle devoid of an intrinsic ganglionated plexus (the retractor penis for example) when heated relaxes and eventually dies, at a temperature between 47°C and 50°C, in heat paralysis (Vincent & Lewis, 1900; De Zilwa, 1901; Evans, 1926). At the point at which devitalisation occurs there is a small temporary contraction due to coagulation of paramyosinogen. With further heating the muscle remains relaxed until somewhere in the range of 60°C to 70°C when a strong contraction or heat rigor appears. This has been variously attributed to coagulation of myosinogen (Evans, 1926) or of connective tissue elements (Vincent & Lewis, 1900). Cooling smooth muscle produced a state of intense contraction which can be maintained for long periods with little deleterious effect on the muscle. On rewarming the original state

and ability to respond to drugs is restored (De Zilwa, 1901; Evans, 1926).

Autonomic nerve fibres & ganglion cells. The useful range of temperature in investigating the effect of the "peripheral mechanism" on the response to stimulation of the extrinsic nerves will be limited to that within which the nerve fibres still conduct and the synapse, where present, still transmits nerve impulses. It is therefore useful to have some idea of the upper and lower limits of temperature for conduction in autonomic fibres and transmission at autonomic ganglia. In the literature the temperature at which cold block in autonomic nerves appears varies quite widely. This is probably partly due to the methods of cooling the tissues. In some experiments it is difficult to know exactly what the tissue temperature was as the temperature given refers to the cooling device (the fluid in a cooling spiral for instance). Fibre size will also play a part: as Widdicombe(1954b) has pointed out the ability of a nerve fibre to withstand cooling is inversely proportional to its diameter. This author in two papers (Widdicombe 1954a, b) has reported vagal afferent fibres whose critical temperature for conduction varied from $0^{\circ}C$ to $19^{\circ}C$. Those requiring very low temperatures for block had also a higher threshold for stimulation. Roger & Bercovitz (1921) reported block of efferent vagal fibres to the stomach at $10^{\circ}C$ while Boycott (1901) found vagal fibres in the rabbit to conduct at a

temperature as low as 0°C. Part of the difference between these authors may be in methods. Roger & Bercovitz lowered the general body temperature so that block may not have been in the nerve fibres but in some more sensitive region of impulse transference. Sympathetic pre-and post-ganglionic fibres were reported by Eve (1900) to be blocked at between 5°C and 7°C. This author also investigated the effect of temperature change on the superior cervical ganglion and ganglionic transmission. The maximum temperature for conduction across the ganglion was as high as the upper limit of viability of smooth muscle (50°C). On cooling, however, transmission was blocked at a temperature between 19°C and 27°C in the rabbitand between 10°C and 18°C in the cat.

Lengths of gut as Magnus Preparations. In such preparations the intrinsic plexuses, comprising ganglion cells, nerve fibres and probably some form of sensory receptor, have considerable autonomy and continue to function in vitro. The extrinsic nerves, both parasympathetic and sympathetic, lose their myelin sheath either before or on entering the gut wall. The parasympathetic fibres are interrupted at a ganglion and the post-ganglionic fibres are again unmyelinated. If it is true that resistance to cooling increases the smaller the fibre, then the resistance of the intrinsic nerve fibres, whatever their origin, ought to be high. If, as appears likely, transmission across a synapse is more sensitive to block than conduction in a nerve fibre then the

parasympathetic outflow will be more sensitive to block than the post-ganglionic sympathetic. These neural elements are said to be continually synthesising and releasing acetylcholine (Dikshit, 1938). Cooling such preparations first reduced and later abolished this production of acetylcholine and the removal of this potent chemical stimulus more than compensated for the direct contracting effect of the lowered temperature on the smooth muscle. On cooling the first effect of the reduced acetylcholine production seems to be disappearance of the peristaltic reflex (Henderson, 1928; Ambache, 1945). That this is due to a failure of ganglionic transmission is shown by the persistence of a contractile response to eserine and the ability of the ganglion cells to respond to nicotine (Ambache, 1945). Further cooling lowers tone and abolishes the rhythmic activity. If these preparations are left in the cold for prolonged periods (4 - 5 days) they lose their ability to contract rhythmically when rewarmed (Ambache, 1945).

It seems then that the weakest link in lengths of gut, with their extrinsic nerves intact, is likely to be the ganglionic transmission affecting only the parasympathetic nerve. It is unlikely that there is any great difference between the muscle and the intrinsic nerve fibres whose resistance to cooling is probably very great: the likeliest structure to fail after the synapse would be the extrinsic nerves themselves.

The response of the rabbit colon to heating and cooling conforms to the pattern which has been described. On lowering the temperature from 36°C there was no effect until about 32°C when the frequency of the rhythmic contractions began to fall. Further cooling produced a fall in tone on which were superimposed slowly developing contractions of progressively diminishing frequency. At first the amplitude of these contractions was large but eventually this too was reduced. Finally, all rhythmic contractions disappeared and, as will be shown later, the preparation also became atonic. The temperature required to reach this arrhythmic, atonic state varied little. It was usually between 17°C and 19°C but on one occasion was 16·5°C. Sometimes rhythmic activity was not completely abolished, an occasional slow contraction and relaxation appearing at intervals of about five minutes. These contractions were so infrequent that, even when present, there was no difficulty in distinguishing them from nerve responses. Rewarming the preparation restored completely the tone, rhythmic activity and responses to nerve and drug stimulation. Heating the preparation above 36°C caused a rise in tone, an increase in the frequency of the pendular movements and a decrease in their amplitude. These effects were reversible up to a temperature of 48°C. Above this the gut did not recover on cooling.

These temperature changes, in spite of the wide fluctuations in tone and rhythmicity which they produced, never reversed the

response from stimulation of the two nerve outflows. The pelvic response remained motor though progressively reduced as the activity and tone of the preparation increased with heating until it finally disappeared at about 45°C. Cooling restored the response. Lowering the tone and activity by cooling the preparation did not reverse the inhibitor response from stimulation of the lumbar colonic nerves. Because the gut was relaxing as a response to cooling it was difficult to distinguish between inhibition from nerve stimulation and from cooling. When the arrhythmic state was reached lumbar stimulation produced no apparent effect (Fig. 30). This is misleading; an inhibitory influence is still exerted but in the absence of rhythmic activity or tone there is no apparent response. The still-present inhibitor effect was shown by superimposing pelvic stimulation on a period of apparently ineffective lumbar stimulation. As shown in Fig. 30 there was over 50% reduction in the motor response. This complete absence of a motor response on stimulation of the lumbar colonic nerves in a preparation whose atonicity made it easy to detect the smallest contraction, and in which pelvic nerve stimulation was still producing large contractions, is striking evidence that alteration of the "peripheral mechanism" in this preparation does not alter the nature of the response.

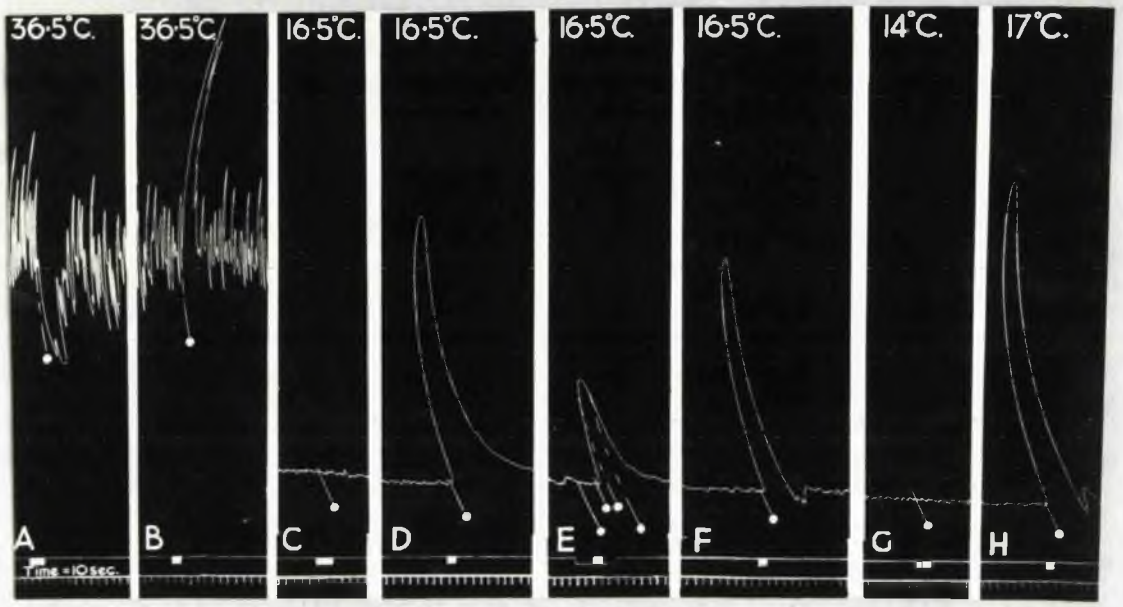


Fig. 30 The colon of the rabbit *in vitro*. Complete abolition of the tone and activity of the preparation by cooling. The bath temperature is indicated above each response. A. & B. show the characteristic responses to stimulation of the two outflows at 36·5°C. C. shows the apparent absence of any effect from lumbar colonic nerve stimulation at 16·5°C; D. the contraction from stimulation of the pelvic nerve still present. In E. pelvic nerve stimulation has been interpolated during a period of apparently ineffective lumbar colonic stimulation: the motor response is reduced. G. & H. demonstrate reversible block of the pelvic response at still lower temperatures.

Pelvic nerve stimulation continued to produce a motor response as the gut was cooled. This response was smaller than that at 36°C in that the final state of contraction was less. Because it was superimposed on inactive and atonic muscle it was, however, easier to detect and the actual amount of shortening was often as great as at the higher temperature. At low temperatures (below 20°C) the latent period of contraction was much prolonged and the rate of contraction and relaxation became much slower so that what appeared on the graphic as a "spike" at 36°C became a slow rounded contracture at lower temperatures (Fig. 30). The temperature at which these nerve responses disappeared fits well with what would be expected from the previous short discussion of cold block in nerve cells and fibres. The ranges in three experiments within which cold block appeared were 15·5°C - 17·5°C: 13·5°C - 16°C: 14°C - 16·5°C giving an average of 15·5°C. An illustration of this blocking effect and its complete reversal is shown in the latter part of Fig. 30. It was not possible to measure the temperature at which the lumbar colonic nerves were finally blocked since stimulation of these nerves could be shown to be effective only by demonstrating a reduction in the motor response from pelvic nerve stimulation. The temperature blocking the pelvic nerve therefore set the lower limit for this method and all that can be said is that the lumbar colonic nerves were not blocked before the pelvic nerves. It is a pity that some other method of detecting function in the

lumbar colonic nerves at lower temperatures was not tried. Possibly reduction in an acetylcholine provoked contraction in place of the pelvic response might have been successful and allowed measurement of the temperature required to block these nerves. Since the threshold for stimulation of both pelvic and lumbar colonic nerves is similar the nerve fibres would not be expected to differ very much in the temperature needed to block them. This might have provided evidence that the pelvic nerve block was located not in nerve fibres but at the synapse.

The action of hexamethonium and of atropine

These drugs are included not as a study in pharmacology but for the light they throw on the organisation of the extrinsic nerves to the colon. Hexamethonium was used to determine whether a synapse interrupted the extrinsic nerve pathway between the point of stimulation and the effector muscle cells. Atropine was used in an attempt to repeat in the colon McSwiney & Robson's (1929a, b) demonstration that, after atropine, stimulation of the vagus nerve innervating muscle strips from the stomach produces inhibition.

The logical complement to the experiments using atropine to uncover an inhibitor component from vagal stimulation would have been to use sympatholytic agents to reveal any non-adrenergic motor component from stimulation of the lumbar colonic nerves. This was not possible; there is no adrenergic blocking agent which has been conclusively shown to abolish inhibitor responses of smooth muscle (Nickerson, 1949). Of the drugs which have been reported at some time or other to block these effects (bulbocapnine, boldine, yohimbine, dihydro-ergot derivatives, certain benzodioxanes, phenoxyethylamines, ephedrine) many have complex side effects or are actually highly toxic to smooth muscle (bulbocapnine). Two blocking agents, ephedrine and C7337 (Rogitine) were used in an attempt to reverse the effects of stimulation of the sympathetic nerves but without success.

The action of hexamethonium (C6). This drug was chosen from the group of ganglionic blocking agents because of its potency and extreme selectivity. Other than blocking ganglionic transmission it appears to have few actions. It does not stimulate ganglion cells before blocking transmission, has no muscarinic or atropine--like effects (Paton & Zaimis, 1952) and slight, if any, histamine releasing ability (Paton & Zaimis, 1949). It effectively blocks transmission at sympathetic ganglia without altering conduction in the post-ganglionic fibres or the ability of the effector muscle to respond (Paton & Zaimis, 1951). Perry & Talesnik (1953) have demonstrated a similar block of a parasympathetic ganglion without modification of post-ganglionic conduction or smooth muscle response. It does, under certain circumstances act at one other site, sensory nerve endings, which may be of importance in experiments on the gut especially when using nicotine as a stimulating agent. Its ability to block the stimulating effect of acetylcholine and of nicotine on the sensory receptors of the carotid body and in the skin have been demonstrated (Douglas, 1951; Douglas & Gray, 1953). The abolition of vasodilator axone reflex reported by Hilton (1954) may have a similar basis. Reports on the action of this drug on the gut show that the peristaltic reflex *in vitro* is weakened and finally abolished by low concentrations which do not affect rhythmic activity or tone. This effect is open to quantitative measurement. A reduction of 50%

is produced by a concentration of 1×10^{-6} of the iodide, equivalent to about 5×10^{-7} of the base. The effects of the drug can be reversed, though not easily, by washing. About ten minutes is required for restoration of the original response (Paton & Zaimis, 1951). The motor effects of nicotine can also be blocked by hexamethonium (Feldberg, 1951).

Hexamethonium added to the bath in which the rabbit colon preparation was suspended blocked completely the response to stimulation of the pelvic nerve without altering the inhibition resulting from stimulation of the lumbar colonic nerves, or the tone and spontaneous activity of the preparation. This effect of the drug is illustrated in Fig. 31.

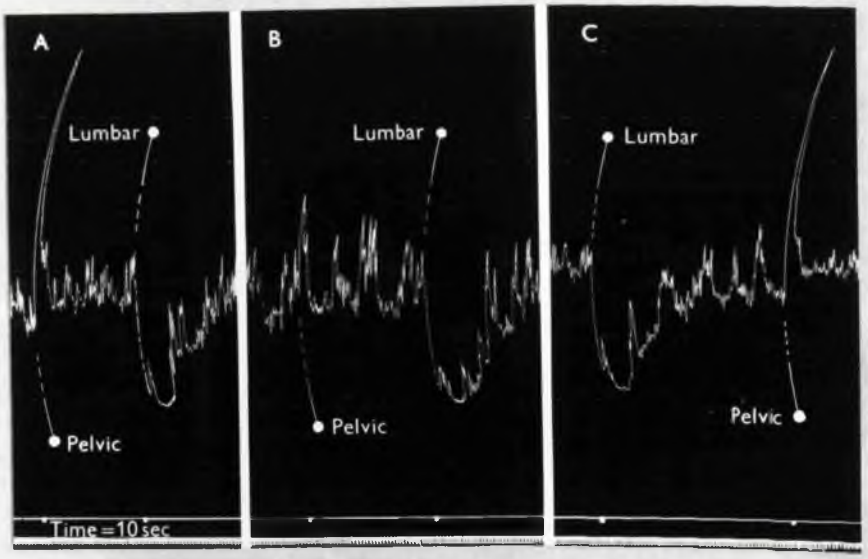


Fig. 31 The colon of the rabbit in vitro. Pelvic nerve stimulation at $10^{P/}_{S}$; lumbar colonic nerve stimulation at $100^{P/}_{S}$. Voltage 15 and pulse duration 1 msec for both. Between A. & B. hexamethonium was added to the bath to give a concentration of 2×10^{-4}. Between B. & C. the preparation was washed several times.

The drug produced a graded block of the response to pelvic nerve stimulation. The threshold concentration was about 1×10^{-5}. A concentration of 5×10^{-5} always produced some reduction in the response but, for complete block, a concentration of 1 or 2×10^{-4} was required. This effect of the drug was completely reversible, but several washes and an interval of as much as half an hour were required to restore the original response. On exposing the preparation for a second time to the drug the response was abolished more rapidly and at a lower concentration, indicating an increase in sensitivity of the preparation or incomplete removal of the drug after the first exposure. Blocking the response from pelvic nerve stimulation did not reveal an inhibitor component i.e., there was no evidence of direct, non-synaptic inhibitor fibres in the parasympathetic outflow.

The concentration required to block the pelvic response is very much greater than that reported to block the peristaltic reflex in the ileum of the same animal (Paton & Zaimis, 1951). Concentrations for comparable effects would be 5×10^{-5} for approximately 50% block of the pelvic nerve and 5×10^{-7} for the same degree of block of the peristaltic reflex (both concentrations refer to the base). Several explanations for this difference could be offerred. The sensitivity of different ganglia is known to vary widely. Such a difference between the ganglion cells of the small intestine and the large intestine might account for the

difference in drug concentrations required in the two regions. Alternatively, the ganglion cells mediating the peristaltic reflex may not be the same cells which act as the post-ganglionic neurone of the parasympathetic outflow and this difference might account for the varying sensitivity to hexamethonium. Secondly, the difference might be more apparent than real, and be due to using two different methods of assessing block in the different regions. It has been shown that the ease with which block is induced by C6 depends to some extent on the activity at the synapse. If this is high block is correspondingly easy to induce. Peristalsis might involve greater synaptic activity than impulses coming through from the extrinsic nerves and so be more easily blocked. Furthermore, since C6 acts as a competitive blocking agent it may be that extrinsic nerve stimulation, by some mechanism of convergence or overlap, can liberate more transmitter than can the impulses mediating the peristaltic reflex, and thereby compete more effectively with C6 for the receptor substrate. A third possibility is that, in abolishing the peristaltic reflex, C6 acts at some point other than ganglionic transmission. This reflex almost certainly has some sensory receptor and afferent link to the ganglion cells. It was pointed out earlier that C6 may be able to act at sensory receptors and it is theoretically possible that this action, rather than ganglionic blockade, is responsible for the disappearance of peristalsis. This explanation is

unlikely. Although C6 can prevent the stimulating effect of acetylcholine and nicotine at sensory receptors in the skin and carotid body it is quite incapable of blocking the response of these structures to their physiological stimulus, touch and anoxia even in the presence of concentrations of hexamethonium sufficient to produce deep ganglionic depression (Douglas, 1951; Douglas & Gray, 1953). The first step in assessing which if any of these mechanisms was responsible was, obviously, to study the effect of C6 on peristalsis in the colon. The results of these experiments were not easy to interpret chiefly because even very high concentrations of C6 did not abolish all expulsive activity. Even after 2×10^{-4} (Fig. 32) waves of contraction could be seen. Those, however, appeared to be given off from tone rings: they were often irregular in rhythm, always shallower than the previous peristaltic waves, and did not empty the colon completely. The receptive relaxation of the longitudinal and circular muscle to distension was abolished. Although these observations favour the view that such waves were not peristaltic in nature, it must be admitted that, after cocaine 2×10^{-5}, even these irregular waves were abolished. The effect of C6 in increasing concentration on both the peristaltic reflex and on the response of the longitudinal muscle to stimulation of the pelvic nerve is shown in Fig. 32.

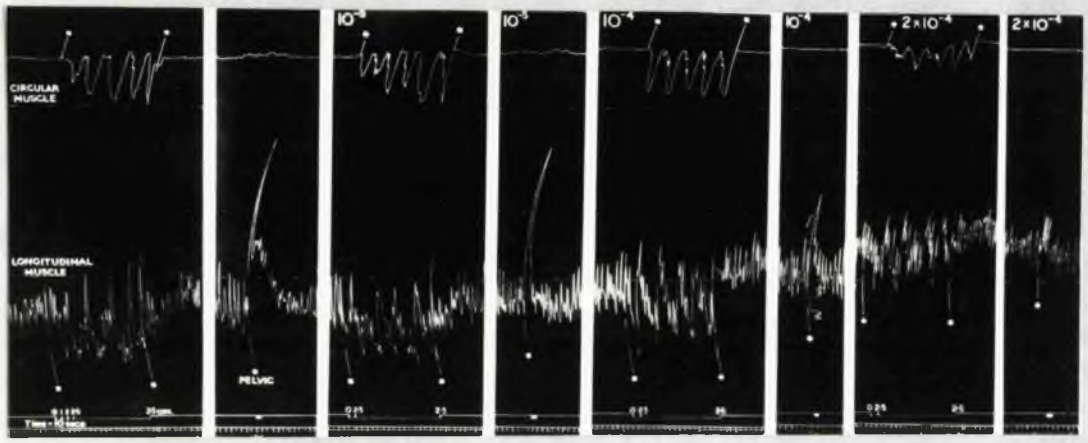


Fig. 32 The colon of the rabbit <u>in vitro</u>; Trendelenburg preparation. The action of hexamethonium on the peristaltic reflex and on the response of the longitudinal muscle to stimulation of the pelvic nerve. The concentration of hexamethonium present in the bath is shown at the top of each illustration.

It is obvious that the peristaltic reflex in the colon is no more sensitive to C6 than is the response from stimulation of the pelvic nerve. Both were unaffected until a concentration of 1×10^{-4} was reached when the pelvic response was reduced; at this concentration little effect on peristalsis was evident. At 2×10^{-4} the pelvic response was almost completely abolished and the regular peristaltic waves, plus the receptive relaxation of

the two muscle coats, had also disappeared. It appears, then, that the difference in sensitivity to C6 between the response of the colon to stimulation of the pelvic nerve and the reflex peristaltic response of the ileum to distension is shared by the reflex peristaltic response to distension of the two regions. In the absence of evidence for some other mechanism, such as ease of access to the ganglion cells, these results imply a difference in sensitivity of the ganglion cells of the two regions to this drug. It might be noted that this difference is not helpful in interpreting the effect of C6 in man since autonomic blockade of the gastro-intestinal tract produced by this drug affects particularly the rectum, producing obstinate constipation (Kay & Smith, 1950). In the small bowel the syndrome of paralytic ileus would be produced but this is rare.

The action of atropine sulphate. This familiar drug has been used extensively in physiological and pharmacological investigations for many years and cosmetically and therapeutically for centuries, yet its mode of actions and even, in certain respects, what that action is, is still not clear. Most text books attribute to it the ability to block the muscarinic action of acetylcholine and those effects of autonomic nerve stimulation which are produced by the peripheral liberation of acetylcholine. In the larger textbooks this is accompanied by a footnote to the effect that the action of the parasympathetic fibres on the smooth

muscle of the gut and bladder, and on certain blood vessels is not blocked, although similar effects produced by acetylcholine are. On looking at part of the literature on this drug it is clear that its ability to block the muscarinic effects of added acetylcholine either *in vivo* or *in vitro* has general support as has its blocking action on the vagus to the heart. The reports, however, of its action on the response of smooth muscle to nerve stimulation contain many contradictions. In fact, almost every possible combination of actions on the two divisions of the autonomic nervous system, with the possible exception of the ability to block the effects of sympathetic stimulation without affecting the response to para-sympathetic stimulation, has been suggested at one time or another.

Bayliss & Starling (1899) found that a dose of atropine six times that required to block the action of the vagus on the heart did not affect the response of the small intestine to stimulation of this same nerve (dog). Langley & Anderson (1895) found that intravenous injection of 20 - 50 mg of the drug did not abolish the movements of the colon on stimulating the pelvic nerve, though it did produce paralysis of the recto-coccygeus muscle (rabbit). Langley (1898) reported that the augmentor action of the vagus on the stomach was only weakened when 15 - 20 mg of atropine was given intravenously to a rabbit. Henderson & Roepke (1934) found atropine ineffective in blocking the parasympathetic fibres to the gut or bladder (dog). It has even been reported that atropine

can augment the response of the bladder of the cat to parasympathetic stimulation (Edge, 1955). On the other hand, there are many reports of the drug successfully blocking parasympathetic nerve stimulation, as for example the motor and secretory response of the colon of the cat on stimulating the pelvic nerve (Wright, Florey & Jennings, 1938); the reflexly excited motor and secretory effect of the vagus on the stomach of the cat (Bachrach, 1953). The assumption that the drug will block cholinergic nerves to blood vessels is implicit in much of the work on vasodilator fibres to the blood vessels of the limb muscles and splanchnic region (Bülbring & Burn, 1935; Folkow, Haeger & Uvnas, 1948). The cholinergic nature of these fibres is demonstrated by augmenting the response with eserine and blocking it with atropine (cats and dogs). Finally, it has been reported that atropine in relatively small amounts (1 - 2 mg) blocked the response of the cardia of the stomach in cats to vagal and to splanchnic stimulation with equal facility (Carlson, Boyd & Pearcy, 1922).
It is difficult to reconcile these variations. Some, no doubt, are due to details of technique. The rabbit, for instance, has the ability to hydrolyse atropine rapidly. Hobbiger & Lessin (1955) demonstrated this in the serum, and in their communication reported hydrolysis rates as high as 11·57 mg/ml serum/hour. It is obvious that, with such rapid destruction, the effect of the drug must be determined within a few minutes of intravenous injection as was pointed out by Marrazzi (1939). Another possible source of

variation is the species of animal used. The cat appears more susceptible to atropine than the dog or rabbit. Ambache & Edwards (1951) found it impossible to block the motor effect of nicotine on the rabbit intestine with atropine yet a concentration of 5×10^{-7} of this drug abolished the motor effect of nicotine on the intestine of the kitten. At the present time, however, it is accepted that the ability of atropine to antagonise the effects of stimulation of different cholinergic nerves varies. An explanation of this variation suggested by Dale & Gaddum (1930) is that atropine acts at the surface of the innervated cell and forms a "barrier" through which acetylcholine cannot pass. The response to nerve stimulation after atropine depends on the site of liberation of acetylcholine in relation to this "barrier". If wholly within the atropine "barrier" then the response is undiminished, if wholly without then the response is completely blocked e.g., the heart, and if the transmitter is liberated partly within and partly without, then the response is diminished.

Atropine sulphate added to the bath had the following actions on the rabbit colon. The first effect, appearing at concentrations as low as 1×10^{-7}, was inhibition of the response to added acetylcholine; at a concentration of 5×10^{-7} inhibition of tone and slight inhibition of the rhythmic contractions also appeared. At 1×10^{-5} there was some reduction in the pelvic response in all preparations, and this response was completely blocked at a

concentration of 1 or 2 x 10^{-4}. After complete block of the pelvic motor response stimulation of the nerve never produced inhibition; the stimulation was simply without effect on the preparation. These concentrations of atropine had no effect on the inhibitor response from stimulation of the lumbar colonic nerves. At the concentration required to block the pelvic nerve completely (1 x 10^{-4} or higher) atropine stimulated the gut causing a rise in tone and an increase in the frequency and amplitude of the rhythmic contractions. This effect appeared within thirty seconds and quickly reached its maximum whereas the blocking effect of the drug on the pelvic nerve deepened gradually over a period of about ten minutes. All of these effects, including the abolition of the motor response to pelvic nerve stimulation, could be removed by washing but this had to be repeated several times and a long interval, up to one hour, allowed. Even then complete restoration of the response to nerve stimulation was not possible in every preparation. These effects are illustrated in Fig. 33.

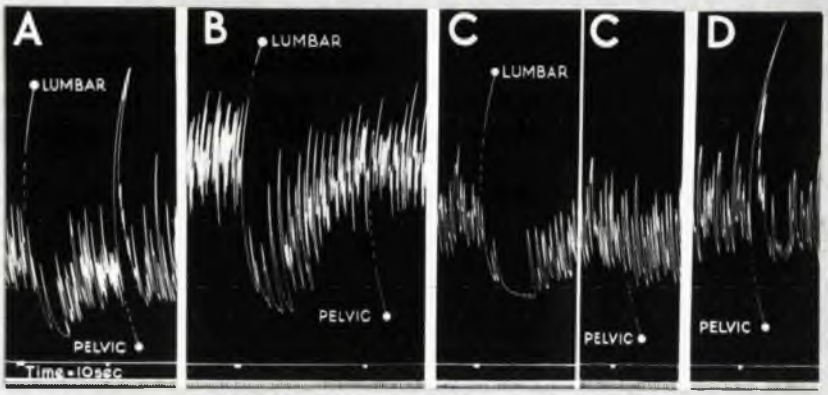


Fig. 33 Rabbit colon <u>in vitro</u>. The effect of atropine on the responses from stimulation of the pelvic and of the lumbar colonic nerves.
A. Before adding atropine.
B. In the presence of atropine sulphate 2×10^{-4}.
C. Shortly after washing.
D. One hour after washing.

Discrepancies were found in the threshold concentrations of atropine required to block the pelvic nerve in different experiments. In some, concentrations as low as 1×10^{-7} reduced slightly the response, whereas in others the first effect did not appear until a concentration of 1×10^{-5} was reached. Whatever the threshold the concentration required to produce complete nerve block was the same, 1 or 2×10^{-4}. This fact made it unlikely that the differences in the threshold concentrations of the drug were due to variations in the potency of different samples of atropine. The ratio between the concentrations producing maximal

and minimal effects in those preparations in which partial block appeared at low concentrations is approximately 1000:1 which seems extraordinarily large. In such preparations (Fig. 34) the first effect is appearing at concentrations which are just effective in blocking added acetylcholine i.e., partial block of the nerve response is as easy to produce as abolition of the response to acetylcholine even though the preparation still shows great resistance to complete block of the pelvic nerve (Fig. 34).

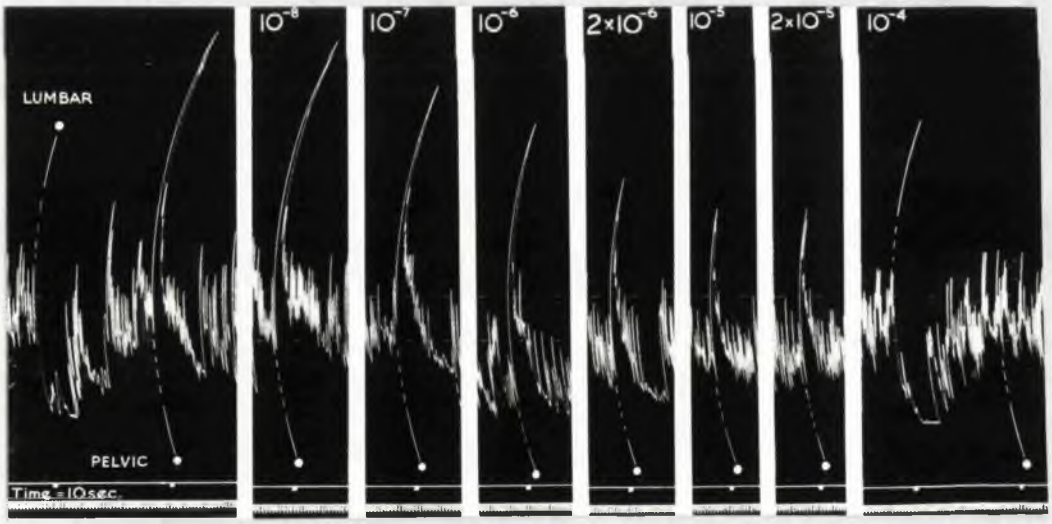


Fig. 34 Rabbit colon <u>in vitro</u>. The effect of atropine on the responses from stimulation of the pelvic and of the lumbar colonic nerves. The concentration of atropine sulphate in the bath is shown at the top of each illustration. The first reduction in the response from stimulation of the pelvic nerve is at 10^{-7} but complete block requires 10^{-4}.

These results suggest that the initial reduction in the response is due to the elimination of the effect of acetylcholine liberated from some site on the nerve-muscle pathway and diffusing to the muscle cells. This diffusion component of the response would, of course, be as open to block by low concentrations of atropine as is the response to acetylcholine added to the bath. The hard core, as it were, of the nerve response, which was blocked only by large concentrations of atropine is produced by some more intimate form of nerve-muscle transmission in which the transmitter is liberated within the atropine "barrier". This idea that the response of smooth muscle to parasympathetic nerve stimulation consists of two parts, one due to diffusing acetylcholine and easily blocked by atropine, the other to direct nerve-muscle transmission and not susceptible to atropine, is not new. Henderson & Roepke (1934) believed that some such mechanism best explained the action of atropine on the response of the bladder to stimulation of the pelvic nerve. There are, it should be admitted, difficulties in applying this hypothesis to the present results. It would have to be assumed that, in those preparations in which atropine was ineffective until high concentrations were reached, the diffusion component of the response was non-existent. It would be reasonable to expect that, under these conditions, the response to stimulation of the pelvic nerve would be small, corresponding to the response which remains after elimination of the diffusion component in

preparations which are sensitive to atropine in low concentration. This was not always the case. In one experiment in particular the initial response to stimulation of the pelvic nerve was large yet atropine produced no reduction in it until a concentration of 1×10^{-5} was reached.

The action of atropine on the tone and spontaneous activity of the colon depends largely on the treatment of the preparation before it is suspended in the bath. Attention was drawn to this by certain differences in the action of atropine in the earliest experiments in the series and those some time later. Although, for technical reasons, no conclusions can be drawn from these early experiments on the effects of atropine on the nerve responses the ability of the drug to inhibit tone and activity was clear. An interval of over a year passed before the drug was re-investigated and it was now found to have little if any effect on tone or rhythmic activity in spite of the fact that, with the improvement in technique, the preparations were more active than before. So notable was this difference that doubt was thrown on the efficacy of the atropine. In one experiment, in which the drug appeared to have no effect on tone, a length of ileum from the same animal which had been covered by a swab moistened with chilled Ringer's solution was set up in the bath. This preparation was rhythmically very active yet again atropine failed to produce inhibition. A frog heart preparation was then set up and the same atropine solution

shown to antagonise the action of acetylcholine thus exonerating the drug. The various changes which had been introduced since the early experiments were reviewed and most excluded as being responsible for the altered response to atropine. These included the presence of glucose in the Ringer's solution, the change to Krebs' saline and changes in the recording system.

In the early experiments the preparation included only one nervous outflow, the parasympathetic. The anatomy was not well understood at that time and the importance of visualising the cranial strand to its junction with the hypogastric nerve was not appreciated. As a result the pelvic nerves were isolated for about 1 cm from their origin only. The dissection was completed by making a bold sweep through the dorsal mesocolon keeping as near to the dorsal wall as possible. However unhappy the results of this technique were for the cranial strand and sacral colonic nerves, it did have the virtue of speed. These preparations with the pelvic nerve only were sometimes suspended in the bath about half an hour after the animal's death. Furthermore, during the dissection the tissues were kept moist with saline at room temperature or even slightly warmed in place of the chilled Ringer's solution introduced later. The possibility that this longer dissection time and the chilling of the preparation was responsible for the altered response to atropine was studied on lengths of ileum and colon, without extrinsic nerves, suspended as Magnus preparations in a double organ bath. An

animal was killed and a length of colon and a length of ileum removed as quickly as possible. Each of these was divided into two equal parts and one section of colon and one of ileum suspended immediately in the double organ bath. The two remaining halves of colon and ileum were kept in chilled Ringer's solution for two hours and then they in turn were suspended in the organ bath. The results of this experiment are illustrated in Fig. 55. A most interesting feature was that the preparations chilled for two hours were rhythmically more active than those suspended immediately yet their sensitivity to acetylcholine was reduced; 10^{-8} produced a smaller contraction in the chilled preparations than 10^{-7} in those suspended immediately. This alteration in sensitivity to acetylcholine was not due to decomposition of the drug in the two hours between tests since on each occasion it was freshly prepared from the solid immediately before use. Nor can these results be explained by differing lengths of gut. True, the original lengths were only approximately halved but there was no gross difference in length such as would explain the great increase not only in amplitude but also in the frequency of contractions. Nor would it explain the fact that the more active preparation, presumably the larger, contracted less with acetylcholine than the shorter.

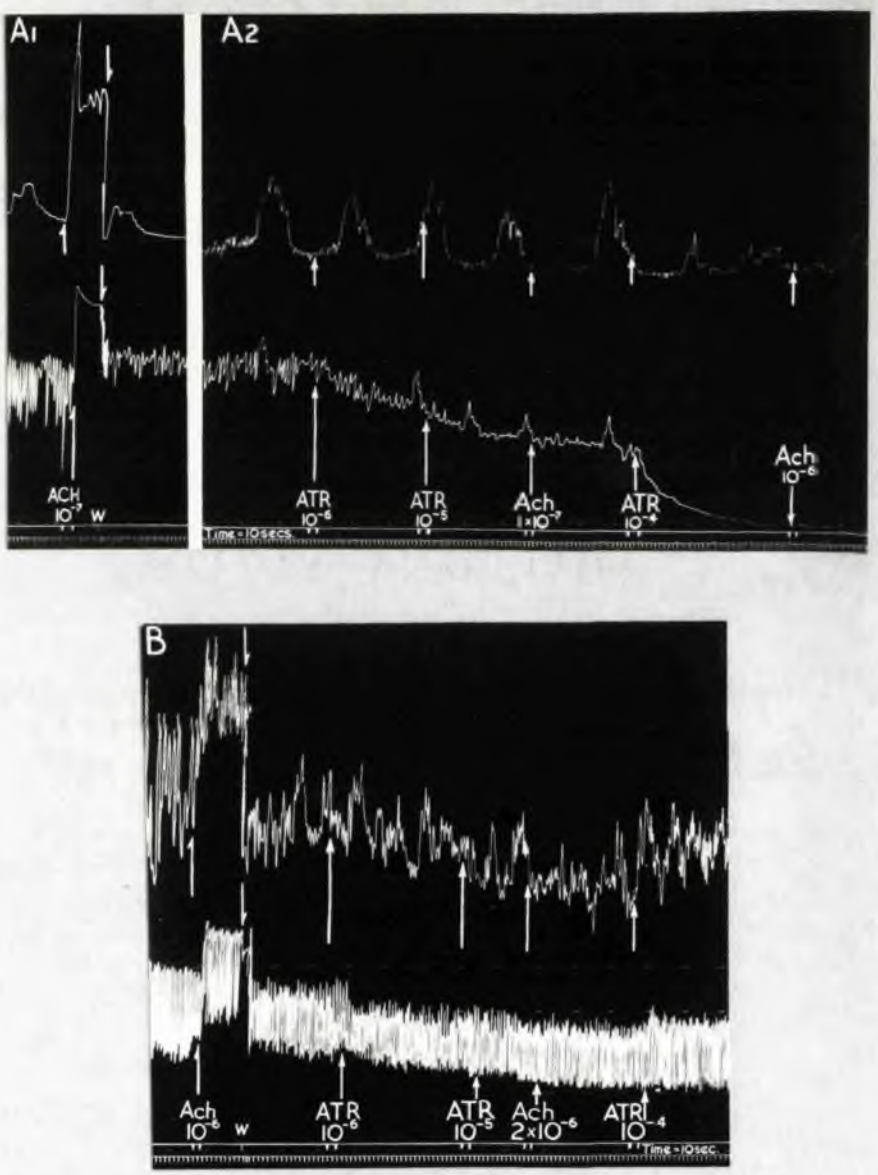


Fig. 35 Rabbit colon and ileum in vitro. In each record the upper trace is of the colon the lower the ileum. The preparations in A. were suspended immediately they were removed from the animal. The preparations in B. were suspended after 2 hr in chilled Ringer's solution. Both ileal preparations and both colon preparations were from the same region and were equal in length. The effect of storing for 2 hr in the cold on the response to atropine and acetylcholine is shown.

The colon was more resistant to atropine than the ileum even in the freshly suspended preparations, requiring high concentrations to inhibit the rhythmic contractions. The freshly suspended section of ileum was very sensitive to atropine. The first addition of the drug, producing a concentration of 1×10^{-6}, lowered tone and this effect increased up to 1×10^{-4} which produced complete arrythmicity and a large fall in tone. After two hours chilling (Fig. 35B) both preparations, in spite of being more active, showed almost no alteration in tone or activity on adding atropine up to a concentration of 1×10^{-4} when there was the customary rise in tone and increase in activity in both. The results with the colon were possibly not very convincing. Both chilled and unchilled preparations showed little response to atropine. This may have been due to a lack of tone as the pattern of activity of these lengths of colon was not typical. The dramatic change in the activity of the ileum, however, and in its response to drugs demonstrates that chilling the gut for two hours may abolish or greatly reduce the inhibitory effect of atropine even when the activity of the preparation would lead one to anticipate a good response. Subsequent to these investigations an article by Vogt (1943) was found in which strips of rabbit jejunum for pharmacological investigation were stored in Ringer's solution at room temperature as in this way their responses to drugs subsequently were only "insignificantly altered" whereas they were "strongly modified" by

storing in the refrigerator for the same length of time.

The concentration of atropine required to block completely the pelvic nerve was so high that it could not be said to be due to a specific peripheral antagonism of acetylcholine at the nerve muscle junction. It is becoming increasingly clear that atropine can antagonise the nicotinic actions of acetylcholine i.e., its stimulant effect at synapses and at the end-plate in striated muscle. The difference between the anti-nicotine and anti-muscarinic action is largely one of degree. The first clear demonstration of this anti-nicotine effect of atropine was by Marrazzi (1939) who showed that, in rabbits, pre-ganglionic impulses could be blocked at the superior cervical ganglion by atropine in quite small doses (2 mg/kg I.V.I.). The stimulating effect of acetylcholine on the perfused superior cervical ganglion of the cat has been shown to be blocked by atropine (Konzett & Rothlin, 1949) and the blocking effect on the same structure and on the end-plate of striated muscle demonstrated by Dutta (1949). The results of this last worker are particularly apposite to the present experimental findings. Using the rat phrenic nerve-diaphragm preparation he found that atropine, at a concentration of $1{\cdot}2 \times 10^{-4}$, facilitated the response to both direct and indirect stimulation. Higher concentrations, $3{\cdot}2 \times 10^{-4}$, depressed the response to nerve stimulation. The similarity to the concentrations required to block the response from indirect stimulation of the colon and the presence of a stimulating effect

at concentrations which would stimulate the colon suggest that the action of atropine on the colon may not be at the periphery as an anti-muscarinic, but at the synapse as an anti-nicotinic agent. Support for this is provided by some experiments with nicotine. Low concentrations of nicotine produce inhibition of the colon, an effect abolished by both hexamethonium and atropine in concentrations which block the pelvic nerve. If this effect of nicotine is due to stimulation of adrenergic inhibitor ganglion cells, as has been suggested for the ileum of the cat (Ambache, 1951), then its abolition by large doses of atropine suggests an action at the ganglion cell. Further indirect evidence for such an action is provided by Langley's (1898) observation that the "weakening" effect of large doses of atropine (15 - 20 mg) on the motor response of the stomach of the rabbit to stimulation of the vagus nerve was additive to the effect of curare. Since the site of action on the autonomic nervous system of curare is the ganglion cell, this suggests that atropine also acts there. Against atropine blocking at parasympathetic ganglia is the inability of the drug to block the action of acetylcholine on the cardiac ganglia of the king crab, its only effect being to stimulate the ganglia at a concentration of 5×10^{-4} (Garrey, 1942).

If the site of action of atropine in concentrations producing complete block of the pelvic response is the ganglion synapse and not the nerve-muscle junction, it means that the pelvic nerve may

contain pre-ganglionic fibres which synapse with inhibitor neurones and whose presence will not be revealed by blocking with atropine. Nor can the possibility be excluded that post-ganglionic transmission is non-cholinergic as suggested by Henderson & Roepke (1934).

This brief consideration of the difficulties of interpreting the action of atropine on an in vitro preparation, with all the advantages of simplification and control that this method offers, may help to explain why the literature on this drug is so contradictory. It is probable that many of the apparent vagaries in action are due to variations in the conditions of the experiment, the species of animal used and unsuspected complexities in the action of a drug which at present is frequently, even for research purposes, over-simplified to "a cholinergic blocking agent".

The response of the colon to simultaneous stimulation of the sympathetic and parasympathetic outflows, and from stimulation of a "mixed" nerve

The preceeding account of the response of this preparation to stimulation of the extrinsic nerves shows that none of the alterations in the "peripheral mechanism" or in the method of stimulation which, in certain other regions of the gut reverse the response, has a similar effect on the colon of the rabbit *in vitro*. The absence of reversal has allowed the characteristics of the response to stimulation, and the effectiveness of various frequencies of stimulation to be studied. The most effective frequency of stimulation of the pelvic nerve is $10^{P}/_{S}$; the most effective frequency of stimulation of the lumbar colonic nerves is $100^{P}/_{S}$. The response to stimulation of the pelvic nerve appears after a short latent period, quickly reaches its maximum and, on stopping stimulation, quickly disappears. The response from stimulation of the lumbar colonic nerves appears after a longer latent period, develops more slowly but, on stopping stimulation, the inhibition persists and, if the period of stimulation has been short, actually increases in the post-stimulatory period. These differences between the nerves could explain the anomalies in the response of other regions such as the stomach, if the nerves to these areas were in fact "mixed", containing both sympathetic and

parasympathetic fibres. Low frequencies of stimulation would evoke preferentially the parasympathetic motor responses and, if the frequency was below the threshold for the sympathetic fibres but above that for the parasympathetic, only these motor effects would appear. High frequencies of stimulation, optimal for sympathetic fibres but above the optimum for the parasympathetic, would favour inhibitory responses with, possibly, a small initial motor response. The magnitude of this motor response would depend chiefly on the difference in latent periods between the two groups of nerve fibres. At the high frequencies, which are optimal for the lumbar colonic nerves, this difference is at its minimum so that the amount of contraction which the quick acting motor fibres could produce in the short interval before the slower inhibitor fibres become effective would be small. Intermediate frequencies, because of the temporal characteristics of the two responses would produce biphasic responses, first contraction then inhibition.

This hypothesis was tested in the rabbit colon by stimulating simultaneously a mixture of sympathetic and parasympathetic fibres. Three methods were used to obtain a mixed nerve:-

1) The electrode on the lumbar colonic nerves was advanced towards the colon in the hope of reaching a position at which sacral colonic fibres, after running freely through the mesocolon, had joined the periarterial nerves. This method, producing a naturally "mixed" nerve, was based on experiments

by Langley & Anderson (1895) in which they obtained inhibition only from stimulation of the upper pair of inferior mesenteric ganglia but both motor and inhibitor responses from the lumbar colonic nerves. Langley & Anderson had the advantage of observing the entire colon. The most cranial of the sacral colonic fibres joining the periarterial nerves are eventually distributed along with the cranial branches of the inferior mesenteric artery to the upper (oral) regions of the colon. The in vitro preparation unfortunately employs only the lower end of the colon and to advance the electrode far enough to include within it sacral colonic fibres actually destined for this region was not easy. The electrode when thus advanced interfered with the movements of the preparation and with its ability to respond to nerve stimulation. Nevertheless, successful preparations were obtained and these gave exactly the same results as preparations stimulated by the other two methods.

2) The pelvic nerve was removed from the lower electrode and pulled into the upper electrode along with the lumbar colonic nerves. This gave an artificially "mixed" nerve but still with all of the fibres simultaneously exposed to the same stimulus. The magnitude of the response to stimulation of the nerves "mixed" in this way showed that they were little damaged compared with the first method. In making the

preparation the pelvic nerves were cleared for a much greater distance than usual of the glutinous mesenchyme in which they lie. A sufficient free length of nerve was obtained to make it possible to pull them into the upper electrode without tension.

3) Both nerves were stimulated each in its own electrode by passing the current through them in parallel. Sometimes the two nerves were simultaneously stimulated using a separate stimulator for each nerve.

Whatever method was subsequently used to obtain a "mixed" nerve the outflows were first stimulated separately. This practice ensured that both nerves were effective and producing characteristic responses. It also allowed a frequency to be selected which produced a motor response when applied to the pelvic nerve but was ineffective for the lumbar colonic nerves. The results of stimulation of a "mixed" nerve were the same whether the nerve was artificially or naturally "mixed". An experiment in which the nerves were artificially "mixed" is illustrated in Fig. 36.

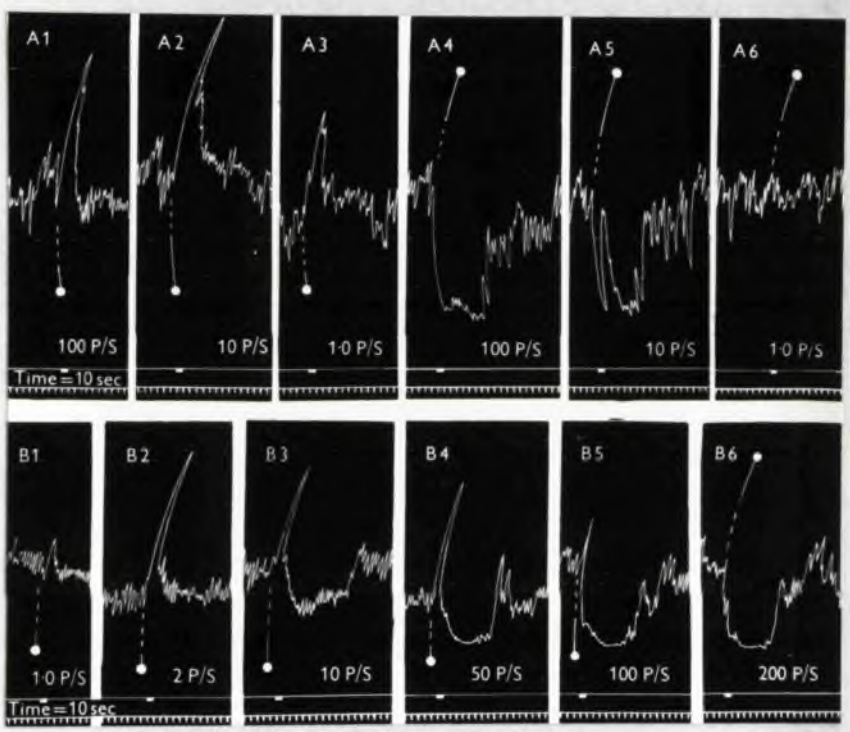


Fig. 36 Colon of the rabbit <u>in vitro</u>. Effect of simultaneous stimulation of a "mixed" nerve containing both sympathetic and parasympathetic fibres.

<u>A</u>. 1, 2, 3 - Stimulation of the pelvic nerves alone

<u>B</u>. 1, 2, 3 - Stimulation of the lumbar colonic nerves alone

<u>B</u>. 1, 2, 3, 4, 5, 6 - The same preparation after artificial mixing of these outflows. Low frequencies cause pure contraction, high frequencies pure inhibition. Intermediate frequencies give biphasic responses.

The upper six responses are from separate stimulation of the two nerves; the first three of the pelvic nerve, the second three of the lumbar colonic nerves. The response from stimulation of the pelvic nerve is contraction irrespective of frequency; from stimulation of the lumbar colonic nerves inhibition irrespective of frequency. The difference in frequency sensitivity is well shown. $10^{P/}S$ is the most effective frequency for stimulation of the pelvic nerve and a frequency of $1^{P/}S$ still produces an obvious response. Stimulation of the lumbar colonic nerves is most effective at $100^{P/}S$, at $10^{P/}S$ the response is reduced and $1^{P/}S$ is ineffective. At this point the pelvic nerve was removed from the lower electrode and drawn into the upper electrode alongside the lumbar colonic nerves. The results of stimulation of this "mixed" nerve are shown in the lower six responses. Frequencies previously shown to be below the threshold for the lumbar colonic nerves, but above threshold for the pelvic nerve, produce pure motor responses (B1, 2). Stimulation at $10^{P/}S$ produced a biphasic response, first contraction, then inhibition. The motor component at $10^{P/}S$ is less than the pure contraction at $2^{P/}S$ i.e., the effect of stimulation of the inhibitor fibres in the "mixed" nerve is to reduce the motor component as well as to produce the subsequent prolonged inhibition. At a frequency of $200^{P/}S$ the motor component, if present, is so small as to be undetectable and the appearance is one of pure inhibition. The relative magnitude of the motor and inhibitor component of the

biphasic responses resulting from stimulation at intermediate frequencies could be altered at will by appropriate variation in the frequency (compare B3, 4, 5 Fig. 36). Increasing the frequency augmented the secondary inhibition; lowering the frequency augmented the initial contraction.

D I S C U S S I O N.

These experiments demonstrate that the response of the colon of the rabbit to stimulation of its sympathetic and parasympathetic nerves shows none of the variations with the frequency of stimulation or with the state of tonus of the muscle which has been described elsewhere in the alimentary canal. The variability in other regions cannot therefore be considered a feature of all smooth muscle but is more likely due to some local peculiarity.

In the colon the sympathetic and parasympathetic outflows are antagonistic in their influence on the smooth muscle cells. This need not mean that under physiological conditions the two are always simultaneously active and opposing one another. Rather their opposing influence would be used to increase the range of activity possible, varying from maximal activity of the sympathetic and inactivity of the parasympathetic through various degrees of activity in both outflows to maximal activity in the parasympathetic and inactivity of the sympathetic.

The results reported here could explain the biphasic responses and the reversal of the characteristic response to nerve stimulation reported in the stomach and elsewhere. The difference found in the frequency sensitivity of the two outflows would provide a basis for the selective stimulation of one or other group of fibres in a

nerve which contained both sympathetic and parasympathetic fibres, at frequencies effective for both fibre groups. The difference in the temporal characteristics of the two responses would allow first the parasympathetic motor fibres and then the sympathetic inhibitor fibres to dominate, so producing a biphasic response. This explanation is supported by the fact that if the two nerve outflows to the colon are combined, then stimulation of this "mixed" nerve can reproduce all the variations in response recorded in the stomach and at similar frequencies. Low frequencies up to about $10^{P/S}$ produce pure contraction; high frequencies over $100^{P/S}$ produce pure inhibition, while frequencies intermediate between these values produce biphasic responses. A similar explanation has been given for the varying response of the blood vessels of the limb musculature to stimulation of the lumbar sympathetic chain. In this nerve both adrenergic and cholinergic nerve fibres are present (Bulbring & Burn, 1935; Folkow & Uvnas, 1948). Greene (1935) offered a similar explanation for the response of the coronary blood vessels to stimulation of the vagus in the neck. Constriction, dilatation and biphasic responses were obtained depending on the initial state of tonus of the vessels. These variations were due, according to the author, to the presence of vagal (constrictor) and sympathetic (dilator) fibres in the nerve stimulated. The sympathetic fibres synapsed in the stellate, middle and superior cervical ganglion, the post-ganglionic fibres crossing to mingle

with the vagal fibres as high up as the nodose ganglion.

It is useful to consider how the present experimental findings fit with each of the main theories which have been put forward in the past to explain the erratic response of the stomach to stimulation of the vagus or splanchnic nerve. These were reviewed in Part I of this thesis.

The "Peripheral Mechanism". In spite of the great alterations produced in the tonus and activity of the colon preparations the nature of the response was unaltered. Even in the arrhythmic and atonic bowel the influence of sympathetic nerve stimulation remained inhibitor, but demonstrable only by its ability to reduce the pelvic motor response. There is therefore no support for the theory that peripheral activity inevitably determines the type of response. It does not necessarily follow from this that a terminal nervous syncytium is impossible. It is true that this nerve net can have only one effect on the muscle cells either inhibitor or excitor. At this level there can no longer be any distinction between sympathetic and parasympathetic for this reason. Many who support this method of innervation have revived in some form or another the theory of the "peripheral mechanism" and no longer consider the sympathetic and parasympathetic to be antagonist nerves. If it is assumed that the nerve network is continually active and the extrinsic nerves act by modifying this activity, then a basis still exists for antagonism between the

sympathetic and parasympathetic outflows. If, as Stöhr (1941) and Meyling (1953) suggest, the transmitter substance of the terminal reticulum is sympathin, then its function might be to hold in check the spontaneous myogenic activity of the smooth muscle. Alvarez (1949) attributed some such function to the nerves of the gut though without invoking a syncytial nerve net. It is interesting that storing lengths of colon or ileum for two hours in chilled Ringer's solution, although impairing the vitality of the smooth muscle as measured by the response to acetylcholine, should also lead to a great increase in activity (Fig. 35).

Wedensky inhibition. This and the related subject of fatigue are reported more fully in Part IV. For the moment it is sufficient to point out that stimulation of the pelvic nerve never produced inhibition though frequencies up to 1000 P/S were used.

The presence of two pharmacologically different types of muscle. It is likely that some at least of the varying responses reported from investigations of the pyloric or cardiac regions of the stomach were due to the presence of two types of smooth muscle responding in opposite ways to nerve stimulation, or to the transmitter substances acetylcholine and adrenaline. The response of the colon of the rabbit showed no similar variation either to extrinsic nerve stimulation or to acetylcholine and adrenaline in spite of the artificial production of circumstances most favourable for reversal of the normal response. It seems that the

smooth muscle of this region is pharmacologically all of one type.

"Mixed" Nerves. The explanation which fits best the present findings is that vagaries in the response of the stomach and of other parts of the gut are due to the presence of motor (cholinergic) and inhibitor (adrenergic) fibres in the nerves stimulated. If the different values for the most effective frequency of stimulation and the threshold to low frequencies found in the colon of the rabbit for the two nervous outflows are applied to the results of previous investigators they explain remarkably well their erratic results. Veach (1925), stimulating the vagus to the stomach in cats, found frequencies as low as 1 pulse every 3 - 5 sec could produce a motor response while high frequencies, usually over $40^{P}/_{S}$, produced inhibition. McSwiney & Robson (1929b), stimulating the vagus nerve, found maximum summation in the response of muscle strips from the stomach of the cat to two single stimuli when the interval between them was 0·1 sec. This corresponds to a frequency of $10^{P}/_{S}$. Finkleman (1930), stimulating the periarterial nerves to the small intestine of the rabbit, occasionally obtained motor responses at frequencies from 2 - $4^{P}/_{S}$. McSwiney & Robson (1931a, b), in two more reports on their in vitro innervated preparation from the stomach of rabbits or cats, found a frequency of 1 - $12^{P}/_{S}$ produced motor effects when applied to periarterial nerves while $50^{P}/_{S}$ produced inhibition. Furthermore, stimulation of these periarterial nerves could inhibit subsequent contraction

from stimulation of the vagus nerve. The higher the frequency of stimulation of the periarterial nerves the more complete was this inhibition. Brown, McSwiney & Wadge (1930), recording in vivo the response of the stomach in the dog and cat to stimulation of the thoracic sympathetic chain, used frequencies from 0·5 $^{P}/_{S}$ up to 65 $^{P}/_{S}$ and reported motor effects at low frequencies and inhibitor effects at high. Brown & McSwiney (1932) stimulating the periarterial nerves to the stomach in the cat obtained motor responses at 1 - 3 $^{P}/_{S}$ and inhibitor responses at frequencies over 20 $^{P}/_{S}$. A similar report by Harrison & McSwiney (1936) quotes a frequency of 1 $^{P}/_{S}$ as effective in producing motor responses and 50 $^{P}/_{S}$ for inhibitor responses. Other smooth muscle which shows similar variations in its response to indirect stimulation shows a similar range of frequencies eliciting these variations. For example Folkow & Uvnas (1948) obtained vasodilatation of blood vessels by stimulating the sympathetic chain at a frequency of 15 $^{P}/_{S}$ but vasoconstriction at a frequency of 60 $^{P}/_{S}$.

Only two reports have been found in which motor and inhibitor responses were obtained with frequencies irreconcilable with this theory. Rogers & Bercovitz (1921) studying the response of the stomach in the dog and the turtle to stimulation of the vagus nerve found weak currents and frequencies from 0·5 $^{P}/_{S}$ to 6 $^{P}/_{S}$ favoured inhibition whereas frequencies above 6 $^{P}/_{S}$ produced contraction. Brown & Garry (1932), studying amytal reversal of

the vagus inhibitory effect on the stomach of the cat, confirmed that variations in the response of the muscle to vagal nerve stimulation depended on the state of tonus of the organ. At that time this had recently been challenged by Veach, Schwartz & Weinstein (1929) who believed these variations to be a form of Wedensky inhibition and therefore dependent solely on the frequency of stimulation. Brown & Garry found that low frequencies (2 - 20 P/S) favoured inhibition and "high frequencies" contraction.

Biphasic responses should always have the motor component first if the explanation that they are due to simultaneous stimulation of sympathetic and parasympathetic fibres is correct. The response at any moment represents the algebraic sum of the opposing influences of the two nerves. As these effects develop at different rates and since the two nerves differ in their susceptibility to fatigue, continued stimulation of the "mixed" nerve produces first a rapid contraction, the effect of the motor fibres, followed by inhibition from the slowly developing but fatigue-resistant effect of the inhibitor fibres. Investigation of the biphasic responses reported in the literature show this to be true in most cases. Biphasic responses in which contraction is followed by inhibition have been reported from vagal stimulation by Courtade & Guyon (1899), Veach (1925), Hsu (1944). Inspection of the traces of McSwiney & Wadge (1928) and McCrea & McSwiney (1926)

show that some responses they describe as inhibitor are really biphasic with a small initial contraction. Similar responses have been reported from splanchnic nerve stimulation by Courtade & Guyon (1899) and from stimulation of the hypogastric nerve to the bladder by Mellanby & Pratt (1939). I have found only three reports describing a biphasic response to nerve stimulation in which the inhibitor component preceeded the motor one. Bayliss & Starling (1899, 1900a), stimulating the vagus nerve to the small intestine and the pelvic nerve to the colon in the dog, describe such a happening. Langley & Anderson (1895), stimulating a peripheral branch (the cranial strand) of the pelvic nerve to the colon (rabbit, cat) and Carlson, Smith & Gibbins (1927), in the dog, are responsible for the other two. These last workers were studying the response of the stomach, small intestine and colon not to nerve stimulation but to intravenous injection of choline. All of these experiments were <u>in vivo</u> and all employed balloon recording except Langley & Anderson who relied mainly on visual observation. It is possible that the inhibition recorded by them was a secondary phenomenon due to the production of peristalsis in the alimentary canal orad to the recording balloon. The descending inhibition from this region of activity would appear on the record as inhibition followed by contraction as the peristaltic wave reached the recording region. Bayliss & Starling (1900b) thought this might account for the preliminary inhibition they had

earlier reported in the colon and small intestine of the dog on stimulating the vagus or pelvic nerve. It is certain that stimulation of either pelvic or lumbar colonic nerves alone do not lead to biphasic responses of the colon of the rabbit in vitro and if biphasic responses are produced by stimulating a mixture of fibres from the two outflows the motor component is invariably first. McSwiney & Brown (1926) studied the action of adrenaline on strips of muscle from the stomach of the rabbit under varying conditions of activity of the muscle. They describe, but do not illustrate, biphasic responses to adrenaline in which the inhibitor component comes first. No simple explanation can be offered for this response.

While variations in the effectiveness of different frequencies of stimulation for sympathetic and parasympathetic fibres provide a ready explanation for variations in the response when stimulating a mixed nerve, they do not explain how the state of tonus also modifies the response. Observations by Bülbring & Burn (1935) may provide a clue. They studied the effect of stimulating the lumbar sympathetic chain on the blood vessels of the hind limb of the dog. Short periods of stimulation produced vasodilatation from stimulation of cholinergic fibres. If the tonus of the smooth muscle was reduced by anaesthesia then this vasodilatation dis--appeared but could be restored, even in the presence of the anaesthetic, by raising the smooth muscle tonus with adrenaline.

It may be that the effectiveness of nerve stimulation is affected by the tonus of the muscle. Where this is high, impulses in the motor nerve are relatively ineffective compared to those in the inhibitor nerve whereas, when tonus is low, the efficacy of the motor impulses is similarly enhanced. Thus there would always be a tendency for the viscus to return to a certain mean state of activity and to eschew extremes of inactivity or activity.

Ambache (1951) and Ambache & Edwards (1951) have produced evidence for the presence of inhibitor neurones in the wall of the small intestine in the rabbit and in the cat. They were able to reveal an inhibitory action of nicotine after blocking the motor effect of the drug either by botulinum toxin (rabbit) or atropine (cat). This inhibitory effect was abolished by hexamethonium and large doses of ephedrine from which it was concluded that the effect was on adrenergic ganglion cells. On the basis of these findings Ambache produced an ingenious diagram showing inhibitor (adrenergic) and motor (cholinergic) ganglion cells as synaptically related couplets. The cholinergic cell sent its axone orally, the adrenergic its axone aborally. Both cells were related to the extrinsic vagal fibres and to afferent fibres in local reflexes. If these cell couplets were made to discharge either by local influences or by impulses in the extrinsic nerves they would automatically increase activity orally and inhibit activity aborally (Bayliss & Starling's Law of the Intestine).

This is an attractive theory. It might explain the effects of parasympathetic stimulation on the smooth muscle of the sphincters where inhibitor neurones might be in the majority. It would certainly explain why vagal stimulation usually promotes peristalsis, a mechanism which probably involves inhibition, rather than a tetanic contraction. Unfortunately the evidence from Ambache's own experiments while it suggests the presence of inhibitor ganglion cells gives no information on their relationship with either intrinsic or extrinsic fibres. The authority he quotes to justify this connection of inhibitor neurones with the vagus nerve (McSwiney & Robson, 1929b) later considered their results to be better explained by the presence of cholinergic and adrenergic fibres in the nerve stimulated (Harrison & McSwiney, 1936).

The present experiments give no support to the theory that parasympathetic fibres have synaptic connection with inhibitor neurones. It was hoped that atropine would provide conclusive evidence on this point. Unfortunately its action would exclude the presence of inhibitor neurones on the pathway only if it is assumed that it acts solely as an anti-muscarinic agent at the terminal nerve-effector junction. It is likely, however, that in the concentrations required to abolish completely the motor component of the response atropine is effective as an anti-nicotinic agent at the ganglion synapse. Consequently it

provides no evidence either for or against the presence of inhibitor neurones in the intramural plexuses and related to the parasympathetic outflow. If such relationship exists it is true to say that no variation in the state of the preparation or in the conditions of stimulation could reverse the domination of the motor neurones. An alternative explanation of Ambache's findings is that the inhibitor neurones are involved in local (peristaltic) reflexes and related to intrinsic nerve fibres only. Some evidence for the presence of such neurones has been obtained from the action of nicotine.

The optimum frequency of stimulation of the sympathetic fibres is about 50 - 100 P/S. Some investigators claim that autonomic fibres cannot conduct impulses at this frequency or that the synapse cannot transmit the impulses. Bishop & Heinebecker (1932), studying transmission at the superior cervical ganglion, stated that fatigue could not be excluded at frequencies above 10 P/S. This was based on a study of the compound action potential. More recently Cannon, Raule & Schaefer (1954) have claimed that above 20 P/S the autonomic fibres from the stellate ganglion are no longer responding to each impulse. This was again based on diminution of a compound action potential. As Rosenblueth & Simeone (1938) have pointed out, a diminution in the compound action potential can be due to increasing temporal dispersion of the individual fibre responses, to a diminution of the individual fibre action potentials which at higher frequencies are travelling in the

relatively refractory period of their predecessor or to a reduction in the frequency of the impulses in each fibre. They found frequencies up to 120 P/S could be transmitted at the ganglion and conducted to smooth muscle. The ability to follow high frequencies diminished rapidly if stimulation was continued. Bishop & Heinebecker (1932) were aware that 10 P/S did not represent the most effective frequency measured by the effect on the smooth muscle. They reported frequencies up to 50 P/S producing a bigger contraction of the nictitating membrane due to more effective peripheral summation.

Most other investigators are agreed that, at least for short periods, frequencies much higher than these quoted by Cannon et al, 1924) can be transmitted by autonomic fibres. Querido (1924) reported the pre-ganglionic fibres of the superior cervical ganglion capable of conducting impulses up to 250 P/S and post-ganglionic fibres up to 160 P/S. Veach & Pereira (1925), on the same test object, reported even higher frequencies being transmitted through the ganglion. It is doubtful if such high frequencies can be carried in these fibres but at least it indicates that Wedensky inhibition does not occur. Katz (1936) found a frequency of 150 P/S more effective than 80 P/S for indirect stimulation of crab muscle. Iggo (1954) reports frequencies up to 100 P/S conducted in the vagus nerve to the stomach of the sheep.

The weight of the evidence is against restricting autonomic

fibres to very low frequencies. It is doubtful, however, if frequencies above $50^{P/S}$ can be transmitted at this frequency for any length of time.

Stimulation of "mixed" nerves containing both sympathetic and parasympathetic fibres will explain many of the inconsistencies in the response of smooth muscle. There remain a number of observations which do not fit this theory. The ability of adrenaline and acetylcholine to produce motor or inhibitor responses from the stomach might be explained by assuming a mixture of smooth muscle cells, some of the sphincter type, the others similar to that lining the alimentary canal generally. It is true that such variations in the alimentary canal are confined almost entirely to the stomach which has a more complicated arrangement of muscle than other regions. The presence of two sphincteric regions in such a relatively short length of gut might predispose to such a mixture of muscle fibre. Furthermore, Brown & McSwiney, (1925) have reported that, after a strip of muscle from the cardia of the cat or dog is made to contract with pilocarpine, the addition of adrenaline superimposes a second contraction suggesting a new group of fibres not activated by pilocarpine. It is more difficult, however, to explain the alteration in the response of the relatively simple muscle of the uterus to adrenaline. In the non-pregnant cat adrenaline causes inhibition but after injections of ovarion hormone this may be altered to

contraction or a biphasic response. This phenomenon is reminiscent of the "peripheral mechanism".

Another difficulty is the origin of the sympathetic fibres in the vagus nerve if the presence of such fibres is responsible for the varying effects of nerve stimulation. It is true that there are numerous connections between the sympathetic chain and the vagus in the neck and even that some of these sympathetic fibres from the stellate ganglion influence the stomach (Carlson, Boyd & Pearcy, 1922). Veach (1925), however, in his careful work took precautions to exclude participation of such fibres in the responses he obtained from stimulation of the vagus to the stomach. After extirpation of the entire cervical sympathetic chain from above the superior cervical ganglion to below the stellate ganglion and allowing 16 days for degeneration, he still obtained inhibitor responses. After sectioning the vagus above or below the ganglion nodosum and allowing time for degeneration, stimulation of the peripheral end produced neither inhibition nor contraction. In acute experiments destruction of the spinal cord did not alter the responses so that these were not due in part to reflexes in the spinal cord and splanchnic nerves. It is not possible to explain these results on the present theory.

The cause of the differences which have been described between the pelvic and lumbar colonic nerves might be related to:-

1) Stimulation of post-ganglionic (sympathetic) versus

pre-ganglionic (parasympathetic) nerves.

2) Stimulation of adrenergic versus cholinergic nerves.

3) The type of response; inhibition versus contraction.

A suitable preparation in which pre-ganglionic sympathetic fibres could be stimulated would demonstrate the part played by (1) above. A preparation in which the parasympathetic fibres were inhibitory and the sympathetic fibres motor, a purely sphincteric region for example, would throw light on (3). The results from stimulation of vasodilator and vasoconstrictor nerve fibres to blood vessels (Folkow & Öwnas, 1948) suggest that it is neither of these two factors but the adrenergic and cholinergic nature of the fibres which accounts for the difference. In the lumbar sympathetic chain cholinergic (vasodilator) and adrenergic (vasoconstrictor) fibres have been demonstrated. These fibres are both supposed to be sympathetic in origin and to leave by the ventral nerve roots so that there is presumably no peripheral cell station on the cholinergic fibres. They show the same differential sensitivity to low frequencies as was found in the colon. Low frequencies stimulate the cholinergic fibres; high frequencies the adrenergic. This difference might rest either on their cholinergic and adrenergic nature or on the direction of the response, inhibition as compared with contraction. Since the direction of the response is opposite to that found in the bowel yet the same difference in the response to frequency of stimulation exists, it

suggests that this is not the determining factor. Possibly differences in the site or rate of release, the rate of diffusion, of re-synthesis or the susceptibility to enzymic destruction of the two transmitter substances is the true determining factor.

Before interpreting the results of Folkow & Üvnas however it would be interesting to know whether ganglion blocking agents modified the vasodilatation produced by low frequency stimulation of the lumbar sympathetic chain in a perfused preparation in which the drug was confined to the limb and did not reach the sympathetic chain. It is possible that these cholinergic fibres are more like parasympathetic fibres with a cell station in the tissues innervated. Hilton (1953) has postulated such an arrangement to explain the vasodilatation which follows contraction of the limb musculature. This is due to an axone reflex in cholinergic nerve fibres but these fibres do not degenerate after posterior root ganglionectomy or lumbar sympathectomy. Gairns & Garven (1952) have demonstrated histologically the presence of ganglion cells in the course of intramuscular nerve fibres in the tongue.

SUMMARY

1. An *in vitro* innervated preparation of the colon of the rabbit is described in which the pelvic and lumbar colonic nerves are retained.

2. Stimulation of the pelvic nerve always causes contraction of the colon. Stimulation of the lumbar colonic nerves always produces inhibition. Neither the frequency of stimulation nor the state of activity of the preparation modifies the type of response.

3. The most effective frequency of stimulation of the pelvic nerve is $10^{P/S}$. Much lower frequencies, for example 1 pulse every 2 sec, will still cause contraction as will frequencies as high as $1000^{P/S}$. The response begins after a latent period of about 0.8 sec, quickly reaches its maximum but is not well maintained. Hexamethonium abolishes this response.

4. The most effective frequency of stimulation of the lumbar colonic nerves is $100^{P/S}$. Frequencies below $5^{P/S}$ are rarely effective. The response appears after a latent period averaging 1.8 sec, develops slowly but persists for a considerable period after cessation of stimulation. Hexamethonium has no effect on this response.

5. Atropine abolishes the motor response from stimulation of the pelvic nerve without revealing an inhibitor component and without affecting the inhibition resulting from stimulation of the

lumbar colonic nerves. The concentration of the drug needed for complete block is fairly constant, about $1 - 2 \times 10^{-4}$. The concentration required to produce the first observable reduction in the contraction varies but may be as low as 1×10^{-7}. The concentration required to abolish the motor effect of acetylcholine added to the bath is also about 1×10^{-7}. In concentrations abolishing the motor response to pelvic stimulation, atropine itself has a stimulating effect on the preparation. The mode and possible sites of action of the drug are discussed.

6. Simultaneous stimulation of both outflows to the colon with low frequencies produces a pure motor response; stimulation with very high frequencies produces pure inhibition; stimulation with intermediate frequencies gives a biphasic response, first contraction then relaxation.

7. The results when stimulating a mixed nerve resemble, and may explain, many of the findings of past workers when they stimulated the extrinsic nerves to the stomach and small intestine. It is suggested that the vagus, splanchnic and periarterial mesenteric nerves contain both sympathetic and parasympathetic fibres for the stomach and small intestine. Low frequencies would selectively stimulate the parasympathetic fibres; high frequencies the sympathetic fibres. Since the time-relations of the responses to stimulation of the two outflows differ, the use of intermediate frequencies, effective for both, would produce biphasic responses.

P A R T IV

THE ORGANISATION OF THE INTRINSIC INNERVATION OF THE COLON

METHODS

The innervated preparation of the rabbit colon described in the last section was used to study the organisation of the para--sympathetic fibres within the bowel wall. Three problems in particular were studied:-

1) The degree of overlap of the extrinsic parasympathetic fibres from the two sides.

2) The nature and site of the fatigue which appears if stimulation of the pelvic nerve is long continued.

3) "Wedensky" inhibition.

The dissection of the preparation has previously been described. As the two pelvic nerves were to be stimulated separately, a longer length of each was desirable to allow easy positioning in two separate fluid electrodes. In isolating these nerves, therefore, they were separated from the glutinous mesenchyme in which they lie for a greater distance than usual. This could be done without damaging the nerves. Simple Magnus preparations of the colon and small intestine, devoid of mesentery or extrinsic nerves, and similar to those described by Paton (1954), were also used to study the effect of passing a current through the entire wall of the gut. Such transmural stimulation may affect any or all of the excitable tissues, and the responses obtained were complex.

Using isotonic recording the colon may contract on stimulation

of one pelvic nerve by as much as 50% of its length, say 3 cm. With such a large change in length it is possible that the non--contractile elements in the gut wall become a limiting factor, preventing or opposing further contraction. If this were so it might give rise to a false impression of overlap. Stimulation of one pelvic nerve might be capable of producing the same state of contraction as stimulation of both nerves in spite of the fact that stimulation of both nerves developed twice as much tension. For this reason the method of recording was altered. A semi--isometric, spring loaded gimbal lever was substituted for the isotonic lever. This measured the tension developed instead of the amount of contraction. With this lever contraction never exceeded 5 mm; the movements of the gut were magnified 20 times. The arrangement in the bath of the innervated colon preparation in these experiments, and the recording system, is shown in Fig. 37.

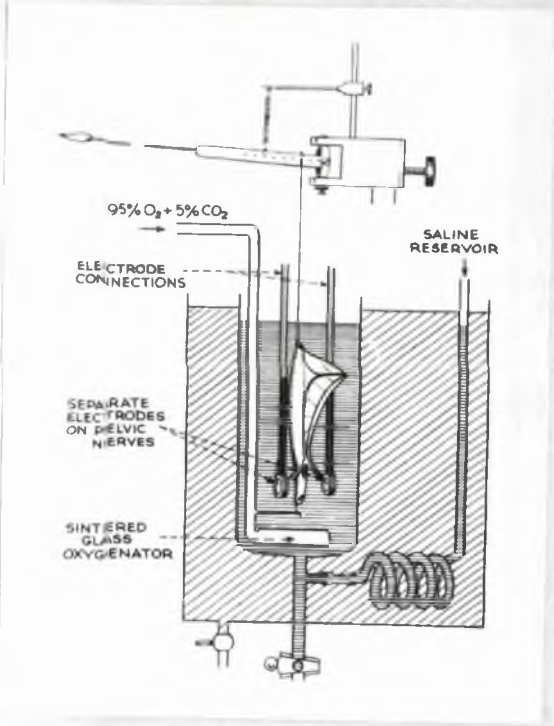


Fig. 37 The innervated preparation of the rabbit colon with separate fluid electrodes on each pelvic nerve. A spring loaded gimbal type lever is used which gives semi-isometric recording.

In studying overlap, fatigue or Wedensky inhibition it was necessary to stimulate both pelvic nerves simultaneously. It was, therefore, no longer possible to have one electrode "floating" i.e., without earth or other connections. This introduced great difficulties in isolating electrically the two electrodes. At first two transformers were used to isolave the two stimulators from their respective electrodes. This failed; current still passed from one electrode to earth via the other, presumably crossing the other transformer by capacitative or inductive pathways. The earth pin of the two stimulators was disconnected in an attempt to improve the isolation of the two circuits. This introduced even greater difficulties. The electrolyte in the inner vessel was no longer at true earth potential whereas the organ bath itself was. Stray $50^{c/}s$ hum picked up in the circuit passed by capacitance between the inner vessel and the bath using the glass wall of the inner vessel as a dielectric. This was sufficient on one occasion to stimulate nerves.

This problem was eventually solved by using a device described by Schmitt & Dubbert (1949) in which the square wave stimulator output is used as a source of power for a midget radio-frequency (R.F.) oscillator. The R.F. energy is transmitted across a smallair gap to the receiving coil where it is rectified producing a pulse which faithfully follows the original stimulator output. The effect of this device on the stimulator pulse form

is illustrated in Fig. 20. The unit used was similar in construction to that described by Schmitt & Dubbert (1949), using two germanium diode rectifiers to increase the maximum output. According to its designers this coupling unit can be regarded as "a completely isolated power source". The circuit finally adopted employed two electronic square wave stimulators, one coupled to the electrode on one nerve through a one for one transformer (Muirhead D-139-C); the other to the electrode on the other nerve through the R.F. coupling unit.

In studying fatigue long periods of continuous stimulation were used. The 1 M.F.D. condensor in series in the circuit in previous experiments was discarded in the present experiments because of the distortion of the pulse form using transformer coupling (see Fig. 20). To detect polarisation a 150Ω resistance was introduced in series in the electrode circuit. The voltage drop across this resistance was displayed on a Cossor oscilloscope. If this remained constant then the current flowing in the circuit was assumed to be constant. In practice even with periods of stimulation as long as one hour there was no diminution in the current flowing. The R.F. coupled circuit was not monitored as it was felt that the introduction of the Cossor oscilloscope might destroy the electrical isolation. Whenever possible the trans-former coupled and monitored circuit was used for long periods of stimulation and only short ten second periods were interpolated through the R.F. coupled circuit.

The arrangement in the bath for transmural stimulation is illustrated in Fig. 38 and corresponds in most respects to that described by Paton (1954). Current was passed from the electrode within the lumen of the gut, through the wall of the organ, to the large outer electrode. The platinum electrodes used by Paton were replaced by Ag-AgCl electrodes. These prevented polarisation. The inner electrode, a length of fine silver wire coated with silver chloride, was soldered to a length of the same wire used for the leads of the fluid electrodes. Eight of the twelve strands of wire were removed to increase the flexibility. Such a lead was easier to handle than one consisting of fine wire running in polythene tubing as described by Paton (1954). The polythene tubing was very slippy which made it difficult to tie firmly the upper end of the gut to it. The P.V.C. sheath was slipped along the wire to cover the soldered joint with the Ag-AgCl electrode and was then sealed off with "Perspex" cement. The level of fluid in the cannula was 0·5 cm above that in the inner vessel so that the gut, when quiescent, was slightly distended with fluid. The activity of the circular muscle was not recorded.

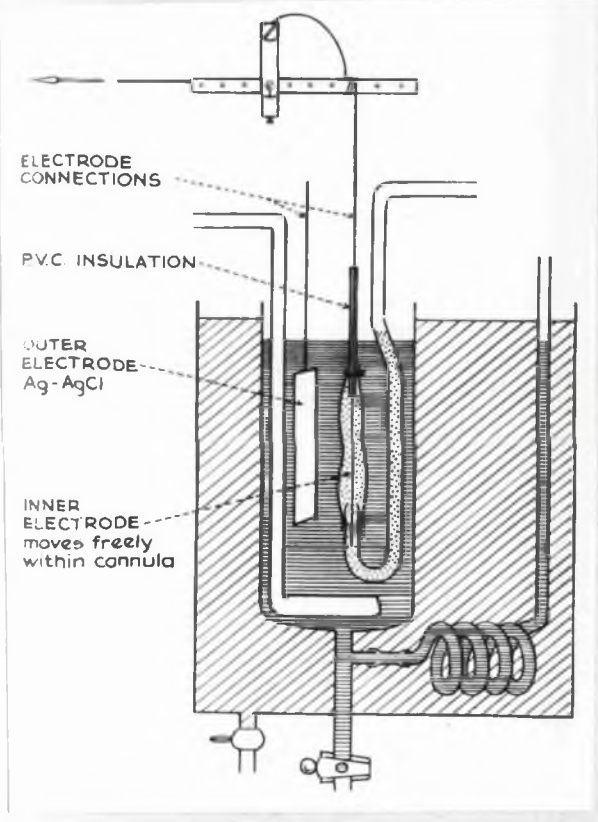


Fig. 38 The arrangement used for transmural stimulation of lengths of bowel.

THE PRESENCE OF OVERLAP

In these experiments each pelvic nerve was first stimulated alone for 10 sec at the optimum frequency of $10^{P}/_{S}$. Both nerves were then simultaenously stimulated for 10 sec at the same frequency. Separate stimulation of each pelvic nerve was then repeated as a control. Isotonic recording was used in the earliest experiments and seemed to show almost complete overlap. The possibility of error due to mechanical factors in this method of recording has already been discussed. The results using isometric recording, however, only confirmed those previously obtained with the isotonic lever. The responses from stimulation of either pelvic nerve alone were usually similar. The response from simultaneous stimulation of the two nerves was only slightly greater than that from either nerve alone. This is illustrated in Fig. 39.

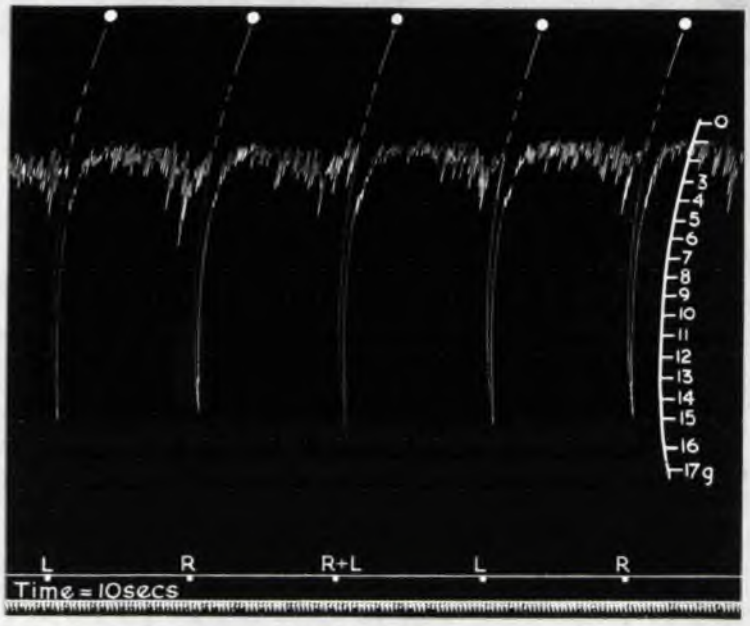


Fig. 39 The response from stimulation of the two pelvic nerves is only slightly greater than the response from stimulation of either alone.

If frequencies lower than the optimum were used the response on stimulating both nerves was greater than that from stimulation of either alone i.e., the appearance approached more that of summation than overlap. The lower the frequency the more noticeable was this. Such an effect is to be expected. If the pool of effector cells is largely common to both nerves, and if the optimum frequency of impulses arriving at these cells is $10^{P/S}$ then a train of impulses at a sub-optimal frequency in each pelvic

nerve may summate at the site of overlap, so that the effective frequency of the stimuli delivered to the muscle will approach closer to the optimum. This effect is illustrated in Fig. 40.

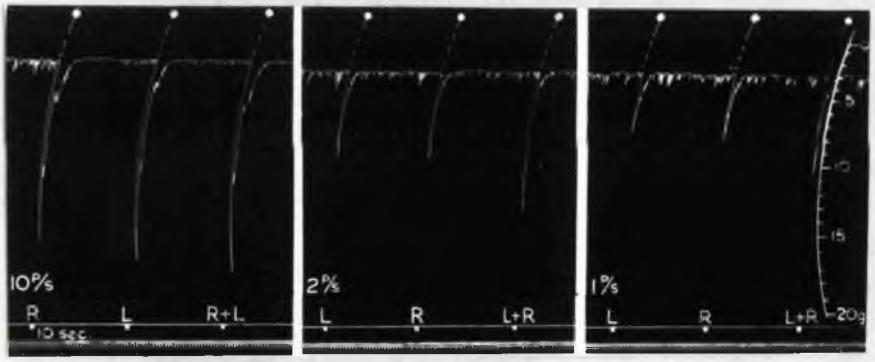


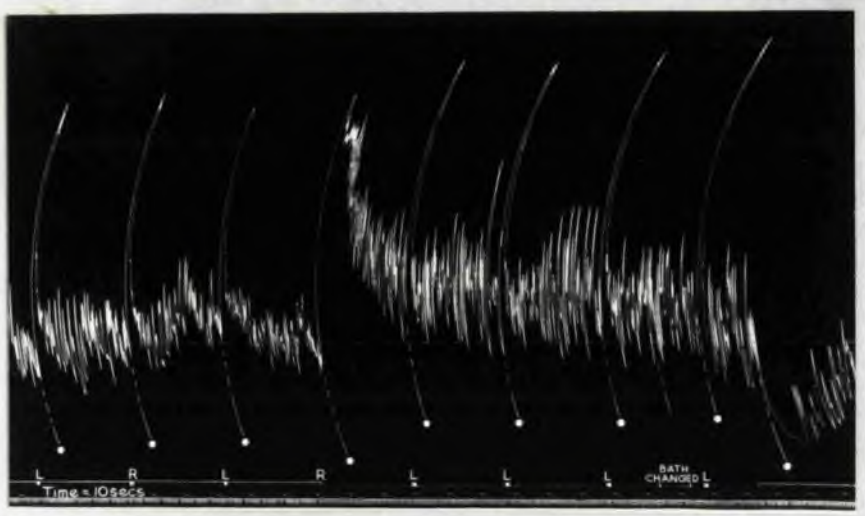
Fig. 40 The response to stimulation of both pelvic nerves compared with the response to stimulation of either alone at varying frequencies. At the optimum frequency of stimulation ($10^{P}/S$) the appearance is of almost complete overlap. At $2^{P}/S$ and $1^{P}/S$ there is an increasing amount of summation, but this is never complete.

At no frequency was the response from stimulation of both nerves equal to the sum of the individual responses i.e., pure summation without occlusion was never seen.

Calibration of the recording lever showed that, within the range of tensions developed (up to about 25 gm), the response was almost linear so that the small increase in contraction which appears when both nerves are simultaneously stimulated does not conceal, as it might otherwise have done, a considerable increase in the tension developed.

FATIGUE AND WEDENSKY INHIBITION

Fatigue. The contraction which follows stimulation of the pelvic nerve was never sustained whatever the frequency of stimulation. The response consisted of an initial spike-like contraction which declined simultaneously with the reappearance of rhythmic activity. Eventually a steady state was reached in which tone was raised and the amplitude of the rhythmic contractions increased. This steady state was attained within five minutes and then remained constant for long periods (up to one hour). The frequency of stimulation, although altering the amplitude of the initial "spike", had no effect on the steady state activity which was similar at all frequencies. If stimulation was continued until this steady state was reached, cessation of stimulation was usually followed by a period of complete loss of tone and loss of rhythmic activity from which the gut recovered in a minute or two.(Fig. 41). All of the steady state activity therefore seems to be dependent on stimulation of the extrinsic nerves. These effects are illustrated in Fig. 41.



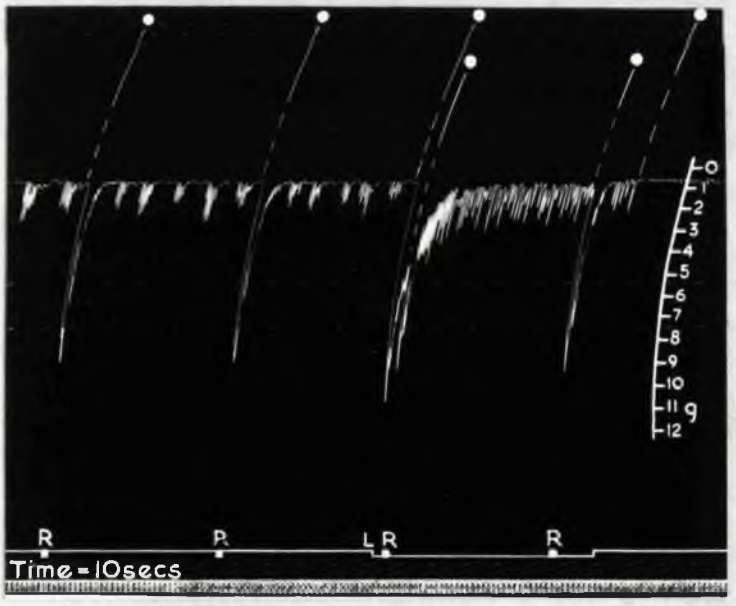


Fig. 41 The appearance of fatigue following continuous stimulation using isotonic and isometric recording. The "steady state" which is reached after a few minutes stimulation and the complete loss of tone and rhythmic activity which follows cessation of stimulation are illustrated. This fatigue does not affect the response to stimulation of the other nerve which is usually increased.

An attempt was made to localise the site of this apparent fatigue more exactly. Since there is a large degree of overlap between the two pelvic nerves it follows that most of the contraction on stimulation of either nerve is produced by the same muscle cells. If therefore the decline in the response with continued stimulation is due to fatigue in the muscle itself or in any part of the pathway common to the two nerves, it should affect the response from stimulation of either nerve, even though induced by stimulation of only one. Experiment showed that this was not so. Fatigue following continued stimulation of one nerve did not reduce the response from stimulation of the other (Fig. 41). In fact the interpolated response was usually facilitated as can be seen in the isotonic recording in Fig. 41. This facilitation increased, up to a point, with time, even though the background activity remained fairly constant. On rare occasions some reduction in the interpolated test response was seen. This occurred only in preparations which had been in use for long periods and the reduction was confined to those test responses interpolated shortly after the beginning of continuous stimulation. Fig. 42 illustrates this and is from the same experiment as Fig. 41 but some hours later. This temporary reduction presumably indicates diminished responsiveness somewhere on the common nerve pathway or in the effector muscle.

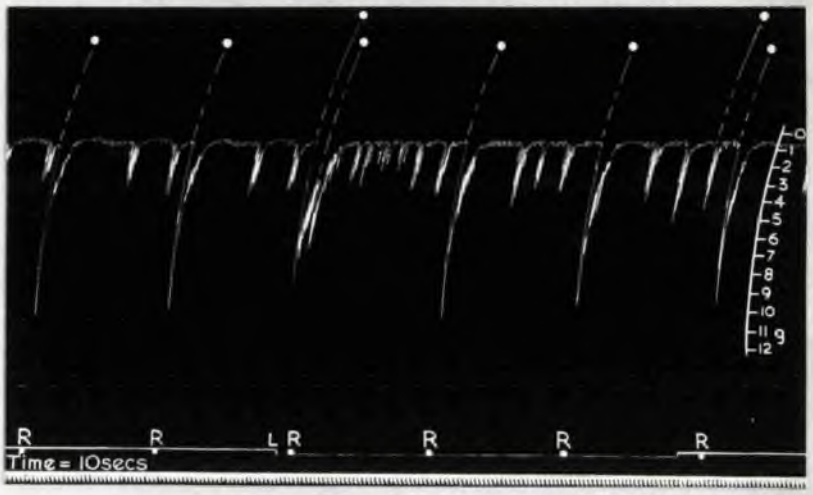


Fig. 42 The test response from stimulation of the R nerve, interpolated immediately after the beginning of continuous stimulation of the L nerve, is reduced. This reduction is absent in subsequent periods of stimulation. Isometric recording.

Recovery from fatigue was rapid. Within 10 sec of the end of continuous stimulation the preparation could respond with a spike contraction approximately 50% of the previous control. Within five minutes the response had returned to normal. Furthermore, the recovery rate was the same even though the other nerve in its turn was being fatigued (Fig. 43).

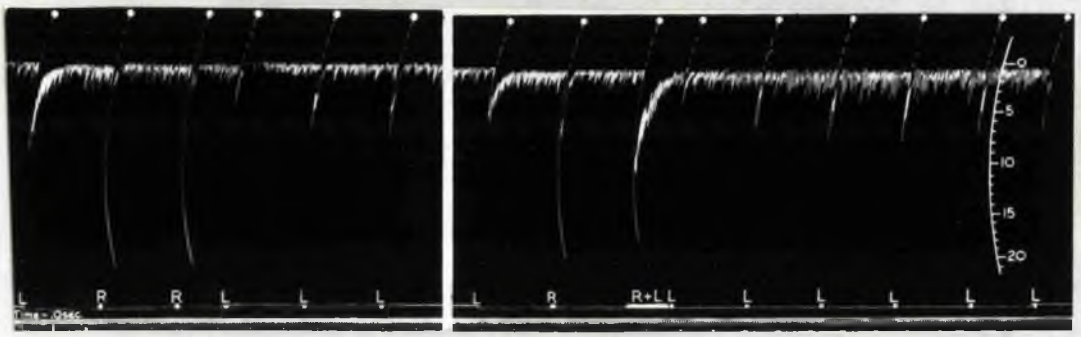


Fig. 43 The recovery of the response to stimulation of the pelvic nerve after inducing fatigue. The first panel shows the recovery of the L. nerve after 12 min continuous stimulation. The second panel shows the recovery of the same nerve at the same time as fatigue is being induced in the R nerve. The recovery in both illustrations is similar. Isometric recording.

The phenomenon of fatigue was therefore strictly localised to some part of the nerve-muscle pathway proximal to the site of overlap. The two nerves, in this respect, were quite independent. Fatigue of one could neither fatigue the other nor delay its recovery. The contractile power of the smooth muscle cells was not reduced in fatigue.

Other possible sites of fatigue were in the nerve itself or at the synapse. From evidence previously quoted (Part III) it seemed unlikely that autonomic nerves were incapable of transmitting a train of impulses at a frequency as low as $10^{P/S}$.

The region most likely to have a recovery period long enough to block impulses at this frequency was the synapse. One other possibility existed. Although the nerve fibres could probably conduct impulses at $10^{P}/S$ the generation of these impulses might have failed because of the artificial conditions of stimulation. The site of fatigue on this theory was the small area of nerve exposed to the stimulating current. This possibility was tested using the electrode, previously described, with three leads and two rubber diaphragms (see Fig. 19). With this electrode it was possible to shift the site of stimulation peripherally. The stimulator output was first applied to the two leads furthest from the preparation; stimulation took place in the region of the first diaphragm. The current was then switched to the two leads nearest to the preparation, the site of stimulation was shifted to the region of the second diaphragm, a point nearer to the preparation. During the experiment each site was first stimulated in turn to show that both were effective. The response from each was similar. The nerve was then stimulated continuously using the two leads furthest from the preparation. When the steady state was reached the current was switched, without interruption, to the leads nearest the preparation. The results of this experiment are shown in Fig. 44.

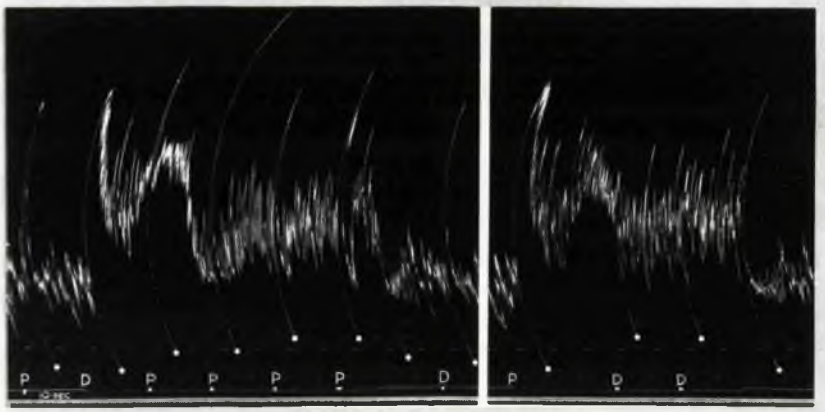


Fig. 44 The effect on fatigue of altering the site of stimulation of the nerve. In the first panel continuous stimulation distally (D) is followed by a decline in the response; moving the site of stimulation towards the preparation (P) abolishes this fatigue. The second panel shows the effect of moving the site of stimulation away from the preparation from the peripheral position (P) to D. The appearance of fatigue is unaltered. Isotonic recording.

Moving the point of stimulation towards the periphery by a distance roughly equal to the distance between the rubber diaphragms (0·5 cm) abolished the fatigue completely. The preparation not only responded once more with a "spike" but this "spike" showed a facilitation similar to what would be expected if, instead of shifting the site of stimulation, the other pelvic nerve had been stimulated. Reversing the procedure i.e., stimulating peripherally

to begin with and then shifting the site of stimulation away from the preparation did not relieve the fatigue. The site of fatigue must be at the site of stimulation. Not only is the generation of propagated impulses interfered with but their conduction is also impaired since moving the site of stimulation away from the preparation does not relieve the fatigue.

Wedensky Inhibition. All effective frequencies of stimulation of the pelvic nerve produced a motor response if applied, without alteration, from the beginning of the period of stimulation. If, however, the nerve was stimulated at a frequency of $10^{P/S}$ and this continued until the response had reached the steady state and then, without interruption of stimulation the frequency was increased to above $30^{P/S}$, a phenomenon very similar to Wedensky inhibition appeared. There was an initial spike followed by a complete disappearance of the previous motor effect of nerve stimulation. On restoring the original frequency of $10^{P/S}$ there was an after-contraction followed by a gradual return to the steady state activity. Fig. 45 illustrates these effects.

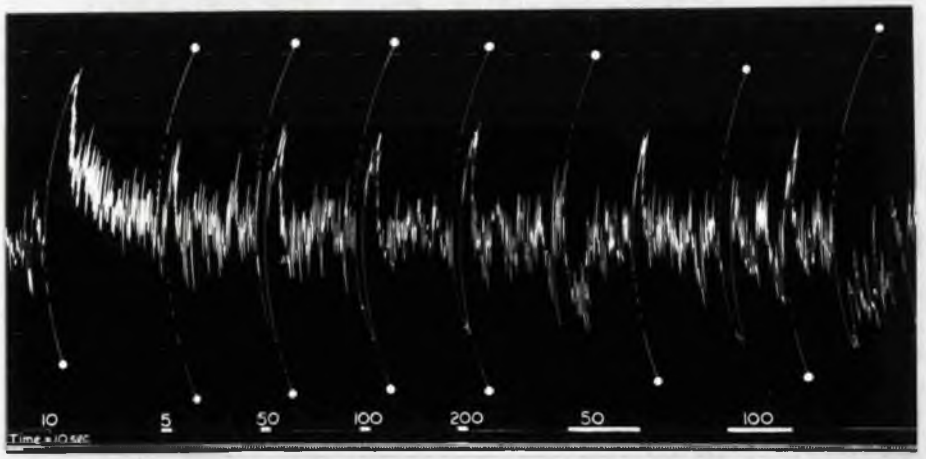


Fig. 45 Wedensky inhibition produced by increasing the frequency of stimulation after conditioning at $10^{P/S}$. When the higher frequency is maintained the preparation escapes from inhibition and returns to the previous steady state; restoration of the low frequency of stimulation at this point produces a "spike" contraction. Isotonic recording.

The inhibition which appears although often complete is in fact a disappearance of the motor effect of nerve stimulation and reproduces the effect of stopping stimulation. This can be seen in Fig. 45 where the inhibition, though dramatic, corresponds to the inhibitory phase following the end of stimulation. In preparations which did not show this complete inhibition of tone and activity on stopping stimulation, complete Wedensky inhibition could not be produced by increasing the frequency.

Escape from the inhibition induced by increasing the frequency of nerve stimulation occurred if the higher frequency was maintained (see Fig. 45). When such escape occurred the gut returned to the same steady state of activity as was present at the low frequency of stimulation. Though the effect at the periphery of the higher frequency was now apparently identical with that of the lower frequency there was some difference. When the original frequency was restored there was an immediate spike like response followed by a return to the steady state (Fig. 45). This difference between high and low frequencies was demonstrated in another way. Stimulation at $100^{P}/S$ was continued until a steady state was reached. If now the frequency was reduced to $10^{P}/S$ the preparation responded with a "spike" contraction and gradual decline as if it were newly stimulated. There was no evidence that the previous stimulation at $100^{P}/S$ had impaired the ability to respond to $10^{P}/S$. In other words, although during steady state

activity stimulation at $10^{P/}S$ and $100^{P/}S$ produce the same effect at the periphery, they have a different effect at some other site. Stimulation at $10^{P/}S$ conditions some region so that a subsequent increase in frequency abolishes all effect of stimulation. Continued stimulation at $100^{P/}S$ does not impair the ability to respond to lower frequencies of stimulation.

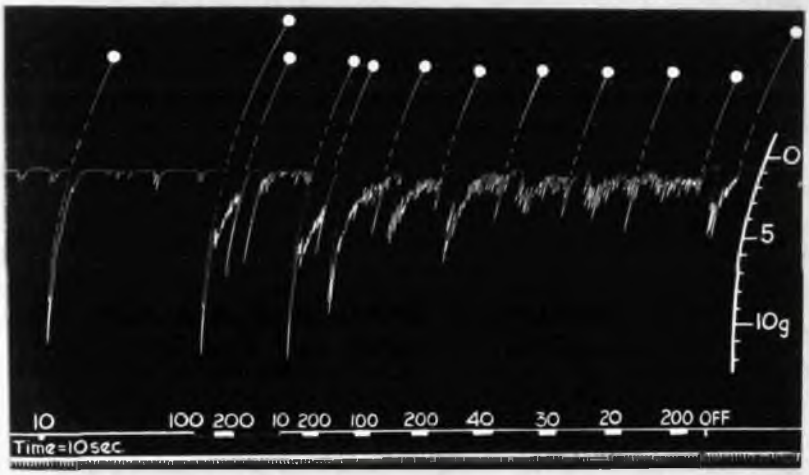


Fig. 46 The effect of varying the frequency during a period of continuous stimulation. The frequency in pulses per sec is shown on the trace. Stimulation at $100^{P/}S$ is followed by fatigue; on changing the frequency to $10^{P/}S$ a response is obtained if anything greater than the previous control. The production of Wedensky inhibition and changing to frequencies above $20^{P/}S$ is also shown. Note the brief spike preceeding inhibition. Isometric recording.

The site of this inhibition was investigated in the same way as the site of fatigue. The preparation was "conditioned" by stimulating one pelvic nerve continually at $10^{P/}S$ and then stimulation at $50^{P/}S$ interpolated on the other nerve. The preparation responded to the second stimulus with a normal contraction; inhibition was not produced. A control in which the frequency of stimulation of the continuously stimulated nerve was increased from $10^{P/}S$ to $50^{P/}S$ produced complete inhibition (Fig.47). The conditioning effect of low frequency stimulation was confined to the nerve stimulated. The site of this Wedensky type inhibition is, therefore, as with fatigue, proximal to the site of overlap.

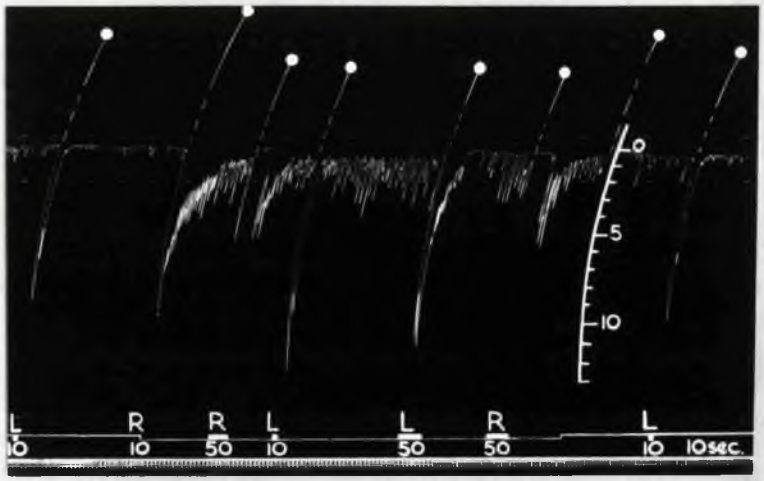


Fig.47 During continuous stimulation of the R pelvic nerve at $10^{P/}S$ altering this frequency in the R nerve to $50^{P/}S$ induces Wedensky inhibition. During this continuous stimulation of the R nerve at $10^{P/}S$, interpolation of $50^{P/}S$ on the L pelvic nerve produces a normal contraction. Isometric recording.

The frequency of stimulation most effective in conditioning the nerve for Wedensky inhibition was 10 - 20 P/S. A frequency of 5 P/S was only occasionally effective. It was possible that the important factor in producing this type of inhibition was not the absolute frequency used but the relative increase in frequency. That if 10 P/S required an increase to 30 P/S to produce inhibition, 5 P/S might only require an increase to 15 P/S and 20 P/S might have to be increased to 60 P/S. This was investigated and found incorrect. No matter what frequency of stimulation was used to condition the preparation the threshold frequency for inhibition was constant at above 30 P/S. An increase to 20 P/S was never effective. Fig. 46 illustrates this.

During continuous stimulation of one pelvic nerve, interpolated stimulation of the other often showed an augmented response. This facilitation might have been related to the steady state activity. If the muscle cells were already in a state of increased activity a normal train of impulses in the unfatigued nerve might be more effective. On the other hand the facilitation might be only indirectly related to this peripheral activity, increased excitability in some structure proximal to the muscle cells, say in the ganglia of Auerbach's plexus, could be the immediate cause. This effect was investigated by abolishing peripheral activity by increasing the frequency of stimulation. The effect of this on the response to short periods of stimulation of the other nerve was then studied.

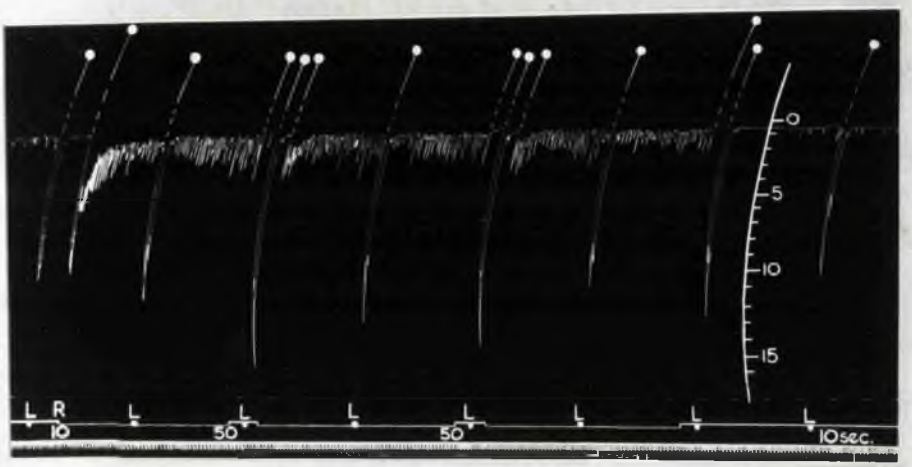


Fig. 48 The R nerve is continuously stimulated at $10^{P/S}$, stimulation of the L nerve at $10^{P/S}$ is interpolated. The facilitation of the test response compared with the control responses is augmented when Wedensky inhibition is induced by increasing the frequency of stimulation of the R nerve to $50^{P/S}$.

The results of these experiments were inconsistent but in one were so definite as to be worth comment. The experiment is illustrated in Fig. 48. Continuous stimulation of the right nerve produced the usual "spike" followed by decline to a steady state. Inter--polated stimulation of the left nerve showed facilitation of the response compared with the previous control. The frequency of stimulation of the right nerve was then increased to $50^{P/S}$ which produced inhibition; interpolated stimulation of the left nerve during this inhibition demonstrated an even greater facilitation of the response than before. This effect could be repeated.

This experiment seems to show that facilitation is not dependent on activity in the muscle and can appear even when no nerve impulses appear to be reaching the periphery. If true this suggests that facilitation is at some more peripheral site.

THE RESPONSE OF THE GUT TO TRANSMURAL STIMULATION

These experiments were performed following the interesting report by Paton (1954) on the results of transmural stimulation of the guinea pig ileum. This investigator obtained motor responses with single electrical stimuli of short duration (0·5 msec). These responses were abolished by very low concentrations of atropine (10^{-8}), potentiated by eserine, reduced by procaine and unaffected by hexamethonium or mepyramine. Paton concluded that they were due to stimulation of post-ganglionic cholinergic fibres. These results are quite unlike what would be expected on the basis of the characteristics of the extrinsic cholinergic nerves to the colon. In this preparation (the rabbit colon) single pulses applied to the pelvic nerves were rarely effective and then produced a just detectable response. The motor response from repetitive stimulation of the nerve was abolished only by high concentrations of atropine. Moreover, since all excitable structures are equally liable to be stimulated by the current crossing the bowel wall, it would be expected that inhibitor responses from stimulation of post-ganglionic sympathetic fibres would occasionally be seen, especially after blocking the motor response with atropine. These were not reported by Paton. Because of these puzzling features the effect of transmural stimulation, in the manner described by Paton (1954), was studied

on the duodenum, mid-ileum and colon of the rabbit.

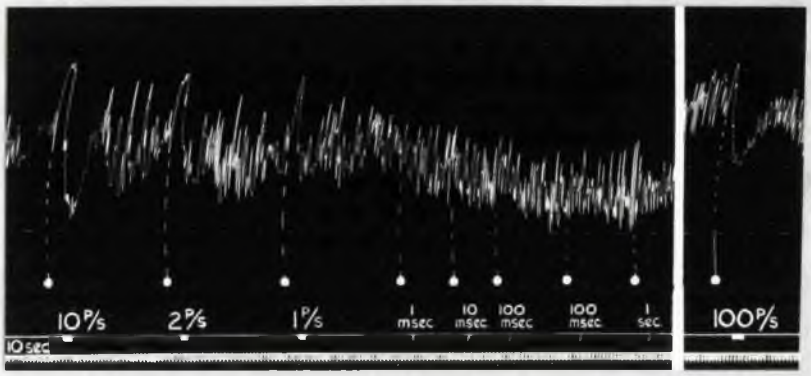


Fig. 49 Transmural stimulation of the colon. Single pulses are ineffective. Low frequencies of stimulation produce pure motor responses, high frequencies biphasic responses, contraction followed by inhibition. The higher the frequency the more obvious is the inhibitor component. Isotonic recording.

The results in the colon were in accord with the known properties of the extrinsic nerves. A fairly high voltage was required for stimulation. Single pulses were never effective. Repetitive stimulation usually produced biphasic responses in which the motor component was always first. High frequencies of stimulation favoured the secondary inhibition and this continued into the post-stimulatory phase. Low frequencies favoured the motor component; at a frequency of about 1 or 2 P/S a pure motor response was produced. These effects are illustrated in Fig. 50.

The effect of atropine also confirmed expectations (Fig. 51). There was no effect on the motor response until a concentration of 10^{-4} was reached. This abolished the motor response and revealed, if previously absent, an inhibitory component. The frequency sensitivity of the inhibitory response thus unmasked corresponded to that of the inhibition produced by stimulating the sympathetic fibres in the lumbar colonic nerves; $100^{P}/_{S}$ was more effective than $10^{P}/_{S}$.

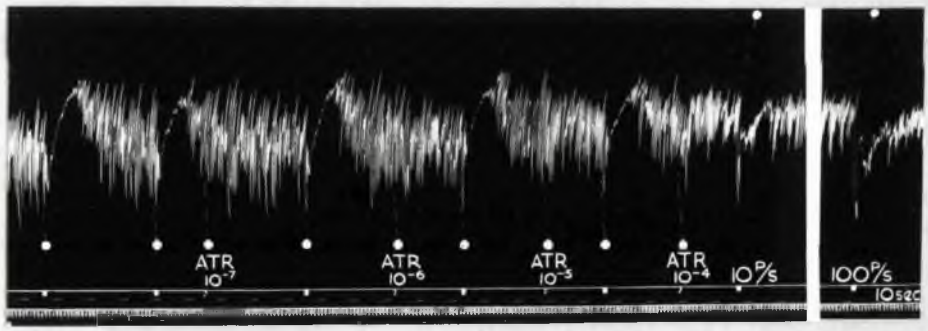


Fig. 50 Transmural stimulation of the colon. The contraction following repetitive stimulation at $10^{P}/_{S}$: 1·0 msec is abolished by atropine 10^{-4} revealing an inhibitor component. Stimulation at $100^{P}/_{S}$ is more effective than $10^{P}/_{S}$ in producing inhibition. Isotonic recording.

The results using a length of duodenum were similar to those from the colon. Single pulses were ineffective, repetitive stimulation had a variable effect depending on the frequency of stimulation. Low frequencies produced a pure motor response, high frequencies a biphasic response, contraction followed by inhibition (Fig. 51). The higher the frequency of stimulation the more marked the inhibition. A feature of interest was that $100^{P}/_{S}$ seemed a more effective frequency of stimulation that $10^{P}/_{S}$ for both motor and inhibitor component. This may indicate post--ganglionic stimulation of both divisions.

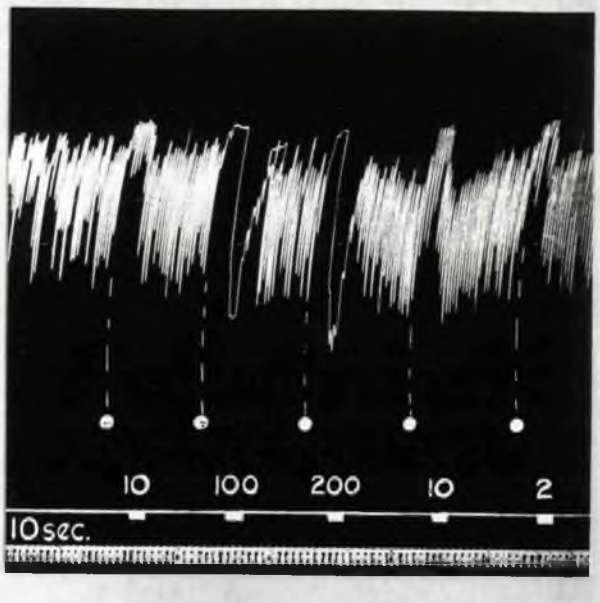


Fig. 51 Transmural stimulation of the duodenum. Below $10^{P}/_{S}$ pure motor responses are produced. At $100^{P}/_{S}$ and $200^{P}/_{S}$ biphasic responses, contraction followed by inhibition, are produced. Isotonic recording. Pulse duration 1·0 msec.

The ileum responded quite differently from the duodenum or colon. The results corresponded closely to those reported by Paton (1954) in the ileum of the guinea pig. The threshold voltage (about 5v) required to elicit a response from the rabbit ileum was lower than for the other two regions. The contraction which followed repetitive stimulation (Fig. 52) to begin with looked like the response of the colon to stimulation of the pelvic nerve. On the assumption that it was due to cholinergic nerve stimulation the frequency sensitivity was investigated. This showed that low frequencies of stimulation were effective but that the optimum frequency was much higher than that of the pelvic nerve, apparently above $100^{P}/_{S}$. These results seemed reasonable if post-ganglionic parasympathetic fibres were being stimulated; the optimum frequency of $10^{P}/_{S}$ with pre-ganglionic stimulation presumably being the result of ganglion transmission.

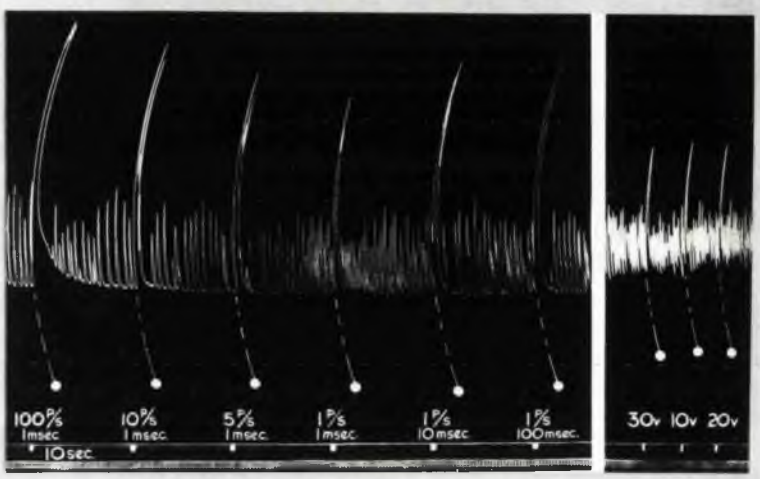


Fig. 52 Transmural stimulation of the ileum. The response in appearance resembles that of the colon to stimulation of the pelvic nerve but is determined not by frequency but by the duration of stimulation; $100^{P}/S$:1·0 msec and $1^{P}/S$:100 msec are almost equal though $1^{P}/S$:1·0 msec is smaller. The intensity of stimulation does not affect the response if above threshold. Isotonic recording.

Acceptance of the nervous basis of these contractions, however, became doubtful when it was found that the response was dependent not only on the frequency of stimulation but on the duration of the individual pulses. This is shown in Fig. 52. The response at $1^{P}/S$ was made as effective as the response at $10^{P}/S$ and $100^{P}/S$ by increasing the pulse duration first to 10 msec and then to 100 msec. Such an effect was never seen on stimulation of the extrinsic nerves to the colon. The next surprising observation was that single

pulses were effective and produced contraction. Once more it was easily shown that the response was dependent on the duration of stimulation. At 1 msec the response was small and rather difficult to detect; increasing the duration first to 10 msec and then to 100 msec caused a progressive increase in the response (Fig. 53). The intensity of stimulation did not affect the response so long as it was above threshold. This is shown in Fig. 52 where voltages from twice the threshold voltage up to six times this value were used without altering the response.

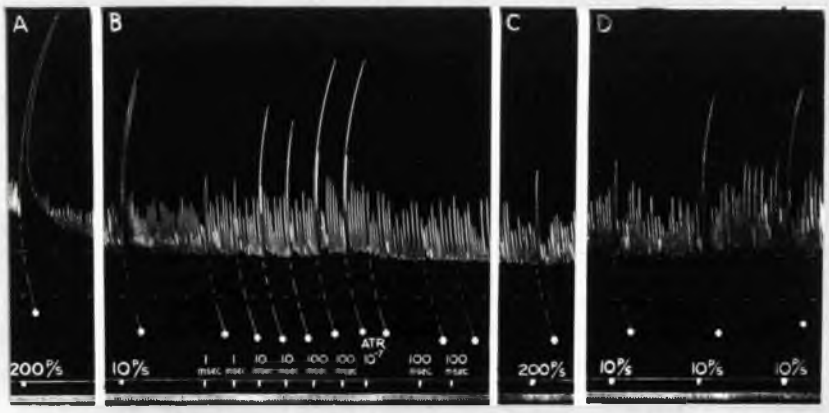


Fig. 53 Transmural stimulation of the ileum. Single pulses produce contraction the extent of which depends on the pulse duration (B). Atropine 10^{-7} completely abolishes the previous motor response to single stimuli (B) or to repetitive stimulation (C). Repetitive stimulation can still produce an after-contraction (D) whose amplitude depends on the pulse duration. The three responses in D were at 1 msec, 10 msec and 50 msec respectively. Isotonic recording.

The action of atropine on these responses was similar to its effect on the guinea pig ileum (Paton, 1954) and is shown in Fig. 53. Both the response to single pulses and to repetitive stimulation were abolished by a concentration (10^{-7}) which would have no effect on the motor response of the colon either to stimulation of the pelvic nerve or to transmural stimulation. The last panel, D, of Fig. 53 shows that a motor response was still obtained to repetitive stimulation after atropine. This appeared only on cessation of stimulation. During stimulation there was a slight inhibition which can just be seen on the trace illustrated but which was more obvious when watching the recording lever during the experiment. The motor component on cessation of stimulation was also dependent on the pulse duration, $10^{P}/_{S}$:1·0 msec was almost ineffective. The same frequency but at 10 and 50 msec produced a progressively increased response.

The results in the colon and duodenum correspond to what would be expected from stimulation of a mixture of nerve fibres with properties similar to the extrinsic sympathetic and parasympathetic nerves of the colon. Responses are obtained only with repetitive stimulation. Biphasic responses are produced in which contraction is always first and either component can be augmented by appropriate variation in the frequency of stimulation. The motor response is resistant to atropine but is finally abolished by a concentration similar to that required to block the pelvic nerve.

After blocking the motor response with atropine a pure inhibitor response is unmasked. The frequency sensitivity of this inhibitor response is similar to the inhibitor response from stimulation of the lumbar colonic nerves. The response of the ileum on the other hand corresponds to that of the same region in the guinea pig. Only motor responses are observed although the gut being active could easily show inhibition. Responses can be obtained to single stimuli and both these and the response to repetitive stimulation are easily blocked with atropine. The additional finding that the response is proportional not to the frequency of stimulation but to the duration of current passage, i.e., the pulse duration alone, using single pulses or the frequency X pulse duration for repetitive stimulation, is difficult to reconcile with nerve stimulation. If 1 msec is sufficient to initiate a propagated nerve impulse and produce a response, it is difficult to see why 10 msec or 100 msec should be any more effective.

D I S C U S S I O N

Of the experiments reported in this section only those showing the presence of overlap are open to a simple interpretation. In these the periods of stimulation were short so that fatigue was absent, the stimulus applied to each nerve was strictly localised to that nerve, and tension, presumably directly related to the number of active muscle units, was measured instead of contraction. Evidence on overlap of the extrinsic parasympathetic outflows has hitherto been confined to observations that vagal fibres from both sides are distributed to all parts of an organ e.g., the stomach (McCrea, 1924). So far as I am aware the presence of overlap of the parasympathetic fibres innervating the smooth muscle of the alimentary tract, so that the outflow from each side has access to the entire mass of contractile tissue, has never previously been demonstrated experimentally. The only suggestion of such an occurrence is Iggo's (1954) observation that after section and degeneration of one vagus nerve there is a sensitisation of the ruminant stomach to subsequent stimulation of the other nerve. The absence of this information is probably explained by the methods used in the past in studying the autonomic outflows to the gut. The vagus and splanchnic nerves were usually employed; the effects of stimulation of these nerves are so complicated that to look for overlap would not have been feasible. The innervated preparation

of the colon allows stimulation of the entire sacral parasympathetic outflow from the two sides to a limited region of the gut. A region moreover whose response to such stimulation has been shown to be consistently motor. These factors together with the increased simplification and control offered by recording only the contraction of the longitudinal muscle coat make this an excellent preparation with which to investigate such a phenomenon. The functional implications of this overlap are obscure. If the outflow from one side is sufficient to activate maximally the muscle cells, what is the effect of activity in both outflows? Speculation is pointless in the absence of any knowledge of the actual frequency of impulses which normally pass along these fibres. It may be that while $10^{p/s}$ is the most effective frequency of peripheral stimulation, this is normally achieved by summation of a lower frequency of impulses in the outflows from either side. The site of overlap was not determined in these experiments. This might be either at the ganglion cells or at the periphery. If it is true that the final common pathway is a nervous syncytium then of necessity there must be overlap at this level.

The finding that the fatigue which appears in the response to long continued pelvic nerve stimulation is located in the small area of nerve within the electrode is surprising. It should be emphasized that there is no question of nerve damage. On cessation of stimulation recovery is rapid and an undiminished response is

obtained in subsequent periods of stimulation. Intermittent stimulation can be repeated for long periods, in one experiment up to 18 hours, and still produce a response. Moreover, during stimulation the nerve still exercises some motor influence on the effector muscle no matter how long stimulation is continued. This influence appears as the "steady state" activity. This is the same irrespective of the frequency of stimulation so that the activity propagated along the nerve in this steady state seems independent of the frequency of stimulation. The conditions at the site of impulse formation, however, are manifestly different at different frequencies. If a high frequency of stimulation is used ($100^{P}/_{S}$ for example) then, although the response declines to the steady state i.e., impulse propagation in the nerve appears to decline, the nerve loses none of its ability to respond to the lower frequency of $10^{P}/_{S}$. If the frequency is altered to this frequency a response similar to that obtained from the unfatigued preparation is obtained. Stimulation at $10^{P}/_{S}$, however, conditions the preparation in some way so that if the frequency is increased there is for a time complete suppression of the effect of stimulation. The nature of the change in the nerve responsible for these effects is unknown. The decline in the response may be a form of adaptation whereby the frequency of the train of impulses set up in the nerve falls as stimulation is continued. If so the changes in the nerve which account for this

adaptation are not the same under all conditions of stimulation. The adaptation to high frequencies of stimulation for example leaves the nerve fully sensitive to low frequencies of stimulation whereas the adaptation which takes place to low frequencies of stimulation is such that subsequent stimulation at a high frequency is, temporarily, completely ineffective. To give this phenomenon a name is pleasant but deceptive. Whether this is a form of adaptation, its relationship to the physiological phenomenon of adaptation in sensory receptors and what the mechanism of the adaptation is, are still unanswered questions.

The inhibitory effect of a high frequency of stimulation after conditioning by a period of stimulation at low frequency is very similar to Wedensky inhibition. There is an initial augmented effect on changing to the high frequency followed by complete suppression of all activity. On changing back to the low frequency, activity is restored. The inhibition at the high frequency mimics the complete loss of rhythmic activity and tonus which follows cessation of stimulation at all frequencies. It is not an active process but a disappearance of the previous motor effect of stimulation. This raises the question of whether Veach was right in attributing inhibition of the stomach on stimulating the vagus at high frequency to Wedensky inhibition. His theory is improbable. No frequency of stimulation applied ab initio will cause inhibition. It is unlikely that activity in the

intrinsic nerves can "condition" the preparation in the same way as stimulation at low frequency does in the present experiments. Stimulation of one pelvic nerve at low frequency activates the muscle and presumably the intrinsic nerves but this does not "condition" the preparation to respond to a high frequency of stimulation of the other pelvic nerve with inhibition. The conditioning effect is limited to the stimulated nerve proximal to the site of overlap with the other nerve. The present findings might explain some of Veach's results if, in studying various frequencies, he had superimposed a period of high frequency immediately after one of low frequency. The resulting inhibition would have been dependent on the effect on the nerve of the previous period of low frequency stimulation.

The site of this Wedensky type inhibition is probably, as with fatigue, in the nerve at the site of stimulation. Bugnard (1934) reported a similar phenomenon in medullated nerve. High frequencies of stimulation alone gave a maximum response but after "conditioning" the nerve with a period of stimulation at low frequency these same high frequencies suppressed all effects of nerve stimulation. This effect was in the region of nerve stimulated and was not propagated.

These changes in the responsiveness of autonomic nerves to artificial stimulation are disturbing and are seldom referred to in reports on investigations into the effect of stimulating such

nerves. It is usually assumed that if a reasonably slow frequency of stimulation is used and if the nerve shows no signs of permanent damage throughout the experiment then the activity propagated along the nerve to an effector is constant. In the present experiments these criteria were certainly fulfilled and I believe that with this type of electrode conditions are as physiological as with most others. Yet it is clear that changes in the response of the smooth muscle due to changes in the response of the nerve at the site of stimulation appear surprisingly quickly. These results, unless the changes are peculiar to the fluid electrode, suggest that the conditions of artificial stimulation of autonomic nerves should be more carefully defined and controlled than is commonly the case.

The results of transmural stimulation of the rabbit gut depend on the region stimulated. The responses of the oral and anal end (duodenum and colon) are similar and correspond to what would be expected if a mixture of nerve fibres with properties similar to the extrinsic sympathetic and parasympathetic fibres of the colon were being stimulated. These responses are quite different from those reported by Paton (1954) from the guinea pig ileum. This investigator's results can, however, be reproduced if the ileum of the rabbit is used. The observation that the response in this region is proportional to the duration of stimulation, whether using single or repetitive pulses, rather than to the frequency of

stimulation is difficult to explain if the response is due to the initiation of nerve impulses in post-ganglionic cholinergic fibres. The ease with which these responses are abolished by atropine compared with the resistance to this drug of the response of the colon to stimulation of known cholinergic nerves is an additional inconsistency. The action of atropine suggests that the motor response in the ileum is due to acetylcholine diffusing to the muscle cells. It is tempting to attribute the source of this acetylcholine to the "sheath" cells of Auerbach's plexus. A neuro--secretory function has been suggested for these (Stöhr, 1955). Unfortunately information on the effect of electrical stimulation on the membrane potential of a secretory cell, and the relationship of this to function, seems to be lacking. If secretion follows membrane depolarisation it is possible that, unlike nerve and muscle, this depolarisation is maintained during the whole of a period of stimulation. The cell would continue to release its secretion throughout this period, the amount released depending on the store available, the rate of diffusion out of the cell, and the duration of stimulation. This would explain the observation that the effectiveness of transmural stimulation of the ileum is dependent on the duration of stimulation.

The difference in behaviour between the colon and duodenum on the one hand, and the ileum on the other, may be related to the extent to which these regions are under the control of the

autonomic nerves. Evidence has already been brought (Part II) which suggests that the stomach (and the first part of the duodenum is functionally allied to the stomach) and the colon are more influenced by, and have a greater supply of, extrinsic nerves. The cellular content of the intrinsic plexuses may also be important. Type II ganglion cells are numerous in the ileum but rare in the colon and stomach. Whatever the explanation for the difference it is clear that the response of the ileum to direct electrical stimulation is quite different from the response of other regions of the gut. It appears also that this response is not due to impulses generated in cholinergic nerve fibres and propagated to the smooth muscle. These results underline again the difficulties in interpreting pharmacological experiments. If a smooth muscle-nerve preparation is required, it is safer to choose a region where the appropriate nerve can be isolated and stimulated directly rather than analyse pharmacologically the response to a stimulus so generalised as to affect multiple structures.

S U M M A R Y

1) The two pelvic nerves have been separately stimulated and their interaction with one another studied.

2) Stimulation of either nerve at the optimum frequency of $10^{P}/_{S}$ develops almost as much tension as stimulation of both. There is, therefore, almost complete overlap of the outflows; both nerves have access to the same mass of contractile tissue.

3) Continued stimulation of one pelvic nerve is followed by a decline in the response. This appearance of fatigue is not due to exhaustion of the muscle since stimulation of the other pelvic nerve elicits, from the same muscle, an undiminished response. If the site of stimulation of the nerve is moved peripherally this fatigue is abolished.

4) A phenomenon similar to Wedensky inhibition is seen if the frequency of stimulation is suddenly increased from $10^{P}/_{S}$ to any frequency above $30^{P}/_{S}$. No frequency, however high, will, if applied by itself, produce inhibition. The "conditioning" effect of the low frequency of stimulation is confined to the nerve stimulated. Interpolation of a period of high frequency stimulation on the other pelvic nerve produces not inhibition but the usual motor response.

5) Experiments were carried out in which a stimulating current was passed through the wall of preparations from the duodenum,

mid-ileum and colon. These preparations were devoid of mesentery or extrinsic nerves. The results in the colon and duodenum were similar. The responses corresponded to what would be expected if a mixture of nerve fibres with characteristics similar to the extrinsic sympathetic and parasympathetic nerves of the colon were being stimulated. The results in the ileum were quite different and would be difficult to explain on the basis of excitation of cholinergic nerve fibres. They could be explained if stimulation releases acetylcholine from some structure which then diffuses to the smooth muscle cells.

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