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# THE RESPONSES OF THE MUSCULATURE OF THE COLON OF THE RABBIT TO STIMULATION, *IN VITRO*, OF THE PARASYMPATHETIC AND OF THE SYMPATHETIC OUTFLOWS

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It is generally accepted that the parasympathetic outflow to the gut has a motor action on both muscle coats except in the region of the sphincters where it is inhibitory. The sympathetic outflow is commonly held to be antagonistic to the parasympathetic outflow. However, many reported observations are in conflict with the above view. Bayliss & Starling (1899) obtained both motor and inhibitor responses from the dog's small intestine when they stimulated the vagi. Carlson, Boyd & Pearcy (1922) also obtained motor and inhibitor responses from the cardiac region of the stomach in cats, in dogs and in rabbits when they stimulated the vagi. Veach (1925) made similar observations on the stomach of the cat and so did McCrea, McSwiney & Stopford (1925) on the stomach of cats, of dogs and of rabbits. Motor and inhibitor responses have also been observed on stimulating the sympathetic outflow to the gut. Both Carlson *et al.* (1922) and McCrea & McSwiney (1928), observing the stomach of the cat, saw motor and inhibitor responses on stimulation of the splanchnic nerves. Brown, McSwiney & Wadge (1930) obtained motor and inhibitor responses from the stomach of the cat when they stimulated the thoracic sympathetic trunk. From these and other observations two factors emerge which seem to have played some part in determining the nature of the response. (*a*) *The frequency of stimulation*: low frequencies favoured motor responses while high frequencies favoured inhibitor responses. This effect was seen chiefly when stimulation was applied to mesenteric nerves, to the splanchnic nerves and to the thoracic sympathetic chain (Brown *et al.* 1930; Harrison & McSwiney, 1936). Veach (1925) reported this same effect of frequency when he stimulated the vagus nerve to the stomach in cats. (*b*) *The degree of activity of the organ, the 'peripheral mechanism'*: when the stomach was active and showing a high degree of tone then stimulation of the

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peripheral ends of the cut vagus nerve or of the splanchnic nerve caused inhibition. A motor response was obtained when the tone was low and the activity slight. This influence of the 'peripheral mechanism' seemed to be more marked with the vagal innervation (Courtade & Guyon, 1899; Carlson *et al.* 1922; McCrea *et al.* 1925; Brown & Garry, 1932).

The observations described above were all made *in vivo*, but similar results have been obtained from *in vitro* preparations of muscle from the stomach of cats, of dogs, and of rabbits. McSwiney & Robson (1931) found that stimulation of the sympathetic nerves at low frequencies had a motor effect while stimulation at high frequencies was inhibitor; in addition, the use of weak currents and of short periods of stimulation was found to favour motor responses. Stimulation of the vagus *in vitro*, however, only rarely caused inhibition but, after addition of atropine, an inhibitory response to nerve stimulation appeared (McSwiney & Robson, 1929). Finkleman (1930) stimulated the periarterial mesenteric nerves to the small intestine of the rabbit *in vitro*. Stimulation at high frequency invariably caused inhibition but stimulation at low frequency sometimes elicited a motor response.

Practically all the work on the influence of the frequency of stimulation and of the state of the 'peripheral mechanism' has been carried out on the stomach. The colon has been little studied. In this region the pelvic nerves supply the parasympathetic outflow and the lumbar colonic nerves the sympathetic outflow. The few observations which have been made suggest that the parasympathetic outflow is predominantly motor and the sympathetic inhibitor. Langley & Anderson (1895) reported motor responses from stimulation of the pelvic nerve in the rabbit *in vivo*. 'Brief' inhibition was occasionally seen but such results were a 'rarity'. The same workers found that stimulation of the caudal pair of the inferior mesenteric ganglia, or of any of the peripheral nerve bundles to the colon (lumbar colonic nerves, ascending bundle, hypogastric nerves), could produce motor, inhibitor or biphasic responses. Langley & Anderson were of the opinion that the motor responses were due to stimulation of parasympathetic fibres of sacral origin which may be present at these sites. Stimulation of the cranial pair of the inferior mesenteric ganglia, on the other hand, produced only inhibition. Bayliss & Starling (1900) found that the sympathetic was purely inhibitor to the colon of the rabbit, while the parasympathetic was purely motor. In the cat they found that the response to stimulation of the pelvic outflow was often biphasic. Only one report on an innervated preparation of the colon *in vitro* has been found in the literature. 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 inhibitor responses.

The present paper describes the effect of frequency of the stimulation, of the voltage of the stimulating current, of the duration of each pulse and of total

duration of stimulation on the responses from *in vitro* preparations of the colon of the rabbit. With this preparation (Garry & Gillespie, 1954*a*, *b*) it is possible to stimulate the parasympathetic and sympathetic outflows separately or simultaneously. With modern rectangular-pulse stimulators it is possible to control the characteristics of the stimulating current in a fashion quite impossible for workers in the past.

## METHODS

Rabbits of either sex were used. The most suitable weight ranged from 1·7 to 2 kg. The method of dissection has already been fully described (Garry & Gillespie, 1954*a*). One modification has been introduced. The lumbar colonic nerves, in the earlier experiments, were tied along with the inferior mesenteric artery and colonic vein at the sharp right-angled bend of that vein. In later experiments this ligature has been tied round the caudal pole of the inferior mesenteric ganglia and the colonic vein; this allows stimulation of the nerves to take place at a greater distance from the colon and reduces the likelihood of including some of the cranial sacral colonic fibres in the electrode on the lumbar outflow. Throughout the dissection the tissues were kept moist and cold by frequent application of chilled Ringer's solution. This had the double advantage of lowering the metabolic demands of the tissue and of inducing flaccidity which facilitated removal of faecal pellets. Even with experience the dissection required about 1 hr but the vitality of the preparation did not seem to be impaired.

The bowel was suspended as a Magnus preparation from a light gimbal lever. The lower end of the colon was fixed to a hook just above the sintered glass disk of a gas distributor. The lever exerted a tension of 0·5 g and had a magnification of 3·5. The gas distributor, gimbal lever and the clamps for the electrodes were all mounted on a common arm which could be raised or lowered by rack and pinion. While the preparation was still suspended above the level of the fluid in the inner vessel of the Burn-Dale bath the ligatures attached to the nerves were threaded, by means of a sharp needle, through the thin condom-rubber diaphragms of the two fluid electrodes. The nerves were pulled into place—the lumbar colonic nerves in the upper electrode and one or both pelvic nerves in the lower electrode. The closed half-cell of each electrode was then filled with Ringer and sealed off with a disk of 'Perspex'. The thread attached to the nerve was caught between the disk and the casing of the electrode, thus holding the nerve in position. When the electrodes were in position on the nerves the whole assembly was racked down into the bath (Fig. 1A).

The fluid electrodes are a modification of those described by Garry & Wishart (1951). The external diameter has been increased to 16 mm and the internal to approximately 10 mm to prevent kinking of the nerves as the preparation moves. The loops of flattened silver wire in the electrode are recessed as deeply as possible in the 'Perspex' walls (Fig. 1B) and are coated with AgCl in conventional fashion. The original multistrand copper wire running in polythene tubing has been replaced by a lead of 12 strands of copper wire, each strand being 0·004 in. in diameter. This lead is covered with a sheath of polyvinyl chloride (P.V.C.). The two leads to each electrode can be sealed together with 'Perspex' cement. Such double leads are still very fine and can be easily manipulated. Such wire, covered by P.V.C., can be obtained from Standard Cables. The electrodes were suspended by clamps from a horizontal bar well above the inner vessel of the bath. These clamps could be swivelled about the bar, moved along the bar and raised or lowered. Thus an electrode could be easily adjusted to the position of its nerve. Final adjustments were made once the preparation had been lowered into the inner vessel.

An electronic rectangular-pulse stimulator, delivering negative pulses, was used for stimulation. As a safeguard against polarization a 1 $\mu$F condenser was inserted in series in the active line of the stimulator. The electrode connexions were so arranged that the cathode was nearest to the preparation. In such a preparation, with two electrodes immersed in Ringer and attached to different

nervous outflows to the gut, stimulation by one electrode can also pass current through the other electrode if that other electrode is earthed. It is thus most important that, when one electrode is used for stimulation, the other should be 'floating', i.e. without earth connexions. Except during actual stimulation the electrodes were short-circuited. The interval between the periods of stimulation was usually 5 min and the duration of stimulation commonly 10 sec.

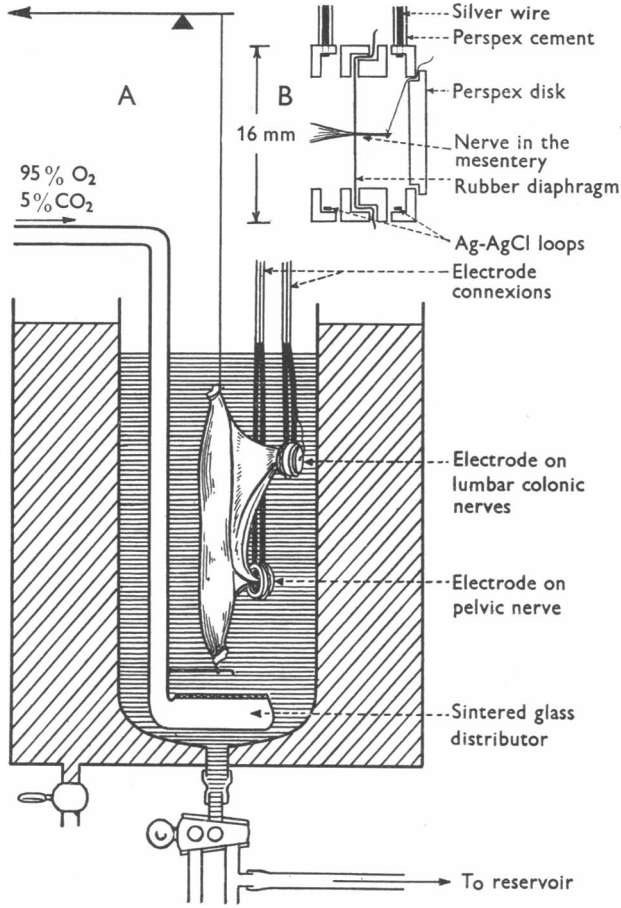


Fig. 1. (A) The Magnus preparation with one electrode on the parasympathetic outflow, the other on the sympathetic outflow. (B) Diagram of one electrode.

The Ringer fluid contained: NaCl, 0·9% (w/v); KCl, 0·4% (w/v); $CaCl_2$, 0·25% (w/v); $NaHCO_3$, 0·05% (w/v). In most experiments glucose was added to give a concentration of 0·1% (w/v). The bath was aerated with a mixture of 95% $O_2$ and 5% $CO_2$ through a sintered glass gas distributor, pore size 40–90 $\mu$. The bath temperature was maintained at 36° C.

Innervated lengths of the colon suspended as Magnus preparations continued to respond to nerve stimulation for many hours. Responses have been obtained after 18 hr in some preparations which were left over night. Once the dissection technique had been mastered a high proportion of successful preparations was obtained.

Observations were also made on Trendelenburg preparations set up in the conventional manner except for the presence of the stimulating electrodes.

Hexamethonium (C6) was used either as the bromide or iodide. Concentrations of hexamethonium are expressed as the base. Atropine was used as the sulphate and concentrations refer to the salt.

## RESULTS

The effects of frequency, of duration of the pulse, of voltage and of duration of stimulation were investigated in over sixty experiments. Most emphasis was placed on the effect of frequency. The majority of the experiments were simple Magnus preparations, the remainder were Trendelenburg preparations. Twenty-eight further experiments on the action of hexamethonium and on the action of atropine were carried out. That the responses to stimulation of the lumbar and of the pelvic nerves were mediated by these nerves and not the result of spread of current through the fluid was demonstrated by tying the nerves between the electrode and the gut after producing typical responses from both outflows. Thereafter stimulation was ineffective even when the voltage was increased sixfold.

### *The response to stimulation of the pelvic and of the lumbar colonic nerves*

Effective stimulation of the pelvic nerve caused rapid contraction of both muscle coats, stimulation of the lumbar colonic nerves a slower inhibition of both coats. At the most effective frequency the response to stimulation of the pelvic nerves began after a short latent period, developed rapidly and quickly reached a maximum. If stimulation were continued there was a gradual decline. When stimulation was stopped there was a fairly rapid relaxation followed by a period in which both tone and rhythmic contractions were depressed. The longer the period of stimulation and the greater the contraction the more marked and prolonged became this phase of post-stimulation depression.

Stimulation of the lumbar colonic nerves at the most effective frequency produced a very different picture. The latent period was longer than with stimulation of the pelvic nerves and the rate of development of inhibition was relatively slow. If stimulation were continued there was no evidence of fatigue or of 'escape' within the periods used in these experiments. When stimulation of the lumbar colonic nerves ceased, there was, after a short period, a sudden sharp contraction which quickly gave way to a prolonged period of increasing inhibition of a greater degree even than the inhibition seen during the actual stimulation. Final recovery was abrupt and complete. Typical responses in the same preparation to stimulation of pelvic and of lumbar colonic nerves are shown in Fig. 2.

*The effect of frequency.* All effective frequencies of stimulation produced motor responses when the pelvic nerves were stimulated and inhibitor responses

when the lumbar colonic nerves were stimulated. Single pulses were used and frequencies of stimulation ranging from one pulse every 2 sec to 1000 pulses per sec (1000 P/S). The uniform nature of these responses is shown in Fig. 3.

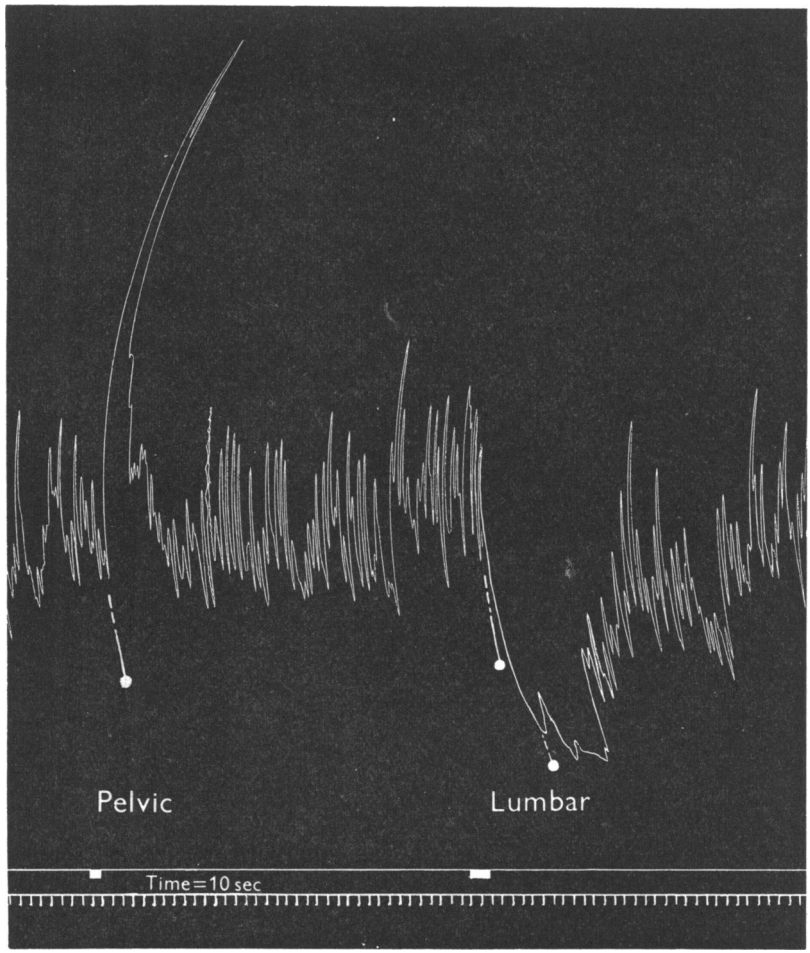


Fig. 2. Colon of the rabbit *in vitro*. Voltage of current 15, duration of each pulse 0·5 msec. Frequency when stimulating pelvic nerve was 10 P/S, when stimulating lumbar outflow 50 P/S.

Although variations in frequency did not affect the character of the response they did alter the magnitude. Stimulation of the pelvic nerves evoked a maximal response when a frequency of 10 P/S was used. The maximum response to stimulation of the lumbar colonic nerves took place when a frequency of 100 P/S was used. When a frequency of stimulation below 10 P/S was used the responses to stimulation of the pelvic nerves were still of considerable

magnitude but the responses to stimulation of the lumbar colonic nerves were greatly reduced: at a frequency of one pulse every 2 sec stimulation of the pelvic nerves was still effective while stimulation of the lumbar colonic nerves was rarely effective at a frequency below 5 P/S.

An attempt has been made to express the effectiveness of the various frequencies quantitatively and the results are summarized in Table 1. In any

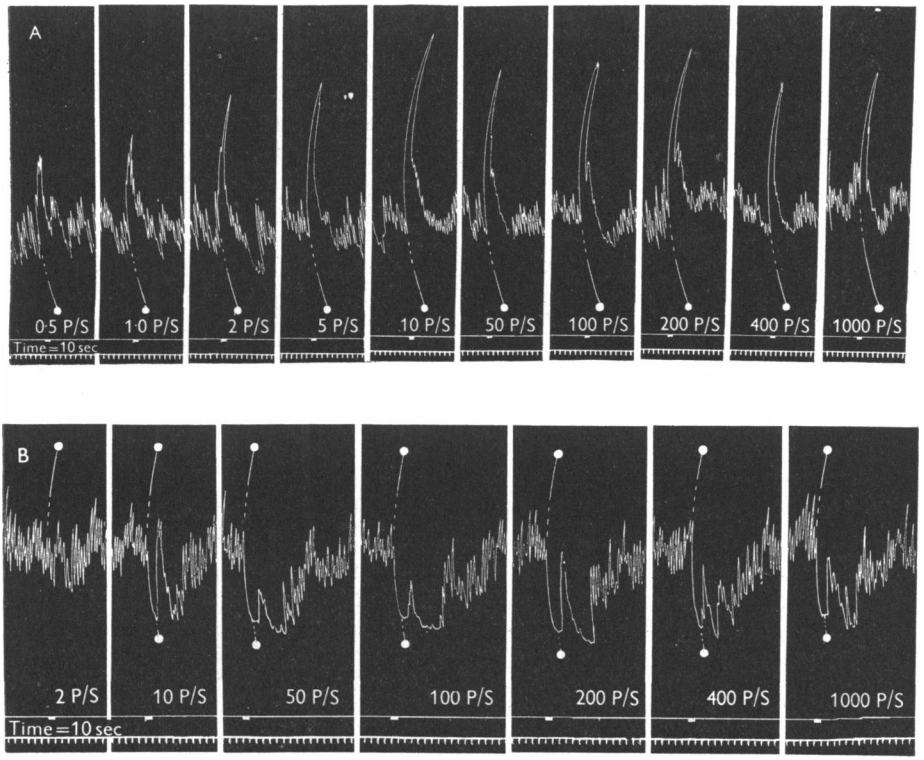


Fig. 3. Colon of rabbit *in vitro*. The effect of varying the frequency of stimulation. Voltage of current 15, duration of each pulse 1·0 msec in all cases except when the frequency was 1000 P/S; then the pulse duration was 0·5 msec. (A) Pelvic nerve: the responses were always motor; stimulation was effective with frequencies well below 10 P/S. (B) Lumbar outflow: the response during stimulation was always inhibitor; stimulation with frequencies below 10 P/S was rarely effective.

one experiment, and on the same graphic, that frequency which produced the maximum response was selected for each nerve and assigned the arbitrary value of six. The remaining responses were then assessed in terms of the maximum response and expressed as some integral fraction of six. During the actual experiment that frequency which appeared to be producing the maximum response was repeated in the middle and at the end of the series of

stimulations. Any gradual variation in the response not associated with frequency was thus detected. Two main components of the response were used in assessing its magnitude: the degree of contraction or inhibition and the duration of this contraction or inhibition. The duration of the response was particularly important in assessing the magnitude of the response to stimulation of the lumbar outflow, the height of contraction was the main factor in assessing the magnitude of the response to stimulation of the sacral outflow.

TABLE 1. The influence of the frequency of recurrence of the stimulating pulse on the magnitude of the response from the colon *in vitro*. An arbitrary figure of 6 is given to the maximum response in any one experiment.

The frequency is indicated as pulses per sec at the head of each column. In all cases the duration of the pulse is 1 msec except at the frequency of 1000 P/S when the duration of the pulse is 0·5 msec.

| | Frequency, P/S | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Expt. | 0·5 | 1 | 2 | 5 | 10 | 20 | 50 | 100 | 200 | 400 | 500 | 1000 |
| 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.

When the total period of stimulation is prolonged beyond 10 sec, stimulation of the pelvic nerve at 10 P/S or less evokes a response which is well maintained and declines very slowly while the stimulation continues: with stimulation at 50 P/S the contraction is not so well maintained even while the stimulation continues. If stimulation of the pelvic nerve at 10 P/S is continued for 30 sec and then, without interruption of stimulation, changed to a frequency of 50 P/S there is diminution in the contraction which rapidly returns practically to its old magnitude when the frequency is restored, again without interruption, to 10 P/S (Fig. 4). The decrease in contraction at the higher frequency can then hardly be attributed to exhaustion of the effector muscle. When the lumbar colonic nerves were subjected to a similar prolonged stimulation there was no sign of 'escape' from the inhibition and change of frequency within the range of 50 to 200 P/S during the actual stimulation made no observable difference to the response.

The effect of frequency of stimulation on the time-relations of the responses was studied by measuring the latent period and the time required for the response to reach its maximum. The results are given in Table 2. The latent

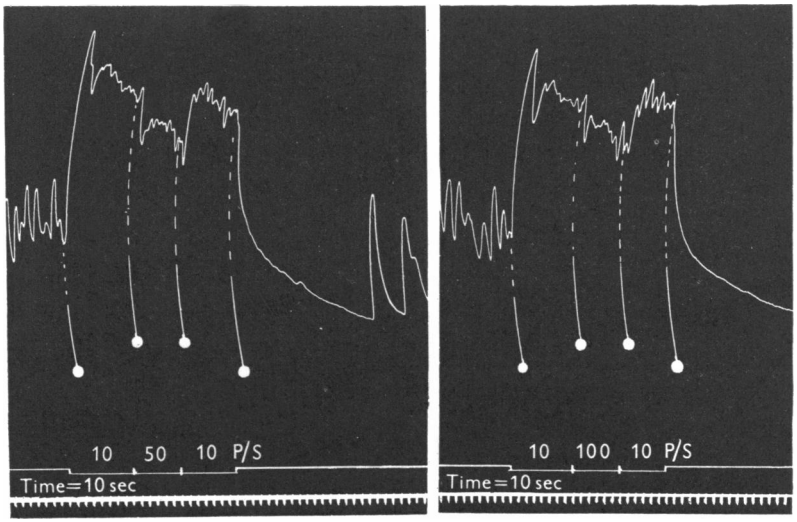


Fig. 4. Colon of the rabbit *in vitro*. Stimulation of the pelvic nerves. Increase in the frequency of stimulation from 10 to 50 P/S or to 100 P/S decreases the amplitude of the contraction: the contraction rises to its old level on restoring the frequency to 10 P/S.

TABLE 2. The influence of the frequency of the recurrence of the stimulating pulse 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) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Expt. | 1 | 2 | 5 | 10 | 50 | 100 | 1 | 2 | 5 | 10 | 50 | 100 |
| 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. | | | | | | | | | | | | |
| 111 | — | — | 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. | | | | | | | | | | | | |

period of the response to stimulation of the lumbar outflow is consistently greater than the latent period when the pelvic outflow is stimulated. This difference is well marked even at the optimal frequencies of stimulation for each outflow. The time which elapses before the response reaches its maximum

also varies with frequency (Table 2). The response to stimulation of the pelvic nerves at all effective frequencies develops more rapidly than the response to stimulation of the lumbar colonic nerves. On stimulation of the lumbar outflow the maximum degree of inhibition occurs well after cessation of stimulation.

The effect of frequency of stimulation was also studied with the Trendelenburg preparation. Stimulation of the pelvic nerves invariably caused contraction of both muscle coats: stimulation of the lumbar outflow caused inhibition of both coats.

*The effect of the duration of the individual electrical pulses.* In a number of experiments single pulses were used of various durations up to a maximum of 1·44 sec. In only one preparation did such single pulses evoke a response from stimulation of the pelvic nerve: there was a slight contraction. Single pulses never evoked a response when the lumbar colonic nerves were stimulated.

An attempt was made to find the threshold for the duration of the pulses. Previous tests showed that increasing the duration of pulse beyond 1·0 msec and up to 100 msec did not lower the voltage required to stimulate the pelvic nerves. Using a pulse duration of 1·0 msec and with the optimal frequency for each outflow, the voltage was gradually increased from zero until a response was obtained. This was taken as the 'rheobase', a value usually between 5 and 8 V. The voltage was then doubled for subsequent use in determining the threshold for pulse duration, our 'chronaxie'. Progressive reduction in the duration of the pulse from 1·0 msec had no effect on the response until a duration of 0·05 msec was reached (Fig. 5). At this level there was a reduction in the response from stimulation of both the pelvic and the lumbar colonic nerves. This reduction in the response appeared at all frequencies of stimulation but was proportionately somewhat greater at low frequencies. When the duration of each pulse was reduced to 0·01 msec there was no response from stimulation of either outflow with double the 'rheobase'. A response with a pulse duration of 0·01 msec could, however, be obtained if the voltage was greatly increased to something of the order of 10 times the 'rheobase'. Variation in the duration of the pulses on no occasion altered the characters of the responses.

*The effect of the total duration of the repetitive stimulation.* McSwiney & Robson (1931), stimulating the mesenteric periarterial nerves *in vitro* of a muscle strip from the stomach of cats and of rabbits, found that 'short periods' of 'faradic stimulation' might produce a motor response instead of the usual inhibition. In the rabbit colon preparation we never found that the duration of the stimulation had an effect on the nature of the response. The response was always motor from the pelvic outflow and inhibitor from the lumbar outflow. The duration of stimulation ranged from 1 sec to 4 min with the pelvic nerve and from 8 sec to 4 min with the lumbar colonic nerves.

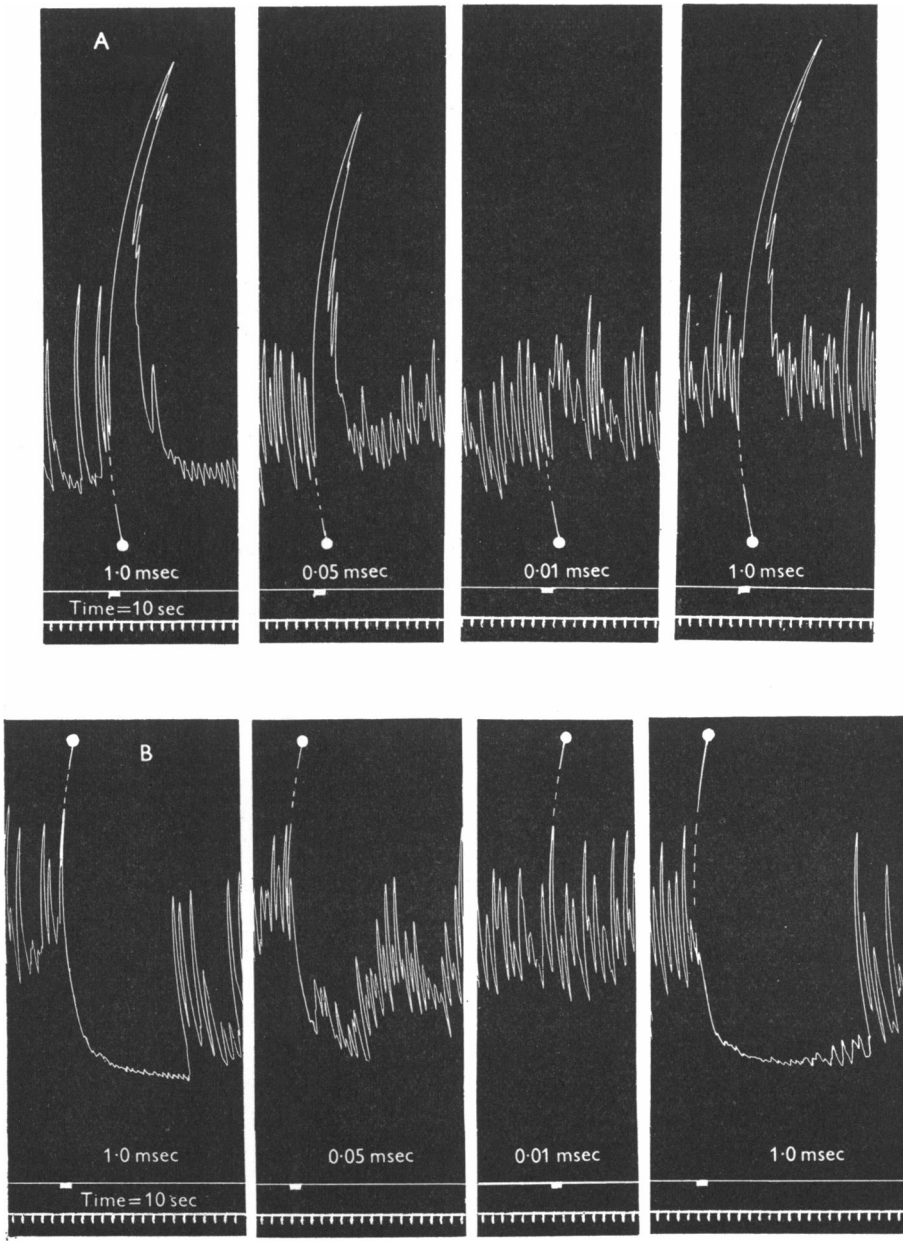


Fig. 5. Colon of the rabbit *in vitro*. The effect of varying the duration of the pulses. Duration of pulse below each graphic. (A) Stimulation of the pelvic nerves at 15 V with 10 P/S. (B) Stimulation of the lumbar outflow at 15 V with 100 P/S.

*The effect of the amplitude (intensity or voltage) of the pulse.* To find the threshold for the amplitude of the pulse the voltage was gradually increased from zero. The first responses were always motor from the pelvic nerves and inhibitor from the lumbar colonic nerves. Further increase in voltage merely increased the magnitude of the response. When the maximum response was attained further increase in voltage was without effect on the observed response.

*The effect of the state of the 'peripheral mechanism'.* Some preparations of the colon show more active rhythmical movements than do others. Stimulation of the lumbar colonic nerves caused a much greater lengthening in some preparations than in others, presumably owing to a greater initial state of tone. It seems probable that these variations were due to a difference in the intrinsic activity and in the tonus of the preparation. Whatever the state of the preparation, however, stimulation of the pelvic nerve always gave a motor response and stimulation of the lumbar colonic nerves always caused inhibition. The state of the 'peripheral mechanism' did not affect the character of the response.

*The action of hexamethonium and of atropine.* Hexamethonium iodide or bromide at concentrations between $1 \times 10^{-4}$ and $3 \times 10^{-4}$ of the base blocked the response to stimulation of the pelvic nerve without affecting the response to stimulation of the lumbar outflow. The tone and rhythmic contractions of the preparation and its response to acetylcholine were unaffected by concentrations of hexamethonium which caused complete block of the pelvic outflow. The lowest concentration ever found to produce a definite decrease in response was $1 \times 10^{-5}$; a concentration of $5 \times 10^{-5}$ always induced some degree of block. The action of C6 could be abolished by washing out the solution of the drug, but a considerable time elapsed before the response to stimulation of the pelvic nerves returned to its previous magnitude (Fig. 6).

In low concentrations the ability of atrophine sulphate to block the motor effect from stimulation of the pelvic nerves was somewhat variable. However, a concentration of $1 \times 10^{-4}$ always caused complete block. Such doses increased the tone and produced a flurry of rapid rhythmic movements. On stimulation of the pelvic outflow there was not the slightest suggestion of inhibition after abolition of the motor response by use of atropine. The inhibitor response from stimulation of the lumbar outflow was, however, unaffected (Fig. 7).

### *The response to stimulation of a 'mixed' nerve*

The optimal frequency for stimulation of the pelvic nerve is in the neighbourhood of 10 P/S; that for the lumbar colonic nerves, on the other hand, is about 100 P/S. Moreover, the contraction elicited by stimulation of the pelvic nerve is rapid and not sustained after cessation of stimulation, while the

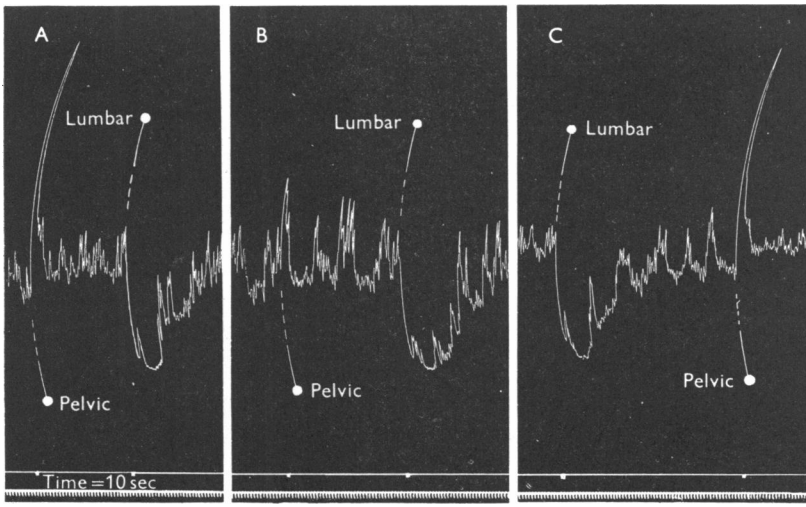


Fig. 6. Colon of the rabbit *in vitro*. In all cases the voltage of the stimulating current was 15, the duration of each pulse 1 msec but the pelvic nerves were stimulated at 10 P/S, the lumbar colonic nerves at 100 P/S. Between A and B hexamethonium was added to the bath to give a concentration of $2 \times 10^{-4}$. Between B and C the preparation was repeatedly washed.

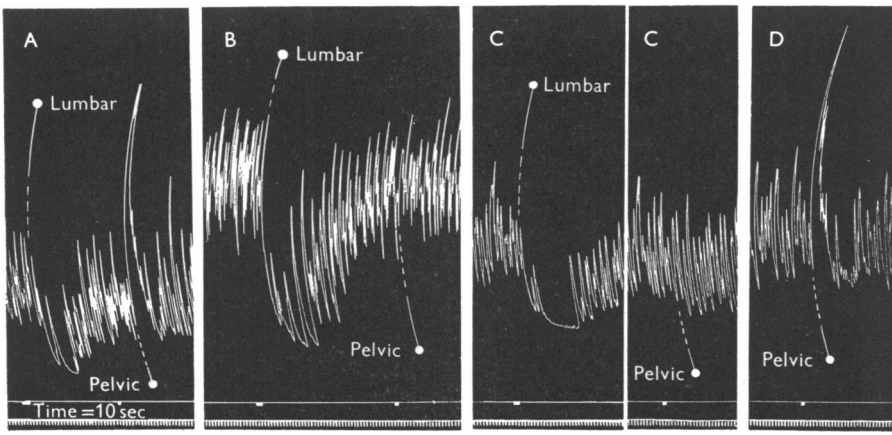


Fig. 7. Rabbit colon *in vitro*. The effect of atropine on the responses from stimulation of the pelvic and of the lumbar colonic nerves. A, before adding atropine; B, in the presence of atropine sulphate $2 \times 10^{-4}$; C, shortly after washing; D, 1 hr after washing.

inhibition produced by stimulation of the lumbar colonic nerves is somewhat delayed in onset and persists for a considerable time after cessation of the stimulation. If a mixture of nerve fibres from both outflows could be simultaneously stimulated then it might be possible to evoke at will either inhibition or contraction according to the frequency of stimulation used. Low frequencies ought to favour motor responses and, if the frequency were at, or above, the threshold for the pelvic nerve and below the threshold for the lumbar colonic nerves, then the response ought to be purely motor. High frequencies should be optimal for stimulation of the lumbar outflow but beyond the optimum for eliciting a response from the pelvic nerves. Then the response from simultaneous stimulation of both outflows should be mainly inhibition with possibly a small initial contraction. With intermediate frequencies one would expect from stimulation of the pelvic component of the 'mixed nerve' initially a contraction of short latent period and developing rapidly. Thereafter should appear inhibition from stimulation of the lumbar component, the inhibition developing later and persisting for a considerable time.

To test this possibility three methods were used to obtain a 'mixed' nerve. (1) The upper electrode on the lumbar colonic nerves was advanced as far as possible along the inferior mesenteric artery towards the colon in the hope of reaching a position at which some of the sacral colonic (parasympathetic) nerves join the periarterial network. In short we were deliberately taking advantage of the old observations of Langley & Anderson (1895). This method gave a naturally 'mixed' nerve, but it had the disadvantage that it was difficult to advance the electrode sufficiently far along the inferior mesenteric artery to reach sacral colonic fibres innervating the actual preparation of the colon. Moreover, when thus advanced close to the colon, the electrode usually interfered to some extent with the free movements of the preparation. (2) This difficulty was overcome by removing the pelvic nerve from the lower electrode and pulling it through the upper electrode along with the lumbar colonic nerves. An artificially 'mixed' nerve was thus produced, still, however, in the one electrode and simultaneously exposed to the same stimulus. (3) While still retaining the two electrodes in position on the separate outflows the stimulating current was passed through them in parallel.

Whichever method was subsequently used the nerves were first stimulated separately at various frequencies. This practice ensured that the two separate outflows were fully effective and giving their characteristic responses and enabled us to find a low frequency of stimulation effective with the pelvic nerve but ineffective with the lumbar colonic nerves. The nerves were then 'mixed'.

The results from both naturally and artificially 'mixed' nerves were similar. Frequencies previously found to be effective for the pelvic nerve but ineffective for the lumbar outflow now gave a pure motor response. High frequencies,

known to be optimal for the lumbar outflow, produced almost pure inhibition with, sometimes, a small initial contraction; intermediate frequencies gave biphasic responses, first contraction then inhibition. This inhibition persisted after cessation of stimulation. By ringing the changes on these intermediate frequencies one could at will augment either the motor or inhibitor response. Fig. 8 shows all these responses in one preparation.

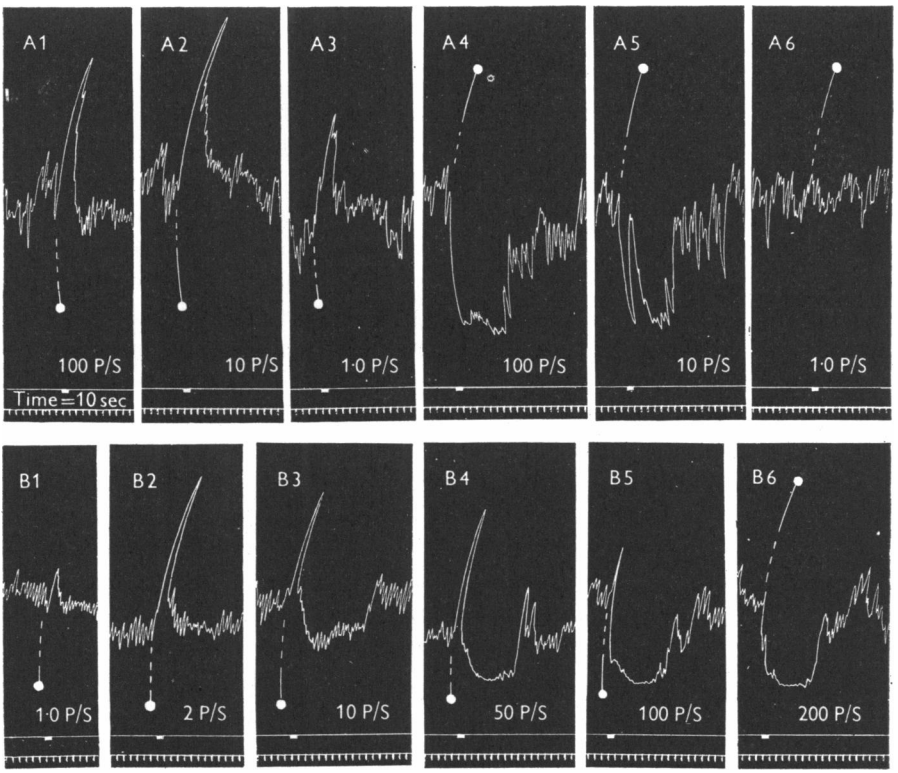


Fig. 8. Colon of the rabbit *in vitro*. Effect of simultaneous stimulation of the pelvic and lumbar outflows in the same electrode. Voltage of current 15, duration of each pulse 1 msec in all cases. Frequency of stimulation is shown on each graphic. A 1, 2, 3: stimulation of pelvic nerve alone. A 4, 5, 6: stimulation of the lumbar outflow alone. B 1, 2, 3, 4, 5, 6: the same preparation after artificial 'mixing' of these outflows. Low frequencies cause pure contractions; high frequencies pure inhibition: intermediate frequencies give a biphasic response. Time marks, 10 sec throughout.

## DISCUSSION

For many years now the old belief that the parasympathetic outflow to the gut is invariably motor in function while the sympathetic is inhibitor has been suspect. There can be few who escape a twinge of conscience when teaching

the old dogma. Yet alternative hypotheses are not attractive. To try to explain the erratic experimental results by invoking the paramount influence of a 'peripheral mechanism' runs counter to the general belief in the dual nature of the autonomic nervous system. Is the state of the 'peripheral mechanism' to predetermine the result irrespective of whether the sympathetic or parasympathetic outflow goes into action?

The fairly general observation that stimuli applied at low frequency favour motor responses while stimuli at high frequency bring about inhibition suggests at once that the inhibition is a manifestation of fatigue. This is a tenable hypothesis until we have more information about the frequency of impulses normally transmitted by the autonomic outflows. Nevertheless, the fact that low frequencies usually have a motor action and high frequencies an inhibitor action both with sympathetic and with parasympathetic outflows once more runs counter to the belief in the dual nature of the autonomic outflows. And Fig. 4 of the present paper shows that the reduction of the response at high frequency of stimulation is not due to exhaustion of the effector muscle cells.

A more hopeful approach is to postulate the existence of motor and of inhibitor fibres in both the sympathetic and parasympathetic divisions of the autonomic system. Langley (1922) had some such idea in mind for the constitution of the vagal supply to the gut. Harrison & McSwiney (1936) found that motor responses in the stomach, from stimulation both of the vagus and of the sympathetic outflows, were augmented by the presence of eserine and abolished by the presence of atropine. They therefore concluded that cholinergic fibres reached the stomach not only by the vagi but also by the sympathetic trunk and by the splanchnic nerves. Ambache (1951) and Ambache & Edwards (1951) bring forward evidence that the cells in the myenteric plexus, conventionally regarded as stations on the parasympathetic outflow, are of two kinds—either motor or inhibitor. In support of this conception it should be remembered that extrinsic autonomic outflows to the gut do have each a dual function. The parasympathetic outflow is motor to the gut proper but inhibitor to sphincters; the action of the sympathetic is in the opposite sense. Moreover, when stimulation of the parasympathetic outflow evokes peristalsis, this is an activity still thought by many to involve 'ascending contraction' and 'descending inhibition'.

However this may be, it is often forgotten that the observations, which are held to be incompatible with the belief that the parasympathetic outflow is essentially antagonistic to the sympathetic outflow, were made by stimulation of quite large nerves such as the vagus, the splanchnics and the periarterial mesenteric branches. These last certainly have fibres both of sympathetic and of parasympathetic origin. Mitchell (1953), in his book, reviews the available evidence. He concludes that certainly the vagus and possibly the sympathetic outflow to the viscera are 'mixed' containing both sympathetic and para-

sympathetic fibres. Kuré, Ichiko & Ishikawa (1931), moreover, put forward the revolutionary idea that 'spinal parasympathetic' fibres emerge in dorsal roots of the spinal cord and run through the sympathetic chain to all of the gut with the exception of the large intestine.

One should never forget, too, that workers in the past were greatly handicapped by poor stimulators. They were very conscious of their handicap and made brave attempts to calibrate induction coils in terms of definite units such as the 'Z units' of Martin (1912). We now have a great advantage over our predecessors and can at will vary the strength, duration and frequency of the stimulating pulses.

Owing to the anatomical arrangement it is probable that the macroscopic nerves supplying the caudal region of the colon are 'pure' parasympathetic or sympathetic, at least close to their origin. If this be so, and if these two outflows are always functionally in opposition, then stimulation should evoke, whatever the frequency, whatever the state of the 'peripheral mechanism', consistently either a motor or inhibitor response. Such in fact proves to be the case with the colon of the rabbit *in vitro*. In addition, the power to alter at will the frequency of the stimulating pulses brought to light a consistent difference in the sensitivity of the sympathetic and of the parasympathetic outflows to the frequency of the pulses.

This difference, which we found, would go far to explain the findings of past workers if the nerves they stimulated were in fact 'mixed', containing axons with the characteristics of the parasympathetic ouflow and also axons with the characteristics of the sympathetic outflow. McSwiney & Robson (1929), eliciting contraction by stimulating the vagus supply to strips of the stomach *in vitro*, found maximum summation from two stimuli when the interval between them was 0·1 sec. This is a frequency of 10 P/S. Brown *et al.* (1930) obtained motor responses from the stomach by stimulating the thoracic sympathetic chain at 1 or 2 P/S but inhibition when the frequency ranged from 10–60 P/S. Veach (1925), stimulating the vagus to the stomach, elicited motor responses with stimuli at frequencies 'as low as 1 pulse in 3 to 5 sec' and inhibition at over 40 P/S. Again, McSwiney & Robson (1931), stimulating the periarterial nerves to strips of the stomach *in vitro*, obtained contraction at 1–12 P/S and inhibition at 4–45 P/S. We were able to mimic what we believe to be the state of affairs in the autonomic outflows to the cranial region of the gut by simultaneously stimulating both the pelvic and lumbar outflow to the colon. We then obtained at will from this 'mixed' nerve a 'pure' parasympathetic effect with low frequencies of stimulation and a 'pure' sympathetic effect with high frequencies.

The time relations of the responses of the colon to stimulation of the two outflows are also distinctive. The response to stimulation of the pelvic nerves is rapid and not sustained, that to stimulation of the lumbar outflow is delayed

and prolonged. This difference enabled us to produce at will, by simultaneous stimulation of the two outflows to the colon, a biphasic response when we used frequencies effective for both outflows. The rapid contraction from the pelvic innervation preceded the later inhibition from the lumbar outflow. If these results from stimulation of an artificially 'mixed' nerve to the colon are valid also for natural, anatomically 'mixed', nerves supplying other regions of the gut then a simple explanation is to hand for many of the past results which were apparently inconsistent with the classical view of the autonomic innervation of the gut. And future work may be helped if the distinctive attributes of the outflows to the colon in the rabbit also apply to the extrinsic innervation of the alimentary canal in general.

Our results, unfortunately, do not begin to explain the differences in the sensitivity of the two outflows: neither do they explain the different time relations of the two responses from the preparation. Stimulation of the pelvic outflow was perforce preganglionic, that of the lumbar outflow was probably largely postganglionic. Yet the response to stimulation of the preganglionic fibres of the pelvic nerves occurred more rapidly than the response to stimulation of the postganglionic fibres of the lumbar outflow. It may be that the different nature of the response, contraction as distinct from relaxation, plays some part in the speed of the response. And one cannot exclude the possibility that the mechanical conditions of the preparation *in vitro* also have an effect. It goes without saying that such intimate details as the sites of liberation of the transmitters, the rates of liberation, of diffusion, of destruction and of resynthesis of these same transmitters may all play a part.

There is a further major gap in our knowledge. We do not yet know the effect of variation in the frequency of the pulses of the stimulating electrical current on the behaviour of the nerve impulses in the actual nerve fibres to the gut. We do not know what is happening in the myenteric plexus, we have no picture of the pattern of the impulses leaving the plexus to the effector muscle cells. It is unlikely that we shall fully understand the functions of the extrinsic innervation of the gut until we have a complete picture of the nature and pattern of the normal outflow of impulses from the spinal cord.

## SUMMARY

1. Stimulation of the pelvic nerves to the colon of the rabbit *in vitro* always causes contraction: stimulation of the lumbar outflow to the colon always causes inhibition. The initial state of the preparation, high tone or low tone, does not affect the results.

2. The maximum contraction from the preparation is obtained when the pelvic nerves are stimulated with frequencies in the neighbourhood of 10 pulses/sec (10 P/S). Nevertheless, frequencies as low as 1 pulse/2 sec and as high as 1000 P/S still cause contraction. The response is rapid but not well

sustained. The presence of atropine or of hexamethonium in high concentration abolishes the response.

3. The maximum inhibition of the preparation is obtained when the lumbar outflow is stimulated with frequencies of 100 P/S or over. Frequencies below 5 P/S are rarely effective. The response is not rapid but persists for a considerable period after cessation of the stimulus. The presence of atropine or of hexamethonium does not abolish the response.

4. Simultaneous stimulation of both outflows to the colon with low frequencies evokes a pure motor response: stimulation with high frequencies causes pure inhibition. Stimulation with intermediate frequencies gives a biphasic response, first contraction then relaxation.

5. Our results suggest that, if extrinsic nerves to the gut contain both parasympathetic and sympathetic fibres, then frequency of stimulation may affect the character of the response. Thus could be explained many of the findings of past workers.

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