J. Physiol. (1976), **260**, *pp.* 425–445
With 1 *plate and* 10 *text-figures*
Printed in Great Britain

AN ELECTROPHYSIOLOGICAL STUDY OF THE SACRAL PARASYMPATHETIC PATHWAY TO THE COLON OF THE CAT

BY WILLIAM C. DE GROAT AND JACOB KRIER

From the Department of Pharmacology, School of Medicine, University of Pitttsbugh, Pittsburgh, Pennsylvania 15261, U.S.A.

(*Received 20 January 1976*)

SUMMARY

1. Electrophysiological techniques were used to study the sacral parasympathetic pathway to the colon of the cat.

2. Electrical stimulation of the sacral ventral roots or the pelvic nerve elicited contractions of the colon and firing in nerve filaments on the serosal surface of the colon. Both responses were markedly reduced by the administration of ganglionic blocking agents. It is concluded that sacral preganglionic fibres to the colon make synaptic contacts with extramural ganglion cells. These cells were identified histologically in small ganglia on the serosal surface of the distal colon and rectum.

3. Transmission in extramural colonic ganglia was cholinergic and mediated by nicotinic receptors. Colonic ganglia did not exhibit large recruiting responses during repetitive (1–4 c/s) preganglionic nerve stimulation or an adrenergic inhibitory mechanism, both of which have been identified in bladder parasympathetic ganglia. It is concluded that colonic ganglia unlike bladder ganglia function primarily as simple relay stations and have little potential for modulating the neural activity arising in the central nervous system.

4. The preganglionic input to colonic ganglia was mediated by C fibres with maximal conduction velocities ranging from 0·5 to 1·4 m/sec. Bladder ganglia, on the other hand, received a preganglionic input composed of B fibres with maximal conduction velocities ranging from 8 to 10 m/sec. The possible physiological significance of different types of preganglionic fibres in the sacral outflow is discussed.

INTRODUCTION

This paper will deal with electrophysiological properties of the sacral parasympathetic pathway to the colon of the cat. It will focus primarily

on the characteristics of the preganglionic efferent fibres and the mechanisms underlying transmission in extramural parasympathetic ganglia. These results will serve as the basis for subsequent papers concerning the central control of colonic motility.

The colon receives an innervation from both divisions of the autonomic nervous system. The sacral parasympathetic pathway (pelvic nerves) is excitatory to this organ and is thought to be active primarily during defaecation (Garry, 1933*b*; Denny-Brown & Robertson, 1935; Hulten, 1969). The lumbar sympathetic innervation (lumbar colonic nerves) exerts an inhibitory influence on colonic motility and an excitatory effect via the hypogastric nerves on the internal anal sphincter (Learmonth & Markowitz, 1929, 1930; Garret, Howard & Jones, 1974).

An anatomic feature of the colonic innervation which played an important role in the success of this investigation was the accessibility of the parasympathetic ganglion cells and post-ganglionic fibres. Unlike most regions of the gastrointestinal tract where the ganglion cells are located in the myenteric plexus, many of the ganglion cells in the sacral outflow to the distal colon and rectum are present in extramural ganglia on the serosal surface of the organ (Langley & Anderson, 1895; Gaskell, 1916). In this location they could be studied with relatively simple electrical recording techniques.

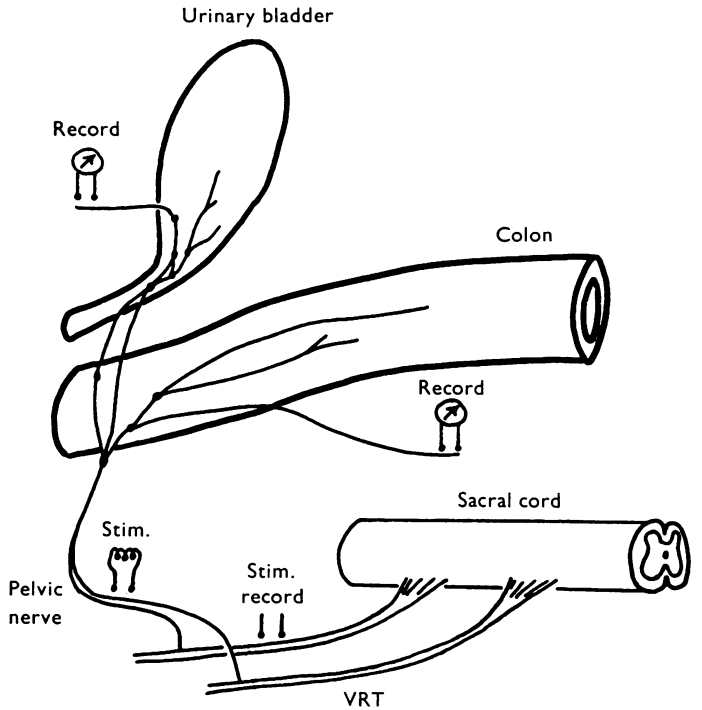
Previous studies of the sacral parasympathetic outflow focused on the pathway to the urinary bladder (Schnitzlein, Hoffman, Hamlett & Howell, 1963; de Groat & Ryall, 1968, 1969; de Groat & Saum, 1972, 1976; de Groat, 1975). Prominent inhibitory (adrenergic) and facilitatory mechanisms were identified in bladder ganglia and considerable information was obtained about the electrical properties of vesical preganglionic neurones and about the reflex pathways underlying micturition.

The present experiments revealed that the sacral pathway to the colon differs considerably from the pathway to the bladder, both in regard to preganglionic fibre characteristics and in regard to mechanisms of transmission in ganglia. This paper will examine these differences. Preliminary reports of these observations have been published (Krier & de Groat, 1974, 1975; de Groat & Krier, 1975).

METHODS

Experiments were performed on seventy cats of either sex anaesthetized with chloralose (50–60 mg/kg, I.V.) following induction with halothane. Three experiments were conducted on cats in which the sacral ventral roots on one side had been sectioned intradurally, 11–15 days before the experiment. Following tracheal intubation, the large intestine, the urinary bladder and the extrinsic nerves to these organs were exposed through a mid line abdominal incision. The pelvic, lumbar colonic, inferior splanchnic, and hypogastric nerves were isolated from the underlying connective

tissue. In some experiments the pelvic nerves (preganglionic) were sectioned central to the point of isolation while in others they were left intact. Nerve filaments arising from extramurally located ganglia on the serosal surface of the bladder and colon were sectioned and prepared for monophasic recording. In addition to the abdominal dissection, a lumbosacral laminectomy was performed in many cats to expose the sacral dorsal and ventral roots. The animal was placed on its side to permit access to the ventral roots and abdominal cavity. In some animals the carotid sinus nerves, the aortic depressor nerves and the vagus nerves were isolated bilaterally so that they could be stimulated or transected during the course of the experiment.



Text-fig. 1. Diagrammatic representation of the preparation used to study synaptic transmission in parasympathetic bladder and colonic ganglia. See text for further description.

The nerves were mounted on bipolar silver electrodes for stimulation or recording. Stimulation was produced by rectangular pulses of 0·05–0·5 msec duration at varying frequencies and intensities. Action potentials recorded on various nerves were displayed on an oscilloscope and photographed on 35 mm film and averaged on a Computer of Average Transients or a PDP-8/E digital computer, the output of which was then plotted on an x-y paper recorder or Cal Comp plotter. The magnitude of the averaged potentials was measured with a planimeter or by a computer program which determined the amplitude or the area of the evoked response.

The arrangement of the stimulating and recording electrodes used to monitor the post-ganglionic discharges to the colon and bladder as well as to measure the conduction velocities of the preganglionic axons is depicted in Text-fig. 1. Colonic motility

was measured by a water-filled condom which was attached to flexible tubing, and inserted into the colon through the anal canal. Intravesical pressure was measured with a saline-filled polyethylene cannula which was passed through the external urethral orifice into the bladder.

End-tidal CO_2 was monitored continuously with a Beckman Medical Gas Analyzer. In many experiments the animals were paralysed with gallamine triethiodide and artificially respired. Experience with unparalysed preparations indicated that the dose of chloralose administered was sufficient to produce surgical anaesthesia for the duration of the experiments. In addition, during the experiments depth of anaesthesia was often checked by discontinuing the administration of gallamine and allowing the animal to recover from the paralysis. In these animals end-tidal CO_2 was maintained between 3·5–4 % by altering the rate and depth of ventilation. Arterial blood pressure was measured in the left common carotid artery or femoral artery via a polyethylene cannula attached to a strain gauge pressure transducer. A heating pad was used to maintain the animal's temperature between 36 and 38° C. Drugs were administered through a cannula inserted into the renal artery and positioned in the abdominal aorta.

The following drugs were used in these experiments: (−)noradrenaline bitartrate (NADR), dopamine hydrochloride, γ-aminobutyric acid (GABA), phenylephrine hydrochloride and tetraethylammonium bromide (TEA). Doses are expressed as the salt.

At the end of some experiments, colonic ganglia were identified on the serosal surface of the distal colon, and removed for histological examination. Ganglia were fixed in 10 % formalin and then sectioned at 50 μM. The sections were stained with either methylene blue or with thionin.

RESULTS

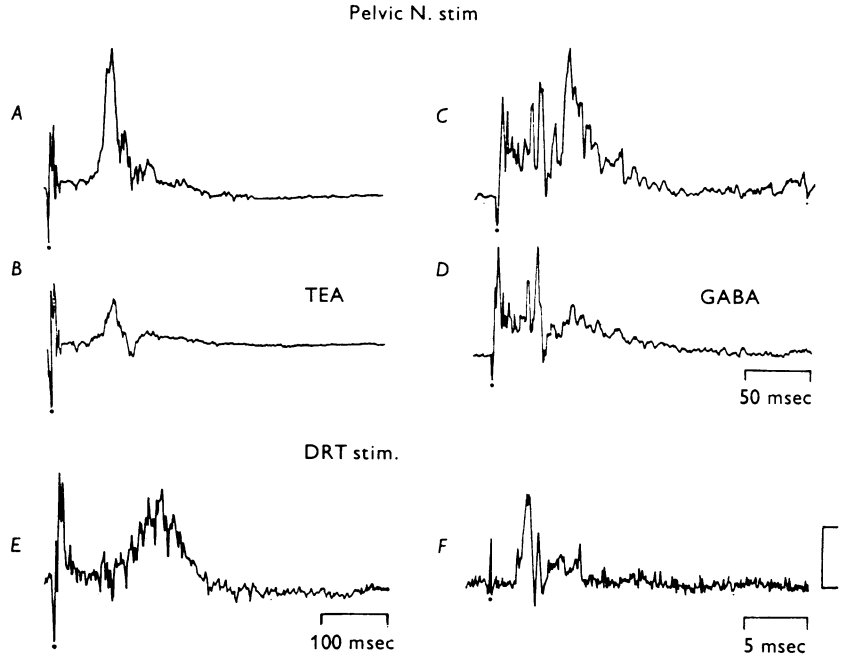
Ganglia and post-ganglionic nerves to the colon

As described by Langley & Anderson (1895) the arrangement of the pelvic nerves varied considerably in different cats but the nerve usually separated into two or three major branches. In many animals it appeared that one or two of these branches passed to the urethra and urinary bladder, whereas another passed to the surface of the distal colon and rectum. Ganglia could be identified along the course of the latter nerves on the serosal surface of the distal colon (Text-fig. 1). The ganglia were usually small (0·5–2 mm in diameter) and variable in number and arrangement in different cats. A section from one of these ganglia in Pl. 1 shows ganglion cells ranging in diameter from 15 to 25 μm. From the pelvic plexus nerves passed rostrally and caudally on the surface of the colon and could be followed for considerable distances (up to 8 cm), particularly in the rostral direction to the middle colon. Usually three or four major filaments could be identified on each side of the colon. Although not examined in detail, it appeared from gross observations that the pelvic plexus on either side was also interconnected by fibres passing over the dorsal surface of the distal colon. Electrical recordings were obtained from the nerve filaments dissected from the surface of the colon at distances ranging from 1 to 4 cm

from the pelvic plexus. Often these filaments were beneath the outer layers of the longitudinal muscle.

The sacral outflow to the colon and urinary bladder

Electrical stimulation of the pelvic nerve elicited a discharge in post-ganglionic fibres on the surface of the colon (Text-fig. 2). The discharge was composed of several components with different stimulus thresholds and latencies. Only one component of the discharge that occurred at latencies of 35–78 msec (mean 53 msec) and at high intensities of stimulation (11–40 V, mean 22 V) was depressed (Text-fig. 2*B*, *D*) by the administration of ganglionic blocking agents, TEA (1–4 mg I.A.) and GABA



Text-fig. 2. Discharge recorded in post-ganglionic nerves to the colon (*A–D*) in response to stimulation of the pelvic nerve. Represented is an early response and late post-ganglionic discharge in *A* and an early and a late bimodal response in *C*. In record *B*, the late post-ganglionic discharge is depressed by the injection of tetraethylammonium (TEA, 1 mg, I.A.) while in record *D* the latter portion of the bimodal discharge is blocked by the administration of gamma-aminobutyric acid (GABA, 0·2 mg, I.A.). Record *E* represents the early and late afferent discharges recorded in colonic nerve filaments during electrical stimulation of the peripheral end of the sectioned S2 dorsal root. The early afferent response is depicted at a faster time sweep in *F*. Vertical calibration is equal to 50 μV, negativity upward. Horizontal calibration in *D* refers to records *A–D*.

(100–500 μg I.A., de Groat, 1970). This response must represent firing in parasympathetic post-ganglionic axons. Non-synaptic responses evoked by stimulation of the pelvic nerve were of two types: (1) short latency (1–4 msec, Text-fig. 2*A–D*) elicited by stimulus intensities of 0·5–2 V and (2) long latency (35–50 msec, Text-fig. 2*C, D*) elicited by stimulus intensities of 7–15 V. Stimulation of the sacral dorsal roots elicited similar activity in colonic nerve filaments (Text-fig. 2*E, F*) suggesting that the axonal responses elicited by pelvic nerve stimulation were in large part mediated by afferents. The estimated axonal conduction velocities in these pathways were 15–40 and 0·75–1·5 m/sec.

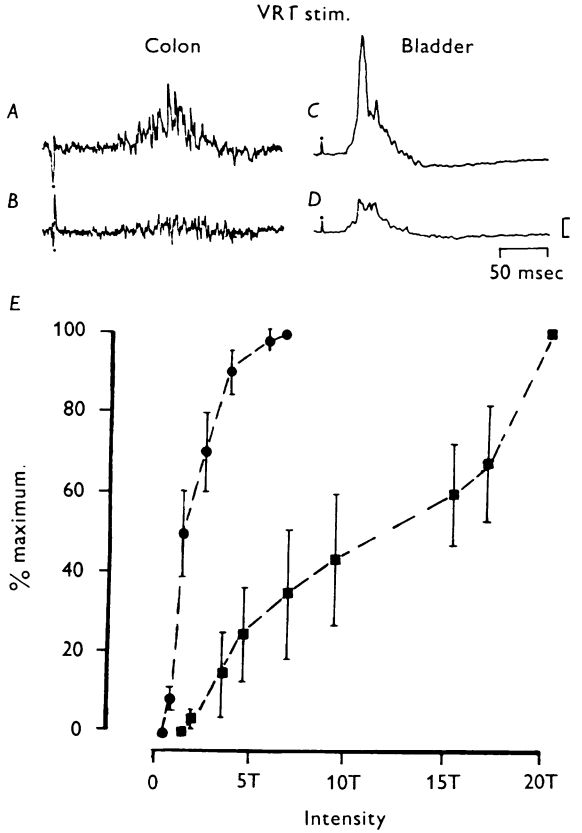
The efferent pathway to the colon could be activated more selectively by stimulation of preganglionic fibres in the sacral ventral roots. Ventral root stimulation elicited a discharge in colonic post-ganglionic fibres at latencies ranging from 70 to 108 msec (mean 91 msec, seven experiments). The discharge represented primarily post-ganglionic activity since 75–90 % of the response was blocked by the administration of ganglionic blocking agents (Text-fig. 3*B*). Stimulus intensities necessary to activate colonic efferents ranged from 1·5 to 10 V which was 2–13 times (mean 6·3 T) the threshold (0·5–1·2 V) for activating preganglionic fibres to the urinary bladder (Text-fig. 3*E*). The latency for evoked discharges in colonic nerves was on the average 3·6 times greater than the latency (12–43 msec, mean 25 msec) of evoked firing on post-ganglionic nerves on the surface of the bladder, even though conduction distances were approximately the same.

Conduction velocities in the two preganglionic pathways were estimated from the latencies for firing to stimulation at two points along the pathway (i.e. ventral roots and pelvic nerve). Maximal conduction velocities determined in this manner from five preparations using different ventral roots ranged from 0·5 to 1·4 m/sec (mean 0·9 m/sec) for preganglionic fibres to the colon and from 8 to 10 m/sec (mean 7 m/sec) for preganglionic fibres to the bladder.

Evidence for two populations of preganglionic axons in the sacral outflow was also obtained from recordings of antidromic volleys in the sacral ventral roots in response to stimulation of the pelvic nerve (Text-fig. 4). Short latency (5–10 msec) responses reflecting axonal conduction velocities of 6–10 m/sec were observed with low intensities of stimulation (1–2 V, 0·05 msec) (Text-fig. 4*A, B, C*) and longer latency (35–50 msec) potentials occurring at estimated axonal conduction velocities of 0·5–1·6 m/sec were evoked at higher intensities of stimulation (15–30 V). It seems reasonable to assume on the basis of traditional neuroanatomical concepts that these volleys occurred in efferent axons. However, there is evidence that the sacral ventral roots of the cat contain C-fibre afferents as well as efferents (Coggeshall, Coulter & Willis, 1973, 1974; Clifton, Vance, Applebaum,

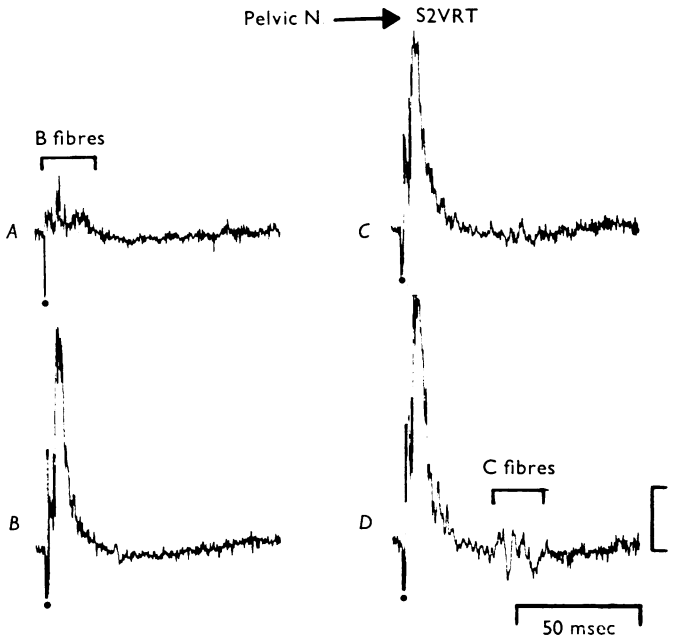
Coggeshall & Willis, 1974). Thus the C-fibre volley observed in our experiments could not be definitely identified as an efferent response.

This problem was analysed further by studying axonal volleys in 'deafferented' ventral roots. A laminectomy was performed 11–15 days before the experiment and the sacral ventral roots on one side were transected intradurally to produce degeneration of the ventral root afferents



Text-fig. 3. Discharge in post-ganglionic fibres to the colon (*A–B*) and bladder (*C–D*) evoked by electrical stimulation of the preganglionic fibres in the S2 ventral root. Records *A* and *C* are the control responses while *B* and *D* depict the responses 1·5 min after the intra-arterial injection of tetraethylammonium (TEA, 2 mg in *B*, 3 mg in *D*), a ganglionic blocking agent. Vertical calibration is equal to 40 μV in *A*, and in *B* and 50 μV in *C* and *D*, negativity upward. *E*, plot of the relationship between intensity of stimulation on sacral roots and the post-ganglionic discharges to the bladder (●----●) and colon (■---■). Ordinate: area of the post-ganglionic discharges expressed as a percentage of the maximum response. Abscissa: intensity of stimulation expressed as multiples of the threshold for evoking a bladder discharge. Each point on the curves represents the mean of five to seven experiments.

arising from the dorsal root ganglia (Clifton *et al.* 1974). In these preparations (three cats) axonal volleys to stimulation of the roots were recorded on the normal and deafferented sides. Fast and slow motoneurone volleys were recorded at low intensities of stimulation (0·1–0·5 V, 0·05 msec duration) (Text-fig. 5*A–D*). At high stimulus intensities (100–150 *T*) potentials occurring at longer latency (13–15 msec) were recorded on

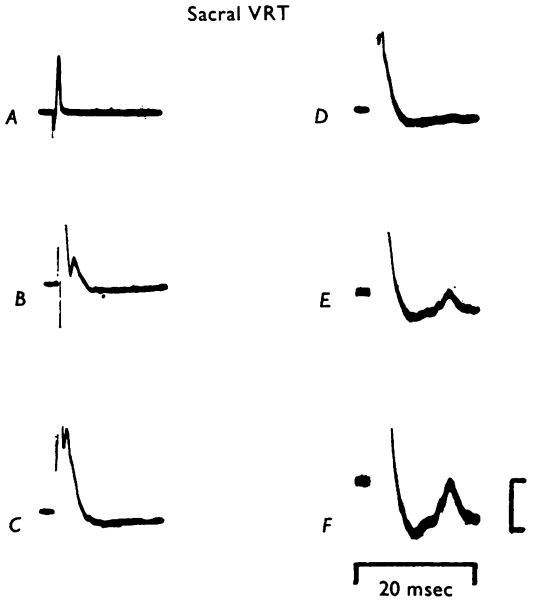


Text-fig. 4. Discharges recorded in the S2 ventral root in response to stimulation of the pelvic nerve at varying intensities. Records *A* and *B* depict the short latency B fibre volley at 3·5 and at 20 V (0·05 msec duration) respectively. A long latency 'C' fibre volley is shown in *C* and *D* at stimulus intensities of 4 V and 10 V (0·5 msec duration), respectively. Conduction distance between the stimulating and recording electrode is 40 mm. Vertical calibration in *D* is 40 μV, negativity upward. Horizontal calibration in *D* refers to all records.

normal and deafferented roots. The calculated conduction velocity for the axons contributing to the late potentials was approximately 1 m/sec. These observations definitely establish the presence of C-fibre efferents in the sacral ventral roots.

The distribution of colonic and bladder efferent pathways in the sacral outflow was studied by recording the effector organ responses or postganglionic discharges to stimulation of individual sacral ventral roots. Increases in intravesical pressure were elicited by repetitive stimulation

(10–20 c/s) at low stimulus intensities (1–4 V) which activated B-fibre preganglionics (de Groat & Ryall, 1968; de Groat & Krier, 1975), whereas excitatory responses in the colon were first noted at 7–8 times these stimulus intensities. Maximal colonic responses were elicited at stimuli ranging from 20–30 *T*. In the course of fifteen experiments excitatory responses in the colon and bladder were observed during stimulation of the three sacral



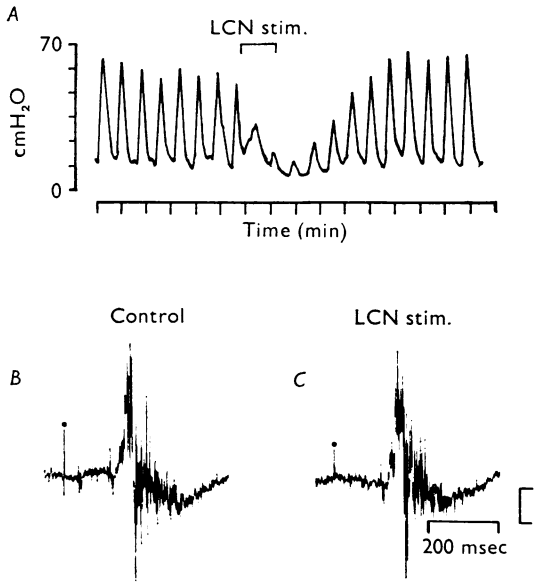
Text-fig. 5. Axonal responses recorded in a 'deafferented' S2 VRT in response to stimulation of the same ventral root at a more peripheral location. The roots had been transected intradurally 15 days before the experiment. Records *A*, *B* and *C* depict the early presumably motor axon response at intensities of 0·1, 1 and 3 V (0·05 msec duration) respectively while records *D* and *E* depict both early and late response at intensities of 10 and 15 V (0·05 msec duration) respectively. Record *F* depicts the early and late responses at same intensity as *E* but at a higher amplification. Conduction distance between the stimulating and recording electrode is 11 mm. Calculated conduction velocities of the early and late responses were 33 and 1 m/sec respectively. Vertical calibration represents 500 μV in *A*–*E* and 250 μV in *F*, negativity upward.

ventral roots; however, in each animal the excitatory outflow was usually present in only one or two roots. The S2 ventral root represented the principal input to the colon containing a portion of the outflow in 90 % of the experiments and being the only root with a detectable outflow in 50 % of the experiments. Occasionally the input to the colon and bladder occurred in different roots, but usually there was overlap. Similar results

were obtained when effector organ activity or evoked responses on peripheral nerves were used to detect the presence of the efferent pathway. Recordings of the neural responses revealed that the preganglionic outflow from one side of the cord only provided an input to ipsilateral ganglia.

Transmission in extramural parasympathetic ganglia

Previous studies of vesical parasympathetic ganglia revealed prominent adrenergic and non-adrenergic inhibitory and facilitatory mechanisms

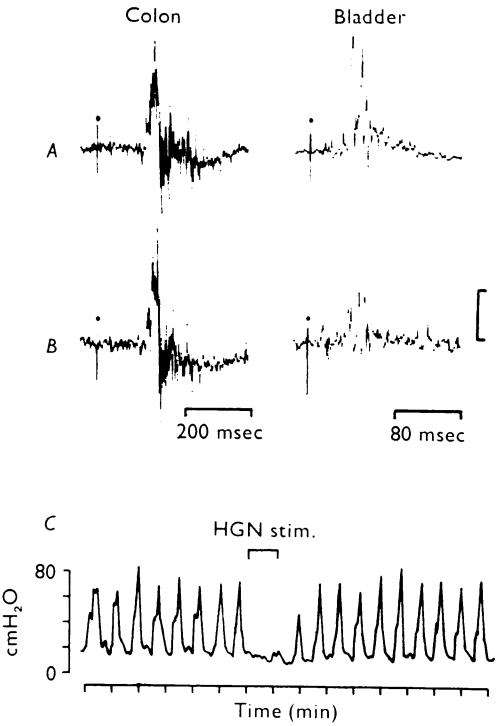


Text-fig. 6. Effects of lumbar colonic nerve stimulation (LCN Stim) on the isovolumetric contractions of the colon (record *A*) and post-ganglionic firing in a colonic nerve filament (record *B*), elicited by electrical stimulation of the S2 ventral root. In *A*, colonic contraction elicited by intermittent stimulation of the S2 ventral root at 10 c/s for 15 sec every 30 sec. The lumbar colonic nerve was stimulated at 30 c/s, 10 V, 0·05 msec duration. Horizontal calibration is 1 min per division and intracolonic pressure is expressed in cmH_2O. Record *C* represents the colonic post-ganglionic discharges during the electrical stimulation of the lumbar colonic nerves (30 c/s, 10 V, 0·05 msec duration). Vertical calibration is equal to 30 μV, negativity upward.

which seem to have an important modulating influence on the neural input to the bladder. Experiments were conducted in this investigation to determine whether similar mechanisms exist in colonic ganglia.

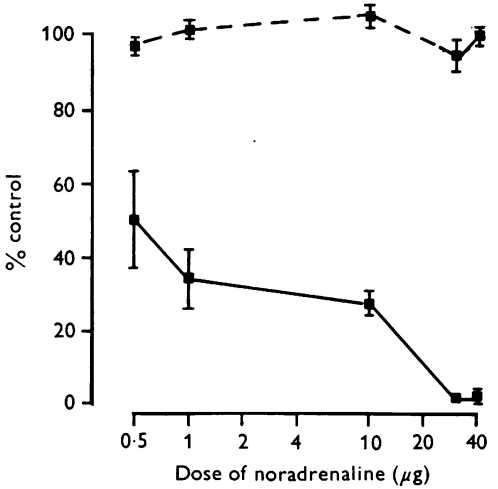
Adrenergic influences on ganglionic transmission. Stimulation of the lumbar colonic nerve at intensities and frequencies which depressed spontaneous and evoked (pelvic nerve stimulation or sacral ventral root

stimulation) contractions of the colon did not depress transmission in colonic ganglia (Text-fig. 6) (ten experiments). Similarly, electrical stimulation of the hypogastric nerve at intensities which depressed bladder and colonic contractions and transmission in bladder ganglia (Text-fig. 7) did not depress transmission in colonic ganglia. Transection of either the hypogastric or lumbar colonic nerves did not alter the amplitude of the colonic post-ganglionic discharge evoked during stimulation of the ventral roots or pelvic nerves but did enhance the contractions and resting tone



Text-fig. 7. Effects of stimulation of the ipsilateral hypogastric nerve (HGN 20 c/s, 20 V) on transmission in colonic and bladder ganglia and colonic contractions. Discharges were recorded in post-ganglionic fibres to the colon and bladder and were elicited by electrical stimulation of pre-ganglionic fibres in the S2 ventral root. Record *A* is the control response while record *B* demonstrates the post-ganglionic discharge during electrical stimulation of the HGN. *C* represents the isovolumetric contractions of the colon during electrical stimulation of the hypogastric nerves. The colonic contractions were elicited by intermittent stimulation of the S2 ventral root at 10 c/s for 15 sec every 30 sec. The hypogastric nerves were stimulated at 20 V, 20 c/s at 0·05 msec duration. Intracolonic pressure is expressed in cmH_2O while the horizontal calibration is 1 min per division. Vertical calibration is 25 μV negativity upward.

(base line pressure) of the colon. Consistent with the above findings the I.A. injection of noradrenaline (1–100 μg) and dopamine (10–100 μg) did not depress transmission in extramural ganglia but did depress transmission in vesical ganglia. Text-fig. 8 depicts dose–response curves for bladder and colonic post-ganglionic discharges during the I.A. administration of noradrenaline. In many experiments the depressant effects of



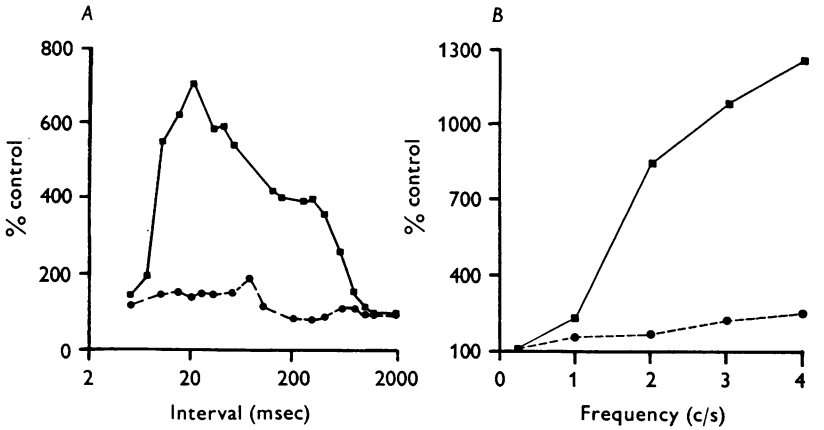
Text-fig. 8. The effects of graded doses of noradrenaline on transmission in parasympathetic colonic and bladder ganglia. The ordinate represents the amplitude of the post-ganglionic discharge expressed as the % control response while the abscissa is the dose of noradrenaline on a log scale. Each point on the colonic (■----■) and bladder curve (■——■) represents the mean of four experiments.

noradrenaline in vesical ganglia were followed by a facilitation of transmission. In contrast, we were unable to demonstrate adrenergic facilitatory mechanisms in extramural colonic ganglia. Thus, unlike other autonomic ganglia (see review by de Groat, 1967) extramural colonic ganglia appear resistant to exogenous catecholamines. However, other ganglionic depressants, GABA (0·1–1·0 mg) and TEA (1–4 mg), depressed both the colonic and bladder post-ganglionic discharges.

Although transmission in extramural ganglia was unaffected by hypogastric nerve stimulation it is noteworthy that such stimulation did depress neurally evoked contractions of the colon. Stimulation of the peripheral end of the sectioned hypogastric nerves inhibited the spontaneous and sacral ventral root-evoked contractions of the mid and distal colon (Text-fig. 7*C*) and enhanced the resting tone in the rectum–anal canal region. The threshold for producing a detectable inhibition of colonic

motility ranged between 7·5 and 10 V at 0·05 msec duration. Hypogastric-medicated inhibition of colonic motility confirms a similar report by Garry (1933*a*) in decerebrate cats.

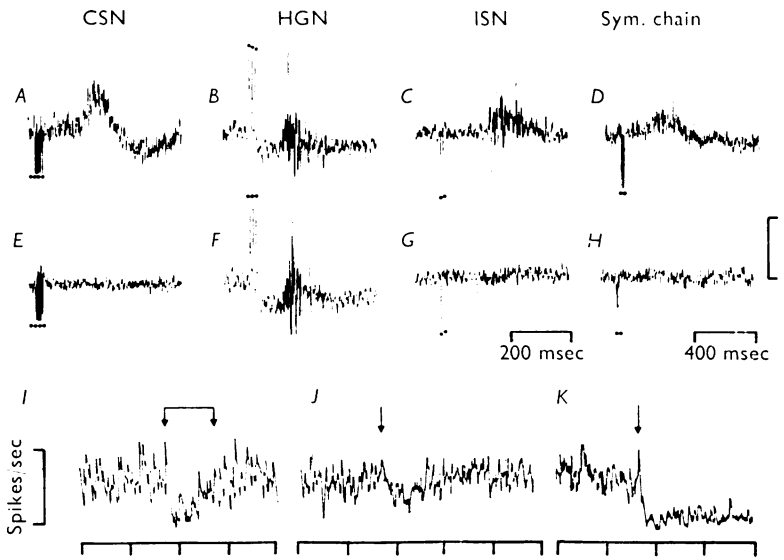
Synaptic transmission in vesical and colonic ganglia. De Groat & Saum (1976) noted a marked facilitation of transmission in vesical ganglia at



Text-fig. 9. *A*, homosynaptic testing in colonic (●——●) and vesical (■——■) ganglia. The test response was elicited by supramaximal stimulation of the preganglionic nerves in the S2 ventral root at frequencies of 0·4 c/s. Single conditioning stimuli were also applied to the same pre-ganglionic nerves at supramaximal intensities at frequencies of 0·4 c/s. The ordinate is the area of the colonic and vesical response expressed as the % control response while the abscissa is the interval in milliseconds between the conditioning and test stimulil. *B*, maximum facilitation obtained in parasympathetic ganglia of the colon (●——●) and bladder (■——■) during different frequencies of stimulation of preganglionic of fibres in the S2 ventral root. The ordinate is the area of the post-ganglionic discharge expressed as the % control response. The abscissa is the frequency of stimulation. Each point represents the computer average of ten responses.

frequencies of preganglionic nerve stimulation between 1 and 5 c/s. A comparison of the frequency–response curves for colonic and vesical ganglia during stimulation of the S1 ventral root (Text-fig. 9) revealed that facilitation was relatively small in the colonic pathway. Evoked responses in colonic nerves increased 1–2 times at frequencies of stimulation between 3 and 4 c/s, whereas discharges in vesical post-ganglionic nerves increased 10–13 times at the same frequencies of stimulation. Experiments were also conducted to measure the duration of the facilitatory responses to a single shock in these two ganglia. Facilitation was observed in both colonic and vesical ganglia when a testing stimulus to a sacral ventral root was preceded by a conditioning stimulus to the same ventral root (Text-fig. 9). However,

facilitation in colonic ganglia was only present at relatively short condition-test intervals (less than 100 msec), whereas facilitation in vesical ganglia was observed at intervals up to 1000 msec after the conditioning stimulus. When trains of stimuli (50–200 c/s intra-train frequency, 50–200 msec duration) were used as the conditioning procedure the facilitation in colonic ganglia was slightly increased in magnitude but of similar duration.



Text-fig. 10. Sympathetic firing recorded in colonic nerve filaments. Records *A–D* represent respectively reflex responses evoked by stimulation of the carotid sinus nerve (CSN), ipsilateral hypogastric nerve (HGN), inferior splanchnic nerve (ISN), and lumbar sympathetic chain (sym. chain, L4). *E*, depression of the CSN-evoked response after sectioning the ipsilateral HGN. *F–G*, depression of responses shown in *B–D* after the I.A. injection of 3, 0·3 and 0·1 mg TEA respectively. *I–K* records of the spontaneous discharge recorded from a colonic nerve filament in a preparation where the pelvic nerves were transected bilaterally. Record *I* depicts the spontaneous discharge during stimulation of the carotid sinus nerve (30 c/s, 15 V, 0·05 msec duration). Records *J* and *K* depict the spontaneous discharge recorded from a colonic nerve filament in another experiment, *J*, the depression of firing after the intravenous administration of phenylephrine (20 μg/kg). *K*, the depression of the firing by transection of the ipsilateral HGN. Vertical calibration is equal to 25 μV, negativity upward in records *B–D*, *F–G* and 15 μV in records *A* and *E*. Horizontal calibration in *G* refers to records *C*, *B* and *F*, while horizontal calibration in *H* also refers to records *A*, *D* and *E*. Horizontal calibration in *I* is 15 sec per division while horizontal calibration in *J* and *K* is 30 sec per division. Vertical calibration in *I* is equal to 50 spikes per sec while vertical calibration in *J* and *K* is equal to 25 spikes per sec.

Three shocks at varying frequencies (100–200 c/s) produced maximal responses. The facilitation was greater in vesical ganglia (2–7 times increase) than in colonic ganglia (1–2 times increase).

Sympathetic pathways in colonic nerve fibres

Since hypogastric nerve stimulation inhibited colonic activity, we examined the hypogastric input to the colonic post-ganglionic nerve filaments.

Evoked responses. Stimulation of preganglionic fibres (the inferior splanchnic nerves) to the ipsilateral inferior mesenteric ganglion evoked firing in colonic post-ganglionic nerves at latencies of 90–100 msec (Text-fig. 10*C*). The firing was abolished by the administration of ganglionic blocking agents (TEA, 100–300 μg, I.A.) (Text-fig. 10*G*). These post-ganglionic responses were mediated entirely via the ipsilateral hypogastric nerve since transection of this nerve abolished the responses and stimulation of the nerve at intensities sufficient to excite post-ganglionic fibres (8–15 V, 0·05 msec duration, mean 13 V) elicited a discharge (latency 50–80 msec, mean 58 msec) in colonic post-ganglionic nerves that was resistant to ganglionic blocking agents (Text-fig. 10*B*, *F*). Stimulation of the lumbar colonic sympathetic nerve (10–30 V) or the contralateral hypogastric nerve (10–30 V) did not produce a discharge. A post-ganglionic input from the sympathetic chain was also evident in many experiments (Text-fig. 10*D*). Depending upon the level at which the chain was stimulated synaptic or non-synaptic responses were obtained.

Spontaneous sympathetic discharge. In four experiments in which the pelvic nerves were transected bilaterally multifibre recordings from colonic nerve filaments revealed a tonic discharge which was not correlated with spontaneous contractions of the colon or with respiration. The firing was reduced or completely abolished after transecting the hypogastric nerves (Text-fig. 10*K*). In animals where the pelvic nerves were intact a reduction in spontaneous colonic efferent firing was also noted in 64 % of the preparations (twelve of nineteen) when the ipsilateral hypogastric nerve was transected. Section of the contralateral hypogastric nerve (five experiments) or the lumbar sympathetic chain (two experiments) did not alter the spontaneous discharge. The firing was depressed by the I.A. administration of a ganglionic blocking agent (TEA, 0·2–1 mg). These results indicated that post-ganglionic sympathetic axons in the hypogastric nerve travel to the colon along with the sacral parasympathetic fibres. This sympathetic innervation may have several functions, including vasomotor control and colonic inhibition. Vasomotor function is suggested by several findings. In two of four experiments repetitive stimulation (30–60 c/s) of the carotid sinus nerve at intensities that elicited a fall in blood pressure depressed the

firing (Text-fig. 10*I*). In the other two experiments the firing was not altered. In addition the administration of pressor agents (phenylephrine, 2–100 μg/kg I.V. or noradrenaline 2–10 μg/kg I.V.) depressed the firing in some experiments (four of six). Electrical stimulation of chemoreceptor afferents in the carotid sinus nerve (de Groat & Lalley, 1974) elicited reflex firing in colonic efferent fibres at latencies 238–390 msec (their fig. 12*A*; four of five experiments). The firing was evoked at intensities of stimulation (1–5 V) that elicited a rise in blood pressure and was abolished by transecting the ipsilateral hypogastric nerve.

DISCUSSION

It was shown in the present experiments that the sacral parasympathetic outflow to the colon of the cat has a ganglionic relay station prior to the myenteric plexus. Electrical stimulation of the sacral ventral roots or the pelvic nerve at intensities which evoked contractions of the colon evoked firing in nerve filaments on the surface of the colon. The firing was depressed 75–90% by competitive ganglionic blocking agents or by GABA and therefore must represent, in large part, activity in parasympathetic post-ganglionic axons.

Ganglion cells which are the likely source of the post-ganglionic fibres were identified histologically in the pelvic plexus and on the serosal surface of the distal colon and rectum. The existence of extramural colonic ganglia was also noted in the early work of Langley & Anderson (1895); however, the anatomic and functional characteristics of these ganglia were never studied in detail. The present results suggest that the extramural ganglion cells provide an excitatory input to the colon; but it is not known whether they directly innervate the colonic smooth muscle or whether they make synaptic connexions with neurones in the myenteric plexus. If the latter arrangement exists, then the efferent parasympathetic pathway to the colon would be composed of at least three neurones instead of two as in other autonomic systems.

Nerve filaments on the surface of the colon also contained (1) a population of axons which passed directly from the sacral ventral roots without synaptic interruption and (2) a population of sympathetic post-ganglionic axons from the lumbar outflow. The former could be either preganglionic through-fibres, which synapse with neurones in the myenteric plexus or non-myelinated afferents which enter the ventral roots (Clifton *et al.* 1974). The latter arose from the inferior mesenteric ganglion although a small input from the sympathetic chain was noted in some experiments. These sympathetic fibres exhibited tonic activity which was abolished by transecting the ipsilateral inferior splanchnic nerves or hypogastric nerve. The

tonic activity could represent firing in cardiovascular efferents, since in some experiments it was reduced by baroreceptor reflexes. It might also represent, in part, activity in sympathetic inhibitory pathways to the colon, since stimulation of the hypogastric nerve depressed spontaneous and neurally evoked colonic contractions (see also Garry, 1933*a*). Thus, a variety of fibre types pass through the pelvic plexus to the colon. However, only preganglionic axons in the sacral ventral roots provided a detectable synaptic input to the extramural ganglion cells in the plexus, indicating that most of these cells can be designated as parasympathetic.

Transmission in colonic parasympathetic ganglia differed in a number of respects from transmission in parasympathetic ganglia on the surface of the urinary bladder. For example, in bladder ganglia transmission was subject to adrenergic inhibitory modulation. Injections of exogenous cathecholamines or the release of endogenous amines by electrical stimulation of the hypogastric nerve depressed transmission. On the other hand, transmission in colonic ganglia was unaffected by injections of large doses of catecholamines or by stimulation of either the hypogastric or lumbar colonic nerves, both of which depressed colonic activity. Thus, colonic ganglia, unlike most other autonomic ganglia (de Groat, 1967; Haefely, 1972) seem to be completely resistant to the inhibitory and facilitatory actions of adrenergic agents. The site of sympathetic inhibition in the colon, therefore, must be in the myenteric plexus (Norberg, 1964; Baumgarten, Holstein & Owman, 1970; Gillespie & Khoyi, 1975) or in the colonic smooth muscle (Bennett, Burnstock & Holman, 1966).

Temporal facilitation and recruitment in colonic ganglia was also relatively small in comparison to facilitatory responses in bladder ganglia. For example, in the latter evoked post-ganglionic potentials gradually increased in amplitude reaching 7–20 times control levels during continuous preganglionic stimulation at frequencies greater than 2–3 c/s (de Groat & Saum, 1976) (Text-fig. 9). On the other hand, recruitment in colonic ganglia was similar in magnitude (1·5–2 times increase) to that observed in sympathetic ganglia (de Groat & Saum, 1976). These data suggest that the subliminal fringe in colonic ganglia is small. Transmission must occur with a high safety factor so that a large percentage of the ganglion cells are activated with single preganglionic volleys. In contrast in bladder ganglia there is a large subliminal fringe which can be modulated by facilitatory and inhibitory mechanisms to provide an important regulatory influence on the neural input to the bladder (de Groat, 1975; de Groat & Saum, 1976). Thus, the extramural colonic ganglia seem to serve as simple relay stations, with very little capability of modifying the neuronal activity arising in the central nervous system. It is possible, however, that these ganglia provide an input into the myenteric plexus which has more

complicated functions (Wood, 1975) and where centrally and peripherally generated activities are integrated to provide a final input to the colonic smooth muscle. The relatively long latency (400–600 msec) for excitatory junctional potentials in the colon in response to pelvic nerve stimulation (Gillespie, 1962; Gonella & Gardette, 1974) is consistent with the concept of a multineuronal efferent pathway.

The preganglionic input to vesical and colonic ganglia also exhibited different properties. The preganglionic pathway to the bladder was composed primarily of B fibres with maximal conduction velocities ranging from 8 to 10 m/sec. On the other hand, the preganglionic outflow to the colon was composed primarily of C fibres having maximal conduction velocities ranging from 0·5 to 1·4 m/sec. It is noteworthy that the colon probably also receives a preganglionic C fibre input from the vagus nerve, since the abdominal vagus contains almost exclusively non-myelinated fibres (Agonstoni, Chinnock, Daley & Murray, 1957; Douglas & Ritchie, 1962).

The latter observations would be unexpected on the basis of traditional neuroanatomical concepts which imply that the ventral roots are composed entirely of myelinated efferents. However, there have been reports that the ventral roots do contain a considerable population of non-myelinated axons (Duncan, 1932; Davenport & Ranson, 1932; Coggeshall *et al.* 1973, 1974). Duncan (1932) suggested that these axons might be autonomic efferents since they were most numerous in spinal segments with a sympathetic outflow. More recent electron microscopic studies indicate that non-myelinated efferent axons are also present in the lumbosacral ventral roots of the cat (Coggeshall *et al.* 1974; Clifton *et al.* 1974). The present experiments confirmed these observations and showed that the non-myelinated efferents provide the major excitatory input to the colon, but no detectable input to the bladder.

Since the bladder and colon have similar excretory functions but receive an innervation from different types of preganglionic fibres, it is of interest to inquire into the physiological significance of these two types of innervation. Rushton (1951) and Douglas & Ritchie (1962) pointed out that in afferent systems, C fibres seem to be utilized in place of larger myelinated fibres when peripheral conduction velocity is not a critical factor. Small diameter axons represent a more efficient conduction system since a greater amount of information can be carried in the same cross-sectional area of nerve. Rushton (1951) proposed that for a particular reflex pathway peripheral conduction time might be matched to the central delay. Thus, the characteristics of the efferent innervation would be expected to reflect the rapidity of an effector organ response. In this sense the innervation to the colon seems to be at one extreme in the autonomic system. The colon

is a slowly responding organ with both its preganglionic and post-ganglionic nerves composed of slowly conducting non-myelinated axons. On the other hand, the parasympathetic pathways to the ciliary muscles in the eye mediate rapid adjustments in muscle tone and seem to represent the other extreme in neural organization where both preganglionic and post-ganglionic fibres are myelinated (Gaskell, 1889; Langley, 1921; Skok, 1973). The innervation to bladder and many other organs assumes a middle position in this spectrum with preganglionic fibres being myelinated and post-ganglionic fibres non-myelinated.

REFERENCES

AGOSTONI, E., CHINNOCK, J. E., DALEY, M. DE B. & MURRAY, J. G. (1957). Functional and histological studies of the vagus nerve and its branches to the heart, lungs and abdominal viscera in the cat. *J. Physiol.* **135**, 182–205.

BAUMGARTEN, G. H., HOLSTEIN, A. F. & OWMAN, C. H. (1970). Auerbach's plexus of mammals and man: electron microscopic identification of three different types of neuronal processes in myenteric ganglia of the large intestine from rhesus monkeys, guinea pigs and man. *Z. Zellforsch. mikrosk. Anat.* **106**, 376–397.

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

CLIFTON, G. L., VANCE, W. H., APPLEBAUM, M. L., COGGESHALL, R. E. & WILLIS, W. D. (1974). Responses of unmyelinated afferents in the mammalian ventral root. *Brain Res.* **82**, 163–167.

COGGESHALL, R. E., COULTER, J. D. & WILLIS, W. D. (1973). Unmyelinated fibres in the human L4 and L5 ventral roots. *Brain Res.* **57**, 229–233.

COGGESHALL, R. E., COULTER, J. D. & WILLIS, W. D., JR. (1974). Unmyelinated axons in the ventral roots of the cat lumbosacral enlargement. *J. comp. Neurol.* **153**, 39–58.

DAVENPORT, H. A. & RANSON, S. W. (1932). Ratios of cells to fibres and of myelinated to unmyelinated fibres in spinal nerve roots. *Am. J. Anat.* **49**, 193–207.

DE GROAT, W. C. (1967). Action of the catecholamines in sympathetic ganglia. *Circulation Res.* **20–21**, suppl. 3, 135–145.

DE GROAT, W. C. (1970). The action of γ-aminobutyric acid and related amino acids on mammalian autonomic ganglia. *J. Pharmac. exp. Ther.* **172**, 384–396.

DE GROAT, W. C. (1975). Nervous control of the urinary bladder of the cat. *Brain Res.* **87**, 201–211.

DE GROAT, W. C. & KRIER, J. (1975). Preganglionic C-fibres: a major component of the sacral autonomic outflow to the colon of the cat. *Pflügers Archiv.* **359**, 171–176.

DE GROAT, W. C. & LALLEY, P. M. (1974). Reflex sympathetic firing in response to electrical stimulation of the carotid sinus nerve in the cat. *Brain Res.* **80**, 17–40.

DE GROAT, W. C. & RYALL, R. W. (1968). The identification and characteristics of sacral parasympathetic preganglionic neurones. *J. Physiol.* **196**, 563–577.

DE GROAT, W. C. & RYALL, R. W. (1969). Reflexes to sacral parasympathetic neurones concerned with micturition in the cat. *J. Physiol.* **200**, 87–108.

DE GROAT, W. C. & SAUM, W. R. (1972). Sympathetic inhibition of the urinary bladder and of pelvic ganglionic transmission in the cat. *J. Physiol.* **220**, 297–314.

DE GROAT, W. C. & SAUM, W. R. (1976). Synaptic transmission in parasympathetic ganglia in the urinary bladder of the cat. *J. Physiol.* **256**, 137–158.

DENNY-BROWN, D. & ROBERTSON, E. G. (1935). An investigation of the nervous control of defecation. *Brain* **58**, 256–310.

DOUGLAS, W. W. & RITCHIE, J. M. (1962). Mammalian nonmyelinated nerve fibres. *Physiol. Rev.* **42**, 297–334.

DUNCAN, D. (1932). A determination of the number of unmyelinated fibers in the ventral roots of the cat, rat and rabbit. *J. comp. Neurol.* **55**, 459–471.

GARRET, J. R., HOWARD, E. R. & JONES, W. (1974). The internal anal sphincter in the cat: a study of nervous mechanisms affecting tone and reflex activity. *J. Physiol.* **243**, 153–166.

GARRY, R. C. (1933*a*). The nervous control of the caudal region of the large bowel in the cat. *J. Physiol.* **77**, 422–431.

GARRY, R. C. (1933*b*). The responses to stimulation of the caudal end of the large bowel in the cat. *J. Physiol.* **78**, 208–274.

GASKELL, W. (1889). On the relation between structure, function, distribution and origin of the cranial nerves; together with a theory of the origin of the nervous system of vertebrata. *J. Physiol.* **10**, 153–211.

GASKELL, W. H. (1916). *The Involuntary Nervous System*, pp. 54–56. London: Longmans.

GILLESPIE, J. S. (1962). The electrical and mechanical responses of intestinal smooth muscle cells to stimulation of their extrinsic parasympathetic nerves. *J. Physiol.* **162**, 76–92.

GILLESPIE, J. S. & KHOYI, M. J. (1975). A pharmacological analysis of the inhibitory effects of the sympathetic nerves on the rabbit colon. *J. Physiol.* **244**, 42–43*P*.

GONELLA, J. & GARDETTE, B. (1974). Etude electromyographique in vivo de la common de nerveuse extrinseque parasympathique du colon. *J. Physiol., Paris* **68**, 395–413.

HAEFELY, W. (1972). Electrophysiology of the adrenergic neuron. In *Handbook of Experimental Pharmacology*, chap. 33, pp. 661–725.

HULTEN, L. (1969). Extrinsic nervous control of colonic motility and blood flow. *Acta physiol. scand.* **335** S, 1–116.

KRIER, J. & DE GROAT, W. C. (1974). Electrophysiological and pharmacological studies of the defecation reflex in the cat. *Pharmacologist* **16**, 684.

KRIER, J. & DE GROAT, W. C. (1975). Sacral parasympathetic reflex pathway to the large intestine of the cat. *Fedn Proc.* **34**, 423.

LANGLEY, J. N. (1921). *The Autonomic Nervous System*, part I. Cambridge: W. Heffer and Sons, Ltd.

LANGLEY, J. N. & ANDERSON, H. K. (1895). On the innervation of the pelvic and adjoining viscera, part I. The lower portion of intestine. *J. Physiol.* **18**, 67–105.

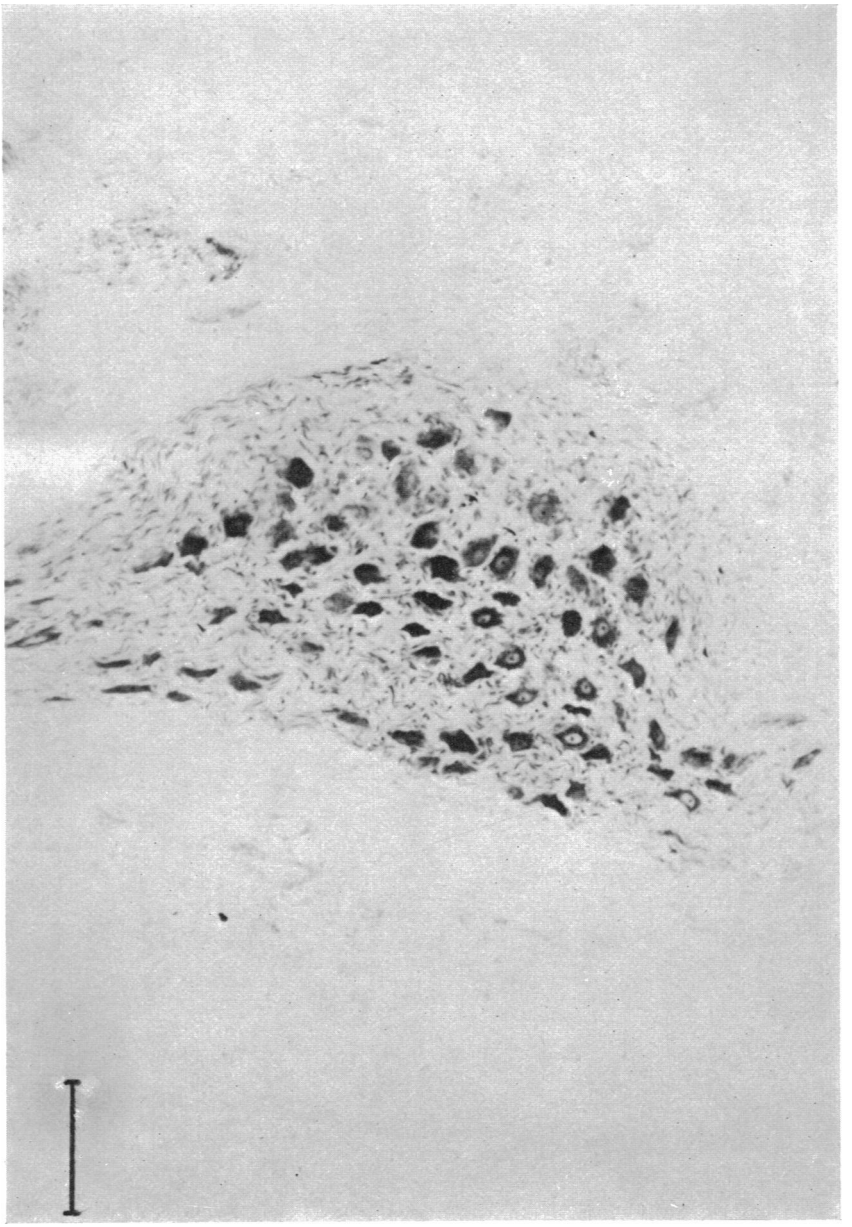
LEARMONTH, J. R. & MARKOWITZ, J. (1929). Studies on the function of the lumbar sympathetic outflow. II. The relation of the lumbar sympathetic outflow to the sphincter ani internus. *J. Physiol.* **89**, 686–691.

LEARMONTH, J. R. & MARKOWITZ, J. (1930). Studies on the innervation of the large bowel. The influence of lumbar colonic nerves on the distal part of the colon. *Am. J. Physiol.* **94**, 501–504.

NORBERG, K. A. (1964). Adrenergic innervation of the intestinal wall studied by fluorescence microscopy. *Int. J. Neuropharmac.* **3**, 379–382.

RUSHTON, W. A. H. (1951). A theory of the effects of fibre size in medullated nerve. *J. Physiol.* **115**, 101–122.

SCHNITZLEIN, H. N., HOFFMAN, H. H., HAMLETT, D. M. & HOWELL, E. M. (1963). A study of the sacral parasympathetic nucleus. *J. comp. Neurol.* **12**, 477–493.



SKOK, V. I. (1973). *Physiology of Autonomic Ganglia*. Tokyo: Igaku Shoin Ltd.
WOOD, J. D. (1975). Neurophysiology of Auerbach's plexus and control of intestinal motility. *Physiol. Rev.* **55**, 307–324.

EXPLANATION OF PLATE

Transverse section of a colonic ganglion dissected from the serosal surface of the distal colon (Toluidine blue, × 133). Horizontal calibration is equal to 100 μm.