CHARACTERISTICS OF THE INTERNAL ANAL SPHINCTER AND THE RECTUM OF THE VERVET MONKEY

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SUMMARY

1. The physiology of the internal anal sphincter of the vervet monkey was investigated.

2. Strips of sphincter *in vitro* contracted to noradrenaline and adrenaline; adrenoceptors were mainly α-excitatory. Strips of rectal circular muscle relaxed to noradrenaline and contained both inhibitory α- and β-adrenoceptors.

3. All strips contracted to acetylcholine. After hyoscine or atropine, high doses of acetylcholine relaxed all strips by stimulating intramural inhibitory neurones as relaxations were blocked by tetrodotoxin and hexamethonium. Nicotine and DMPP gave relaxations with similar characteristics.

4. It was concluded that relaxations to acetylcholine, nicotine and DMPP were not adrenergic as relaxations still occurred in strips from sympathetically denervated or reserpinized animals. The block of these relaxations by propranolol and guanethidine was considered to be unrelated to their actions as adrenergic blocking drugs.

5. All strips relaxed to field electrical stimulation (1–5 Hz) through stimulation of intramural inhibitory neurones as tetrodotoxin blocked these relaxations. Adrenergic blocking drugs, prior reserpinization or prior section of the hypogastric nerves did not block these responses. The relaxations were not therefore adrenergic.

6. 5-Hydroxytryptamine relaxed all strips but was not the transmitter in relaxations to acetylcholine, DMPP or nicotine, nor to field electrical stimulation, as desensitization of strips to 5-HT did not alter these responses.

7. The circular smooth muscle of the internal anal sphincter had a dense terminal adrenergic innervation which rapidly decreased orad.

8. *In vivo*, hypogastric nerve stimulation relaxed the rectum but contracted the sphincter. Sacral nerve root stimulation caused an after-contraction in both rectum and sphincter. *In vivo*, a close arterial injection of adrenaline or noradrenaline inhibited the spontaneous contraction waves of the rectum, but contracted the sphincter. Both these responses were blocked by phentolamine.

9. It was concluded that the internal anal sphincter is a discrete high pressure zone which has excitatory cholinergic and adrenergic innervations and an inhibitory non-adrenergic innervation.

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INTRODUCTION

Defaecation and the maintenance of continence are controlled by the different reflex responses of the internal and external anal sphincters. The reflexes of these sphincters have recently been reviewed (Schuster, 1975). Efferent cholinergic neurones of the pudendal nerves innervate the voluntary muscle of the external sphincter. The smooth muscle of the internal sphincter receives a dual innervation, sympathetic, in some species through the hypogastric nerves, parasympathetic through the pelvic nerves. Traditionally the sympathetic innervation of the sphincters is excitatory, the parasympathetic inhibitory – the reverse of non-sphincteric regions.

This paper reports work that confirms the presence of a functionally distinct internal anal sphincter in the final 6 mm of the circular muscle of the rectum of the vervet monkey (*Cercopithicus ethiops*). This conclusion is based on four results, namely the contraction of sphincteric muscle *in vivo* and *in vitro* to some sympatho-mimetic amines, the contraction of the sphincter *in vivo* to stimulation of the hypogastric nerves, the absence of an after-contraction on field electrical stimulation of sphincteric strips and the different distribution of terminal adrenergic nerves in the circular smooth muscle of the sphincter. Some of these results have already been published (Rayner, 1971) in a preliminary form.

METHODS

In vitro *experiments*

Thirty vervet monkeys of either sex weighing 1·3–4·2 kg were anaesthetized with phencyclidine 2 mg/kg body wt. I.M. ('Sernylan' 20 mg/ml., Parke, Davis and Co.). A mid line incision of the skin of the lower abdomen was made and was extended to the anus avoiding the external genitalia. In males the spermatic cords and associated blood vessels were clamped and cut. The symphysis pubis was split and the muscles of the pelvic floor were dissected away to expose the terminal rectum which was then carefully separated from the bladder, urethra, genital organs and skin. The terminal rectum was cut along its mesenteric border, emptied of any contents, and washed in cold Krebs bicarbonate saline.

The external sphincter and mesentery were dissected away. Full thickness strips of rectum 2–3 mm wide were cut parallel to the circular muscle starting from the anal margin and numbered as in Text-fig. 1. In many experiments a portion of the strip was removed for histology. The mucosa and sub-mucosa were dissected off the strip, and the strips were set up in 50 ml. organ baths containing Krebs bicarbonate saline, of the following composition: NaCl 118 mM, NaHCO$_3$ 25 mM, KCl 4·7 mM, CaCl$_2$ 2·5 mM, KH$_2$PO$_4$ 1·2 mM, MgSO$_4$ 1·2 mM, glucose 5·6 mM and gassed with 95 % O$_2$ and 5 % CO$_2$. The bath temperature was 37 °C. Recording was by isotonic levers on smoked paper. The levers were loaded to exert a tension of 1 g on the tissues. Drugs were dissolved in isotonic saline (0·9 %, w/v) except for sympathomimetic amines where 2 ml. 0·01 N-HCl was used and the volume made up to 10 ml. with saline.

The following drugs were used: acetylcholine hydrochloride (B.D.H.), adenosine-5′-triphosphoric acid (disodium dihydrogen salt) (B.D.H.), adrenaline (B.D.H.), atropine sulphate (Apoteket Kronan Uppsala), L-β-(3,4-dihydroxyphenyl)-alanine (L-DOPA) (Koch-Light), 1,1-dimethyl-4-phenylpiperazinium iodide (Ralph N. Emanuel), guanethidine (Ciba), hexamethyl-tetra-ammonium bromide (hexamethonium bromide) (Harrington Brothers), 3-hydroxytyramine hydrochloride (dopamine hydrochloride) (Koch-Light), hyoscine hydrobromide (Burroughs Wellcome), isoprenaline sulphate (Burroughs Wellcome), nicotine hydrogen tartrate (B.D.H.), noradrenaline bitartrate (Koch-Light), phenoxybenzamine hydrochloride (Smith, Kline and French), phenotolamine mesylate (Ciba), phenylephrine hydrochloride (Koch-Light), propranolol (I.C.I.), reserpine (Ciba), tetrodotoxin (Sankyo). Grateful acknowledgement is made of the gifts of drugs from Burroughs Wellcome, Ciba and I.C.I.

Concentrations of drugs are given as g base/ml. Five animals were given reserpine (5 mg/kg body wt. I.M.) on each of 2 days before experiment. Three other animals had their hypogastric nerves chronically sectioned 2 weeks before experiment.

Falck and Hillarp fluorescence histochemical technique

Full thickness pieces of tissue approximately 3 mm square cut from the strips as described above were frozen in isopentane cooled to − 160 °C with liquid nitrogen. They were dried overnight in an Evans–Pearse tissue freeze-drier at a vacuum of 0·01 torr. Tissues were treated with formaldehyde vapour following the standard technique (Corrodi & Jonsson, 1967) except that paraformaldehyde (B.D.H. or Merck) was not equilibrated with an atmosphere of known relative humidity as this did not give more reproducible results. Ten micron sections were cut and examined under U.V. and short wavelength blue light using a 3 mm BG-12 primary filter, dark ground condenser and a barrier filter with a cut off at 530 nm.
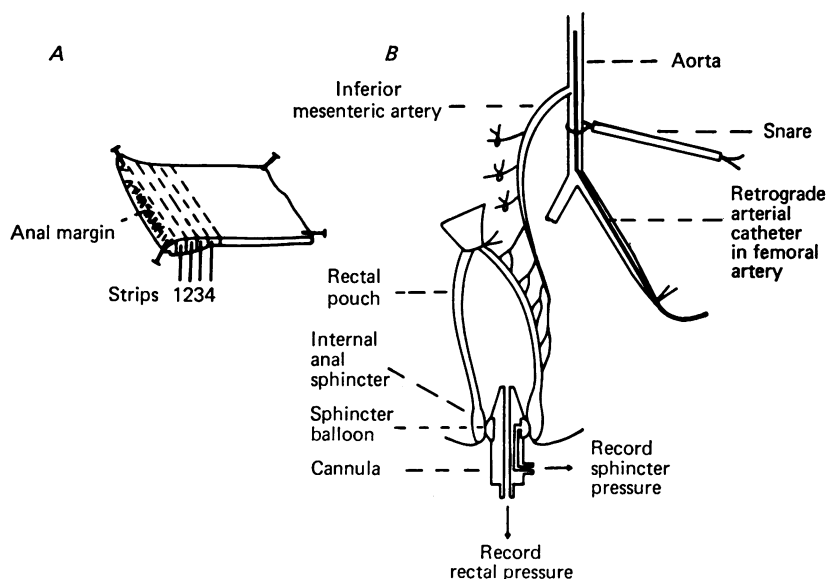
Text-fig. 1. *A*, the location of strips 1–4 in the terminal rectum. For *in vitro* experiments, the terminal rectum is dissected out, opened along the mesenteric border, and pinned out. Four strips 2–3 mm wide are cut to record from the circular muscle, numbering the strips 1–4, strip 1 being nearest the anal margin. The mucosa and sub-mucosa are then removed from each strip, strips 1 and 2 showed the distinctive sphincteric responses. *B*, the preparation for recording from the terminal rectum and internal anal sphincter *in vivo*. Most of the large intestine is removed leaving a tied off pouch of terminal rectum, including the internal anal sphincter. The blood supply through the inferior mesenteric artery remains intact. A cannula with a rubber balloon around its circumference to record from the sphincter is inserted through the anus. The rectal pouch is filled with 0·9 % saline or liquid paraffin and intra-rectal pressure recorded. Pressure from the water filled balloon in the sphincter is also recorded. A retrograde arterial catheter is inserted through one femoral artery so that when the snare around the aorta is tightened, drugs go selectively through the inferior mesenteric artery to the terminal rectum. Not shown are the venous catheter and the drum electrodes used in some experiments for nerve stimulation.

In vivo *experiments*

Vervet monkeys of either sex, weight 2·1–4·2 kg, were anaesthetized with warm chloralose urethane (2 ml./kg I.V.) prepared on the day of experiment by mixing 1 + 1 stock solutions of 5 % chloralose in 5 % borax and 20 % urethane in 0·9 % saline. A mid line incision was made

from the xiphisternum to the symphysis pubis. The branches of the inferior mesenteric artery and vein supplying the transverse, sigmoid and descending colon were tied off and the large intestine from the transverse colon to the sigmoid colon removed leaving a 5 cm stump of rectum protruding through the pelvic floor. The end of this stump was tied and a cannula inserted through the anus. The rectal cannula was filled with liquid paraffin or 0·9 % saline and connected to a 100 cm manometer. Pressure changes at constant volume were registered on this manometer and recorded on a smoked drum using a float recorder. The activity of the internal anal sphincter was independently recorded by a condom rubber balloon attached to the circumference of the anal cannula. Pressure changes in the balloon were registered on a small water manometer and recorded on the drum using a float recorder.

A catheter was inserted into the femoral vein. A retrograde catheter was inserted through the femoral artery until its tip was just rostral to the branching of the inferior mesenteric artery. A snare was placed around the abdominal aorta caudal to the inferior mesenteric artery. When the snare was tightened, drugs administered retrogradely through the femoral artery went selectively to the terminal rectum. Saline was continuously infused through both catheters to keep them patent. In some experiments the hypogastric nerves and sacral nerve roots were dissected out and cut; then they were drawn through small drum electrodes. Before recording the abdomen was filled with liquid paraffin at 37 °C. The preparation is shown diagrammatically in Text-fig. 1.

RESULTS

Pharmacology of isolated strips

Responses of strips 1 and 2 to sympathomimetic amines

Strips 1 and 2 were strongly contracted by noradrenaline, adrenaline, phenylephrine (all 10^{-7} g/ml.) and dopamine (10^{-5} g/ml.). DOPA (10^{-5} g/ml.) was without effect. The α-adrenoceptor blocking agent phentolamine (10^{-6} g/ml.) completely blocked (Text-fig. 2*A*) and the β-adrenoceptor blocking agent propranolol slightly potentiated these contractions. Strips 1 and 2 did relax to the β-agonist, isoprenaline (10^{-7} to 10^{-6} g/ml.) but were less sensitive to this drug than strips 3 and 4. The relaxations were blocked by propranolol (Text-fig. 2*B*). It was concluded that strips 1 and 2 contained mainly excitatory α-adrenoceptors, a distinctively different finding from strip 4, and also some inhibitory β-adrenoceptors.

Responses of strip 3 to sympathomimetic amines

Unlike strips 1 and 2, strip 3 relaxed to noradrenaline, adrenaline and phenylephrine but still contracted to dopamine (10^{-5} g/ml.). The relaxation to isoprenaline occurred at a lower concentration of the drug than in strips 1 and 2 (10^{-7} as opposed to 10^{-6} g/ml.). The relaxation to phenylephrine only occurred at a high concentration of the drug (10^{-6} g/ml.) while the relaxations to noradrenaline and adrenaline occurred at the same concentrations (10^{-7} g/ml.) as the contractions of strips 1 and 2 to these drugs. In three out of six strips propranolol (10^{-6} g/ml.) converted the relaxations to noradrenaline and adrenaline to contractions demonstrating that this strip contained both inhibitory β-adrenoceptors and excitatory α-adrenoceptors. Phenylephrine is supposed to stimulate α-adrenoceptors only, but in these experiments, propranolol converted the relaxation to a contraction showing that part of the relaxation to phenylephrine must be mediated by β-adrenoceptors. Results are illustrated in Text-fig. 3. It was concluded that strip 3 was cut from the transition zone between the internal anal sphincter and the rectum.

Responses of strip 4 to sympathomimetic amines

The four agonists that relaxed strip 3 also relaxed strip 4, but dopamine (10^{-6} to 10^{-5} g/ml.) still caused a contraction. Phentolamine (10^{-6} g/ml.) reduced the relaxations to adrenaline and noradrenaline and the contraction to dopamine but abolished the relaxation to phenylephrine (Text-fig. 4A). In four out of six strips, propranolol (10^{-6} g/ml.) reduced the relaxation to noradrenaline and adrenaline,
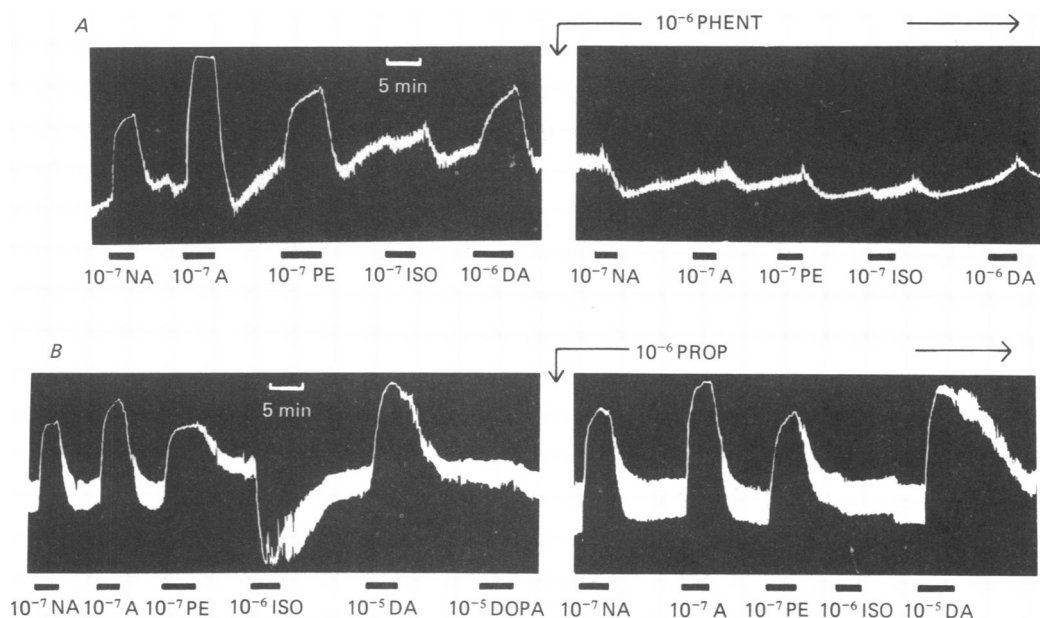
Text-fig. 2. Effects of the α-blocking agent phentolamine (PHENT) and the β-blocking agent propranolol (PROP) on the responses of muscle strips from area 1 (the internal sphincter) of the terminal rectum to applied sympathomimetic drugs. A, contractions are induced by noradrenaline (NA), adrenaline (A) and phenylephrine (PE) (all 10^{-7} g/ml.) and to dopamine (DA) (10^{-6} g/ml.). Isoprenaline (ISO) (10^{-7} g/ml.) induces a small relaxation. Phentolamine (10^{-6} g/ml.) converts the contractions to small relaxations, suggesting that the preparation has excitatory α-receptors. B, as in A, contractions are seen to NA, A, PE and DA, but DOPA has no effect. At a concentration of 10^{-6} g/ml., isoprenaline causes a marked relaxation, suggesting the presence of some inhibitory β-receptors. The relaxation is blocked by propranolol, which also slightly potentiates the contractile responses to the other drugs.

slightly reduced the relaxation to phenylephrine (Text-fig. 4B), but abolished the relaxation to isoprenaline. These results are characteristic of rectal circular muscle and can be contrasted with the 'sphincteric' response of strips 1 and 2. However, in 2 experiments strip 4 behaved like strip 3, and propranolol blocked inhibitory β-adrenoceptors and allowed contractions mediated by α-excitatory adrenoceptors to occur.

Responses of strips 1–4 to field electrical stimulation

Strips 1 and 2 only relaxed to field electrical stimulation (0·5 msec pulse width, 1–20 Hz, 20–30 V for 30 sec) both with and without hyoscine (10^{-7} to 10^{-6} g/ml.)

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in the organ bath (Text-fig. 5A). At frequencies of 1–10 Hz, strips 3 and 4 gave biphasic responses both before and after block of muscurinic receptors by hyoscine. At 20 Hz, either a motor or a biphasic response was seen before treatment with hyoscine and a biphasic response occurred after hyoscine (Text-fig. 5B). The relaxations to field electrical stimulation were never blocked by adrenergic blocking agents (5×10^{-6} to 10^{-5} g guanethidine/ml., 10^{-6} g propranolol/ml. or 10^{-6} g phentolamine/ml.), but were blocked by tetrodotoxin (10^{-7} g/ml.), but not hexamethonium
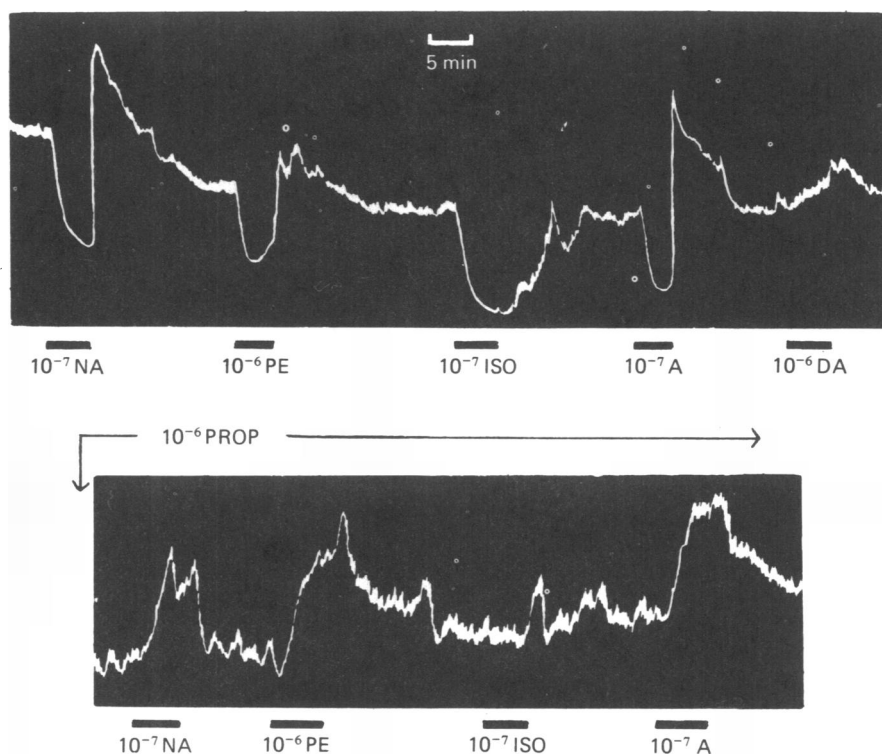

Text-fig. 3. The response of muscle strip 3 (from the transition zone between the internal anal sphincter and rectal circular muscle) to sympathomimetic drugs. This area shows a biphasic response to noradrenaline, adrenaline (both 10^{-7} g/ml.) and phenylephrine (10^{-6} g/ml.), a small contraction to dopamine (10^{-6} g/ml.), and a relaxation to isoprenaline (10^{-7} g/ml.). β-receptor blockade with propranolol converts the biphasic responses into contractions (mediated through excitatory α-receptors) and blocks the effect of isoprenaline.

(10^{-4} g/ml.). Strips which contained no fluorescent varicose fibres because of prior chronic hypogastric denervation (Text-fig. 5B) or treatment with reserpine (5 mg/kg) on each of 2 days before the experiment still relaxed to field electrical stimulation. It was concluded that relaxations were caused by the excitation of non-adrenergic inhibitory nerves.

Responses of strips 1–4 to nicotinic stimulation

All strips contracted to acetylcholine although strips 1 and 2 were less sensitive than strips 3 and 4. After block of the contraction with hyoscine (10^{-7} g/ml.), higher

doses of acetylcholine (10^{-4} g/ml.) relaxed the strips (Text-fig. 6). The relaxations were blocked by guanethidine (5×10^{-6} to 10^{-5} g/ml.) (Text-fig. 6), propranolol (5×10^{-7} to 2×10^{-6} g/ml.) (Text-fig. 7), by hexamethonium (10^{-4} g/ml.) and tetrodotoxin (10^{-7} g/ml.). Relaxations to DMPP and nicotine (3×10^{-8} to 3×10^{-6} g/ml.) were also obtained. These relaxations were blocked by the same drugs (Text-fig. 8).
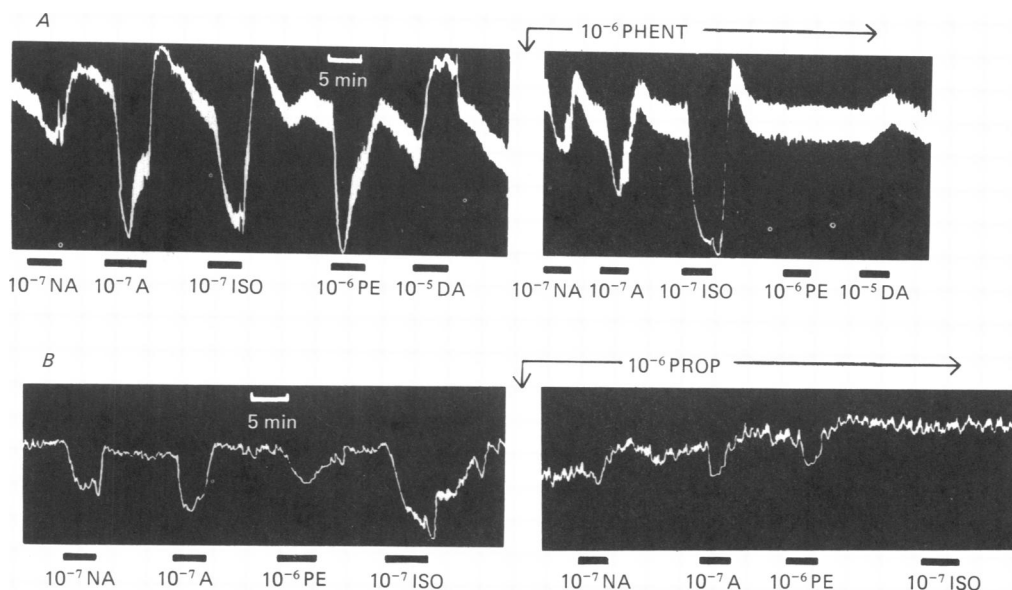

Text-fig. 4. Effects of phentolamine and propranolol on responses of muscle strips from rectal circular muscle (muscle strip 4) to sympathomimetic drugs. *A*, relaxations are induced by noradrenaline, adrenaline, isoprenaline (all 10^{-7} g/ml.) and phenylephrine (10^{-6} g/ml.) but dopamine (10^{-5} g/ml.) still contracts this strip. α-Receptor blockade with phentolamine (10^{-6} g/ml.) reduces the relaxation to adrenaline, blocks the relaxation to phenylephrine but does not alter the response to isoprenaline. The contraction to dopamine is reduced too. This strip evidently contains inhibitory α-adrenoceptors although the block of contraction to dopamine indicates the presence of excitatory α-adrenoceptors as well. *B*, relaxations are induced by noradrenaline, adrenaline, phenylephrine and isoprenaline. β-Receptor blockade with propranolol (10^{-6} g/ml.) reduces the relaxations to noradrenaline and adrenaline, but, unlike strip 3, does not convert the response to these drugs to contractions. The response to phenylephrine is slightly reduced and the relaxation to isoprenaline is blocked. Strip 4 also contains inhibitory β-adrenoceptors as well as α-adrenoceptors.

Phentolamine or phenoxybenzamine (10^{-6} to 2×10^{-6} g/ml.) slightly reduced relaxations to nicotinic stimulants, but did not block. Relaxations to nicotinic stimulants still occurred in strips from animals in which the hypogastric nerves had been chronically cut (Text-fig. 7) or from reserpinized animals (Text-fig. 8.). It was unlikely that nicotinic stimulants released noradrenaline which stimulated β-adrenoceptors.

Responses of strips 1–4 to 5-hydroxytryptamine

Strips 1–4 relaxed to 5-hydroxytryptamine (10^{-10} to 3×10^{-8} g/ml.). After contact with a high concentration of 5-hydroxytryptamine (10^{-5} g/ml.) the strip was
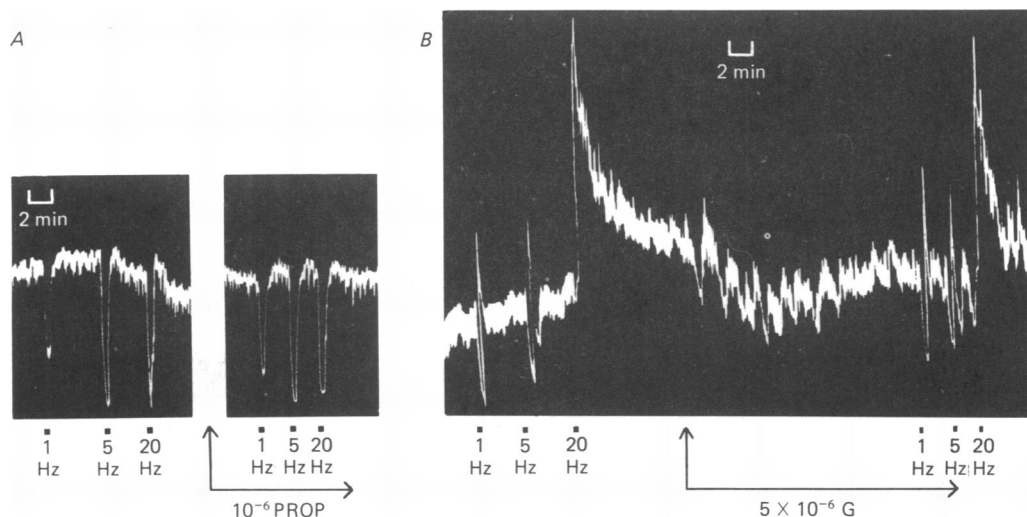
Text-fig. 5. Effects of field electrical stimulation (20 V; pulse width 0·5 msec; pulse train 30 sec; 1, 5 and 20 Hz) on muscle strips from the terminal rectum. *A*, strip 1 (the internal sphincter) only relaxes to field stimulation. The relaxations are not blocked by β-blocker propranolol (10^{-6} g/ml.). Hyoscine (10^{-7} g/ml.) is present throughout. *B*, strip 4 (circular smooth muscle) has a biphasic response to field stimulation. The relaxation is not blocked by the adrenergic neurone blocker guanethidine (G) (5×10^{-6} g/ml.). This particular strip was from an animal whose hypogastric nerves had been chronically cut 2 weeks before the experiment.
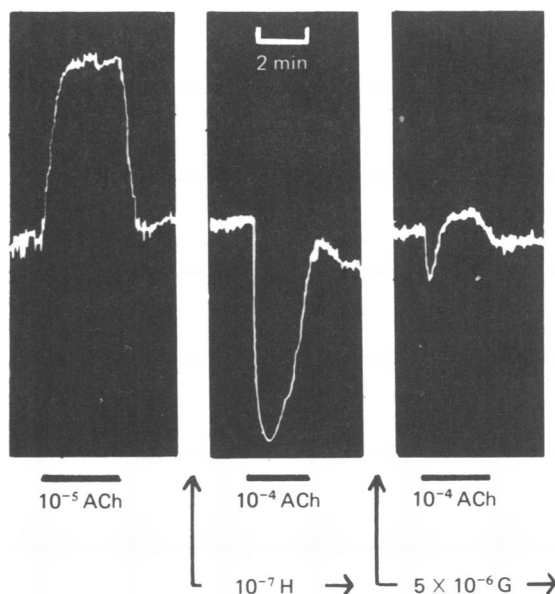
Text-fig. 6. Effects of hyoscine (H) and guanethidine (G) on responses of strip 1 (the internal sphincter) to acetylcholine (ACh). Acetylcholine (10^{-5} g/ml.) contracts the strip. Hyoscine (10^{-7} g/ml.) blocks muscurinic receptors and acetylcholine (10^{-4} g/ml.) now relaxes the strip. This relaxation is then blocked by the adrenergic neurone blocking drug guanethidine (5×10^{-6} g/ml.). This result seems to show that high doses of acetylcholine release noradrenaline from inhibitory adrenergic nerves, but see text and Text-fig. 7.

desensitized to further doses. After desensitization both field electrical stimulation and nicotinic drugs still relaxed the strip (Text-fig. 9). It was unlikely that tryptaminergic receptors were involved in relaxations which seemed to involve non-adrenergic nerves.
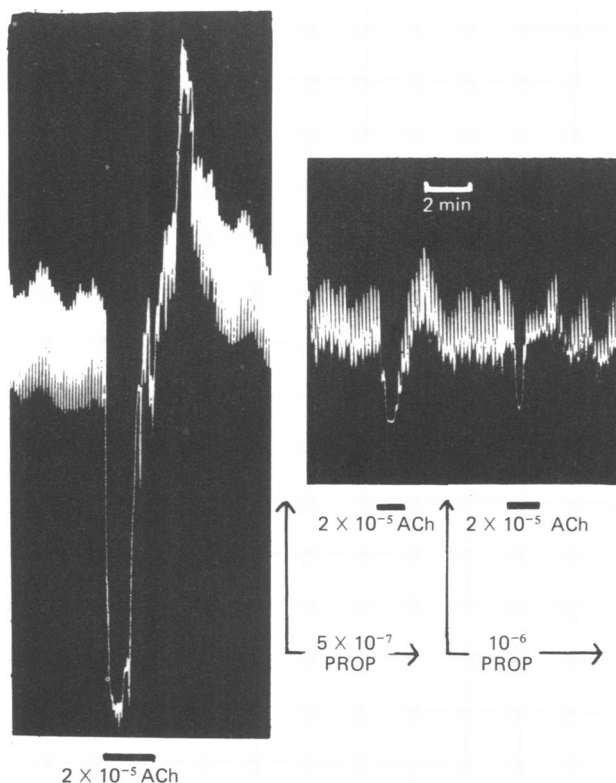

Text-fig. 7. The effect of hypogastric denervation on relaxations to acetylcholine in strip 4 (rectal circular muscle). In the presence of hyoscine (10^{-7} g/ml.), acetylcholine relaxes this strip from a monkey whose hypogastric nerves were chronically cut 2 weeks before experiment. No adrenergic nerves were seen by the Falck and Hillarp fluorescence technique yet the β-adrenoceptor blocker propranolol (5×10^{-7} and 10^{-6} g/ml.) reduces the relaxations to acetylcholine. Probably propranolol is acting as a local anaesthetic rather than as a specific β-blocker.

The distribution of adrenergic nerves

In strips 1 and 2 there was a dense adrenergic innervation (Pl. 1*A*). There were virtually no adrenergic nerves in the longitudinal muscle in these strips (Pl. 1*B*). In strip 3, there was still a dense adrenergic innervation in the circular muscle (Pl. 1*C*) while only a few terminal adrenergic nerves were seen in the longitudinal muscle. Adrenergic nerves could be seen around the ganglia of Auerbach's plexus in strip 4 (Pl. 1*D*) but the density of adrenergic nerves in the circular muscle layer was now much reduced from that found in strip 1.

The hypogastric nerves were surgically removed from three animals two weeks prior to the experiment. No adrenergic nerves were visible in the circular muscle of strip 1, although an occasional fluorescent fibre was seen around blood vessels (Pl. 1*E*).
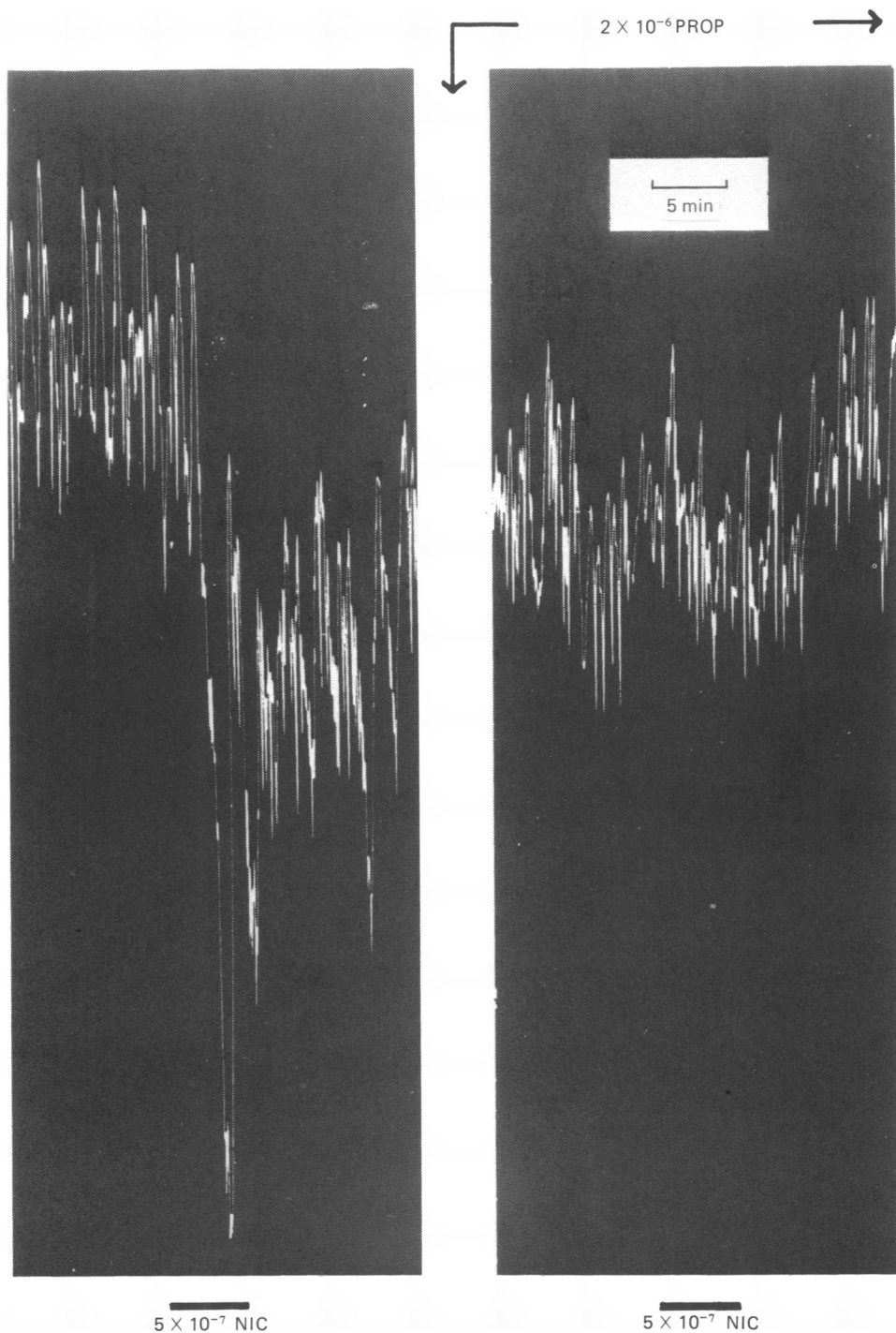
Text-fig. 8. The effect of pre-treatment with reserpine (5 mg/kg I.M. on each of 2 days before experiment) on responses of strip 3 to nicotine (5×10^{-7} g/ml.). Nicotine (NIC) still relaxes this strip and the relaxation is blocked by propranolol (2×10^{-6} g/ml.). No adrenergic nerves were seen in this preparation by the Falck and Hillarp fluorescence technique and its is unlikely that nicotine was releasing noradrenaline from adrenergic nerves even though propranolol blocks the relaxation.

Three other animals were given reserpine (5 mg/kg) on each of two days before the experiment. No adrenergic nerves were now seen, and there was little yellow fluorescence characteristic of 5-hydroxytryptamine from enterochromaffin cells (Pl. 1*F*).

In vivo *experiments*

Motility pattern of the terminal rectum and sphincter

Most preparations (seventeen out of twenty-three) showed peristaltic contractions propagating caudad from rectum to internal anal sphincter (Text-fig. 11) at an intra-rectal pressure of 5–10 cm water while six preparations showed no spontaneous
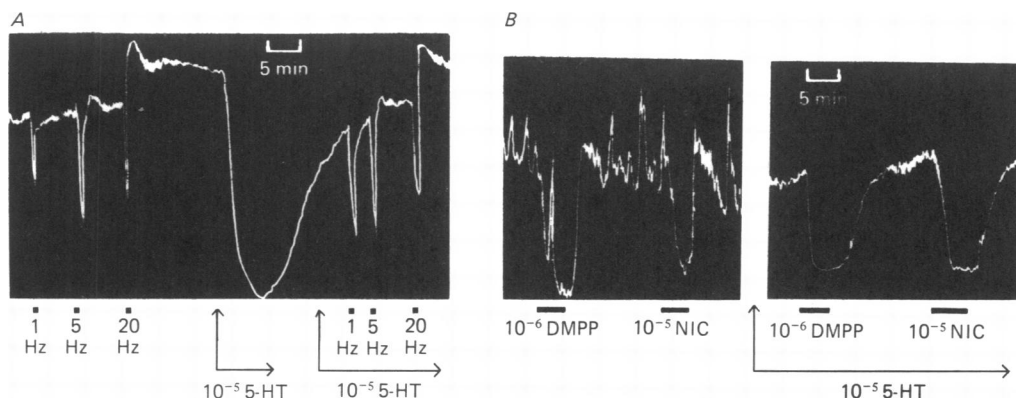
Text-fig. 9. The effects of desensitizing doses of 5-hydroxytryptamine (5-HT) on relaxations to field electrical stimulation and nicotinic stimulation. *A*, field electrical stimulation (20 V; pulse width 0·5 msec; pulse train 30 sec; 1, 5, 20 Hz) relaxes strip 2 (the internal sphincter). 5-Hydroxytryptamine (10^{-5} g/ml.) also relaxes this strip but a second dose of 5-HT has no effect. Field stimulation at the same stimulus parameters as previously still relaxes the strip. As 5-HT receptors are desensitized the relaxation to field stimulation is not mediated by 5-HT. *B*, nicotine (10^{-5} g/ml.) and DMPP (10^{-6} g/ml.) relax strip 2. Relaxations are unaltered by desensitizing the strip to 5-HT by a large dose of 5-HT (10^{-5} g/ml.) and these relaxations are unlikely to be caused by the release of 5-HT.

activity. The rate of peristaltic contractions seemed to vary with intra-rectal pressure. For instance, in one experiment contraction frequency increased from 0/min to 1·0/min as intrarectal pressure was raised from 4 to 13 cm of water. Atropine (1 mg/kg I.V.) blocked these peristaltic contractions.

*Responses to stimulation of the sacral outflow and the hypogastric nerves*

Stimulation of the hypogastric nerves (20 Hz, pulse width 0·5 msec, duration 15–30 sec, stimulus strength 8–20 V) either had no effect on the rectum (three experiments) or caused a small decrease in rectal pressure (two experiments). In five out of six experiments, hypogastric nerve stimulation contracted the internal anal sphincter (Text-fig. 10). The contracton of the sphincter was not blocked by propranolol (1–2 mg/kg I.V.), but was reduced by phentolamine (1–2 mg/kg I.V.). The relaxation of the rectum was reduced by both α- and β-adrenoceptor blocking drugs.

The rectum and sphincter either had no response or contracted during stimulation
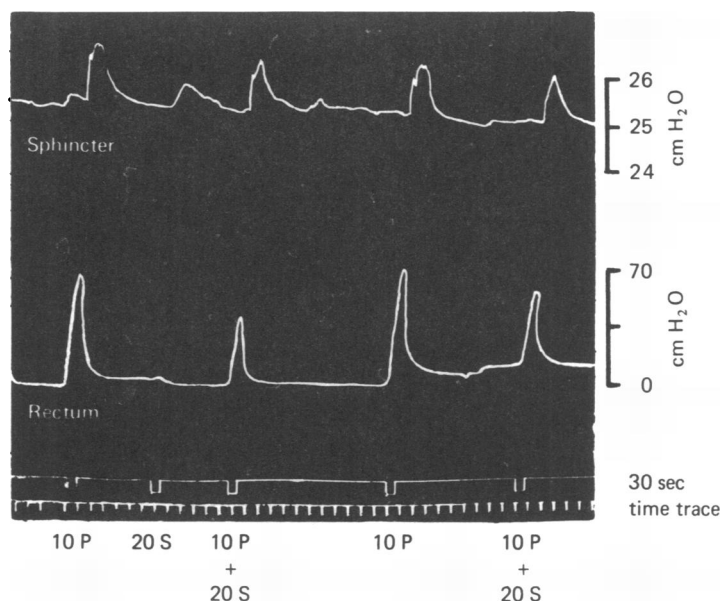
Text-fig. 10. *In vivo* recording of pressure from the internal sphincter and the rectum during stimulation of the parasympathetic sacral nerve roots (P) and the cut peripheral ends of the sympathetic hypogastric nerves (S). Stimulation of the parasympathetic nerve at 10 Hz contracts the rectum and causes an after-contraction in the sphincter. Stimulation of the sympathetic nerve at 20 Hz contracts the sphincter, but slightly relaxes the rectum. Simultaneous stimulation of both nerves reduces the contraction of the rectum. Stimulus strength 20 V, pulse width 0·5 msec, duration 30 sec for both nerves.
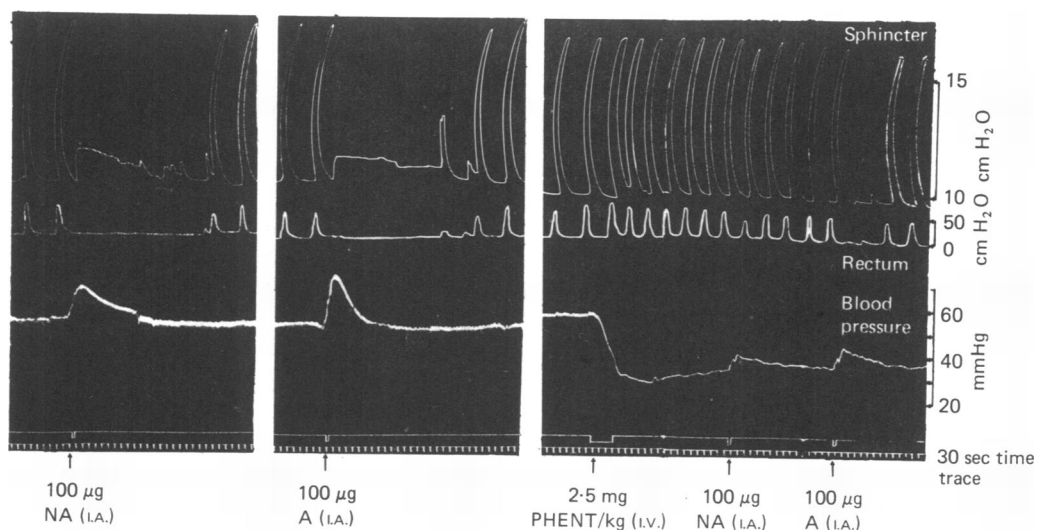
Text-fig. 11. Effects *in vivo* of noradrenaline and adrenaline on the terminal rectum and internal anal sphincter. Retrograde intra-arterial injections of noradrenaline (100 μg I.A.) and adrenaline (100 μg I.A.) inhibit the spontaneous peristaltic contraction waves of the rectum and sphincter, cause a maintained contraction of the sphincter, and raise arterial blood pressure. α-Receptor blockade with phentolamine (2·5 mg/kg I.V.) blocks or reduces all these effects of noradrenaline and adrenaline and causes a prolonged fall in blood pressure too.

of the sacral parasympathetic outflow (10 Hz, pulse width 0·5 msec, voltage 8–20 V, duration 15–30 sec). At the end of the stimulation, there was an after-contraction. The contraction of the rectum was reduced by simultaneously stimulating both the hypogastric nerves and the sacral nerve roots. This effect of hypogastric nerve stimulation was reduced by phentolamine (1–2 mg/kg I.V.) but not by propranolol (1–2 mg/kg I.V.).

Responses to sympathomimetic amines in vivo

Retrograde intra-arterial injections of noradrenaline or adrenaline (20–100 µg in 30 sec) contracted the sphincter and reduced the spontaneous contraction waves of the rectum and sphincter (Text-fig. 11). Both the contraction of the sphincter and the inhibition of rectal motility were blocked or reduced by phentolamine (2·5 mg/kg I.V.).

Isoprenaline (100 µg, I.A.) had no effect on the sphincter in two experiments, and caused a small relaxation in one experiment, but did completely inhibit the spontaneous contraction waves of the terminal rectum.

DISCUSSION

Does the internal anal sphincter have an innervation different from the rest of the large intestine? Langley & Anderson (1895) showed that in the cat, electrical stimulation of the lumbar sympathetic outflow contracted the internal anal sphincter, but relaxed the rectum; electrical stimulation of the sacral outflow relaxed the sphincter but contracted the rectum. More variable results were found in the rabbit. It became accepted that parasympathetic stimulation relaxed, while sympathetic stimulation contracted the sphincters (e.g. Koelle, 1975).

The vervet monkey was chosen to re-investigate the control of the internal anal sphincter because the gross anatomy of its large intestine, unlike that of the cat, corresponds closely with that of the human. This species was also easy to obtain in Uganda.

In the vervet monkey, it is an oversimplification to say that sympathetic and parasympathetic stimulation have opposite effects on the internal anal sphincter and rectal circular muscle. *In vitro*, parasympathetic drugs and field electrical stimulation had both inhibitory and excitatory effects on both sphincteric and non-sphincteric strips. The inhibitory responses were more pronounced in the strips of sphincter. This difference was of degree rather than kind. But the internal sphincter of the vervet monkey did respond to sympathetic stimulation differently from the non-sphincteric circular muscle. Noradrenaline and adrenaline *in vivo* and *in vitro* and hypogastric nerve stimulation *in vivo*, contracted the sphincter but relaxed rectal circular muscle. There was a difference in adrenergic innervation too. The circular muscle of the sphincter had a dense adrenergic innervation, unlike the circular muscle of the rest of the rectum.

The inhibitory responses to field electrical stimulation were not adrenergic, as they were not reduced by adrenergic blocking drugs. Inhibitory responses to nicotine, DMPP and acetylcholine were reduced by adrenergic blocking agents at high concentrations. No adrenergic ganglion cells were seen but nicotinic stimulants could

have released catecholamines from adrenergic nerves (Burn, Leach, Rand & Thompson, 1959). Relaxations occurred in the sphincter which contracts to most sympatho-mimetic amines; relaxations to nicotinic stimulants and field electrical stimulation persisted after the elimination of specific fluorescence by reserpine or chronic hypo-gastric nerve section. These relaxations are unlikely to be adrenergic. The block of nicotinic stimulants by adrenergic blocking agents was probably non-specific. Propranolol is a potent local anaesthetic (Barrett & Cullum, 1968). Guanethidine blocked contractions of the guinea-pig rectum to acetylcholine and pelvic nerve stimulation (Boyd, Burnstock, Campbell, Jowett, O'Shea & Wood, 1963).

5-Hydroxytryptamine relaxed all strips from both sphincter and rectum. But it is unlikely that 5-hydroxytryptamine is the transmitter released by parasympatho-mimetic drugs and field stimulation as they still relaxed the strips after desensit-ization to further doses of 5-hydroxytryptamine. The responses were neither adrenergic nor tryptaminergic, but were probably mediated by the inhibitory 'third transmitter' (Burnstock, Campbell & Rand, 1966). This 'third transmitter' might be adenosine triphosphate (Su, Bevan & Burnstock, 1971) released from intramural nerve fibres.

Biphasic responses to field electrical stimulation were only seen in strips 3 and 4. After hyoscine the relaxation to stimulation at 20 Hz increased showing that there was a small motor response mediated through excitatory cholinergic nerves. But the after-contraction was unchanged and was probably a secondary rebound con-traction similar to that seen after field stimulation of the guinea-pig colon (Furness, 1970). Although the monkey internal anal sphincter only relaxed to field electrical stimulation, muscurinic receptors were present. *In vitro* both sphincteric and non-sphincteric strips contracted to acetylcholine in the absence of hyoscine; *in vivo*, peristaltic contraction waves in both rectum and sphincter were blocked by atropine.

The internal anal sphincter showed pronounced relaxations mediated by non-adrenergic nerves. Do these nerves have connexions with the sacral nerve roots? Although no relaxation was seen when the sacral nerve roots were stimulated, motor responses were delayed until stimulation ceased. Shepherd & Wright (1968) sometimes obtained relaxation on stimulation of the sacral nerves in this monkey. In both the guinea-pig and the cat stimulation of the sacral nerves produced non-adrenergic relaxation of the internal sphincter (Costa & Furness, 1974; Garrett, Howard & Jones, 1974).

Stimulation of the hypogastric nerves at the same time as sacral nerve root stimu-lation reduced the contraction of the rectum to sacral root stimulation, but had no effect on the after-contraction seen in the sphincter. The inhibition of the rectal contraction was mediated by α-adrenoceptors. In the sphincter α-adrenoceptors were excitatory; stimulation of sympathetic and parasympathetic outflows together should give an enhanced motor response in the sphincter. This was not so, suggesting there might be inhibitory non-adrenergic connexions with the sacral nerve roots.

The distinctive feature of the internal anal sphincter of the vervet monkey was the rapidity of transition from sphincteric muscle with its distinct innervation and responses to normal rectal circular muscle. Other species do not have such a rapid transition. In the cat, the rat and guinea-pig the adrenergic innervation progressively decreased orad from the internal anal sphincter (Howard & Garrett, 1973; Gillespie

& Maxwell, 1971; Furness & Costa, 1973). The cat rectum sometimes contracts to noradrenaline (Rayner, 1973) and hypogastric nerve stimulation (Langley & Anderson, 1895).

One interesting feature of responses to sympathomimetic amines was that the transition zone between sphincter and normal rectal circular muscle contracted to dopamine while it relaxed to noradrenaline, adrenaline and phenylephrine. Recently excitatory dopamine receptors have been found in the oesophagus of the opossum (de Carle & Christiansen, 1976; Rattan & Goyal, 1976). It is possible that dopamine was exciting specific dopaminergic receptors as well as α-adrenergic receptors in this region.

How does the physiology of the terminal rectum of man and the vervet monkey compare ? Unfortunately the monkey's sphincter did not respond identically to the human sphincter. First, stimulation of the presacral nerve relaxed the human sphincter (Shepherd & Wright, 1968); hypogastric nerve stimulation contracted the monkey sphincter. Secondly, *in vitro* strips from only the orad part of the human internal sphincter relaxed to nicotinic stimulants (Friedmann, 1968; Parks, Fishlock, Cameron & May, 1969) while all strips from the monkey sphincter relaxed. Thirdly, both human and monkey sphincters have dense adrenergic innervations (Baumgarten, 1967) and strips from sphincters of both species contract to noradrenaline and adrenaline (Friedmann, 1968; Parks *et al.* 1969). But it is not clear whether the human sphincter has a discrete innervation like the vervet monkey or whether it gradually decreases in density orad.

Do other gastro-intestinal sphincters have a distinctive sympathetic innervation ? The lower oesophageal and pyloric sphincters probably do not, as *in vitro* strips of these sphincters from human and dog did not respond to adrenaline differently from adjacent circular muscle (Bass, Ustach & Schuster, 1970); only strips of the ileo-colic and anal sphincters contracted to adrenaline. These workers found that all sphincters, except the internal anal sphincter, contracted to acetylcholine; the internal anal sphincter did not respond.

Sphincters resist the passage of intestinal contents. By manometric recording techniques, a high pressure zone can often be demonstrated. Such a zone has been shown in the lower oesophageal sphincter (Code & Schlegel, 1968). The ileo-colic sphincter has been shown to require a high opening pressure (Pahlin & Kewenter, 1976) before flow through the sphincter occurred. The pyloric sphincter resists the regurgitation of intestinal contents into the stomach (Fisher & Cohen, 1973). As for the vervet monkey internal anal sphincter, a high pressure zone in the rectal lumen was shown in some experiments. High pressure zones corresponding to the internal anal sphincter have been found in humans (Hill, Kelly, Schlegel & Code, 1960; Bennett & Duthie, 1964) and the cat (Garrett *et al.* 1974).

In conclusion, the internal anal sphincter of the vervet monkey showed a distinctive adrenergic innervation. This region of the rectum showed a higher intra-luminal pressure too. The sphincter showed a similar pattern of non-adrenergic inhibitory innervation and cholinergic innervation to the rectum, although the non-adrenergic inhibitory responses were more prominent and cholinergic excitatory responses less prominent in the sphincter. The internal anal sphincters of other species have broadly the same characteristics with some species differences, the

human internal sphincter showing some differences in the response to parasympathomimetic drugs.

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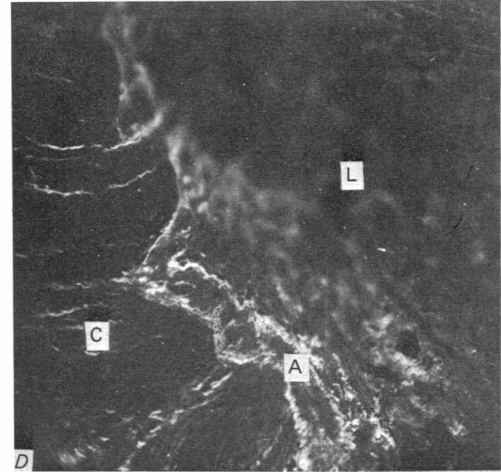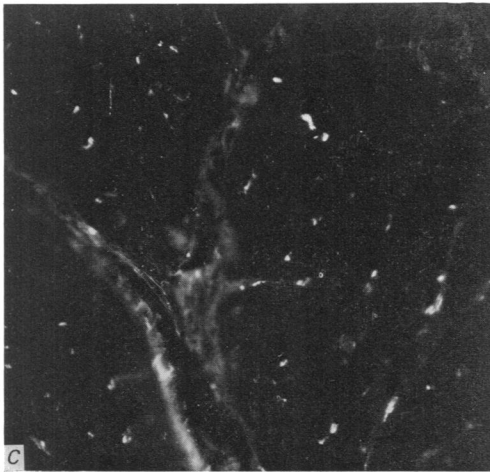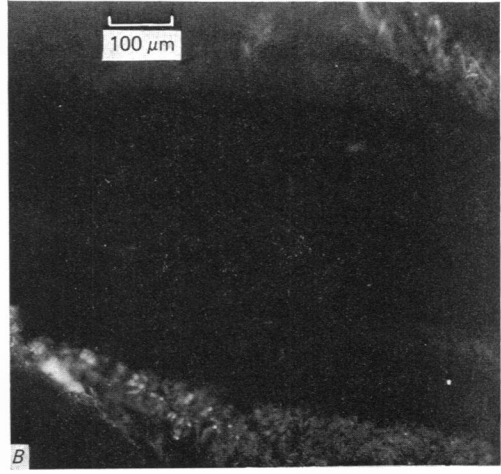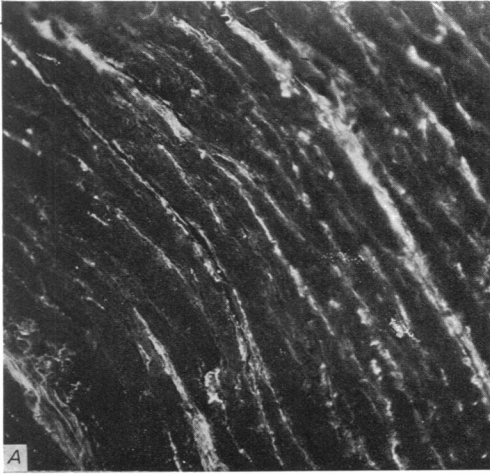
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EXPLANATION OF PLATE

Adrenergic nerves shown by the Falck and Hillarp fluorescence technique in 10 μm sections of vervet monkey terminal rectum. *A*, the internal anal sphincter (that is the circular muscle of strip 1) had a dense adrenergic innervation. This region showed distinctive responses to sympathomimetic amines. *B*, the longitudinal muscle of strip 1 did not show a dense adrenergic innervation. The dense innervation is limited to the internal anal sphincter. *C*, the circular muscle of strip 3 (that is the transition zone between the sphincteric muscle and the normal rectal muscle) contained a moderate adrenergic innervation. *D*, strip 4 which usually showed responses of normal rectal circular smooth muscle had a dense adrenergic innervation of Auerbach's plexus (A) but less innervation of the circular muscle (C) and very little in the longitudinal muscle layer (L). *E*, if the hypogastric nerves and plexuses were chronically cut 2 weeks before the experiment no terminal adrenergic nerves were found in a section of internal anal sphincter. *F*, no adrenergic nerves were seen in a section of internal anal sphincter from a monkey treated with reserpine (5 mg/kg I.M.) on each of 2 days before the experiment.