Brit. J. Pharmacol. (1964), 23, 151–163.

INNERVATION OF THE LARGE INTESTINE OF THE TOAD (BUFO MARINUS)

BY

HELEN BOYD, G. BURNSTOCK AND D. ROGERS

From the Department of Zoology, University of Melbourne, Parkville, Victoria, Australia

The morphology, physiology and pharmacology of the innervation of the toad (*Bufo marinus*) large intestine have been studied. The large intestine can be divided into the regions colon, rectum and cloaca, on morphological grounds, but acts as a unit in response to nerve stimulation. Of the right and left nerves, each appears to supply the entire large intestine. Autonomic innervation of the large intestine of *Bufo marinus* is as follows: (1) The 9th and 10th spinal nerves (pelvic) contain predominantly excitatory preganglionic cholinergic fibres, but some inhibitory adrenergic fibres are also present in most preparations. (2) The splanchnic nerves contain inhibitory postganglionic adrenergic fibres from the 3rd to 5th sympathetic ganglia, and a small number of excitatory cholinergic fibres. The pathway of adrenergic inhibitory fibres to the large intestine alongside the posterior mesenteric artery as seen in mammals is rarely present in the toad. Several nonspecific actions of autonomic drugs on the large intestine are discussed. The functional organization of the autonomic innervation of the toad large intestine is similar to that in mammals, that is the large intestine is controlled by antagonistic cholinergic and adrenergic nerves. However, the separation of these two types of nerve fibres into anatomically distinct nerves does not appear to be as complete as in mammals. It is suggested that inhibitory autonomic control of the alimentary canal in vertebrates first appears in the hind-gut region.

The terminology applied to the posterior part of the amphibian gut is confusing. Langley & Orbeli (1910) divided the large intestine into upper colon, lower colon and rectum (which includes the cloaca), according to the distribution of blood vessels and nerves. However, Ecker & Wiedersheim (1899) and Marshall (1914) have called the whole of this region the "rectum." On the basis of anatomical and histological studies, we have distinguished three regions in the large intestine, namely colon, rectum and cloaca. The present investigation has been confined to the innervation of the colon and rectum, which are regions homologous to those defined in mammals.

Gaskell (1886), Steinach & Wiener (1895), Horton-Smith (1897) and Langley & Orbeli (1910) all studied the innervation of the large intestine in the frog (*Rana temporaria*), but their findings were contradictory. This was partly due to the lack of stimulators capable of generating pulses with controlled durations and frequencies, and partly because earlier studies were made by direct observation *in situ*, so that inhibition, in particular, was difficult to detect.

The work described here is a reappraisal and extension of the earlier work, using modern techniques. It includes an investigation of such features as the response

of the innervated organ to different parameters of nerve stimulation, the pre- or postganglionic nature of the nerve fibres, and the identities of the transmitter substances released from the nerves.

METHODS

Morphology

Observations of the anatomical details of innervation were made with a binocular dissecting microscope after staining the nerves *in situ* with 0.5% osmium tetroxide solution.

The large intestine was fixed in Helley's fluid, dehydrated in ethanol and embedded in paraffin. Sections 8 μ thick were taken along the entire length of the organ and studied after staining with Ehrlich's haematoxylin and eosin, Giemsa or Masson's trichrome stain. Preparations of the large intestine were also stained with methylene blue at *p*H 6.0 and *p*H 5.0 for nerve fibres and ganglion cells in the myenteric plexus.

It is possible to subdivide the large intestine into colon, rectum and cloaca on the basis of the following characteristics: the relative thickness of longitudinal and circular muscle coats; differences in the type and inclusions of the mucosal epithelial cells; distribution of lymph nodules; and the degree of mucosal folding. A bundle of striated muscle forms a prominent part of the wall of the cloaca.

The electron microscopic observations were made on material fixed in Palade's solution, embedded in Araldite, stained with lead hydroxide (Watson, 1958) and examined in a Siemens Elmiskop 1 electron microscope.

Physiology

Experiments in situ. The skin and the ventral body wall musculature of the pithed toad were cut in the midline and deflected to the sides, to expose the body cavity. The anterior abdominal vein was tied and removed, and the pelvic girdle was split in the midline to expose the entire large intestine. The 6th to 10th spinal nerves inclusive were severed in the region of their emergence from the vertebral column and separated out. The splanchnic nerves were also exposed. The heart beat persisted during these experiments, and the blood circulation to the large intestine remained intact. A drip of oxygenated toad saline (Boyd, Burnstock, Campbell, Jowett, O'Shea & Wood, 1963) was allowed to fall on the large intestine to keep the organ and nerves moist. The various nerves supplying the large intestine were stimulated by two platinum electrodes connected to a Grass rectangular wave stimulator. The pulse strength and duration were set at 10 V and 1 msec respectively. The pulse frequency was varied.

Experiments in vitro. The initial dissection was as described above. When the large intestine was removed from the body cavity, care was taken not to interfere with the fine branches of the 9th and 10th nerves lying close to the dorsal body wall. Since striated muscle is present in the wall of the cloaca, as little as possible of this region was included in the isolated preparation. The organ was emptied of faeces, mounted on a Perspex holder, and suspended in a 50 ml. organ-bath containing aerated toad saline at a temperature of 22 to 23° C. The free end of the organ was attached to an isotonic frontal-writing lever and length changes were recorded on a kymograph. The nerves were looped over stimulating electrodes embedded in the Perspex holder. When nerves were closely associated with an artery, both were looped over the electrode. In most experiments, only one nerve was investigated. In some instances, a doubly innervated preparation was used, one nerve being looped over the electrodes in the Perspex holder and the other being passed through an electrode of the type described by Burn & Rand (1960). A Grass stimulator delivering rectangular wave pulses was used to stimulate the nerves. Ten-second bursts of stimuli of controlled strength, duration and frequency were applied at intervals of 6 to 10 min, as shorter intervals between stimulations caused fatigue. Drugs dissolved in either distilled water or isotonic saline were added to the 50 ml. organ-bath in volumes up to 1 ml. Drug concentrations are expressed in g/ml. of salt.

Treatment with reserpine. In some experiments toads were treated with reserpine given intraperitoneally for three days, and were killed on the third. Doses of 50 mg/kg/day were used. Samples of large intestine from these animals were examined with the electron microscope.

RESULTS

Morphological studies

Macroscopic innervation and blood supply of large intestine. The most common anatomical arrangements of nerves and arteries to the large intestine are illustrated in Fig. 1.

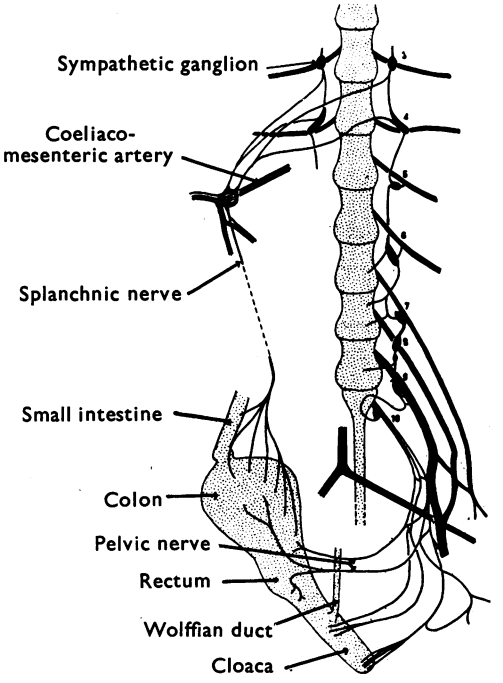

Fig. 1. Diagram of the anatomy of the large intestine (colon, rectum and cloaca) of *Bufo marinus*, showing its divisions and the distribution of nerves and blood vessels. The *in vitro* experimental preparation included only the colon and rectum.

The large intestine may receive its blood supply from both the anterior mesenteric and posterior mesenteric arteries. However, in most individuals, all of the latter vessel was absent. Where the posterior mesenteric artery was present, the main branches of the two arteries anastomosed on the surface of the organ. There is no evidence that haemorrhoidal arteries as such exist in *Bufo marinus*. Thus in individuals where the posterior mesenteric artery is absent, the entire blood supply to the large intestine is through the anterior mesenteric artery.

It is possible to distinguish the following general features of the nerve supply, despite variation between individuals, and the fact that there may be differences in details between the right and left sides of one individual.

The large intestine is innervated from each side by (1) the pelvic nerves, which take their origin usually from the 10th, and/or occasionally the 9th spinal nerves, and course along the lateral margin of the Wolffian duct to the large intestine; and (2), the splanchnic nerves, which emerge from the 3rd, 4th and 5th ganglia of the sympathetic chains, and join at the level of the coeliaco-mesenteric artery in a mass of ganglion cells before associating with the anterior mesenteric artery. Fine branches of these splanchnic nerves, consisting mainly of nonmyelinated fibres, run to the large intestine with branches of the anterior mesenteric artery. No nerves were seen running with the posterior mesenteric artery, when present, in the preparations studied.

Innervation of smooth muscle cells. After staining with methylene blue, an extensive plexus of nerve fibres and neurons can be seen in the *muscularis externa* of the colon and rectum. The neurons are not grouped into small ganglia but are distributed as isolated elements in association with nerve fibres (Fig. 2). Electron

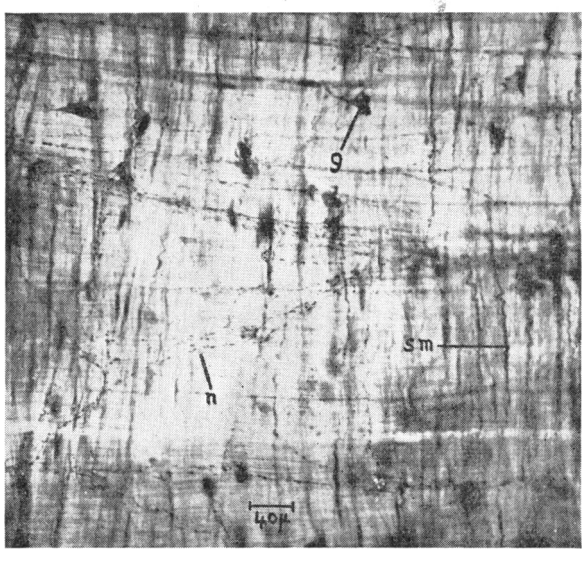

Fig. 2. Whole mount of large intestine (colon-rectum region) stained with methylene blue to show the distribution of ganglion cells. g=ganglion cell ; n=nerve fibre ; sm=smooth muscle cell.

micrographs show that the majority of the nerve fibres from this plexus pass to the smooth muscle cells of the circular layer (Fig. 3,*b*). Only a few large nerve bundles and single nerve fibres traverse the longitudinal smooth muscle. The muscle cells are more closely packed in the longitudinal coat and intermuscular intrusions are abundant (Fig. 3,*a*). Nerve-muscle junctions (with a separation between opposing plasma membranes of nerve fibre and smooth muscle cell of 200 to 500 Å) are rare in the longitudinal muscle layer compared with the circular coat. Many axons contain membrane-bounded vesicles, 400 to 1,000 Å in diameter with an internal electron density slightly greater than the general axoplasm (Fig. 3,*b*).

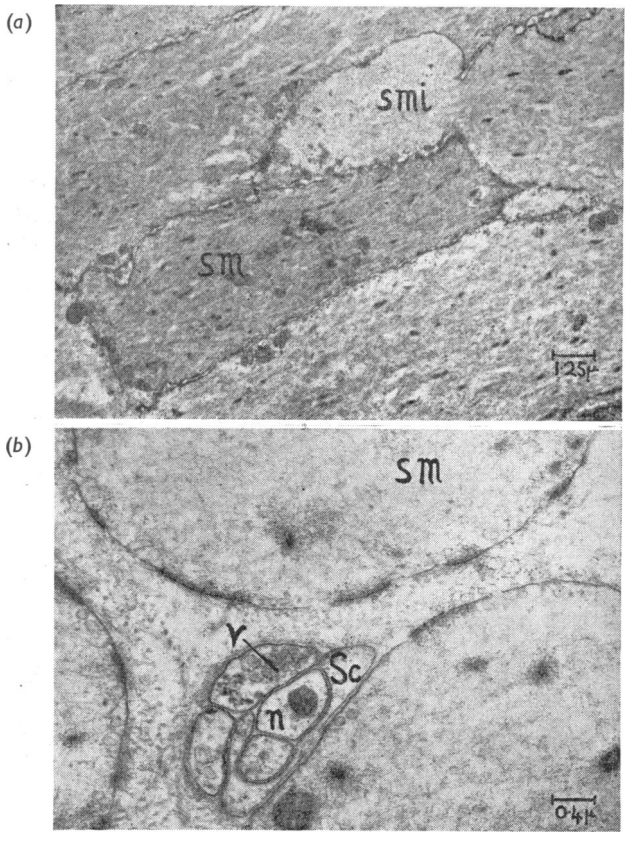

Fig. 3. Electron micrographs showing transverse sections of the *muscularis externa* at the level of the colon-rectum region of the large intestine. (*a*), longitudinal coat ; (*b*), circular coat. sm= smooth muscle cell ; n=nerve fibre ; Sc=Schwann cell ; v=vesicles ; smi=smooth muscle cell intrusion.

Effects of nerve stimulation

Although it is possible to distinguish the various regions of the large intestine anatomically and histologically, this organ acted as a unit in response to nerve stimulation. Responses of the large intestine to nerve stimulation were similar whether observed *in situ* or *in vitro,* so that isolated preparations could be used for the detailed physiological and pharmacological analyses.

The large intestine was never affected by stimulation of the 6th, 7th and 8th spinal nerves.

Stimulation of the 9th spinal nerves usually caused both circular and longitudinal contractions, whose relative strengths varied between individuals. In general the circular contraction was the more noticeable.

Stimulation of the 10th spinal nerves caused a weak circular contraction followed by a strong longitudinal contraction of the large intestine. The longitudinal contraction appeared to originate in the posterior region and to pass anteriorly. The

strengths and durations of the stimulating pulses were varied, and threshold values established for the individual nerves in isolated preparations. For the 9th and 10th spinal nerves, the pulse duration which gave maximal responses was usually from 0.5 to 1 msec (Fig. 4,*a*). Supramaximal stimuli (strength and duration) were

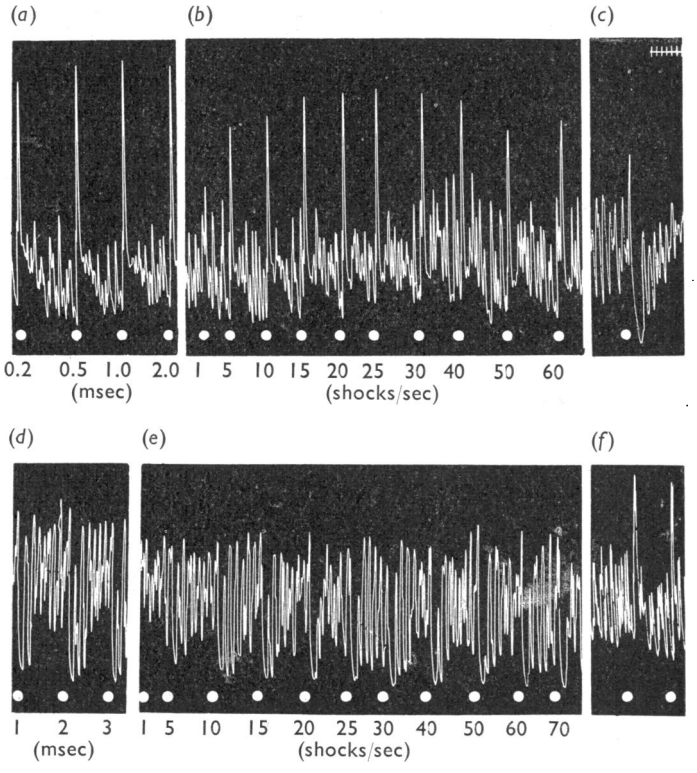

Fig. 4. Responses of the longitudinal muscle of the large intestine to stimulation (at white dots) of : (*a*, *b*), 9th and 10th spinal nerves; (*d*, *e*) splanchnic nerves. (*a*), 25 shocks/sec, variable pulse duration ; (*b*), 1 msec, variable pulse frequency ; (*d*), 30 shocks/sec, variable pulse duration ; (*e*), 2 msec, variable pulse frequency. Examples of atypical responses of the large intestine to : (*c*), spinal nerve stimulation (25 shocks/sec and 1 msec); (*f*), splanchnic nerve stimulation (30 shocks/sec and 2 msec). Time marks are at 1 min. intervals ; the temperature was 22·5° C

used in all experiments. Stimulation with these parameters was ineffective after the nerves had been cut between the electrodes and the organ ; thus the possibility of direct stimulation of the muscle by current spread was eliminated. At constant strength and duration, a stimulus frequency giving a maximal response was always seen. This optimal frequency was 25 shocks/sec at 22.5° C (Fig. 4,*b*). It was not possible to ascertain the optimum frequency of the inhibition which often followed the contraction (Fig. 4,*c*).

The right and left 10th spinal nerves each appeared to supply the entire large intestine, that is, if the two nerves were stimulated simultaneously with supramaximal

pulses, no summation of responses was seen. However, submaximal stimulation of the two nerves together caused a greater contraction than submaximal stimulation of the nerves individually.

Stimulation of the splanchnic nerves alongside the anterior mesenteric artery caused an inhibition, a relaxation and a cessation of spontaneous activity which commenced 8 to 10 sec after the onset of stimulation. This inhibition was sometimes preceded by a small rapid contraction which commenced 4 to 6 sec after the onset of stimulation (Fig. 4,*d*). The pulse duration required to produce maximal inhibition was 2 msec (Fig. 4,*d*) and the optimal pulse frequency for inhibition was 30 shocks/sec at 22.5° C (Fig. 4,*e*). No relationship between stimulus frequency and the amplitude of the initial contraction was found. In two out of thirty experiments, contraction but no inhibition was recorded after stimulation of these nerves (Fig. 4,*f*).

Stimulation applied to the posterior mesenteric artery, where present, usually had no effect on the large intestine. However, in 5% of the individuals investigated, a contraction or biphasic response similar to that caused by stimulation of the pelvic nerves was obtained. This result may indicate the presence of nerve fibres along the posterior mesenteric artery in some animals, although none was seen histologically ; or the response may have been due to spread of current down the artery.

Direct action of possible transmitter substances on the large intestine

Acetylcholine (10^{-8} to 10^{-5} g/ml.) caused a sustained contraction of the large intestine.

Adrenaline (10^{-8} to 10^{-6} g/ml.), noradrenaline (5×10^{-8} to 10^{-6} g/ml.), and dopamine (5×10^{-7} to 10^{-5} g/ml.) caused inhibition (relaxation of the large intestine, and cessation of spontaneous activity).

5-Hydroxytryptamine (10^{-9} to 10^{-8} g/ml.) caused sustained contraction of the large intestine. Tachyphylaxis was observed with successive applications of the drug.

Histamine (10^{-9} to 10^{-4} g/ml.) had no effect on the large intestine.

Effects of autonomic drugs on the nerve mediated responses

In the following experiments the nerve supply was stimulated with pulses of supramaximal strength and duration, and with optimal frequency. Bursts of stimulation were given for 10 sec at 6 to 10 min intervals. Responses to acetylcholine were usually used as controls in association with pelvic nerve stimulation, and responses to noradrenaline as controls with splanchnic nerve stimulation. The splanchnic nerves were stimulated distal to the ganglionic mass at the coeliaco-mesenteric junction.

Responses to stimulation of the pelvic nerves

Cholinergic drugs. Physostigmine sulphate (10^{-7} to 10^{-5} g/ml.) caused no potentiation of the response of the large intestine to stimulation of the pelvic nerves or to applied acetylcholine (Fig. 5,*a*).

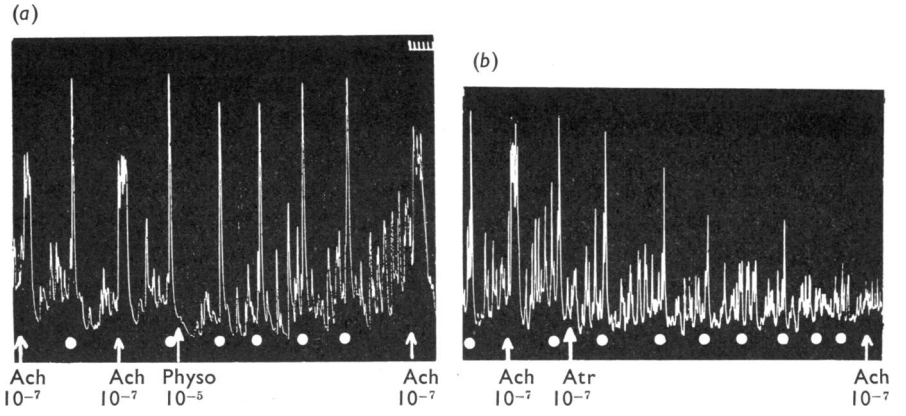

Fig. 5. Effects of : (*a*) physostigmine (Physo 10^{-5} g/ml.) and (*b*), atropine (Atr, 10^{-7} g/ml.) on the responses of the large intestine to pelvic nerve stimulation at white dots and to added acetylcholine (Ach, 10^{-7} g/ml.). Time marks are are at 1 min intervals ; the temperature was 22° C.

Atropine sulphate (10^{-8} to 10^{-6} g/ml.) usually completely blocked the excitatory response to stimulation of the 9th and 10th nerves (Fig. 5,*b*) ; block was not as rapid as the abolition of the response to applied acetylcholine. In some experiments where the response to pelvic nerve stimulation was diphasic, block of the excitatory phase revealed a distinct inhibitory component (Fig. 6,*a*).

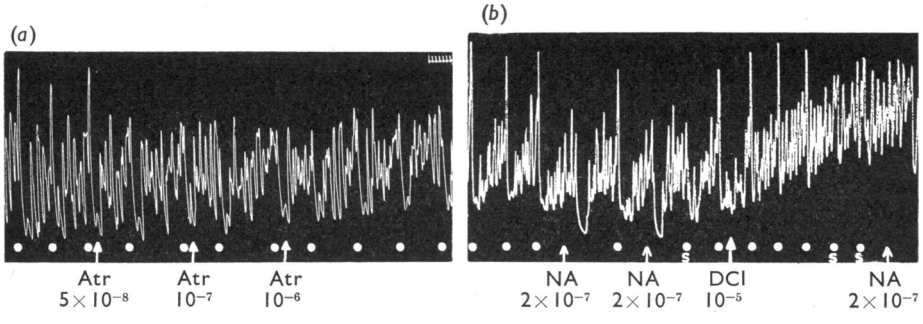

Fig. 6. Diphasic response of the large intestine (where inhibition follows contraction) to stimulation of the pelvic nerves (at white dots). Effect of (*a*) atropine (Atr, 5×10^{-8}, 10^{-7} and 10^{-6} g/ml.) ; (*b*), dichloroisoprenaline (DCI, 10^{-5} g/ml.) ; and noradrenaline (NA, 2×10^{-7} g/ml.). Control stimulations of the splanchnic nerve are denoted by S at white dots. Time marks are at 1 min. intervals ; the temperature was 22° C.

Adrenergic blocking drugs. The adrenergic blocking drugs used were the α-receptor blocking agents phenoxybenzamine hydrochloride (Dibenzyline) (10^{-5} g/ml.), piperoxan hydrochloride (933F ; 8×10^{-5} g/ml.), phentolamine methanesulphonate (Rogitine ; 10^{-4} g/ml.), yohimbine hydrochloride (5×10^{-5} g/ml.), tolazoline hydrochloride (Priscol ; 10^{-5} g/ml.). The β-receptor blocking agent dichloroisoprenaline (10^{-5} g/ml.), and the presynaptic blocking agents quanethidine sulphate (Ismelin ; 10^{-5} g/ml.) and bretylium tosylate (Darenthin ; 10^{-4} g/ml.) were also used.

All the α-receptor blocking agents used blocked the excitatory responses to stimulation of the 9th and 10th spinal nerves. However, the response to acetylcholine was also blocked by each of these drugs, except yohimbine which only reduced it. The concentrations of adrenergic blocking agents used were comparable with those required to block the responses of smooth muscle to stimulation of adrenergic nerves (Boyd *et al.*, 1963). Dichloroisoprenaline reduced the excitatory responses both to nerve stimulation and to acetylcholine by not more than 25%.

When inhibition was produced on stimulation of the pelvic nerves, it was blocked by dichloroisoprenaline (Fig. 7,*b*), guanethidine, bretylium and yohimbine. Dichloroisoprenaline often caused an initial potentiation of the excitatory phase of the mixed response. Drugs (other than yohimbine) which block α-receptors did not affect the inhibition caused by nerve stimulation or by applied noradrenaline.

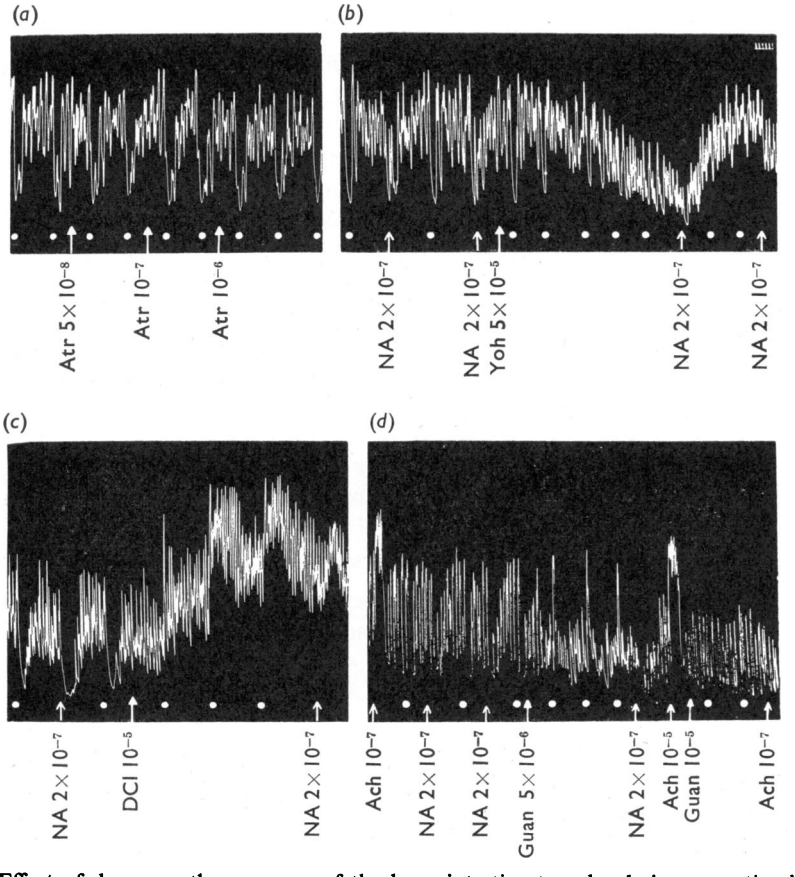

Fig. 7. Effect of drugs on the response of the large intestine to splanchnic nerve stimulation at white dots. (*a*), atropine (Atr, 5×10^{-8}, 10^{-7} and 10^{-6} g/ml.) ; (*b*) yohimbine (Yoh ; 5×10^{-5} g/ml.) ; and noradrenaline (NA, 2×10^{-7} g/ml.) ; (*c*), dichloroisoprenaline (DCI, 10^{-5} g/ml.) ; and noradrenaline (2×10^{-7} g/ml.) ; (*d*), guanethidine (Guan, 5×10^{-6} and 10^{-5} g/ml.) ; acetylcholine (Ach, 10^{-7} and 10^{-5} g/ml.) ; and noradrenaline (2×10^{-7} g/ml.). Time marks are at 1 min intervals ; the temperature was 23° C.

Ganglion-blocking agents. Those used were hexamethonium bromide (5×10^{-4} to 10^{-3} g/ml.), pentolinium tartrate (5×10^{-6} to 5×10^{-5} g/ml.) and mecamylamine hydrochloride (10^{-6} to 5×10^{-6} g/ml.). Hexamethonium and pentolinium each blocked the contractile response to applied acetylcholine before reducing the response to nerve stimulation. Mecamylamine, on the other hand, completely blocked the excitatory response to nerve stimulation before affecting the response to applied acetylcholine.

Responses to stimulation of the splanchnic nerves

Atropine (5×10^{-8} to 10^{-6} g/ml.) had no effect on the inhibition caused by stimulation of the nerves accompanying the anterior mesenteric artery (Fig. 7,*a*), but always blocked the initial contraction of a diphasic response.

Of the adrenergic blocking agents, yohimbine (5×10^{-5} g/ml.) was the only α-receptor blocking drug to affect the inhibitory response to nerve stimulation. It blocked the nerve response but only reduced the response to added noradrenaline (Fig. 7,*b*). Dichloroisoprenaline (10^{-5} g/ml.) reversed the inhibitory response both to nerve stimulation and to applied noradrenaline (Fig. 7,*c*). Bretylium (10^{-4} g/ml.) completely blocked the nerve-mediated inhibition. Guanethidine (5×10^{-6} to 10^{-8} g/ml.) reversed the response from inhibition to contraction (Fig. 7,*d*). Neither of these drugs reduced the inhibitory response to applied noradrenaline.

Ganglion-blocking agents potentiated the inhibitory responses to nerve stimulation and to added noradrenaline.

Effects of treatment with reserpine

On dissection of the treated animals, the gastro-intestinal tract appeared enlarged, flaccid and very congested. Blood vessels generally were dilated but the heart beat was strong. However, the isolated large intestine behaved normally to stimulation of both nerve supplies. The inhibitory responses could be blocked by dichloroisoprenaline (10^{-5} g/ml.). No fatigue of either response was seen, nor was there any change in sensitivity to applied noradrenaline and acetylcholine.

Electron-micrograph studies of the large intestine from animals treated with reserpine showed no change in the appearance of the nerve-muscle junctions or the vesicles contained in the nerve endings.

DISCUSSION

Our morphological observations support the view of Langley & Orbeli (1910) that the posterior portion of the amphibian intestine can be divided into regions analogous to those of the mammalian large intestine. Such features as lymph nodes, points of entry of the anterior mesenteric artery and splanchnic nerves and gross asymmetry of the anterior part of the sac-like portion of the large intestine indicate that this region is equivalent to the mammalian colon. The median region, designated the rectum, has been shown on histological (abrupt thinning of circular smooth muscle layer, absence of hyaline inclusions in epithelial cells and presence

of spathiform villi) and anatomical (points of entry of posterior mesenteric artery, when present, and pelvic nerves) grounds to be distinct from the colon. The cloacal region is anatomically caudal to the point of entry of the Wolffian duct and contains striated muscle fibres.

We have found that the large intestine is innervated both by fibres passing from the sympathetic chain in the splanchnic nerves (from the 3rd to 5th spinal nerves) and by fibres running in the 9th and 10th spinal nerves. These observations agree with those of Langley & Orbeli (1910). However, contrary to Langley & Orbeli's experimental observations on *Rana temporaria,* our investigations on *Bufo marinus* did not reveal any fibres passing to the large intestine in nerves 6 and 7 ; nor is the posterior mesenteric artery a major pathway to this organ for fibres from the sympathetic chain. These observations can probably be explained in terms of species differences. The present experiments have shown a predominance of excitatory fibres to the large intestine in the pelvic nerves whilst the majority of fibres in the splanchnic nerve are inhibitory.

The actions of ganglion-blocking agents suggest that the excitatory fibres to the large intestine are preganglionic, and that the inhibitory fibres are postganglionic. Mecamylamine, in a concentration which did not greatly affect the response to applied acetylcholine, completely blocked the excitatory response to stimulation of the 9th and 10th nerves, but failed to block the inhibition caused by stimulation of these or of the splanchnic nerves. Hexamethonium and pentolinium cannot be considered as reliable tools for analysis of the pre- or postganglionic nature of excitatory nerves in this preparation, since they blocked the excitatory response to nerve stimulation only in concentrations which also blocked the response to acetylcholine. This might indicate an atropine-like action of these drugs (see Mason & Wein, 1955 ; Burnstock & Campbell, 1963) or it might be taken as evidence for the nicotinic action of acetylcholine on myenteric ganglia. A predominantly nicotine-like action of acetylcholine on fish gut and bladder has also been postulated (Young, 1936 ; Burnstock, 1958). None of the ganglion-blocking agents blocked the inhibitory response to splanchnic nerve stimulation ; instead they all potentiated inhibitory responses both to nerve stimulation and to applied noradrenaline. This potentiation of the response to splanchnic nerve stimulation may be due to an increased sensitivity of the smooth muscle to the splanchnic nerve transmitter ; or possibly to block of the initial excitation which is a component of the response and which may be mediated by preganglionic fibres. These results suggest, however, that most of the nerve cells seen in the *muscularis externa* of the large intestine after staining with methylene blue are associated with the excitatory fibres in spinal nerves 9 and 10.

The transmitter released at the endings of the excitatory postganglionic nerve fibres might be acetylcholine, or a related choline ester, since acetylcholine mimicked the effects of stimulation of these nerves and both responses were blocked by atropine. However, physostigmine potentiated neither. This finding poses the problem of the mode of destruction of the transmitter substance. The fact that a similar result was obtained in the toad bladder by Burnstock, O'Shea & Wood (1963) suggests that resistance to physostigmine is common in toad smooth muscle.

Research in this laboratory (Bell & Burnstock, 1964) indicates that there is a "physostigmine-resistant" true cholinesterase in toad bladder smooth muscle, similar to that found in the frog brain by Hawkins & Mendel (1946). The results of experiments with adrenergic blocking agents appear at first to be ambiguous, since they blocked the responses to pelvic nerve stimulation and to added acetylcholine as effectively as they blocked adrenergic nerves. However, these agents have been shown to have anti-acetylcholine actions (Boyd *et al.*, 1963), so that their block of responses to stimulation of toad pelvic nerves does not indicate that these nerves are adrenergic.

The inhibitory nerve transmitter appears to be noradrenaline since added noradrenaline caused an inhibition similar to the response to nerve stimulation, and both these responses were blocked by dichloroisoprenaline.

It is of interest to find that toads could be given much higher sublethal doses of reserpine (50 mg/kg/day for 3 days) than could mammals (Muscholl & Vogt, 1958). However, even with this concentration the inhibitory responses of the large intestine to nerve stimulation remained strong and did not fatigue. This is in contrast to the effect of reserpine (0.2 mg/kg/day for 1 to 10 days) on the response of rabbit colon to sympathetic nerve stimulation, where the inhibition was not only abolished but was replaced by a motor response (Gillespie & Mackenna, 1961). These results suggest that reserpine may not deplete the local catechol amine stores in the toad as it does in the mammal. This question is being investigated further by quantitative assay of catechol amines before and after treatment with reserpine, and by fluorescent histochemical localization of the catechol amines.

On the basis of their anatomical findings, Langley & Orbeli (1910) divided the amphibian autonomic nervous system into sympathetic and parasympathetic divisions which, they stated, were not as clearly defined as in the mammal. Our observations support their conclusion that the general arrangement of the posterior autonomic nervous system in the amphibian is similar to that of the mammal but is less differentiated. In the toad, the pelvic nerves contain many inhibitory adrenergic nerve fibres, whereas in the mammalian pelvic nerve inhibitory adrenergic fibres are either absent (Garry & Gillespie, 1955) or rarely seen (Boyd, unpublished). Moreover, in the toad as in the frog, the sympathetic chain extends as far posteriorly as the pelvic nerves. In view of this lack of differentiation in the amphibian autonomic nervous system, it seems preferable to classify visceral efferent nerve fibres as "excitatory, preganglionic, cholinergic" and "inhibitory, postganglionic, adrenergic" rather than "parasympathetic" and "sympathetic" respectively. The lack of differentiation of the autonomic nervous system seen in these amphibians may be a primitive feature associated with the incomplete development of the inhibitory component of the sympathetic nervous system. Burnstock *et al.* (1963) could find only excitatory fibres in the autonomic nerves supplying the toad (*Bufo marinus*) bladder, and in the teleost fish (*Salmo trutta*) the only visceral organ to possess both an excitatory and an inhibitory nerve supply was the hind-gut (Burnstock, 1958). Preliminary experiments in this laboratory indicate that the role of the splanchnic nerves supplying the toad stomach and small intestine is predominantly excitatory (Burnstock & O'Shea, unpublished). These results, in

conjunction with our own, suggest that inhibitory autonomic control of the alimentary canal first appeared in the hind-gut region.

We would like to express our thanks to Miss Siew Tan, for assistance with some experiments, and to Ciba (Basle) and the Wellcome Research Laboratories for their kind donations of drugs. This investigation was supported in part by Public Health Service Research Grant No. NB-02902 from the National Institute of Neurological Diseases and Blindness, and by the National Health and Medical Research Council of Australia. One of us (D. C. R.) was in receipt of a General Motors Holden's Postgraduate Research Fellowship.

REFERENCES

BELL, C. & BURNSTOCK, G. (1964). Cholinesterases in the bladder of the toad (*Bufo marinus*). *Biochem. Pharmacol.* in the press.

BOYD, H., BURNSTOCK G., CAMPBELL, G., JOWETT, A., O'SHEA, J. A. & WOOD, M. (1963). The cholinergic blocking action of adrenergic blocking agents in the pharmacological analysis of autonomic innervation. *Brit. J. Pharmacol.*, **20**, 418–435.

BURN, J. H. & RAND, M. J. (1960). The relation of circulating noradrenaline to the effect of sympathetic stimulation. *J. Physiol. (Lond.)*, **150**, 295–305.

BURNSTOCK, G. (1958). The effect of drugs on spontaneous motility and on response to stimulation of the extrinsic nerves of the gut of a teleostean fish. *Brit. J. Pharmacol.*, **13**, 216–226.

BURNSTOCK, G. & CAMPBELL, G. (1963). Comparative physiology of the vertebrate autonomic nervous system II : Innervation of the urinary bladder of the ringtail possum (*Pseudocheirus peregrinus*). *J. exp. Biol.*, **40**, 421–436.

BURNSTOCK, G., O'SHEA, J. & WOOD, M. (1963). Comparative physiology of the vertebrate autonomic nervous system. I : Innervation of the urinary bladder of the toad (*Bufo marinus*). *J. exp. Biol.*, **40**, 403–419.

ECKER, A. & WIEDERSHEIM, R. (1899). *Anatomie des Frosches.* Braunschweig : F. Vieweg und Sohn.

GARRY, R. C. & GILLESPIE, J. S. (1955). The responses of the musculature of the colon of the rabbit to stimulation, *in vitro*, of the parasympathetic and the sympathetic outflows. *J. Physiol. (Lond).*, **128**, 557–576.

GASKELL, W. H. (1886). On the structure, distribution and function of the nerves which innervate the visceral and vascular systems. *J. Physiol. (Lond).*, **7**, 61–80.

GILLESPIE, J. S. & MACKENNA, B. R. (1961). The inhibitory action of the sympathetic nerves on the smooth muscle of the rabbit gut, its reversal by reserpine and restoration by catechol amines and by dopa. *J. Physiol. (Lond).*, **156**, 17–34.

HAWKINS, R. D. & MENDEL, B. (1946). True cholinesterases with pronounced resistance to eserine. *J. cell. comp. Physiol.*, **27**, 69–85.

HORTON-SMITH, R. J. (1897). On efferent fibres in the posterior roots of the frog. *J. Physiol. (Lond.)*, **21**, 101–111.

LANGLEY, J. N. & ORBELI, L. A. (1910). Observations on the sympathetic and sacral autonomic system of the frog. *J. Physiol. (Lond.)*, **41**, 450–482.

MARSHALL, A. M. (1914). *The Frog : Introduction to Anatomy, Histology and Embryology.* Ed., GAMBLE, F. W., 2nd edit. London : MacMillan.

MASON, D. F. J. & WIEN, R. (1955). The actions of heterocyclic biquaternary compounds, especially of a pyrrolidinium series. *Brit. J. Pharmacol.*, **10**, 124–132.

MUSCHOLL, E. & VOGT, M. (1958). The action of reserpine on the peripheral sympathetic system. *J. Physiol. (Lond.)*, **141**, 132–155.

STEINACH, E. & WIENER, H. (1895). Motorische Functionen hinterer Spinalnerven-Wurzeln. *Pflügers Arch. ges. Physiol.*, **60**, 593–622.

WATSON, M. L. (1958). Staining of tissue sections for electron microscopy with heavy metals. *J. biophys. biochem. Cytol.*, **4**, 475–479.

YOUNG, J. Z. (1936). The innervation and reactions to drugs of the viscera of a teleostean fish. *Proc. roy. Soc. B*, **120**, 303–318.