J. Physiol. (1978), **283**, *pp.* 319–330
With 10 *text-figures*
Printed in Great Britain

ELECTRICAL ACTIVITY OF HUMAN COLONIC SMOOTH MUSCLE *IN VITRO*

BY H. L. DUTHIE AND D. KIRK*

From the University Department of Surgery, Royal Infirmary, Sheffield S6 3DA

(*Received 17 February 1978*)

SUMMARY

1. Extracellular and intracellular recordings were made of the electrical activity of isolated strips of human colonic smooth muscle, cut from sixty surgical specimens.

2. Strips of taenia were spontaneously active. The myogenic activity consisted in half the strips of intermittent periods of regular spike activity (frequency 22 ± 5 (S.D.) c/min) accompanied by tetanic contractions; in the other half of the strips activity was continuous. In half the specimens, slow potentials were recorded between periods of spike activity. Slow potentials were not accompanied by contractions.

3. Spikes in taenia were abolished by verapamil. Spikes disappeared in low Ca and low Na solutions, but in low Na solution spikes could be stimulated by 15 mM-KCl.

4. ACh and physostigmine produced tetanic contractions in taenia.

5. Circular muscle was not spontaneously active within 1 h of incubation in the water bath, possibly due to inhibition by prostaglandins.

6. Circular muscle responded to ACh with irregular bursts of spikes associated with discrete contractions. Similar activity was seen after inhibition of prostaglandin synthesis with indomethacin. After treatment with tetrodotoxin, ACh produced regular spikes and tetanic contractions in circular muscle.

7. The possible relationships of these results to the myoelectrical activity of the human colon *in vivo* are discussed.

INTRODUCTION

Information about the *in vitro* behaviour of human colonic smooth muscle is needed (Daniel, 1975), particularly to aid the interpretation of the recordings of myoelectrical activity of the human colon which have been made *in vivo* (Taylor, Duthie, Smallwood & Linkens, 1975). In this paper, the results are presented of an electrophysiological study of isolated strips of smooth muscle, obtained from sixty surgically resected specimens of colon. A preliminary report of this work has been presented to a meeting of the Physiological Society (Kirk, 1976).

* Present address: Royal Devon and Exeter Hospital (Wonford), Barrack Road, Exeter EX2 5DS.

METHODS

Muscle strips were obtained from sixty specimens of colon resected surgically, fifty-three for colonic or rectal carcinoma, four for diverticular disease and three for ulcerative colitis. Immediately after removal from the body, a segment of bowel a few centimetres in length was cut from one or both ends of the specimen. With the exception of the three cases of ulcerative colitis, these segments were taken from macroscopically normal bowel. After washing they were placed in Krebs solution. Unless the segment was to be studied immediately, it was stored in Krebs solution at 4 °C. After a variable period from 15 min to 24 hr, the bowel was opened along its antemesenteric border and pinned out in fresh Krebs solution. Muscle strips were cut with the long axis in the direction of the fibres. They measured 1·5 × 0·2 × 0·1 cm. Strips of taenia were cut from the serosal surface, leaving the serosa *in situ*. Circular muscle strips were taken from the intertaenial region and were cut from the mucosal aspect, after removal of mucosa and submucosa by sharp dissection. Strips were mounted individually in a heated tissue bath at 37 °C. The volume of the bath was 1·7 ml., and it was perfused with Krebs solution at a rate of 2·0 ml./min. Four fluid inputs allowed rapid changes of solution to be made. One end of the strip was attached to a strain gauge. An initial tension of 1 g was applied in most experiments.

Extracellular recordings were made with glass pore electrodes (Christensen, Caprilli & Lund, 1969). Monopolar recordings were made with reference to a silver/silver chloride electrode placed in the corner of the bath. Immediately over the surface of the muscle strip, a pore electrode produced a recording approximating to that in a volume conductor. If it was pressed lightly on the surface of the strip, a recording was obtained in which the major deflection produced by an action potential was positive. These latter recordings, which were similar to those made with pressure electrodes, as described by Bortoff (1961*a*), tended to be more stable and of greater amplitude, and in most experiments the electrode was used in this mode. The output was amplified 100-fold by a purpose built amplifier.

Intracellular recordings were made with glass micro-pipettes drawn from 1·0 mm borosilicate glass, containing a glass filament (Clarke Electromedical). They were filled with 3 M-KCl by direct injection (Tasaki, Tsukahara, Ito, Wayner & Yu, 1968). Electrodes of impedance 25–50 MΩ were used. Output was taken by short leads to a high impedance differential amplifier (Fenlow AD55). A backing-off device was incorporated in the circuit, and the output could be switched to a meter to monitor the impedance of the electrode.

Signals from the extracellular and intracellular electrodes and the strain gauge were displayed on three channels of a fibre optic recording oscilloscope (Medilec FOR 4), the fourth channel being used for a time marker. Recordings were made on ultra-violet paper and were photographed for illustration purposes. Illustrations have been retouched to the extent of removing defects resulting from the photographic process.

A modified Krebs solution of the following composition was used. Na^+ 151 mM, K^+ 4·7, Ca^{2+} 2·5, Mg^{2+} 1·2, Cl^- 142, HCO_3^- 16·3, $H_2PO_4^-$ 1·4, SO_4^{2-} 1·2, dextrose 7·1, equilibrated with 95 % O_2 + 5 % CO_2. 'Low Na Krebs' contained 17 mM-Na^+, the deficit being made up with an equimolar quantity of Tris chloride. The following drugs were used: acetylcholine (ACh), atropine sulphate, hexamethonium bromide, indomethacin, physostigmine sulphate, tetrodotoxin (TTX) and verapamil hydrochloride. Where relevant, concentrations given in the text are those of the base.

RESULTS

Taenia coli

Eighty-five strips of taenia have been studied. In fifty-four spontaneous activity was seen within 1 hr of incubation at 37 °C in the water-bath.

Spikes. The dominant electrical event in spontaneously active taenia was a spike action potential. This was usually single, but double or treble spikes were observed on occasions. In extracellular recordings, spikes had an amplitude of 0·1–0·5 MV. Spikes occurred in a regular rhythm (Fig. 1). The rate of this rhythm was characteristic of a given strip.

The rhythm of each strip was recorded when a stable pattern had been established and prior to any pharmacological interference. The mean rate was 22 ± 5 (S.D.) c/min ($0{\cdot}37 \pm 0{\cdot}08$ Hz).

Slow potentials. In addition to spikes, some strips also produced sinusoidal oscillations of potential, of approximate amplitude 0·1 mV (Fig. 2). Under the standard conditions used, strips which showed slow potentials also produced spikes, although in many strips spikes were seen without slow potentials (Table 1).

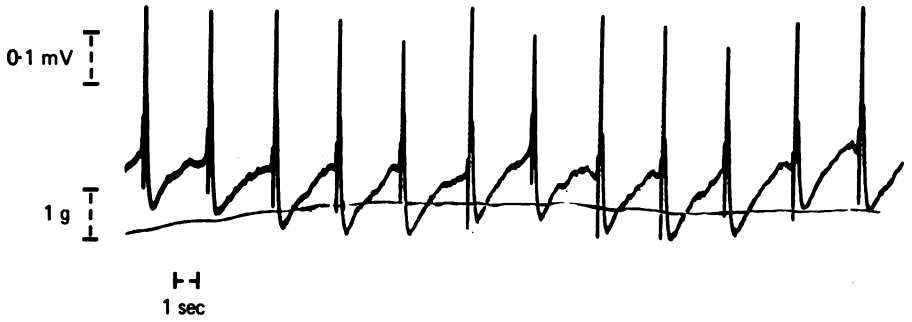

Fig. 1. Recording of spontaneous activity of a typical strip of taenia. Upper: extracellular recordings of electrical activity. Lower: isometric tension recording.

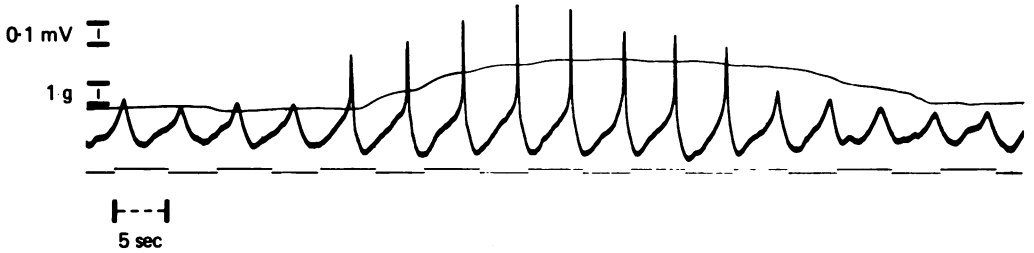

Fig. 2. Extracellular recording, showing slow potentials. Contraction of muscle occurs only when spike activity appears.

TABLE 1. Patterns of spontaneous activity recorded in spontaneously active taenia

	Electrical		Mechanical	
	Spikes	Slow waves	Contractions	Number
1	Intermittent	Absent	Tetanic	14
2	Intermittent	Present	Tetanic	11
3	Continuous	—	Continuous	29
4	Absent	Absent	Absent	31

Mechanical activity. Spikes were associated with a contraction of the muscle. There was no mechanical activity in the absence of spikes; no contractions were seen in association with slow potentials alone.

Patterns of spontaneous activity. Four types of activity were seen in strips of taenia (Table 1).

(1) Intermittent periods of regular spike activity, each period lasting from a few

seconds to several minutes. Mechanical activity took the form of tetanic contractions coinciding with the periods of electrical activity, an increment of tension occurring with each spike. Between these periods, no activity was present in either electrical or mechanical recordings.

(2) Spikes and mechanical activity present as in (1), with slow potentials in the intervening periods. The slow potentials might continue from one episode of spike activity to the next, or be interrupted by a period of zero activity. In the latter case, the slow potentials always reappeared before the spikes. The slow potentials had the same over-all frequency as the spikes, in a given strip, although the timing of the spikes in relation to the slow potentials sometimes was variable. In this case, the slow potential would remain regular, with a beat to beat fluctuation of the frequency of the spikes. This can be seen in Fig. 3.

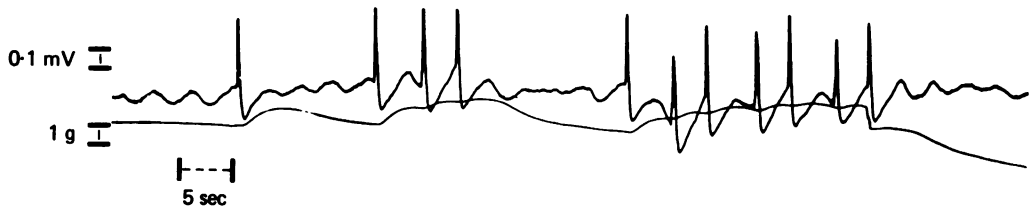

Fig. 3. Extracellular recording, demonstrating early activity in a specimen in which spikes later became continuous. Note slow potentials, and inconsistent relationship of spikes to slow potentials.

(3) Continuous spike activity. Regular spikes, each associated with a rise and fall in tension, occurred throughout the recording period, once the initial stabilization period had elapsed after mounting the strip in the bath. At slow recording speeds, a regular rise and fall of the amplitude of the spikes would often be seen in the extracellular records (Fig. 4). During the initial stabilisation period, a short episode of intermittent activity was seen in many recordings, and slow potentials sometimes were seen at this time (Fig. 3).

(4) No electrical or mechanical activity occurred spontaneously.

Intracellular recordings. For reasons to be discussed, satisfactory penetrations have been difficult to achieve in human taenia coli. The penetrations that have been made confirm that spikes recorded extracellularly represent typical action potentials. The recording illustrated (Fig. 5) was made from the intermittently active strip which provided extracellular recording shown in Fig. 1. Slow potentials were not recorded from this strip.

Effect of tension changes. The frequency of spikes in taenia was not affected by altering the tension applied to the tissue. Many strips showed no spike activity at zero tension, activity appearing at the rate characteristic for the strip when tension was applied. The frequency then remained constant until it disappeared at tensions in excess of 6 g. In strips in which activity was periodic, the proportion of time during which spike activity was present increased with tension, becoming continuous at tensions above 4 g (Fig. 6).

Effect on spikes of alterations in ionic composition of Krebs solution. Loss of spike activity occurred within a few minutes of replacing the standard Krebs solution

with a solution containing 17·5 mM Na or one containing 0·5 mM Ca. If strips in low Na Krebs solution were treated with a solution containining 15 mM-K, normal amplitude spikes appeared transiently.

Effect of TTX. Spikes in taenia persisted in the presence of TTX (10^{-7} g/ml.). However, TTX produced intermittent activity in four of six cases in which it was applied to continuously active preparations (Fig. 7).

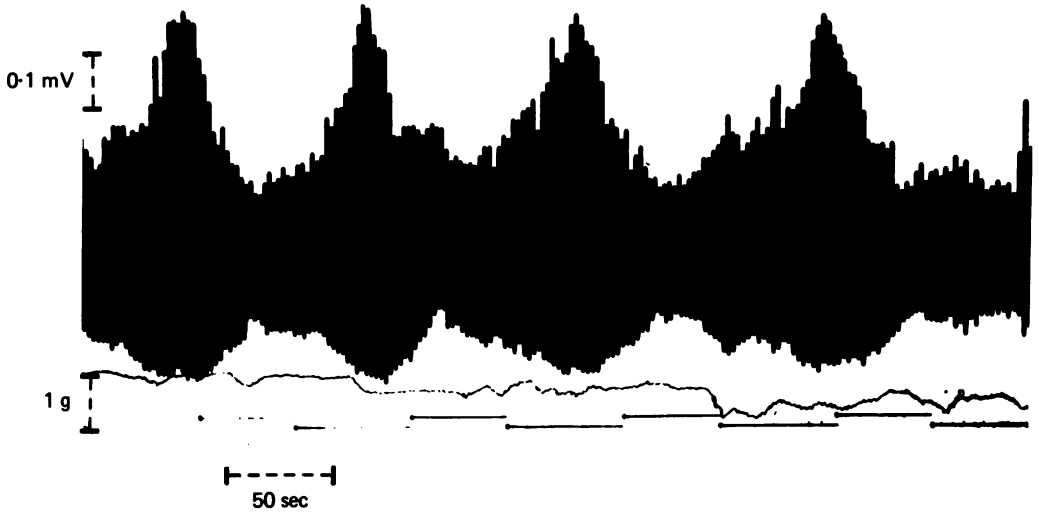

Fig. 4. Extracellular recordings, at slow recording speed, to demonstrate rise and fall in amplitude of spikes in a continuously active specimen.

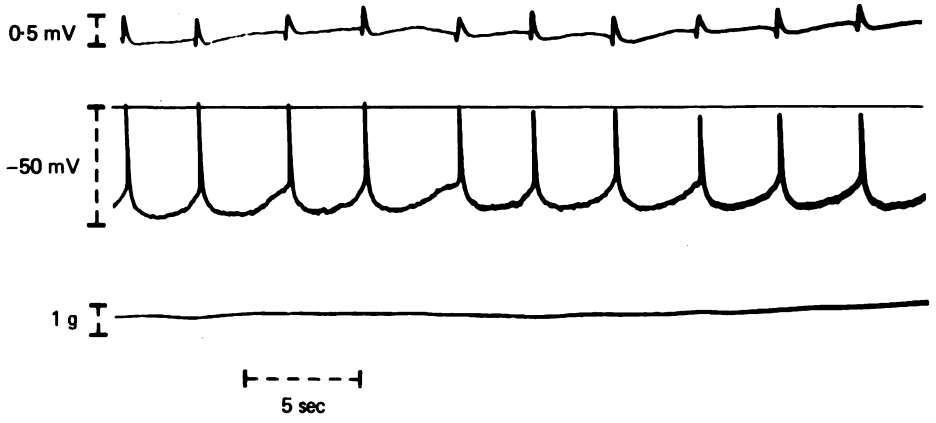

Fig. 5. Intracellular recordings (middle tracing) from taenia. Same strip as extracellular recording shown in Fig. 1. Extracellular record at reduced gain at top.

Effect of verapamil. Treatment with verapamil (10^{-6} g/ml.) inhibited spike production within 20 min (Fig. 8). The effect of verapamil was ıeversible, although it will be noted that in the experiment illustrated in Fig. 8 spikes appeared on recovery at approximately half the original rate. Also it can be seen that after 10 min treatment with verapamil there was a period when the original continuous activity became intermittent.

Effects of cholinergic stimulants and inhibitors. ACh was applied to twelve spontaneously active strips, and in all cases a stimulatory effect was noted. In strips showing intermittent activity a low concentration (10^{-7}–10^{-6} g/ml.) produced continuous activity with little or no effect on the actual spike rate. ACh at 10^{-5} g/ml. accelerated spike rate progressively, causing a sustained contraction. Spikes were usually abolished at the height of the contraction which then persisted in their

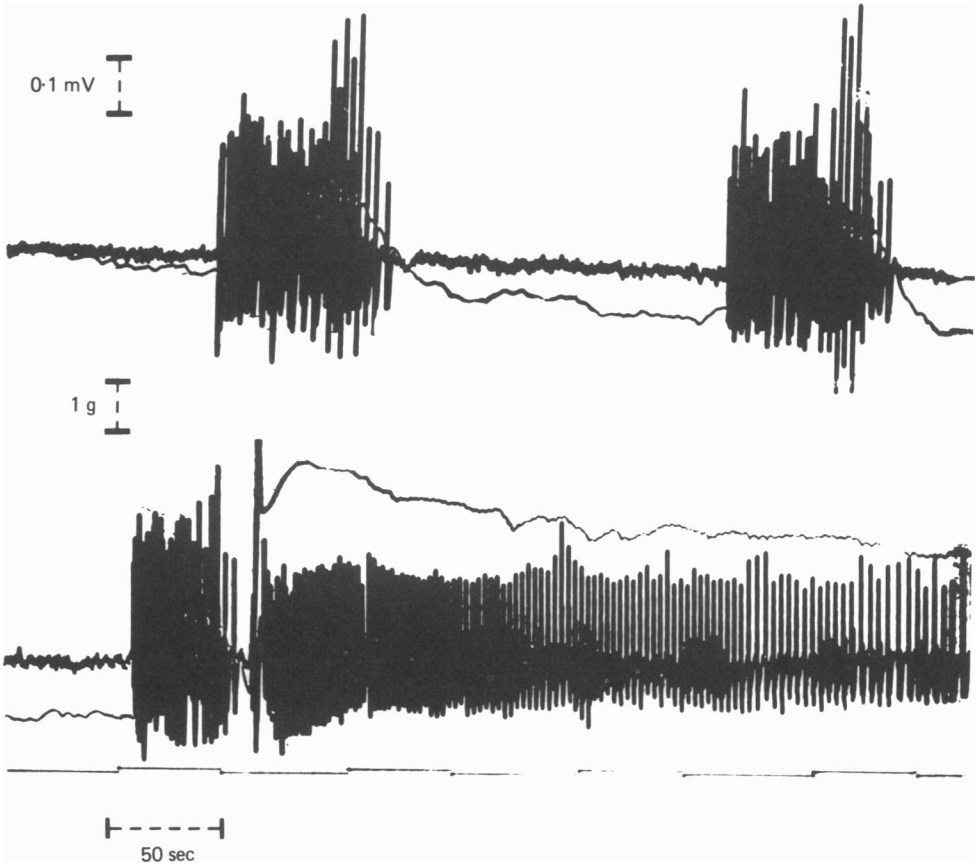

Fig. 6. Continuous activity induced by increasing tension exerted on strip of taenia. Upper trace initial tension 1 g. Lower trace initial tension 4 g.

absence. Similar contractions were seen with physostigmine (10^{-5} g/ml.) (Fig. 9). Eight of the thirty-one strips in which spontaneous activity was not seen also were treated with ACh. Six responded with regular spikes. The minimum concentration required to stimulate these strips varied from 10^{-7} to 5×10^{-5} g/ml., and in all cases accompanying mechanical activity was very weak.

The action of ACh was inhibited by atropine (10^{-5} g/ml.) but not by hexamethonium (10^{-5} g/ml.). In continuously active strips, atropine alone could produce intermittent activity, an effect similar to that of TTX, as illustrated in Fig. 7.

Effect of indomethacin. Indomethacin was mainly used in studies of circular muscle

(see below). It was applied to six strips of taenia. In four of these there was a small reduction in spike rate, the significance of which is uncertain. Indomethacin did not appear to have a stimulatory effect on taenia.

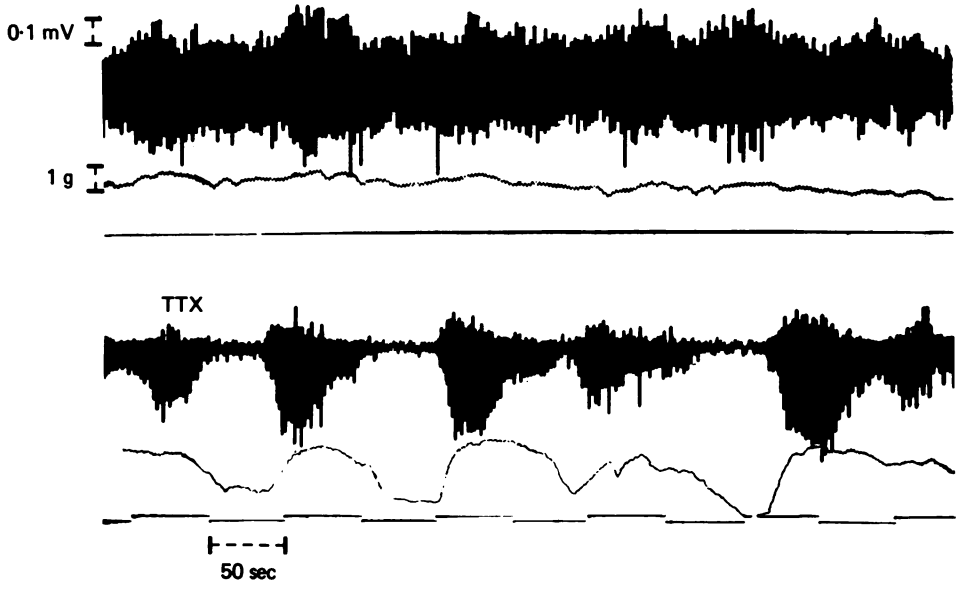

Fig. 7. Effect of tetrodotoxin (TTX) (10^{-7} g/ml.) on a continuously active strip of taenia.

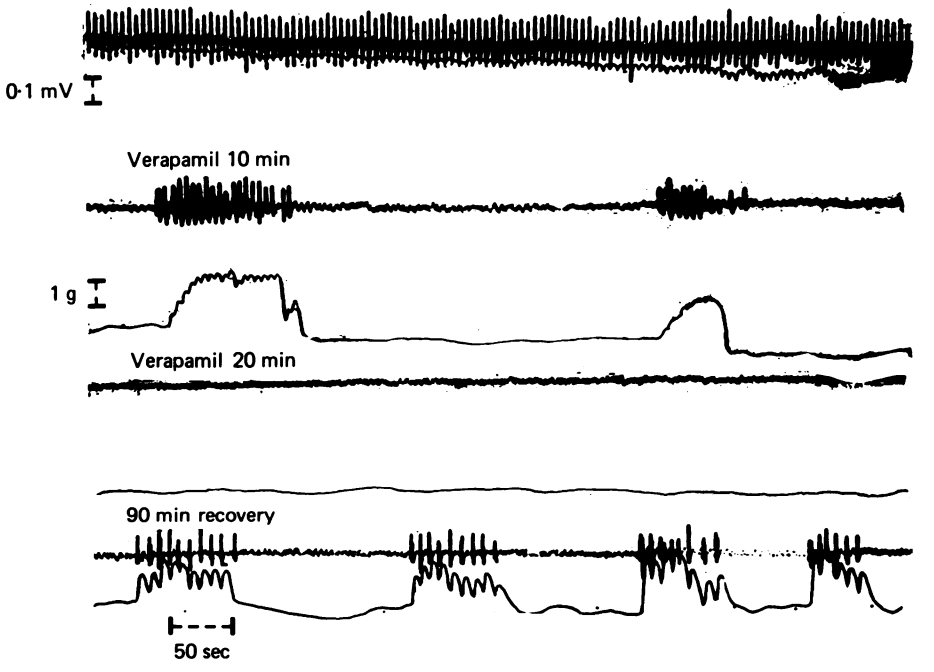

Fig. 8. Effect of verapamil (10^{-6} g/ml.) on activity of taenia. Times in middle two tracings are from introduction of verapamil to bath. Time in bottom tracing from withdrawal of verapamil.

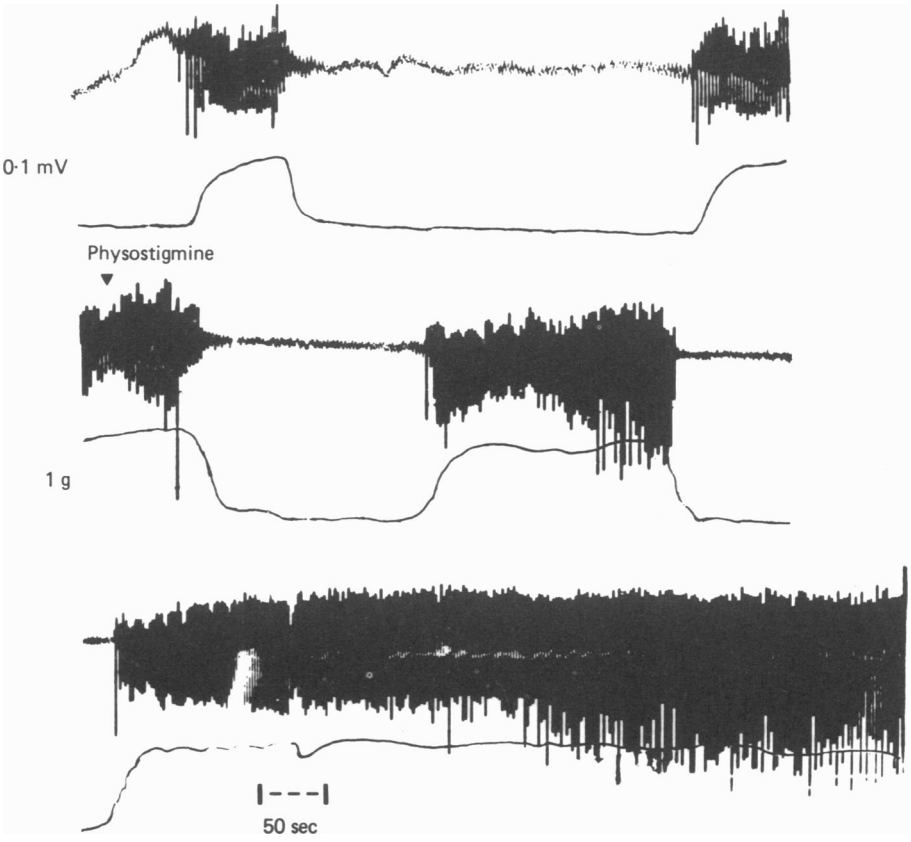

Fig. 9. Contracture in taenia produced by physostigmine (10^{-6} g/ml.).

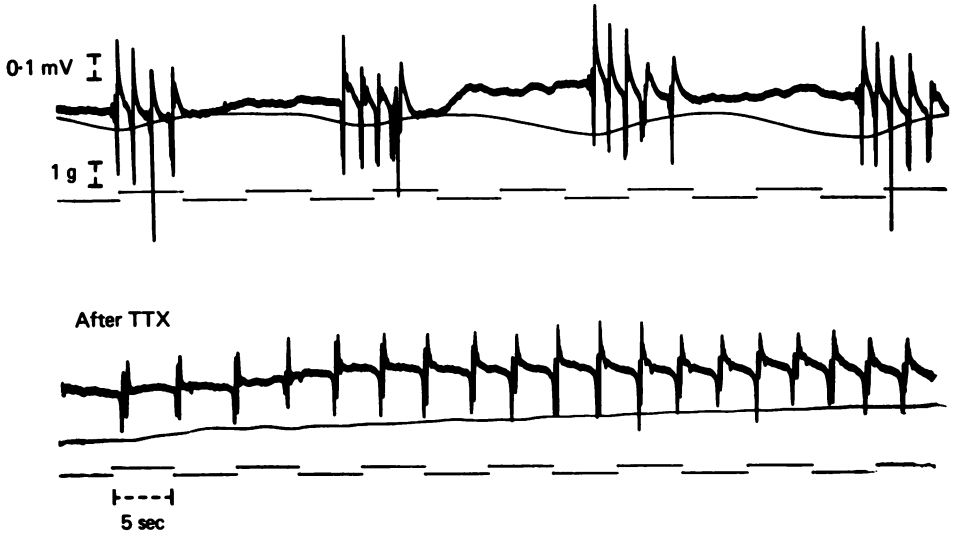

Fig. 10. Effect of TTX on response of circular muscle to ACh. Upper: response to ACh prior to treatment with TTX (10^{-7} g/ml.). Lower: response to ACh after treatment with TTX.

Circular muscle

Twenty-six strips of circular muscle have been studied. Of these, only five showed spontaneous activity within 1 hr of incubation in the water bath. In two, periods of regular spikes occurred, in a pattern resembling that obtained with taenia. In the other three there were spontaneous bursts of spikes, similar to those seen in the majority of circular muscle strips in response to ACh (see below). In the remaining twenty-one strips, single spike impulses and contractions were occasionally seen immediately after the tension on the strip had been increased.

Reponse to ACh. Circular muscle was less sensitive to ACh than taenia. Responses to concentrations of less than 10^{-6} g/ml. were rarely seen. Of the twenty-one strips which did not show spontaneous activity, two failed to respond to ACh. Two responded with regular runs of spikes, but in the remaining seventeen the response was entirely different from the activity that has been described for taenia. Within one minute of introducing ACh to the bath, irregular short bursts of spikes occurred, each burst being followed by a single contraction (Fig. 10). The pattern of response did not alter as the concentration of ACh was increased, although at higher concentrations activity was often short-lived. The duration of individual bursts of spikes varied and the rate of the bursts varied from 1/min to 12/min. A contraction followed each burst with a complete relaxation before the next. Even if ACh concentration as high as 10^{-4} g/ml. were used, tetanic contractions were rarely seen in circular muscle. The action of ACh was abolished by atropine but not by hexamethonium.

Effect of TTX. After treatment with TTX (10^{-7} g/ml.), the action of ACh on circular muscle was different. Instead of isolated bursts of spikes, regular spikes and tetanic contractions occurred (Fig. 10).

Effect of indomethacin. Strips of circular muscle which responded to ACh by producing isolated bursts of spikes (as described above) were returned to plain Krebs solution for a period of 15 min and then treated with indomethacin (10^{-7} g/ml.). After 10–15 min activity appeared without further introduction of ACh. This activity closely resembled that seen in the same strip in response to ACh. It persisted for at least 30 min after withdrawal of indomethacin.

Intertaenial longitudinal muscle

Four strips of muscle from the thin layer of longitudinal muscle from the intertaenial region were studied. No spontaneous activity was recorded from them, and they did not respond to ACh.

DISCUSSION

Entirely normal human colon is rarely removed at surgical operations, and in obtaining specimens for physiological study, reliance has to be placed on the normality of the bowel at the 'margins of specimens resected for focal lesions such as carcinoma. During the surgical resection of the specimen there is inevitably a period of exposure, handling and ischaemia, which would be unacceptable in a study of animal tissue, and might have physiological consequences. Throughout the study a degree of variability in behaviour was seen, as has been indicated in reporting the results. This may be due to the adverse circumstances in collection

of the specimens. In particular, a number of muscle strips appeared particularly resistant to the effects of drugs. These were often strips of taenia in which spikes were of low voltage or of bizarre form. Considerable caution must be exercised in interpreting results obtained under these circumstances. The findings described have not been uniform in all strips, but were the most consistent patterns to emerge during the study. Tissue obtained and handled in a different manner might well have not behaved in the same way, but the ethical constraints placed on the study of human tissue make these problems inevitable.

Spontaneous activity in taenia. The majority of strips of taenia were spontaneously active (Table 1). Many of the inactive strips responded to stimulation with ACh. However, the resistance of spontaneous activity to TTX (10^{-7} g/ml.) suggests that it may be independent of neural elements, and may be predominantly myogenic. Half of the strips were active intermittently (Table 1). Intermittent activity is present in recordings from the human colon *in vivo* (Taylor *et al.* 1975) and has been found in previous studies *in vitro* (Lynen, 1973; Vanasin, Ustach & Schuster, 1974). The conversion of continuous activity to intermittent activity by atropine and TTX (Fig. 7) raises the possibility that continuous activity may result from ACh release by intrinsic parasympathetic nerves, as occurs in strips of longitudinal muscle from guinea-pig jejunum (Kuriyama; Osa & Toida, 1967). Many continuously active strips had a periodic fluctuation in spike amplitude in the extracellular records (Fig. 3). The first effect of verapamil was to produce intermittent activity in these specimens in which spikes had been continuous (Fig. 8). These responses suggested that in taenial muscle the myogenic pattern of activity was intrinsically intermittent, which could be an example of the smooth muscle 'minute rhythm' described by Golenhofen (1976). This does not take into account any lack of precision with which the extracellular technique used registers spike potentials. Variations in the closeness with which the pore electrode made contact with the tissue could have given an impression of action potentials appearing and disappearing. However, the contractions of the strips were intermittent and there was an invariable direct relationship between spikes and contraction, and since phasic contractions were never seen in the presence of slow waves alone, nor in the absence of all electrical activity, it seemed that there was at least a variation in the intensity of spike activity. However, the possibility remained of residual spike activity, perhaps with contractions too weak for the sensitivity of the strain gauge, or simply maintaining the tone of the tissue.

Slow potentials. In many of the intermittently active preparations, sinusoidal oscillations were recorded between the periods of spike production. The absence of such slow potentials in some muscle strips, the activity of which was in other ways similar (Table 1) made it difficult to assess their significance. In one previous study (Vanasin *et al.* 1974) such oscillations, described as 'slow waves' appear to have been a constant feature, although they did not appear at all in Lynen's (1973) recordings, perhaps due to differences in technique. *In vivo*, slow waves were the major component of myoelectrical activity, spikes being rare events (Taylor *et al.* 1975). That in the present study they could be artifacts, perhaps due to tissue injury, has to be considered. However, they were often present in these strips which appeared to be particularly healthy, with large amplitude spikes and powerful mechanical

activity. The possibility that they represented spikes which are being inadequately registered by the pore electrode is unlikely since phasic muscular contractions were never seen in the presence of slow potentials alone (see above). The regularity of the slow potentials and their relationship to the spikes was in many ways similar to the slow waves described in the cat small intestine (Bortoff, 1961*b*). It has been noted that in many continuously active specimens slow potentials were recorded in the initial period before activity stabilized (Fig. 4). Although quantitative values cannot be deduced with any confidence (see above) intracellular recordings did confirm that 'spikes' in the extracellular record represented typical action potentials. The mechanism of production of action potentials in smooth muscle has yet to be completely elucidated in guinea-pig or other animal tissues (Kuriyama, 1970). It is known to be different from that in nerve and skeletal muscle and is thought that Ca^{2+} ions are involved in the initial rising phase. The observations reported here of the effects of low Na and low Ca solutions (Brading, Bulbring & Tomita, 1969) the resistance of the spikes to TTX (Kao, 1966) and their suppression by verapamil (Golenhofen & Lammel, 1972) indicate that a similar mechanism is likely in human taenia.

Circular muscle. The absence of spontaneous activity in the circular layer has been noted before (Vanasin *et al.* 1974), resembling the small intestine of the cat (Bortoff, 1965) but not the cat colon (Christensen *et al.* 1969). Indomethacin, in the concentration used in these studies, is an inhibitor of prostaglandin synthesis (Ferriera & Vane, 1974). Prostaglandins of group E are known to inhibit the circular muscle of the colon (Schuster & Vanasin, 1971; Bennett, 1975). The action of indomethacin on circular muscle is unlikely to be due to direct stimulation, since the effect takes some 15 min to appear, and persists for a long period after withdrawal of the drug. In addition, indomethacin did not stimulate taenial strips. In a more recent study (Van Merwyk A., & Duthie, H. L. unpublished observations) it has been found that after prolonged incubation at 37 °C, activity can appear spontaneously in circular muscle strips; this activity is similar to that described here in response to ACh. As yet, it is impossible to rule out that prostaglandin release may be a response of the tissue to the trauma of surgery rather than of physiological significance, although inactivity of the circular muscle layer is seen in many animals tissues, the preparation of which is subject to less trauma than human surgical specimens.

When stimulated with ACh, or after treatment with indomethacin, the activity of the circular layer differed from that of taenia. The latter produced prolonged, tetanic contractions while the circular muscle produced short isolated contractions. The different behaviour of the circular muscle may be due to an inhibiting neural influence since tetanic contractions occurred after treatment with TTX (Fig. 10).

REFERENCES

BENNETT, A. (1975). Pharmacology of colonic muscle. *Gut*, **16**, 307–311.

BORTOFF, A. (1961*a*). Electrical activity of intestine recorded with pressure electrode. *Am. J. Physiol.* **201**, 209–212.

BORTOFF, A. (1961*b*). Slow potential variations of small intestine. *Am. J. Physiol.* **201**, 203–207.

BORTOFF, A. (1965). Electrical transmission of slow waves from longitudinal to circular intestinal muscle. *Am. J. Physiol.* **209**, 1254–1260.

BRADING, A., BULBRING, E. & TOMITA, T. (1969). The effect of sodium and calcium on the action potential of the smooth muscle of the guinea-pig taenia coli. *J. Physiol.* **200**, 637–654.

CHRISTENSEN, J., CAPRILLI, R. & LUND, G. (1969). Electric slow waves in circular muscle of cat colon. *Am. J. Physiol.* **217**, 771–776.

DANIEL, E. E. (1975). Electrophysiology of the colon. *Gut* **16**, 293–306.

FERRIERA, S. H. & VANE, J. R. (1974). New aspects of the mode of action of non-steroid anti-inflammatory drugs. *A. Rev. Pharmac.* **14**, 57–73.

GOLENHOFEN, K. (1976). Spontaneous activity and functional classification of mammalian smooth muscle. *Physiology of Smooth Muscle*, ed. BULBRING, E. & SHUBA, M. F., pp. 92–97. New York: Raven Press.

GOLENHOFEN, K. & LEMMEL, E. (1972). Selective suppression of some components of spontaneous activity in various types of smooth muscle by Improveratril (Verapamil). *Pflügers Arch.* **331**, 233–243.

KAO, C. Y. (1966). Tetrodotoxin, Saxitoxin and their significance in the study of excitation phenomena. *Pharmac. Rev.* **18**, 997–1049.

KIRK, D. (1976). Electrical activity of human colonic smooth muscle *in vitro*. *J. Physiol.* **259**, 28*P*.

KURIYAMA, H., OSA, T. & TOIDA, W. (1967). Nervous factors influencing the membrane activity of intestinal smooth muscle. *J. Physiol.* **191**, 259–290.

KURIYAMA, H. (1970). Effects of ions and drugs on the electrical activity of smooth muscle. *Smooth Muscle*, ed. BULBRING, E., BRADING, A. F., JONES, A. W. & TOMITA, T., pp. 366–395. London: Edward Arnold.

LYNEN, F. K. (1973). Motor function of the large bowel in megacolon. *Proc. IVth Int. Symp. G.I. Motility, Banff*, pp. 191–195. Vancouver: Mitchell Press.

SCHUSTER, M. M. & VANASIN, B. (1971). Prostaglandin effects in electrical and motor activity of colonic muscle. *Rend. Gastroenterol.* **3**, 109–110.

TASAKI, K., TASUKUHARA, Y., ITO, S., WAYNER, M. J. & YO, W. Y. (1968). A simple direct and rapid method of filling microelectrodes. *Physiol. and Behav.* **3**, 1009–1010.

TAYLOR, I., DUTHIE, H. L., SMALLWOOD, R. & LINKENS, D. (1975). Large bowel myoelectrical activity in man. *Gut*, **16**, 808–814.

VANASIN, B., USTACH, T. J. & SCHUSTER, M. M. (1974). Electrical and motor activity of human and dog colon *in vitro*. *Johns Hopkins Med. Jnl* **134**, 201–210.