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ELECTRICAL ACTIVITIES OF THE MUSCLE LAYERS OF THE CANINE COLON

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SUMMARY

1. The spontaneous electrical and mechanical activities of the circular and longitudinal muscle layers of the canine colon were studied.

2. The smooth muscle cells of the circular muscle layer exhibited regular, omnipresent myogenic slow-wave activity at a frequency ranging from 4 to 7 c/min. With intracellular micro-electrodes, the slow-wave amplitude was 21–38 mV and its duration 3–6 sec. The 'resting' membrane potential was −60 to −76 mV. Some slow waves had superimposed spike bursts on their peak depolarizations and only these were associated with phasic contractions. It is concluded that they serve a pace-maker function similar to their counterpart in the small intestine.

3. The longitudinal muscle layer exhibited periods of electrical activity alternating with periods of electrical quiescence. During the activity periods electrical oscillations occurred at a frequency of 13–35 c/min with spikes on top of them. Each electrical activity period was associated with a prolonged 'tonic' contraction. The duration of these periods was 30–120 sec and their frequency 0·4–1·1 period/min. This activity is similar to that recorded from the longitudinal muscle of the guinea-pig caecum despite the anatomical differences.

4. The electrical activity periods of the longitudinal muscle appeared to require an excitatory input (stretch and/or acetylcholine release). Provided the strips were not excessively stretched, atropine abolished all electrical and motor activity. Stretching prolonged the electrical activity periods until they eventually fused together and the muscle developed maintained tone.

5. Simultaneously recording from both layers showed that, although electrotonic spread between the two layers is probably insignificant, the activity of the two layers was co-ordinated. Only those slow waves of the circular layer that occurred during the electrical activity periods of the longitudinal layer had superimposed spikes. It is suggested that this co-ordination may indicate that the two muscle layers may be commanded by a common input from periodically active, cholinergic intramural neurones.

It is proposed that the complex patterns of colonic electrical and motor activities may be explained as consisting of two major components: one arising from the longitudinal (long spike bursts, high-frequency oscillations and tonic contractions) and the other from the circular layer (slow waves, short spike and phasic contractions).

7. Simultaneous electrical records from the two muscle layers and the mucosa failed to show a consistent relationship between the mucosal record and the activity of either layer. Caution should be exercised in the interpretation of intraluminally derived electrical recordings.

INTRODUCTION

The patterns of motility in the stomach and small intestine are regulated by omnipresent myogenic slow waves of depolarization of the smooth muscle cell membrane (slow waves, pace-setter potentials or electrical control activity (e.c.a.)). Acting as a pace-maker, they predispose the membrane to another type of activity, the electrical response activity or e.r.a., which triggers muscle contraction (Daniel, 1973; Szurszewski, 1981). In the small intestine, the e.r.a. consists of a burst of spikes occurring on the depolarized phase of the slow wave (El-Sharkawy & Daniel, 1975) while in the non-fundic region of the stomach, it consists of a plateau-type depolarization (plateau potential) which follows the pace-maker potential (initial potential). Spike activity is not seen in the corpus and body of the stomach but in the terminal and pyloric antrum, spike-like oscillations may occur on the plateau potential (El-Sharkawy, Morgan, Szurszewski, 1978; El-Sharkawy & Szurszewski, 1978).

In the colon, the electrophysiological basis of contractile activity is poorly understood. Both slow-wave activity and spike discharges have been recorded from this organ in a number of species including the cat (Christensen, Caprilli & Lund, 1969; Christensen & Hauser, 1971; Wienbeck & Christensen, 1972), dog (El-Sharkawy, 1978; Kocylowski, Bowes & Kingma, 1979), rabbit (Gillespie, 1968; Julé, 1974), mouse (Wood, 1973) and man (Taylor, Duthie, Smallwood & Linkens, 1975; Sarna, Bardakjian, Waterfall & Lund, 1980; Sarna, Waterfall, Bardakjian & Lund, 1981; Sarna, Latimer, Campbell & Waterfall, 1982). However, in most studies the relationship between the slow waves and spike bursts and of both of these to contractions were variable and inconsistent with the pace-maker role advanced for their counterpart in the small intestine. Spike discharge occurs both as short bursts associated with the slow waves and as long bursts spanning a number of slow waves (cat: Christensen, Anuras, & Hauser, 1974; man: Sarna *et al.* 1981, 1982). Furthermore, periods of high frequency electrical oscillations (30–60 c/min) were recorded from both feline (Christensen *et al.* 1969) and human (Sarna *et al.* 1981, 1982) colon. It is thus not surprizing that no conceptual image of the electrophysiological mechanisms controlling colonic motility has emerged.

The purpose of the present study was to test the hypothesis that, unlike the stomach and small intestine where the electrical activity of both muscle layers are similar and in phase, the two muscle layers of the colon have distinctly different patterns of electrical (and motor) activities. This hypothesis, if proven, can explain the multiplicity of the patterns of slow wave and spike activities, the variability of motor patterns, and the lack of consistent relationships between slow waves, spikes and contractions. Preliminary accounts of some aspects of this work have been presented (El-Sharkawy, 1978; El-Sharkawy & MacDonald, 1980; El-Sharkawy, MacDonald & Diamant, 1980; El-Sharkawy, Bardakjian, MacDonald & Diamant, 1982).

METHODS

Tissue preparation

Dogs of either sex were anesthetized using pentobarbitone (35 mg/kg) given intravenously. The colon was exposed by a mid-line incision and three 5-cm-long segments were removed from the proximal, mid- and distal colon. The segments were then placed on cotton-gauze cloth generously moistened with oxygenated Krebs solution and immediately opened flat. Colonic contents were removed as rapidly as possible, taking care to prevent contact of faecal matter with the muscularis propria. The segment was then pinned flat to the Sylgard bottom of a dissecting dish filled with oxygenated Krebs solution and the mucosa and submucosa removed. Strips (5 mm × 2·5–3 cm) of the entire muscularis propria were cut with their long axes parallel either to the circular (transverse

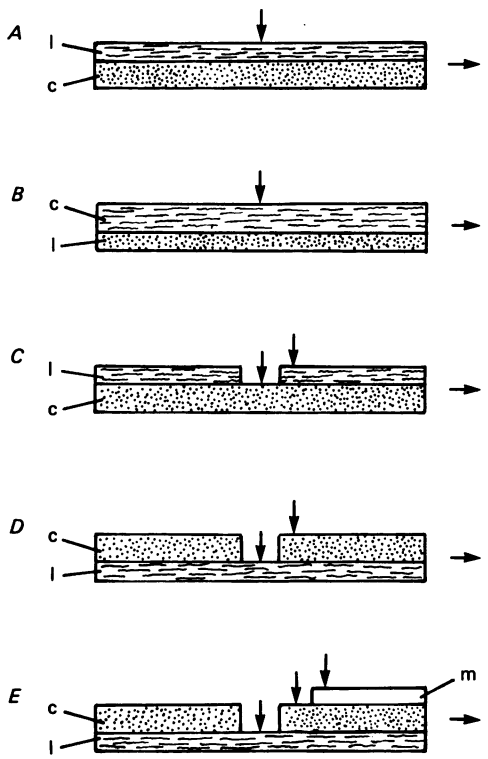

Fig. 1. Diagrammatic representation of vertical sections in the long axes of the preparations used in this study. Symbols l, c and m indicate longitudinal and circular muscle layers, and mucosa respectively. Vertical arrows indicate where suction electrodes were applied and horizonal arrows indicate the direction in which tension was recorded.

strips) or longitudinal (longitudinal strips) muscle bundles. Fine silk threads were then tied to both ends of each strip before mounting in the recording bath. One thread was attached to a micrometer and the other to a force displacement transducer (Grass F.03). In some longitudinal strips, one of the two muscle layers was carefully removed from a small patch in the centre of the strip to allow simultaneous recording of the electrical activity of both muscle layers from the same side of the preparation. Another set of longitudinal strips was prepared before removing the mucosa and, in this set, the mucosa was removed from one half of the strip and a 'window' was made in the exposed circular layer to allow access to the longitudinal layer. Such a preparation was designed to allow simultaneous electrical recording from the mucosa and the two muscle layers. Fig. 1 shows schematic diagrams of these preparations. Unless otherwise indicated in the text, all strips were studied stretched to 120% of the initial length (the maximum length to which a strip could be stretched without developing passive tension). All experiments were performed at 37°C.

Recording techniques

The spontaneous electrical activity was recorded using extracellular monopolar suction electrodes (Bortoff, 1975) connected to the couplers (9853A) of an eight channel ink writing recorder (Beckman, model R-612). The electrical activity was recorded either d.c. or a.c. (time constant = 1 sec) amplified. Muscle contractions were recorded isometrically using force-displacement transducers (Grass, model F.03) with the transducers output recorded on the Beckman Dynograph through model 9853A couplers.

For intracellular recording of the electrical activity of the circular muscle layer, smaller muscle strips (2 × 4 mm) were prepared and pinned to the rubber floor of a continuously perfused muscle chamber with the circular muscle facing upwards. The electrical activity was recorded from single cells in these strips using 3 M-KCl-filled glass micro-electrodes as described previously (El-Sharkawy *et al.* 1978).

Solutions and drugs

A modified Krebs solution was used throughout this study; it had the following composition (mM): Na^+, 137·4; K^+, 5·9; Ca^{2+}, 2·5; Mg^{2+}, 1·2; Cl^-, 134; HCO_3^-, 15·5; $H_2PO_3^-$, 1·2 and glucose, 11·5. It was bubbled with a carbogen gas mixture consisting of 95% O_2 and 5% CO_2 before and during use in the experiments. At 37 °C, this solution had a pH of 7·35–7·4.

The following drugs were used in the study: tetrodotoxin, atropine sulphate, indomethacin, and DL-propranolol, all from Sigma, and phentolamine (a gift from Ciba-Geigy). Drug concentrations reported in the results represent the final molar concentration of the base reaching the tissue.

Data analysis

Throughout this paper the data are presented as the statistical mean ± S.E. of the mean. n represents the number of preparations from which records were analysed statistically.

RESULTS

The circular muscle layer

Electrical activity. In all types of preparations studied, the circular muscle layer exhibited periodic waves of depolarizations (slow waves) occurring in any one preparation at a remarkably regular frequency. This type of activity could be recorded from each and every preparation studied, irrespective of the location along the colon from which it was removed. Their frequency varied somewhat in different preparations ranging from 4·1 to 7·0 (mean = 5·09 ± 0·76, $n = 95$) c/min. No significant difference in the slow-wave frequency was observed between preparations taken from the proximal, mid-, or distal colon. When recorded with intracellular micro-electrodes (Fig. 2*A*1, *A*2) each slow wave consisted of a relatively rapid depolarization followed by a plateau lasting 3–6 sec which was terminated by a slow repolarization. In all cells studied so far ($n = 71$) the period between slow waves remained isopotential. The slow-wave amplitude averaged 26·8 ± 2·3 mV (range 21–38 mV). These arose from a 'resting' membrane potential of 68·9 ± 3·1 mV (range: 60–76 mV). In some cells, especially those with fast rates of depolarization and higher slow-wave amplitude, the wave appeared to consist of two components: the rapid depolarization was followed by partial repolarization before the plateau started. Using extracellular suction electrodes with d.c. amplification (Fig. 2*B*1, *B*2), the slow-wave configuration was identical to that recorded intracellularly although the amplitude was much smaller (1–5 mV). In the circular muscle of dog colon, more than one type of slow wave activity has not been found, and no sign of a fast and slow rhythm, as has been reported in *in vivo* recrds from the human colon (Sarna *et al.* 1980, 1982) has been seen. On a few occasions, however, the records revealed two

populations of cells falling in and out of phase under the suction electrode. When the phase lag between the two populations exceeded the duration of the slow-wave depolarization, the waves of each population occurred during the inter-slow-wave periods of the other giving the impression that the frequency has doubled. In the majority of such situations, and if the signal is d.c. amplified, the activity of the two populations could easily be distinguished by the relative amplitudes of the slow waves (Fig. 2*C*2, *C*3). Fig. 3 illustrates the electrical activity recorded with suction electrodes with a.c. amplification and the associated motor activity of the circular layer in two transverse strips.

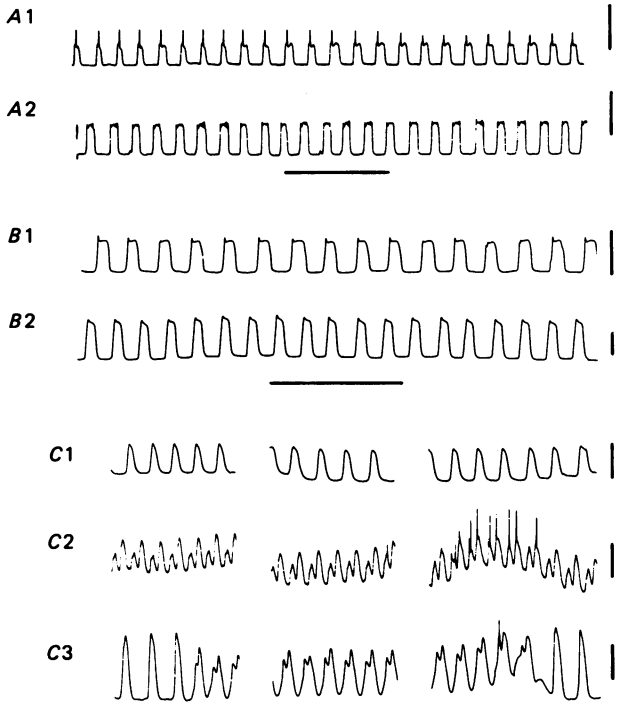

Fig. 2. Electrical activity of smooth muscle cells of the circular muscle layer of canine colon. Records *A*1 and *A*2 were obtained by intracellular micro-electrodes from transverse strips removed 6 (*A*1) and 28 (*A*2) cm from the ileocaecal junction. Records *B*1, *B*2 and *C*1–*C*3 were obtained by monopolar suction electrodes with d.c. amplification. *B*1 and *B*2 are records from strips removed 25 and 3 cm from the ileocaecal junction, respectively. Notice that the configuration of the slow waves recorded with suction electrodes are similar to those recorded intracellularly. Records *C*1, *C*2 and *C*3 represent the activities recorded from three strips removed from adjacent locations in the proximal colon. For each strip, the three traces are parts of a continuous record with the centre and right-hand traces taken 5 and 15 min after the records on the left-hand trace. Notice that while the first strip (*C*1) exhibited synchronous activity, the second (*C*2) and third (*C*3) strips showed asynchronous slow-wave activity. In these two strips the electrodes detected two populations of cells, the slow waves of which were permanently out of phase in the second strip (*C*2) and those of one population occurred during the 'inter-slow-wave period' of the other. In the third step (*C*3), the slow waves of one population fell in and out of phase with those of the other population. Vertical calibration bars are 50 mV for *A*1 and *A*2 (top of bar represents zero potential) and 2 mV for *B*1, *B*2, *C*1, *C*2 and *C*3. Horizontal bars represent 1 min interval.

Spike activity was also recorded from the majority of preparations studied, at least for the first 30–60 min (see below). In preparations where the slow waves were synchronized, spike activity occurred only on the peak depolarizations of the slow waves but not in any other phase of the slow-wave cycle. When present, spikes occurred either as a single spike or a burst of two to several spikes per slow-wave (Figs. 2*A*1 *and* 3). In the few preparations where the slow waves were not synchronized, the relationship between these and the spike potentials was difficult to establish although, on occasions, one could relate spiking to the multiple slow-wave activities detected by the suction electrodes. I have not seen any evidence of loss of synchrony

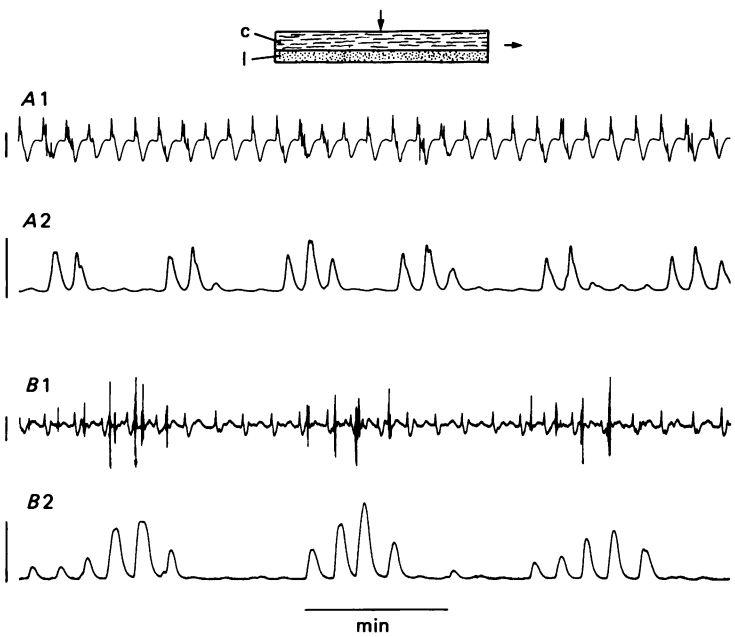

Fig. 3. Electrical (*A*1 and *B*1) and mechanical (*A*2 and *B*2) activities of the circular muscle of two transverse strips from the proximal (*A*) and mid-colon (*B*). Electrical records were obtained from suction electrodes (*A* – *C*, time constant = 1 sec) and calibration bars are 1 mV in *A* and 2 mV in *B*. In *A*2 and *B*2 calibration bars are 5 g.

in preparations studied with intracellular micro-electrodes. The temporal distribution of spike activity exhibited two patterns. In the majority of preparations (69 out of 105), spiking occurred with a remarkable periodicity; runs of two to six slow waves having superimposed spikes alternated with runs of slow waves (also two to six in number) with no spikes (Fig. 3). In the rest of the preparations, spiking, although still associated with the slow waves, occurred more or less randomly. Furthermore, in most preparations, the spike activity gradually declined with time and often completely disappeared within the first hour. This is in contrast to the slow wave activity which remained unaltered throughout the duration of the experiments (5–7 h).

Motor activity. Those preparations which showed electrical activity indicative of good synchrony exhibited phasic contractions in association with those slow waves with superimposed spike activity. The time interval between contractions was equal

to or a multiple integer of the slow-wave cycle time. Although all slow waves with superimposed spikes were associated with contractions, the reverse was not universally true; contractions were occasionally recorded in association with slow waves which did not bear spikes. This is most likely due to the fact that the electrical activity was recorded from a small area of the preparation from which contractile activity was recorded. It is thus possible that in such situations spiking occurred in association with the slow waves generated in other areas.

In the majority of preparations, the phasic contractions occurred with remarkable periodicity governed by the periodicity of spike discharge noted above (Fig. 3). Also, in parallel with the decline of spiking activity (see above), the contractile activity decreased with time and in many cases became undetectable 30–60 min after mounting the preparation. Replacing the Krebs solution in the bath with fresh solution caused the reappearance of spiking and contractions, but these declined again with time.

The longitudinal muscle layer

Electrical activity. The electrical activity recorded from the longitudinal muscle layer of each type of preparation studied was characteristically different from that recorded from the circular muscle layer. The longitudinal muscle exhibited cyclically recurring periods of electrical activity alternating with periods of quiescence (Fig. 4). Each activity period lasted 35–110 sec (mean = 61·2 ± 5·6 sec, $n = 78$) and was followed by a period of 40–100 sec (mean = 57·6 ± 6·9 sec, $n = 78$) of electrical quiescence. During each activity period, slow electrical oscillations occurred initially starting at low amplitude and frequency. As their amplitudes increased, spike activity appeared on their peaks. Towards, the end of the activity period, their amplitudes and frequency diminished rapidly leading to the period of electrical quiescence. The frequency of these electrical oscillations was much more variable than that of the circular muscle slow waves, ranging from 13 to 32 ct/min (mean = 21·3 ± 3·9 c/min, $n = 78$). With extracellular suction electrodes, their amplitudes ranged from 0·1 to 0·8 mV. Such type of activity is similar to that recorded from the longitudinal muscle layer of the guinea-pig caecum (Golenhofen & v. Loh, 1970) and human colon (Duthie & Kirk, 1978; Van Merwyk & Duthie, 1980) where the electrical oscillations were called pace-maker or prepotentials and slow potentials, respectively. Furthermore, with the use of intracellular micro-electrodes, a wave of depolarization lasting throughout the activity period was found to occur in the guinea-pig muscle (Golenhofen & v. Loh, 1970). Such depolarizations are likely to be present in the longitudinal muscle of the dog colon but could not be observed with the extracellular suction electrodes used in the present study. In this paper, the longitudinal muscle oscillations will be referred to as prepotentials to distinguish them from the slow waves of the circular muscle.

Unlike the circular muscle activity, the periodic electrical activity of the longitudinal layer persisted throughout the recording time and no observable decline in spiking activity occurred.

Motor activity. Each period of electrical activity was associated with a 'tonic' contraction (Fig. 4). As the prepotentials started to appear and when they became large enough to produce spike potentials, the longitudinal muscle tension began to develop and increased progressively. In most circumstances, tension appeared to

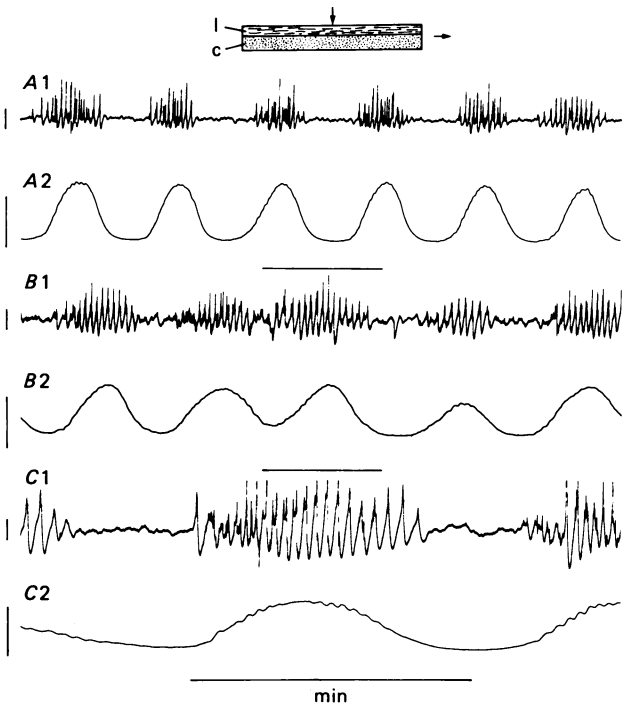

Fig. 4. Electrical (*A*1, *B*1 and *C*1) and motor (*A*2, *B*2 and *C*2) activities of the longitudinal muscle in three longitudinal strips from the proximal (*A*), mid- (*B*) and distal (*C*) colon. Electrical records were from suction electrodes (*A–C*, time constant = 1 sec). Calibrations are 0·5, 0·2 and 0·1 mV for *A*1, *B*1 and *C*1, and 5, 10 and 5 g for *A*2, *B*2 and *C*2, respectively.

increase in steps, each increment of tension being clearly associated with a prepotential and its accompanying spike activity. As the spike activity declined towards the end of the activity period, tension decreased progressively towards its resting level. Prepotentials, when not associated with spike activity, were not associated with tension development.

Co-ordination between the two layers

Since the spike discharges on top of the slow waves in the circular muscle layer, in most preparations, exhibited some periodicity which was temporally similar to the periodicity of the longitudinal muscle activity, it was of interest to determine the relationships between longitudinal and circular muscle activity. This was studied in preparations in which the electrical activity could be recorded simultaneously from both muscle layers (preparations *C–E* in Fig. 1). Additionally simultaneous records were needed to establish whether the observed differences in activity between the two layers were true differences, i.e. did not result from technical problems. Two factors complicated the study of the relationships between the activities of the two layers: (a) in the preparations used in this study, mechanical activity activity could not be recorded simultaneously from the two muscle layers and (b) the spiking and motor activity of the circular layer declined with time. Thus this aspect was examined primarily by studying the electrical activities of both layers in the early part of the

experiments or in the presence of indomethacin (see below). In all three types of preparations, a consistent relationship between the two layers was observed. In the circular layer, spiking activity (and phasic contractions) occurred only in association with those slow waves generated from the circular muscle during the electrical activity periods (and contractions) of the longitudinal muscle as if the two layers were commanded by a common excitatory input. This pattern of co-ordination is illustrated in Fig. 5.

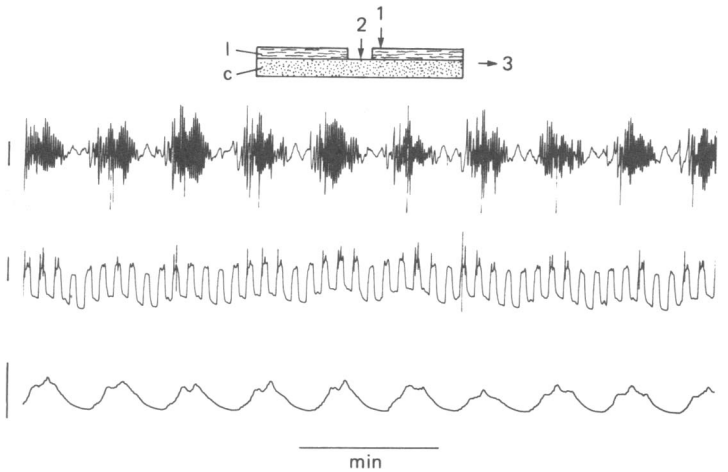

Fig. 5. Electrical activities recorded simultaneously from the circular (c) and longitudinal (l) muscle layers of a type *C* preparation using suction electrodes. Top and middle traces are the electrical activities of longitudinal (*A–C*, time constant = 1 sec, 1 mV calibration) and circular (*D–C*, 0·5 mV calibration) muscle layer, and bottom trace is longitudinal muscle contractions (10 g calibration). Notice that the tension record reflects the contractile activities of the two muscle layers as predicted from the electrical records.

Effects of atropine

Whether spontaneous release of acetylcholine underlies the proposed periodic excitatory input to the two muscle layers was examined by studying the effects of atropine. On circular muscle strips, atropine ($0{\cdot}1 - 1{\cdot}0\ \mu$M) suppressed the spiking activity and contractions but left the slow-wave activity virtually unaltered. The effect on longitudinal muscle varied depending on the length to which the preparation was stretched at the time of atropine application. When the strips were stretched to less than 110% of their initial length, atropine abolished or greatly suppressed the electrical (both prepotentials and spikes) and motor activity. When stretched to longer lengths, the ability of atropine to antagonize longitudinal muscle activity diminished; the more the stretch, the weaker the ability of atropine to antagonize longitudinal muscle activity. Fig. 6 illustrates the effect of atropine on the activity of a longitudinal muscle strip stretched to 105% of its initial length.

Effects of stretch

The dependence of the inhibitory effect of atropine on basal tension of longitudinal muscle prompted us to examine the effect of stretch on the activities of the longitudinal and circular muscle. Stretch was without effect on circular muscle

activity except for an occasional small enhancement of the spiking activity associated with the slow waves. The force of phasic contractions increased progressively as the preparations were stretched to longer lengths (up to 200 % of their initial length). The longitudinal muscle activity responded differently. As the tissues were stretched to longer lengths, the duration of the electrical activity periods increased at the expense of the quiescent periods. The duration of the contractions associated with them was thus prolonged. Finally, at sufficiently high stretch (130–150 % of initial length depending on the preparation) the electrical activity became continuous and contractions fused into a truly tonic contraction. The effects of stretch on the longitudinal muscle activity are illustrated in Fig. 7.

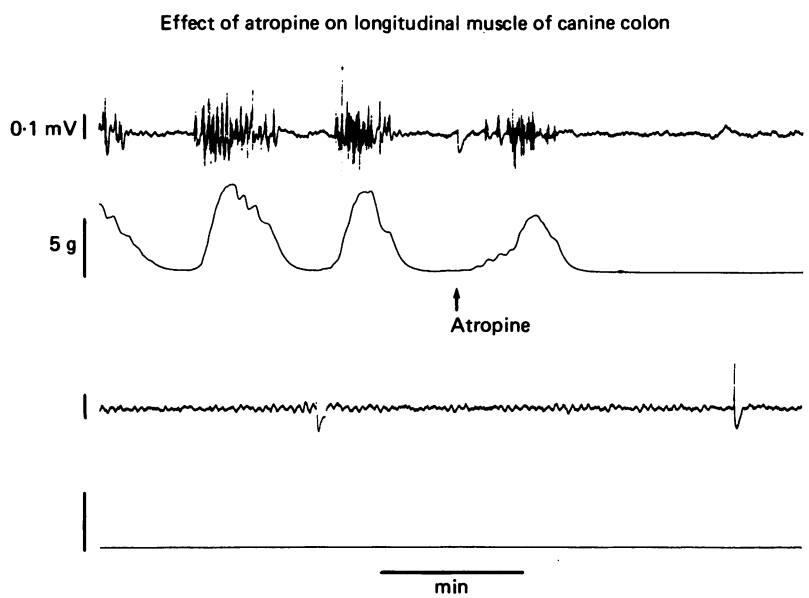

Fig. 6. Effect of atropine (1 μM) on the electrical activity recorded with suction electrodes (top trace) and mechanical activity (botton trace) of the longitudinal muscle layer. The bottom panel is continuous with the top panel.

Effects of tetrodotoxin

Application of tetrodotoxin (0·1–0·5 μM) had an excitatory effect on circular muscle activity. It caused a dramatic enhancement of spiking activity; both in the percentage of slow waves bearing spikes and in the number of spikes associated with the slow waves. In its presence, each slow wave succeeded in initiating a burst of spikes and was associated with a strong phasic contraction. However, it had no significant effect on the slow-wave activity (Fig. 8*A*). On the longitudinal muscle, tetrodotoxin progressively enhanced the amplitude of the prepotentials and increased the period during which they occurred until finally they occurred continuously. This effect was associated with the disappearance of the periodic contractile activity and, in most strips, with an increase in muscle tone (Fig. 8*B*).

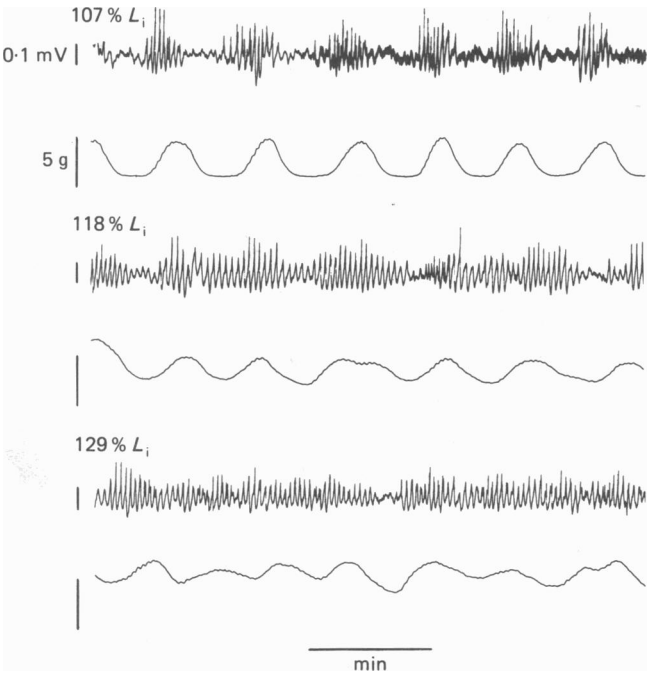

Fig. 7. Effect of stretch on the electrical activity recorded with suction electrodes (top trace) and motor activity (bottom trace) of the longitudinal muscle layer. The lengths to which the strip was stretched are indicated as percent of initial length (L_i).

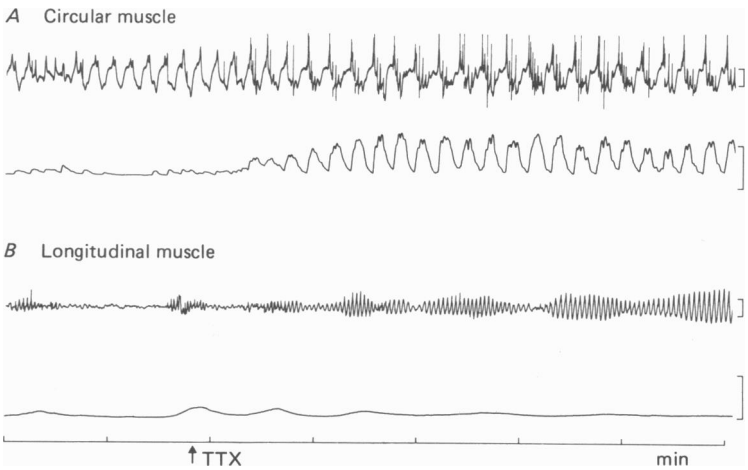

Fig. 8. Effect of tetrodotoxin (TTX) (0·2 μM) on the electrical activities recorded with suction electrodes (top traces) and motor activities (bottom traces) of the longitudinal and circular muscle layers. Vertical calibrations are 0·5 and 0·2 mV for the first and third trace, and 5 and 10 g for the second and fourth trace, respectively.

Effects of adrenergic antagonists

Blockage of α- or β-adrenergic receptors by phentolamine (1 μM) or propranolol (1 μM), respectively, had no effect on the electrical and motor activities of the circular and longitudinal muscle layers (Fig. 9).

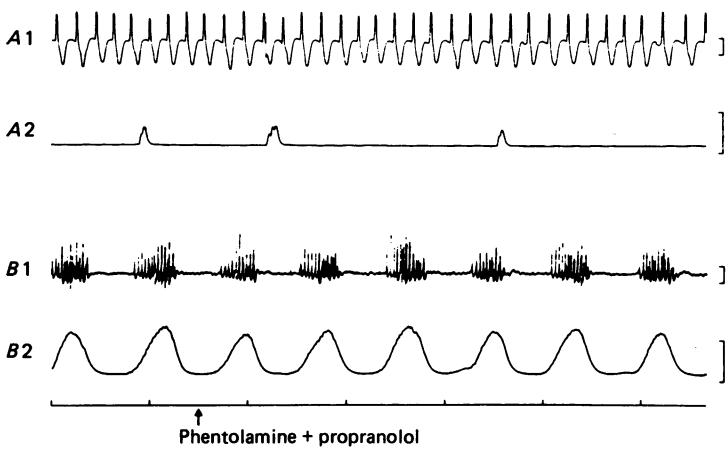

Fig. 9. Effect of propranolol (1 μM) and phentolamine (1 μM) on the electrical and motor activities of the longitudinal and circular muscle layers. Traces *A*1 and *B*1 are the electrical activities recorded from the circular and longitudinal layers with suction electrodes, respectively. Traces *A*2 and *B*2 are the associated contractions.

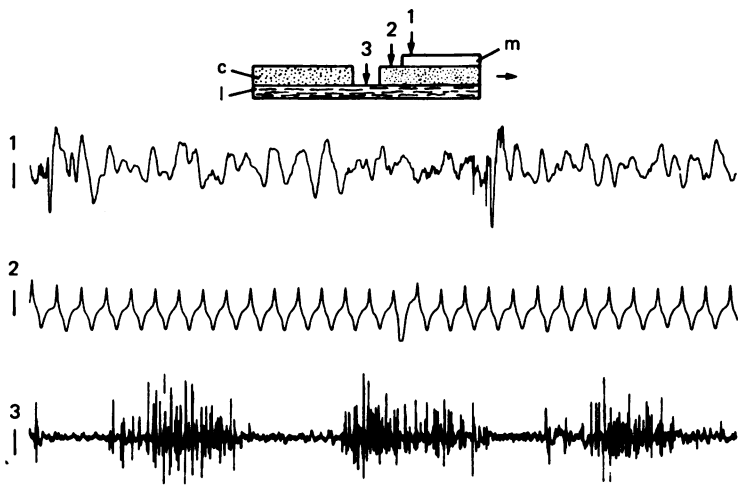

Fig. 10. Simultaneous electrical activities recorded from the longitudinal (trace 3) and circular (trace 2) muscle layers, and the mucosa (trace 1) in a type *E* preparation. Suction electrodes were used (a.c. amplification, time constant = 1 sec). Vertical calibration bars were 0·05 mV in 1, 1 mV in 2 and 0·2 mV in 3. The horizontal bar represents a 1 min interval.

Electrical activity recorded from the mucosa and its relation to muscular activities

Because intraluminal electrodes have been used to obtain information on the electrical activity of the muscle coat of the colon *in vivo* (Taylor *et al.* 1975; Snape, Carlson & Cohen, 1977; Sarna *et al.* 1982), it was of interest to determine the extent to which the electrical activity recorded from the mucosa reflects the activities of the muscle layers. This question was examined in preparations which allowed simultaneous recording from the mucosa and the two muscle layers (preparation *E* in Fig. 1). In

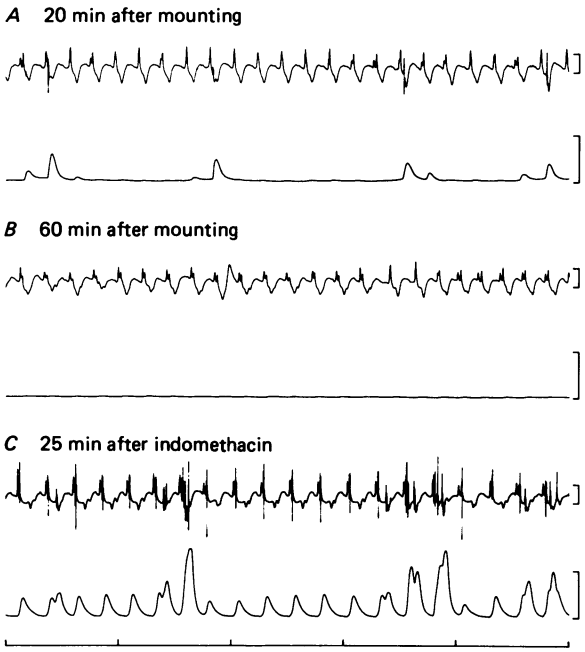

Fig. 11. The decline of spiking activity recorded with suction electrodes (top traces) and contractions (bottom traces) in the circular muscle layer with time and its reversal by indomethacin (15 μM). Vertical calibration bars are 1 mV in top traces and 5 g in bottom traces. Marks on the horizontal line at the bottom indicate 1 min intervals.

such preparations, spontaneous voltage fluctuations could be recorded from the mucosa but these had no obvious relation to the electrical activity recorded from either layer (Fig. 10), even though the electrodes were only 3–4 mm apart.

Effect of indomethacin

Our finding that the spiking and contractile activities of the circular, but not those of the longitudinal, layer declined with time and that this could be reversed by a simple change of the bathing medium led us to suspect that the tissue preparations released a substance inhibitory to the circular, but not to the longitudinal muscle, probably a prostanoid (see Crofts, Stockley & Johnson, 1980). To test this possibility, we studied the effect of indomethacin, an inhibitor of prostaglandin synthesis. When the tissues were dissected and later bathed in Krebs solution containing indomethacin (15 μM), no decline in circular muscle activity occurred even after 5 or 6 hr incubation with no change of solution. Furthermore, when indomethacin was added to the bathing medium after the circular muscle activity had been allowed to decline and the solution changed to a fresh solution containing indomethacin 10–15 min later, intense spiking activity and contractions reappeared in association with the slow waves in the circular muscle (Fig. 11). Indomethacin had little effect on the longitudinal muscle activity.

DISCUSSION

The results of the present study establish that the spontaneous electrical and motor activities of the longitudinal and circular muscle layers of the dog colon are fundamentally different, with the differences of electrical activity underlying the differences in motor activity. Although the electrical activity in both layers consists of pace-maker activity and spike potentials, the nature of pace-maker activity is quite different. In the circular layer, the smooth muscle cells produce omnipresent slow waves at a single frequency varying from 4 to 7 c/min. These waves persisted virtually unaltered in the presence of tetrodotoxin, atropine, phentolamine and propranolol. This suggests that they are myogenic in origin, i.e. they are an inherent property of the smooth muscle cell in this layer. When spikes occur, they invariably occur at or near the plateau phase of the slow wave. This pattern of spike distribution during the slow-wave cycle is similar to that observed in the small intestine (El-Sharkawy & Daniel, 1975) and suggests that they may serve a similar pace-maker function. The contractile activity in this layer consists of phasic contractions associated with those slow waves that initiated spike discharge. In this respect, the circular layer of the colon is similar to the small intestine. The activity of the canine colonic circular muscle reported here is qualitatively similar to that recorded from the circular muscle of cat colon (Christensen *et al.* 1969) and rabbit proximal colon (Gillespie, 1968; Julé, 1974).

In the longitudinal muscle layer, the electrical activity consists of periods of activity (0·5–2 min) alternating with periods of quiescence (also 0·5–2 min). The periods of activity start with slow membrane potential oscillations which rapidly increase in amplitude and frequency and begin to develop spike potentials on their peaks. Towards the end of the period, the amplitude and frequency of the oscillations decline and spikes cease to occur in association with them, ending in a period of quiescence. The motor activity consists of a prolonged contraction associated with, and lasting as long as the period of electrical activity. This is in contrast to the circular layer where each oscillation (slow wave) with associated spikes leads to a phasic contraction. The pattern of electrical and motor activity of the longitudinal muscle is virtually identical to that recorded from the taenia of the guinea-pig caecum (Golenhofen & v. Loh, 1970), pig colon (Huizinga, Diamant & El-Sharkawy, 1982*a*, *b*) and is probably similar to that recorded from the human taenia coli (Duthie & Kirk, 1978; Van Merwyk & Duthie, 1980). In the guinea-pig taenia, intracellular records show that a wave of depolarization occurs during each activity period; the electrical oscillations or prepotentials arising from the depolarized level during this wave. Such waves of depolarization most likely occur in the longitudinal layer of the dog colon but could not be recorded with extracellular electrodes. Intracellular recording from this layer is needed to establish their presence. Thus, it appears that in the longitudinal muscle of the colon, the spontaneous electrical and motor activities are similar in all species studied irrespective of whether this layer is 'taeniated' or not. Waves of membrane depolarization lasting up to several minutes with superimposed prepotentials represent the pace-maker activity in this layer; the prepotentials leading to spike potentials. The effects of atropine and stretch on the longitudinal layer indicate that a depolarizing stimulus (e.g. acetylcholine or stretch) may be required for the prepotentials and the spikes associated with them.

The pharmacological studies indicate that (a) the slow-wave activity of the circular muscle layer is myogenic whereas the pace-maker (prepotential) activity of the longitudinal layer requires an excitatory input (stretch and/or acetylcholine release), (b) the inhibitory innervation to both muscle layers is probably tonically active, and (c) under the *in vitro* conditions of these experiments, the strips release a substance which selectively inhibits spiking and contractions in the circular muscle layer. This substance may be a prostanoid.

The results of this study also suggest that there is no significant propagation of the electrical activity of one layer into the other. The pattern of electrical activity of the longitudinal layer was never observed in records obtained from the circular muscle. The highly attenuated circular muscle slow-wave activity seen in some records obtained with suction electrodes from the longitudinal layer, are most likely due to the electrode having partially penetrated the relatively thin longitudinal layer. The absence of significant electrotonic spread of activity between the two layers is supported by our finding that the motor activity recorded from either layer never reflected the electrical activity of the other. However, the activities of the two layers were co-ordinated. Spike activity (and hence contractions) of the circular layer occurred most commonly on those slow waves generated during the electrical activity period of the longitudinal layer. Conversely, those slow waves recorded from circular muscle during the periods of electrical quiescence were less likely to produce spike potentials. This pattern of co-ordination appears to be mediated by myenteric cholinergic neurones since atropine was found to abolish, or greatly antagonize, the electrical and mechanical activity of the longitudinal layer and to suppress the spike activity and contractions of the circular muscle. These neurones may be spontaneously active in a periodic fashion with their periodicity governing the activation of circular muscle spiking and the generation of longitudinal muscle prepotential and spike activities.

The information obtained in the present *in vitro* investigation may provide an explanation for the variability of the electrical and mechanical activities recorded *in vivo* from the colon in man and other species. The feline (Christensen *et al.* 1974), canine (Kocylowski *et al.* 1979) and human (Sarna *et al.* 1982) colon exhibits both phasic and tonic contractions. In these studies, it was shown that the phasic contractions were related to the electrical slow waves and short spike bursts occurring in association with them while the more prolonged or tonic contractions were accompanied by longer spike bursts spanning a number of slow waves. In the cat these were called migrating spike bursts since they migrated along the colon (Christensen *et al.* 1974). In the human colon and rectosigmoid, Sarna *et al.* (1981, 1982) classified the spike activity patterns into two types: short spike bursts associated with slow waves (discrete electrical response activity, d.e.r.a.) and spike bursts occurring continuously for prolonged periods (continuous electrical response activity, c.e.r.a.). Furthermore, he described periods in which electrical oscillations occurred at a frequency range of 25–40 c/min and reported that these periods were associated with contractions (contractile electrical complex or c.e.c.). Short and long bursts of spike activity were also recorded from pig colon *in vivo* (Fioramonti & Bueno, 1980). Furthermore, in the cat periods of electrical oscillations were recorded. The oscillations occurred at a frequency of 30–60 c/min and occasionally had spikes superimposed on them (Christensen *et al.* 1969). It is tempting to speculate that the complexity of

the *in vivo* recorded colonic activity reflects the distinctly different patterns of activity in the two muscle layers; the gross recording techniques used under such conditions being unable to record selectively from only one of the two layers. In the motility records, phasic contractions may represent activity arising from the circular layer whereas more prolonged and tonic contractions may originate from longitudinal muscle. In the electrograms, the slow waves, and the short spike bursts associated with them, may be the circular muscle component whereas the more prolonged spike bursts (migrating spike bursts, and c.e.r.a.) as well as the high frequency oscillations in the cat and the c.e.c. in man may be the longitudinal muscle component of the electrogram. Finally, in view of our finding that longitudinal muscle activity could not be detected in records obtained from the circular muscle side and that no consistent relationships existed between the activity of either layer and the electrical fluctuations recorded from electrodes applied to the mucosa, caution must be exercised in the interpretation of records obtained from intraluminal electrodes.

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REFERENCES

BORTOFF, A. (1975). Recording of extracellular electrical activity. In *Methods in Pharmacology*, vol. 3, *Smooth Muscle*, ed. DANIEL, E. E. & PATON, D. M., pp. 185–199. New York: Plenum Press.

CHRISTENSEN, J., ANURAS, S. & HAUSER, R. L. (1974). Migrating spike bursts and electrical slow waves in the cat colon: effect of sectioning. *Gastroenterology*, **66**, 240–247.

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

CHRISTENSEN, J. & HAUSER, R. L. (1971). Circumferential coupling of electric slow waves in circular muscle of the cat colon. *Am. J. Physiol.* **221**, 1033–1037.

CROFTS, T. J., STOCKLEY, H. L. & JOHNSON, A. G. (1980). Regulation of human colonic muscle activity by endogenous prostaglandins. In *Gastrointestinal Motility*, ed. CHRISTENSEN, J., pp. 469–472. New York: Raven Press.

DANIEL, E. E. (1973). A conceptual analysis of the pharmacology of gastrointestinal motility. In *International Encyclopedia of Pharmacology and Therapeutics*, Sect. 39a, *Pharmacology of Gastrointestinal Motility and Secretion*. ed. HOLTON, P., pp. 457–545. Oxford: Pergamon Press.

DUTHIE, H. L. & KIRK, D. (1978). Electrical activity of human colonic smooth muscle *in vitro*. *J. Physiol.* **283**, 319–330.

EL-SHARKAWY, T. Y. (1978). Electrophysiological control of motility in canine colon. In *Gastrointestinal Motility in Health and Disease*, ed. DUTHIE, H. L., pp. 387–396. Lancaster: MTP Press.

EL-SHARKAWY, T. Y., BARDAKJIAN, B. L., MACDONALD, W. M. & DIAMANT, N. E. (1982). Origins of the multiple patterns of electrical control activity in the colon. In *Motility of the Digestive Tract*, ed. WIENBECK, M., pp. 491–497, New York: Raven Press.

EL-SHARKAWY, T. Y. & DANIEL, E. E. (1975). Electrical activity of small intestinal smooth muscle and its temperature dependence. *Am. J. Physiol.* **229**, 1268–1276.

EL-SHARKAWY, T. Y. & MACDONALD, W. M. (1980). Electrical and mechanical activities of the longitudinal and circular muscle layers of the canine colon in vitro. *Proc. int. Union. physiol. Sci.* **XIV**, 397.

EL-SHARKAWY, T. Y., MACDONALD, W. M. & DIAMANT, N. E. (1980). Electrophysiological control of motility in longitudinal (LML) and circular (CML) muscle layers of canine colon. *Gastroenterology*, **78**, 1162.

EL-SHARKAWY, T. Y., MORGAN, K. G. & SZURSZEWSKI, J. H. (1978). Intracellular electrical activity of canine and human gastric smooth muscle. *J. Physiol.* **279**, 291–307.

EL-SHARKAWY, T. Y. & SZURSZEWSKI, J. H. (1978). Modulation of canine antral circular smooth muscle by acetylcholine, noradrenaline and pentagastrin. *J. Physiol.* **279**, 309–320.

FIORAMONTI, J. & BUENO, L. (1980). Motor activity in the large intestine of the pig related to dietary fibre and retention time. *Br. J. Nutr.* **43**, 155–162.

FIORAMONTI, J., GARCIA-VILLAR, R., BUENO, L. & RUCKEBUSCH, Y. (1980). Colonic myoelectrical activity and propulsion in the dog. *Dig. Dis. Sci.* **25**, 641–646.

GILLESPIE, J. S. (1968). Electrical activity of the colon. In *Handbook of Physiology*, sect. 6, *Alimentary Canal*, vol. 4, *Motility*, ed. CODE, C. F., pp. 2093–2120. Washington, D.C.: Am. Physiol. Soc.

GOLENHOFEN, K. & v. LOH, D. (1970). Elektrophysiologische Untersuchungen zur normalen Spontanaktivitat der isolierten Taenia Coli des Meerschweinchens. *Pflügers Arch.* **314**, 312–328.

HUIZINGA, J. D., DIAMANT, N. E. & EL-SHARKAWY, T. Y. (1982*a*). Activity of pig colonic muscle and the role of cholinergic innervation. *Fedn Proc.* **41**, 1745.

HUIZINGA, J. D., DIAMANT, N. E. & EL-SHARKAWY, T. Y. (1982*b*). On the nature of the slow wave activity in pig colonic circular muscle. *Dig. Dis. Sci.* **27**, 658.

JULÉ, Y. (1974). Étude in vitro de l'activité electromyographique du colon proximal et distal du lapin. *J. Physiol., Paris* **88**, 305–329.

KOCYLOWSKI, M., BOWES, K. L. & KINGMA, Y. J. (1979). Electrical and mechanical activity in the ex vivo perfused total canine colon. *Gastroenterology* **77**, 1021–1026.

SARNA, S. K., BARDAKJIAN, B. L., WATERFALL, W. E. & LUND, J. F. (1980). Human colonic electrical control activity (ECA). *Gastroenterology*. **78**, 1526–1536.

SARNA, S. K., LATIMER, P., CAMPBELL, D. & WATERFALL, W. E. (1982). Electrical and contractile activities of the human rectosigmoid. *Gut*, **23**, 698–705.

SARNA, S. K., WATERFALL, W. E., BARDAKJIAN, B. L. & LUND, J. F. (1981). Types of human colonic electrical activities recorded postoperatively. *Gastroenterology* **81**, 61–70.

SNAPE, W. J., CARLSON, G. M. & COHEN, S. (1977). Human colonic myoelectric activity in response to prostigmine and the gastrointestinal hormones. *Am. J. dig. Dis.* **22**, 881–887.

SZURSZEWSKI, J. H. (1981). Electrical basis for gastrointestinal motility. In *Physiology of the Gastrointestinal Tract*, ed. JOHNSON, L. R., pp. 1435–1466. New York: Raven Press.

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

VAN MERWYK, A. J. & DUTHIE, H. L. (1980). Characteristics of human colonic smooth muscle in vitro. In *Gastrointestinal Motility*, ed. CHRISTENSEN, J., pp. 473–479. New York: Raven Press.

WIENBECK, M. & CHRISTENSEN, J. (1972). Electromyography of the colon in the unanesthetized cat. *Am. J. Dig. Dis.* **17**, 356–362.

WOOD, J. D. (1973). Electrical activity of the intestine of mice with hereditary megacolon and absence of enteric ganglion cells. *Am. J. Dig. Dis.* **18**, 477–488.