*J. Physiol.* (1986), **372**, *pp.* 501–520
*With* 10 *text-figures*
*Printed in Great Britain*



# ELECTROPHYSIOLOGY OF SMOOTH MUSCLE OF THE SMALL INTESTINE OF SOME MAMMALS

BY Y. HARA*, M. KUBOTA AND J. H. SZURSZEWSKI

*From the Departments of Physiology and Biophysics and Pharmacology, Mayo Medical School, Rochester, MN 55905, U.S.A.*

(*Received 10 April 1985*)

## SUMMARY

1. Intracellular recordings were made from cells located in the longitudinal, inner and outer circular muscle layers of the dog, cat, rabbit, opossum and human small intestine.

2. In whole-thickness preparations in all five species, longitudinal muscle cells generated slow waves and spikes. However, in isolated longitudinal muscle preparations, all cells tested were electrically silent.

3. In whole-thickness and in isolated preparations, cells in the inner circular muscle layer generated spontaneous spikes superimposed on slow potentials. However, the occurrence of spikes and slow potentials was more regular in whole-thickness preparations.

4. In whole-thickness preparations, cells in the outer circular muscle layer generated slow waves which were coupled with phasic contractions. However, in isolated outer circular muscle preparations, all cells tested were electrically silent and spontaneous phasic contractions were absent.

5. In whole-thickness preparations, non-neural cells located on the serosal side of the outer circular muscle layer generated slow waves.

6. The data suggest that spontaneous slow waves of the small intestine of the dog, cat, rabbit, opossum and human are generated in non-neural cells located between the longitudinal and outer circular muscle layer and by non-neural cells located between the outer and inner circular muscle layers.

## INTRODUCTION

The membrane properties of longitudinal and circular smooth muscle of the canine stomach (El-Sharkawy, Morgan & Szurszewski, 1978; Morgan & Szurszewski, 1980; Morgan, Muir & Szurszewski, 1981) and canine large intestine (El-Sharkawy, 1983) have been described. The electrical activity of longitudinal and circular smooth muscle layers of the canine small intestine have been studied extensively but with extracellular recording techniques (see Szurszewski, 1981). A single exception is the

* Present address: Hamanomachi Hospital, Surgery Unit, Maizuru 3-5-27, Chuoku, Fukuoka City 810, Japan.

report of a few intracellular recordings made *in vivo* where impalements could be maintained for limited periods of time (Daniel, Honour & Bogoch, 1960). The results from this latter study suggest that both longitudinal and circular smooth muscle layers are required for generation of slow waves, a situation which, if correct, would be different from the cat small intestine where it has been suggested that slow waves are generated only by the longitudinal muscle layer (Bortoff, 1961; Bortoff, 1965; Kobayashi, Nagai & Prosser, 1966; Bortoff & Sachs, 1970; Connor, Prosser & Weems, 1974; Connor, Kreulen, Prosser & Weiger, 1977; Bortoff, Michaels & Mistretta, 1981).

Little is known regarding the types of intracellular potential changes in the longitudinal and circular smooth muscle layers of the canine small intestine. The circular muscle layer can be subdivided into an inner and outer layer (Li, 1937, 1940; Gabella, 1972; Thuneberg, 1982). In the dog small intestine, the inner layer is the thinner of the two, consisting of ten to twenty cells (Duchon, Henderson & Daniel, 1974). We are unaware of any detailed studies which have characterized the intracellular potentials of these different layers of circular muscle.

The present study describes the electrical activity of the longitudinal, inner and outer circular muscle layers of the canine jejunum, and differentiates between activity which is characteristic of each layer in the intact intestine and of each layer in isolation. It will be shown that the electrical basis for contraction in the inner circular and longitudinal muscle layers is spike-dependent whereas it is spike-independent in the outer circular muscle layer. During the course of this study it became apparent that slow waves did not originate in the longitudinal muscle layer of the dog. Thus the study was expanded to include the small intestine of the cat, rabbit, opossum and human. Evidence will be offered in support of the hypothesis that spontaneous slow waves in the intestine of these mammals also originates in non-neural tissue located on the serosal surface of the outer circular muscle layer and in non-neural tissue located between the inner and outer circular muscle layers.

A prelimary report of this study has been published (Hara & Szurszewski, 1981).

## METHODS

### *Preparation of canine muscle*

Sixty-one dogs (10–22 kg) of either sex were used. A segment of jejunum (35 cm distal to the gastroduodenal junction) was removed and a piece of external muscularis with attached mucosa was dissected from the antimesenteric border and pinned to a transparent rubber floor of a dissecting dish filled with Krebs solution. With the aid of a dissecting microscope, the mucosa and submucosa were carefully dissected away, taking care not to damage the inner layer of circular muscle. Strips of muscle (2 × 6 mm) were dissected and pinned to the floor of a Sylgard-coated recording chamber. For simultaneous measurement of tension and intracellular potential, a 2 × 2 mm area of one end of the strip was securely pinned to the floor of the chamber for intracellular impalement. The remaining portion of the muscle strip (2 × 4 mm) was left unpinned and attached to a force transducer. Glass micro-electrodes filled with 3 M-KCl and having resistances ranging from 40 to 80 MΩ were used to measure membrane potential intracellularly. The standard electrophysiological techniques employed throughout have been described previously (El-Sharkawy *et al.* 1978). The criteria used to judge an impalement as successful were the same as those listed by Kao & Nishiyama (1964). In preparations which consisted of both muscle layers, the hook attached to the force transducer was passed through the muscle layer from which intracellular recordings were made. A variety of different preparations were prepared surgically. They varied with regard to their thickness, orientation of the individual muscle layers to the long axis of the muscle strip and to

whether the serosal or mucosal side faced upward. The type of preparation used in any particular experiment is schematically identified in each Figure illustrating voltage and tension recordings. The location of the tip of the micro-electrode in each schematic diagram indicates the muscle layer from which the intracellular recording was made. The location of the bracket indicates the muscle layer to which the force transducer was attached. In order to record intracellularly from cells located on the serosal side of the outer circular muscle, overlying bundles of longitudinal muscle were carefully spread apart in a small region, thereby exposing these cells. The tip of the micro-electrode was placed on the surface of the exposed outer circular muscle and without further advancement 'tapped' into cells. Recording from cells in this region was difficult. The number of successful impalements of these cells was the lowest and the duration of impalements the shortest. Values for resting membrane potential for all cells tested were determined upon withdrawal of the electrode from the cell.

*Preparations of cat, rabbit, opossum and human muscle*

Ten male cats (2·5–3·5 kg), five male rabbits (2·5–3·5 kg) and four male opossums (2·5–3 kg) were used. A segment of jejunum from each species was removed 20–25 cm distal to the gastroduodenal junction. The dissection procedures and the techniques employed to record simultaneously tension and intracellular electrical activity were similar to those described above. Normal human muscle from the lower jejunum and ileum were obtained from patients undergoing small bowel surgery. The tissue used in this study was removed normally during the course of the prescribed surgical procedure; it did not entail excising any additional muscle, or cause any additional disadvantage, inconvenience or risk to the patient. Use of such tissue was approved by the Institutional Review Board. Preparation of strips of human muscle was similar to that described above with the exception that preparations of isolated inner circular muscle were not used.

*Solution and drugs*

Krebs solution at 37 ± 0·5 °C flowed through the chamber at 8–11 ml/min. The solution contained (mM): $Na^+$, 137·4; $K^+$, 5·9; $Ca^{2+}$, 2·5; $Mg^{2+}$, 1·2; $Cl^-$, 134; $HCO_3^-$, 15·5; $H_2PO_4^-$, 1·2; dextrose, 11·5; was equilibrated with a 97 % $O_2$ and 3 % $CO_2$ gas mixture and had a pH of 7·3–7·4.

The following drugs were used (concentrations in the text refer to bath concentrations): acetylcholine hydrochloride (Sigma), atropine sulphate (Sigma), hexamethonium bromide (Sigma), ± phentolamine tosylate (Ciba), ± propranolol hydrochloride (Sigma) and tetrodotoxin (Sigma).

*Histology*

Strips of muscle were dissected as described above, fixed in buffered formalin for 24 h and blocked in paraffin. Sections 6 μm thick were cut and stained using hematoxylin–eosin and the trichrome–Masson techniques. A number of preparations which consisted of isolated longitudinal muscle were stained with Methylene Blue (0·03 %) following the particular experiment to check for the presence of neural tissue.

*Statistics*

Student's paired *t* test was used to test for significance of differences between groups of data.

## RESULTS

### *Histology*

Muscle strips that were used for histological studies were selected at random from strips prepared daily for the physiological studies described below. In all, twenty-six strips of dog small intestine were examined. The circular muscle layer of the dog small intestine could be subdivided anatomically into an inner thin layer and an outer thick layer as described in other species (Cajal, 1893; Li, 1937, 1940; Gabella, 1972, 1974; Duchon *et al.* 1974; Thuneberg, 1982). The inner thin layer ranged in thickness from 85 to 285 μm. The outer layer ranged from 710 to 1080 μm. These two layers were separated from each other by connective tissue containing the plexus muscularis profundis.

TABLE 1. Electrical parameters measured intracellularly in the jejunum of the dog

| | Maximum membrane potential (mV) | Frequency (cycles/min) | Amplitude of slow wave or potential* (mV) | Total rate of rise of slow wave (V/s) |
|---|---|---|---|---|
| Whole-thickness preparations | | | | |
| Longitudinal ($n = 41$) | $-59{\cdot}8 \pm 2{\cdot}5$ | $13{\cdot}0 \pm 0{\cdot}9$ | $3{\cdot}3 \pm 0{\cdot}7$ | — |
| Circular | | | | |
| Superficial–outer ($n = 50$) | $-59{\cdot}8 \pm 2{\cdot}5$ | $13{\cdot}0 \pm 0{\cdot}9$ | $17{\cdot}5 \pm 1{\cdot}3$ | $0{\cdot}05 \pm 0{\cdot}01$ |
| Deep–outer ($n = 70$) | $-60{\cdot}7 \pm 2{\cdot}3$ | $13{\cdot}0 \pm 1{\cdot}0$ | $19{\cdot}0 \pm 2{\cdot}6$ | $0{\cdot}05 \pm 0{\cdot}01$ |
| Inner ($n = 34$) | $-50{\cdot}8 \pm 2{\cdot}6$† | $13{\cdot}0 \pm 1{\cdot}0$ | $17{\cdot}4 \pm 2{\cdot}1$* | — |
| Isolated preparation | | | | |
| Longitudinal ($n = 39$) | $-61{\cdot}3 \pm 2{\cdot}7$ | 0 | 0 | 0 |
| Inner circular ($n = 34$) | $-52{\cdot}5 \pm 4{\cdot}0$ | Range: 0–18 | Range: 0–10* | Range: 0·0–0·01 |
| Outer–deep circular ($n = 84$) | $59{\cdot}7 \pm 2{\cdot}6$ | 0 | 0 | 0 |
| Partial-thickness preparations | | | | |
| *Outer*† circ. and long. ($n = 24$) | $-56{\cdot}1 \pm 2{\cdot}9$ | $12{\cdot}9 \pm 1{\cdot}2$ | $5{\cdot}2 \pm 2{\cdot}2$ | $0{\cdot}05 \pm 0{\cdot}01$ |
| *Outer*† and inner circ. ($n = 44$) | $-59{\cdot}7 \pm 2{\cdot}6$ | Range: 1–10 | Range: 0–15 | Range: 0·0–0·003 |
| *Inner*† and outer circ. ($n = 11$) | $-51{\cdot}9 \pm 4{\cdot}0$ | Range: 1–18 | Range: 0–12* | — |

* $P < 0{\cdot}01$ compared to all other values for maximum membrane potential.
† Italicised, layer from which recordings were made.
Circ., circular; long., longitudinal.

Circular muscle of the cat, opossum and rabbit small intestine were also examined. An inner circular muscle was present in the rabbit (three of five) and opossum jejunum (two of five). The thickness ranged from 34 to 101 $\mu$m. In the cat, the inner circular muscle in four preparations ranged from 225 to 315 $\mu$m.

Five strips of isolated longitudinal muscle from dog and three strips from cat intestine were examined for evidence of completeness of removing all circular muscle fibres. In all preparations there was no adherent circular muscle. Auerbach's plexus was removed as revealed by Methylene Blue staining. Seven strips of whole thickness circular muscle from dog and four strips from cat intestine were examined after surgical removal of the longitudinal muscle layer. All preparations were devoid of longitudinal muscle and the superficial layer of the outer circular muscle cells was damaged as evidenced by numerous nicks, cuts and tears. Thus we were unable to spare the integrity of the superficial cells of the outer circular muscle when surgically removing the longitudinal muscle layers.

*Electrophysiological recordings from canine jejunum*

Values for membrane potential and for some characteristics of slow waves, slow potentials and spikes in the different layers of the canine jejunum are summarized in Table 1. None of the electrical characteristics presented in Table 1 were affected by atropine, hexamethonium, phentolamine, propranolol and tetrodotoxin (each $10^{-6}$ M).

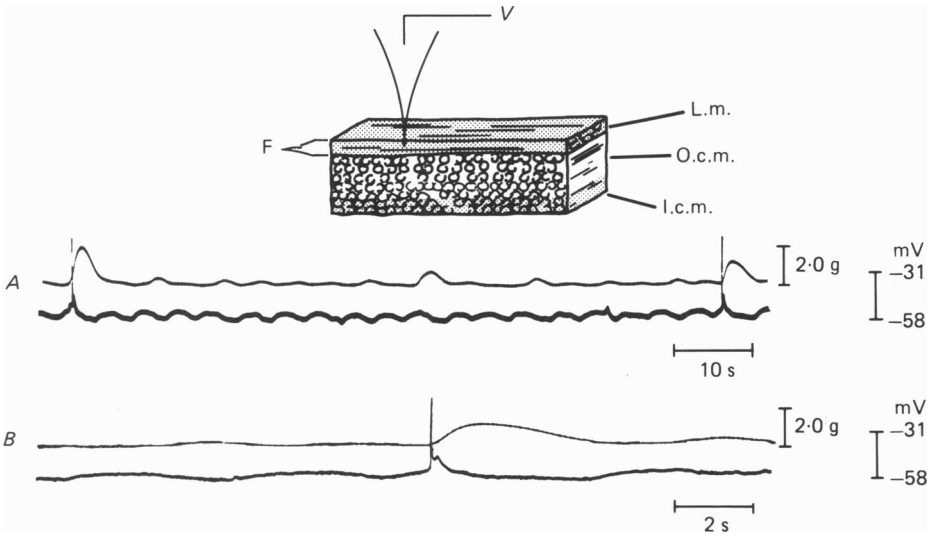


Fig. 1. Spontaneous activity in longitudinal muscle of a whole-thickness preparation of the dog jejunum. Traces in panel *B* made at a faster time base compared to those in panel *A*. In each panel, top trace contraction, bottom trace electrical record. In this and some of the following illustrations the following abbreviations are used: L.m., longitudinal muscle; O.c.m., outer circular muscle; I.c.m., inner circular muscle; F, Force transducer.

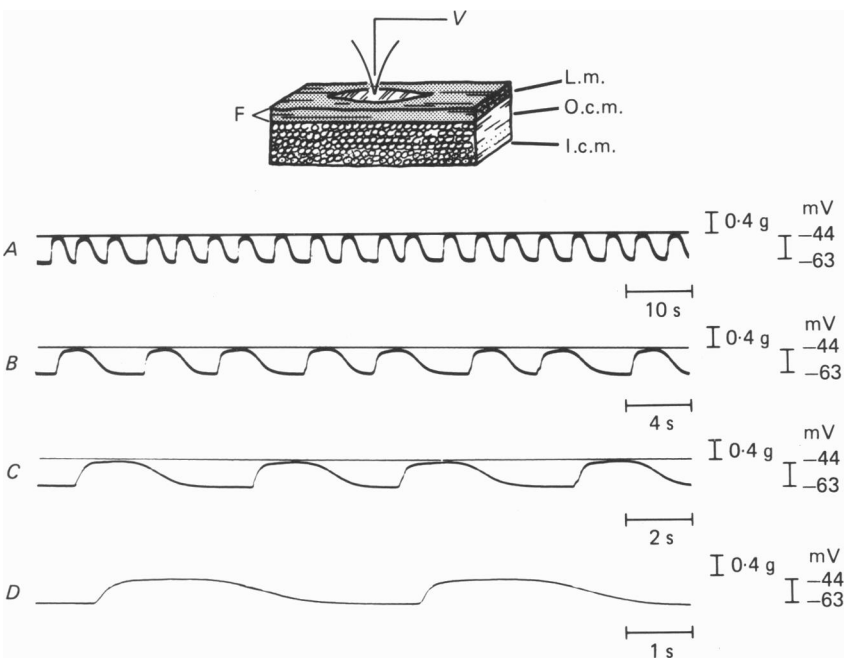


Fig. 2. Spontaneous activity recorded from a cell on the serosal side of the outer circular muscle of a whole-thickness preparation of the dog jejunum. Serosal side of the outer circular muscle was exposed by gently separating overlying bundles of longitudinal muscles. Traces in panels *B–D* made at successively faster time bases compared to those in panel *A*. In each panel, top trace contraction, bottom trace electrical record.

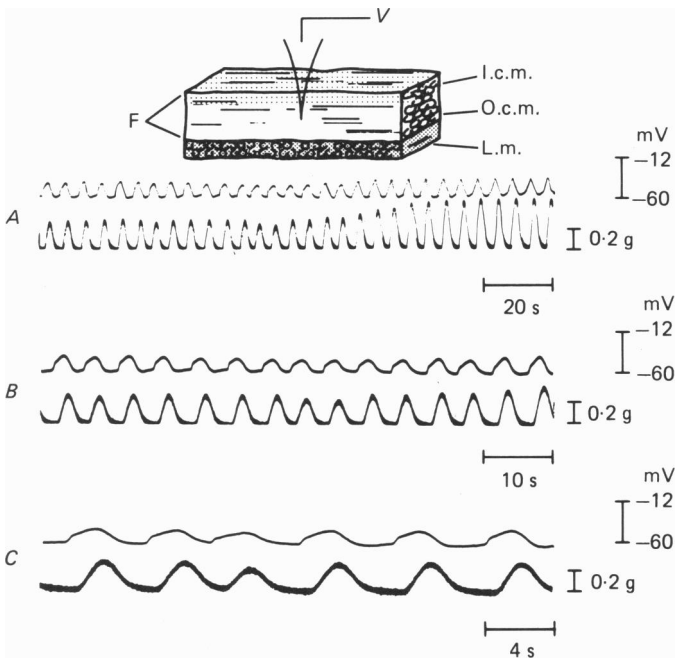


Fig. 3. Spontaneous activity recorded in outer circular muscle of a whole-thickness preparation of the dog jejunum. Traces in panels *B* and *C* made at successively faster time bases compared to those in panel *A*. In each panel, top trace electrical record, bottom trace contraction.

*Whole-thickness preparations*

*Membrane potential.* The maximum membrane potential recorded between spontaneous electrical activity was least negative in the inner circular muscle (Table 1). The maximum membrane potential of any particular muscle layer was similar when it was part of an intact whole-thickness preparation, part of a partial-thickness preparation or isolated from adjacent muscle layers (Table 1).

*Slow waves and slow potentials.* Slow changes in membrane potential which always carry spike potentials have been referred to as slow potentials (Kuriyama, Osa & Toida, 1967), whereas changes in membrane potential which do not always carry spike potentials have been referred to as slow waves (Bolton, 1971). This terminology will be used in the present report. In intact whole-thickness preparations, slow waves were distinguishable in longitudinal and outer circular muscle layers and in superficial cells located on the serosal side of the outer circular muscle layer. In the inner circular muscle layer of whole-thickness preparations, all muscle cells tested generated slow potentials which carried a burst of spikes. Typical examples of simultaneously recorded electrical and mechanical activity in the different muscle layers of intact whole-thickness preparations of the jejunum are shown in Figs. 1–4. In the longitudinal layer, slow waves without spikes consisted of a monotonic voltage change, whereas in the outer circular muscle slow waves consisted of two or more voltage changes differing from one another in rate of rise (Figs. 1–4). A comparison of slow waves recorded from different layers of the same intact preparation is shown in Fig. 5.

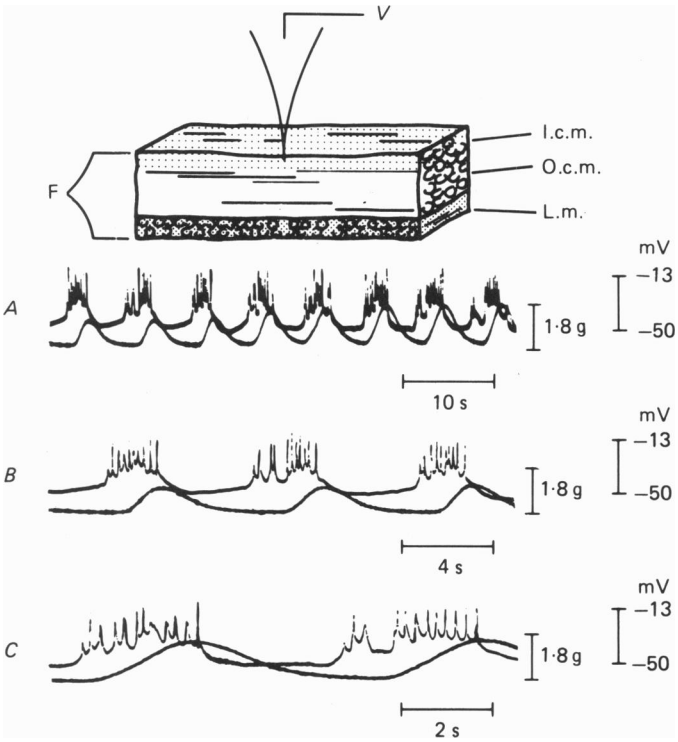


Fig. 4. Spontaneous activity recorded in inner circular muscle of a whole-thickness preparation of the dog jejunum. Traces in panels *B* and *C* made at successively faster time bases compared to those in panel *A*. In each panel, top trace electrical record, bottom trace contraction.

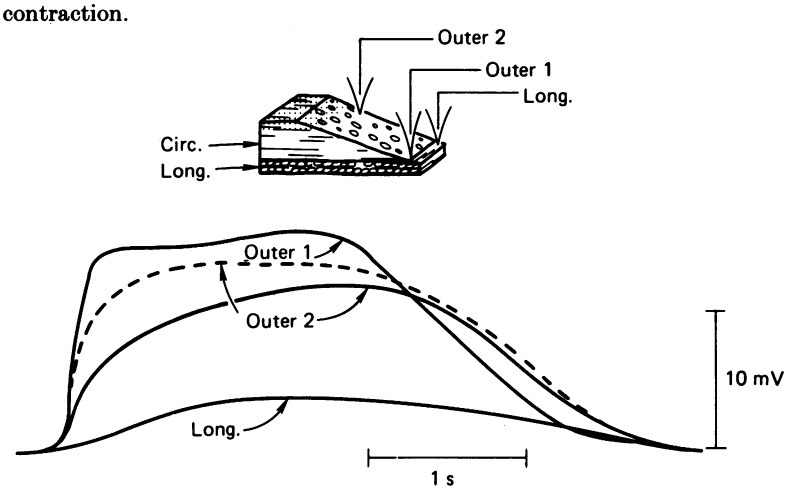


Fig. 5. Comparison of slow waves recorded in longitudinal and outer circular muscle layers of the dog jejunum. All recordings were made from the same preparation. Slow wave labelled Outer 1 was recorded from a cell located on serosal side of the outer circular muscle layer. Slow waves labelled Outer 2 were recorded from the same muscle cell in the outer circular muscle cell but at different times. Circ., circular; Long., longitudinal. For additional details see text and Table 1.

Compared to other layers of the intestinal wall, the maximum rate of rise of the slow wave was slowest in longitudinal muscle cells (Fig. 5 and Table 1). In the outer circular muscle layer, slow waves had a relatively rapid initial rate of rise which gave way to a slower developing depolarization. In superficial cells on the serosal side of the outer circular muscle layer the slower depolarization reached a plateau (Fig. 5, Outer 1), whereas in deeper cells of the outer circular muscle the sustained depolarization varied in amplitude and duration (Fig. 5, Outer 2). The total rate of rise of slow waves measured from the base line to the initial peak was obtained for each layer (see Table 1). The total rate of rise of slow potentials in inner circular muscle was not determined because each slow potential was initiated by a spike-like potential (cf. Fig. 4).

The total amplitude of slow waves was smallest in longitudinal muscle. In cells deep within the outer circular muscle, the total amplitude at times varied during the recording period (cf. Figs. 3 and 5). Variation in slow-wave amplitude was associated with changes in the amplitude of the phasic contraction (Fig. 3). The frequency of slow waves and of slow potentials which carried bursts of spikes (inner circular muscle layer) were similar (Table 1). The frequency of activity in each cell was obtained by determining the inter-slow-wave or burst interval during at least 10 min of continuous recording. The mean frequency observed *in vitro* in this study corresponds well with the mean frequency recorded *in vivo* from the whole wall of the transected small intestine (Code & Szurszewski, 1970).

*Spikes.* In intact whole-thickness preparations, spontaneous spikes were recorded from longitudinal and inner circular muscle layers. In longitudinal muscle, spike potentials occurred singly and in bursts on the peak of slow waves. Spike amplitudes ranged from 45–54 mV. Rarely, they crossed zero potential. When a spike occurred the amplitude of the resulting phasic contraction was larger than when there were no spikes (Fig. 1). In the inner circular muscle layer spike potentials always occurred in bursts on top of slow potentials in all cells tested ($n = 34$). In 51 % of all bursts recorded in the inner circular muscle layer ($n = 881$) a slow diastolic-type depolarization preceded each slow potential (Fig. 4). Within the same cell, the number of spikes in each burst varied but the interval between the leading edge of successive slow potentials was regular, never exceeding 8 % of the average interval between slow potentials. The amplitude of individual spikes measured from the base of the underlying slow potential to the peak of the spike ranged from 20 to 34 mV. Phasic contractions were coupled to each burst. Although not analysed in detail, the amplitude of contraction appeared to depend upon the number of spikes.

Spike potentials were not recorded from cells located either deep within ($n = 148$) or on the serosal side ($n = 72$) of the external circular muscle. However, phasic contractions and slow waves of the outer circular muscle were coupled.

*Isolated preparations*

*Longitudinal muscle.* None of the muscle cells tested in preparations of isolated longitudinal muscle ($n = 44$ preparations) exhibited spontaneous electrical activity. However, slow potentials, spikes and phasic contractions could be induced by acetylcholine. The effect of acetylcholine in concentrations ranging from $10^{-7}$ to $5 \times 10^{-6}$ M was tested in twenty-seven cells in twenty preparations. In all cells tested

acetylcholine caused slow potentials, spikes and phasic contractions. A complete analysis of the effect of acetylcholine on preparations of isolated longitudinal muscle is described elsewhere (Hara & Szurszewski, 1986).

*Inner circular muscle.* Successful impalement of muscle cells in preparations of isolated inner circular muscle was made in thirty-four cells from twenty preparations. In all cells tested, spontaneous activity was recorded. However, in marked contrast to recordings from cells in inner circular muscle of whole-thickness preparations (cf. Fig. 4), spontaneous activity was irregular in occurrence and consisted of spike potentials which occurred either singly or in bursts and often without a slow potential.

*Outer circular muscle.* Preparations of isolated outer circular muscle were obtained by removing the inner circular muscle layer and the longitudinal muscle layer together with Auerbach's plexus and a thin layer of muscle from the serosal side of the outer circular muscle. Intracellular recordings were made from eighty-four cells in twenty preparations. No spontaneous activity was recorded in any of the cells tested. However, in the presence of acetylcholine ($10^{-5}$ M), slow waves and phasic contractions were recorded in all cells tested ($n = 21$). Typically, acetylcholine depolarized the membrane by 6–11 mV and induced slow waves. The frequency and amplitude of these slow waves was 6–11/min and 18–26 mV, respectively. Each acetylcholine-induced slow wave caused a phasic contraction even though spike potentials were absent. A complete analysis of the effect of acetylcholine on this muscle layer is described elsewhere (Hara & Szurszewski, 1986).

*Partial-thickness preparations*

In five experiments, recordings were made from longitudinal muscle attached to a thin layer of outer circular muscle. In these preparations, the thickness of the remaining outer circular muscle ranged from 82 to 168 $\mu$m as determined histologically. A total of nineteen longitudinal muscle cells were tested in these five preparations. All cells tested in the longitudinal layer generated spontaneous slow waves with characteristics similar to those observed in longitudinal muscle cells of whole-thickness preparations. An example of slow waves recorded from a cell in the longitudinal muscle layer in one of these preparations is shown in Fig. 6*A*. In three of these preparations, bundles of longitudinal muscle were carefully spread apart in a small area, exposing the serosal side of the underlying outer circular muscle. Spontaneously occurring slow waves were recorded in four of four cells. An example of slow waves recorded from one of these cells is shown in Fig. 6*B*. Note that the slow waves were similar in appearance to those recorded from superficial cells located on the serosal side of the outer circular muscle of whole-thickness preparations (cf. Fig. 2). Finally, in two of these three preparations, the longitudinal muscle layer was very carefully teased away bundle by bundle under microscopic control, leaving a preparation which consisted of the superficial cells of the outer circular muscle. Intracellular recordings were especially difficult to achieve. However, in four of four cells tested, slow waves were recorded. An example of one of the recordings is shown in Fig. 6*C*. Note that the configuration and frequency of slow waves are similar to those illustrated in Fig. 6*B*.

In twenty preparations, intracellular recordings were made from muscle cells located in the outer circular muscle layer with either the longitudinal or the inner

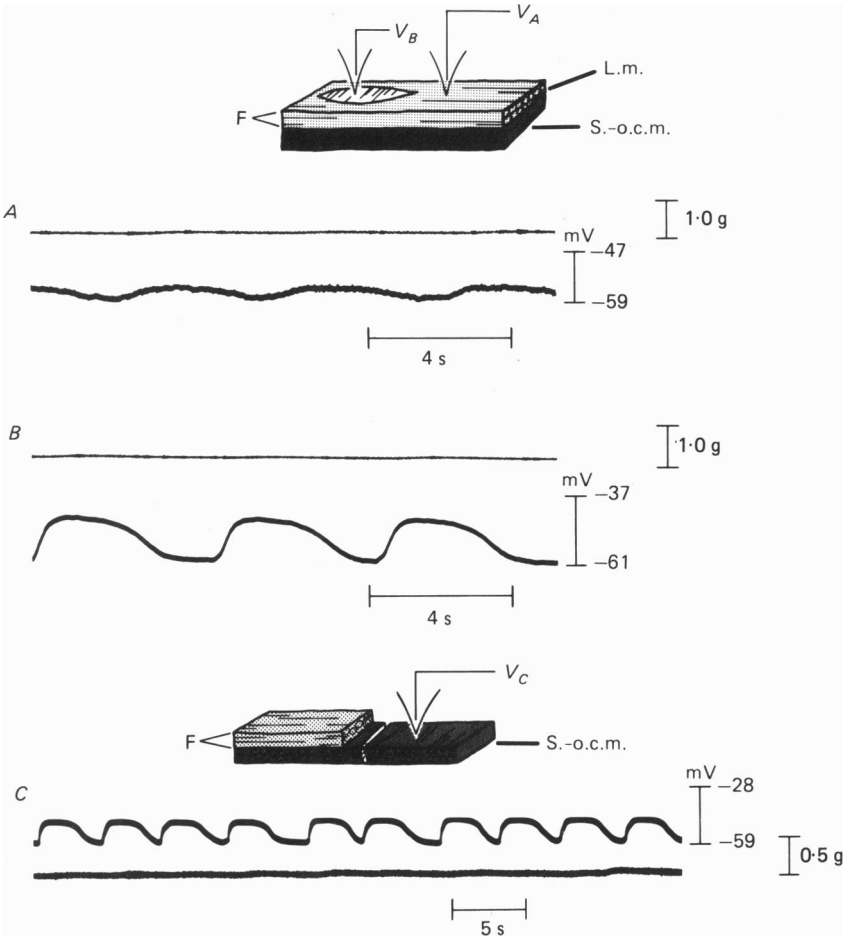


Fig. 6. Spontaneous mechanical and intracellular electrical activity recorded from two layers of the jejunum. In the top schematic, the location of the tip of micro-electrodes $V_A$ and $V_B$ indicates the muscle layer from which the voltage traces in panels *A* and *B*, respectively, were made. The voltage recording from $V_A$ (panel *A*) was made from a muscle cell in the longitudinal layer whereas the voltage recording from $V_B$ (panel *B*) was made from a cell located on the surface of the outer circular muscle. In panels *A* and *B*, force was recorded from the longitudinal muscle layer. In the bottom schematic, the tip of micro-electrode $V_C$ was in a cell located on the serosal side of a thin layer of outer circular muscle. The voltage trace in panel *C* was obtained by $V_C$. Note that in the bottom schematic, the longitudinal muscle layer was removed from the right half of the strip and that a cut was made in the middle of the strip and through its thickness thereby completely separating the right half from the left half. Thickness of the remaining outer circular muscle was 108–116 $\mu$m. All recordings were made from the same preparation. In each panel, top trace electrical record, bottom trace contraction. L.m., longitudinal muscle. S.–o.c.m., superficial cells on serosal side of outer circular muscle. For additional details see text.

TABLE 2. Electrical parameters measured intracellularly in the jejunum of four species

| | Cat | Opossum | Rabbit | Human |
|---|---|---|---|---|
| Intact muscle | | | | |
| Longitudinal | | | | |
| R.m.p. (mV) | −61·4 ± 2·4 | −58·5 ± 2·2 | −58·5 ± 4·0 | −64·8 ± 3·1 |
| S.p.a. (mV) | 28·2 ± 1·9 | 11·5 ± 0·6 | 11·5 ± 1·3 | 9·4 ± 0·7 |
| S.p.f. (cycles/min) | 14·8 ± 1·7 | 39·1 ± 4·7 | 8·7 ± 0·2 | 8·8 ± 0·5 |
| $dV/dt$ (V/s) | 0·09 ± 0·01 | 0·025 ± 0·001 | 0·031 ± 0·003 | 0·09 ± 0·02 |
| $n$ | 22 | 11 | 6 | 4 |
| Superficial–outer circular | | | | |
| R.m.p. | −63·6 ± 2·2 | −61·2 ± 1·4 | −59·9 ± 2·8 | N.r. |
| S.w.a. | 28·9 ± 1·1 | 11·9 ± 0·5 | 15·1 ± 0·6 | N.r. |
| S.w.f. | 14·9 ± 1·8 | 15·2 ± 0·3 | 8·5 ± 0·2 | N.r. |
| $dV/dt$ | 0·09 ± 0·01 | 0·050 ± 0·003 | 0·053 ± 0·002 | N.r. |
| $n$ | 8 | 11 | 6 | – |
| Deep–outer circular | | | | |
| R.m.p. | −68·3 ± 1·2 | −59·9 ± 1·5 | −61·2 ± 1·4 | −67·4 ± 2·0 |
| S.w.a. | 15·0 ± 0·8 | 6·4 ± 0·4 | 6·0 ± 0·8 | 5·9 ± 0·07 |
| S.w.f. | 14·7 ± 1·9 | 15·3 ± 0·3 | 8·7 ± 0·3 | 12·6 ± 0·5 |
| $dV/dt$ | — | 0·049 ± 0·002 | 0·050 ± 0·001 | 0·006 ± 0·000 |
| $n$ | 15 | 11 | 6 | 9 |
| Inner circular | | | | |
| R.m.p. | −55·4 ± 1·4* | Abs. | — | −60·0 ± 1·1 |
| S.p.a. | 6·1 ± 0·6 | 11 | — | 5·8 ± 0·8 |
| S.w.f. | 14·7 ± 0·9 | 11 | — | 12·0 ± 0·4 |
| $n$ | 15 | — | — | 4 |
| Isolated muscle | | | | |
| Longitudinal | | | | |
| R.m.p. | −64·4 ± 1·2 | −60·8 ± 1·4 | −60·1 ± 1·4 | −64·4 ± 2·3 |
| S.p.f. | 0 | 0 | 0 | 0 |
| $n$ | 23 | 3 | 3 | 4 |
| Deep–outer circular | | | | |
| R.m.p. | −67·6 ± 1·1 | −62·0 ± 1·4 | −62·0 ± 1·0 | −67·1 ± 1·8 |
| S.w.f. | 0 | 0 | 0 | 0 |
| $n$ | 15 | 4 | 4 | 4 |
| Inner circular | | | | |
| R.m.p. | −56·4 ± 0·9 | Abs. | — | N.r. |
| S.w.f. | Range: 0–9 | 11 | — | — |
| $n$ | 15 | — | — | N.r. |

R.m.p., maximum resting membrane potential; S.p.a., slow potential amplitude; S.p.f., slow potential frequency; S.w.a., slow wave amplitude; S.w.f., slow wave frequency; $dV/dt$, initial rate of rise; N.r., no recordings made; Abs., layer absent.

* $P < 0·01$ compared to all other values for maximum resting membrane potential.

circular muscle layer attached. When the longitudinal layer remained attached, spontaneous slow waves were recorded in all muscle cells tested ($n = 37$). Their frequency of occurrence was similar to that seen in this muscle layer in whole-thickness preparations. The initial rapid rate of rise of slow waves was also similar to that seen in cells in this muscle layer of whole-thickness preparations. However, the amplitude was significantly lower and more variable (Table 1: outer circular and longitudinal

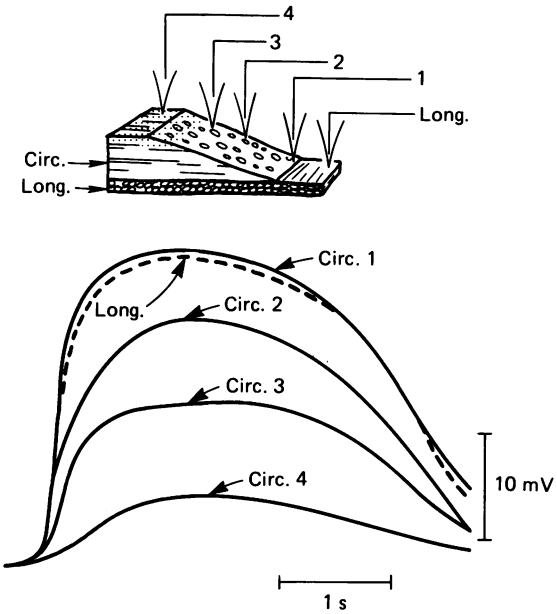


Fig. 7. Comparison of slow electrical activity recorded from different muscle layers of the cat jejunum. All recordings made from the same whole-thickness preparation. Slow waves labelled Circ. 1–3 were recorded from cells located at different depths of the outer circular muscle layer. Slow wave labelled Circ. 1 was recorded from a cell located on serosal side of the outer circular muscle layer. Slow wave labelled Circ. 4 was recorded from a cell located in inner circular muscle layer. Slow wave labelled Long. was recorded from a cell located in the longitudinal muscle layer. For additional details see text and Table 2.

muscle). When only the inner circular muscle layer remained attached to the outer circular muscle layer, spontaneous slow waves were recorded in all muscle cells tested ($n = 74$) located in the outer circular muscle layer. However, in 74 % of the cells tested (forty-four of seventy-four), there were frequent periods during which slow waves and contractions were absent. When slow waves occurred their frequency was lower, their amplitude was smaller and their initial rate of rise was slower (cf. Table 1, outer and inner circular muscle) when compared to slow waves recorded from cells in outer circular muscle of whole-thickness preparations.

In six preparations, intracellular recordings were made from inner circular muscle cells when only the outer circular muscle layer was left attached to the inner layer. Recordings were made from eleven cells in the inner circular muscle layer. As in isolated inner circular muscle, spike potentials occurred irregularly, either singly or in bursts. The frequency of bursts was 1–18/min (Table 1: inner and outer circular muscle).

*Electrophysiological recordings from cat, rabbit and opossum jejunum*

Values for maximum resting membrane potential and for some of the characteristics of slow waves and slow potentials recorded from each muscle layer of the jejunum of these three animals are listed in Table 2.

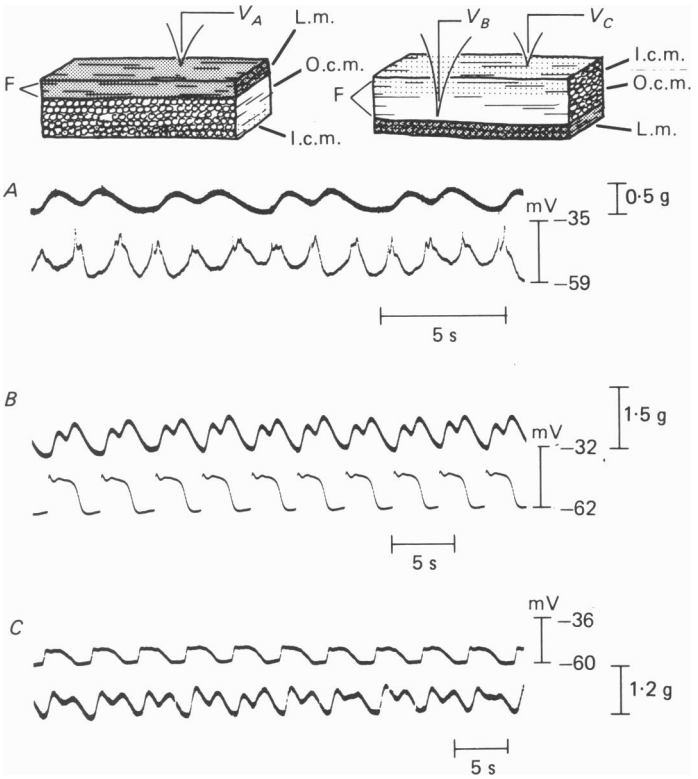


Fig. 8. Spontaneous activity recorded from different layers of the opossum jejunum. *A*, recording from a cell located in the longitudinal muscle layer. *B*, recording from a cell located on the serosal side of the outer circular muscle. *C*, recording from a cell located deep in the outer circular muscle. The location of the type of micro-electrodes $V_A$, $V_B$ and $V_C$ indicate the muscle layers from which the voltage traces in panels *A*, *B* and *C*, respectively, were made. Recordings made from three cells in three different whole-thickness preparations. For additional details see text.

*Intact preparations of cat jejunum.* The mean maximum resting membrane potential in the inner circular muscle was significantly ($P < 0{\cdot}01$) less negative than that recorded in any of the other layers. Spontaneous slow waves were recorded in all muscle layers. An example of slow waves recorded in four different regions is shown in Fig. 7. Except for recordings from cells in the inner circular muscle, slow waves did not always consist of a simple monotonic voltage change. The initial rate of rise was fastest for slow waves recorded from cells located in the longitudinal layer and from cells located on the surface of the serosal side of the outer circular muscle layer (Table 2). The mean total amplitude of slow waves declined from outer to inner regions of the circular muscle layer (Fig. 7 and Table 2). These results confirm previous observations (Connor *et al.* 1977; Bortoff *et al.* 1981). Spontaneously occurring spikes were recorded occasionally in muscle cells in the longitudinal and inner circular muscle layers. Spikes were not recorded in any of the cells tested ($n = 14$) in the outer circular muscle layer.

*Intact preparations of opossum jejunum.* There were no significant ($P > 0.05$) differences between the mean maximum resting membrane potential in the different muscle layers (Table 2). Spontaneous electrical activity was recorded in all layers. A typical example of spontaneous electrical and mechanical activity recorded in cells located in the longitudinal and circular muscle layers of the same preparation is shown in Fig. 8. In the longitudinal layer, slow potentials and spikes were recorded. Slow potentials in the longitudinal muscle were irregular in amplitude and gave rise to spike potentials of variable amplitude. The mean frequency of slow potentials in eleven cells in eleven preparations was $39.1 \pm 1.7$ cycles/min (mean $\pm$ S.E. of mean). In circular muscle, slow waves consisted of an initial rapid rate of rise separated from a plateau-type potential by a negative giving notch. In one of twelve preparations, spike potentials occurred on top of the plateau potential. Phasic contractions, which often consisted of two peaks, occurred with and without spikes. The amplitude of slow waves was largest in cells located on the serosal side of the outer circular muscle (Table 2, cf. Fig. 7).

*Intact preparations of rabbit jejunum.* There were no significant differences in the mean maximum resting membrane potential between cells in the different muscle layers (Table 2). Spontaneous slow potentials and spikes were recorded in all longitudinal muscle cells tested ($n = 11$). Spontaneous slow potentials with spikes were recorded in four of ten cells located on the mucosal side of the inner circular muscle. In the other six cells, only slow waves were recorded. Only slow waves were recorded from cells located on the serosal side of the outer circular muscle ($n = 16$) and from cells located deep in the outer circular muscle ($n = 10$). The shape of slow waves in these two latter regions of the outer circular muscle layer was similar to the shape of slow waves observed in the dog jejunum. The mean total amplitude of slow waves was largest when recorded from cells located on the serosal side of the outer circular muscle (Table 2). The results obtained confirm those previously described for the rabbit intestine (Taylor, Daniel & Tomita, 1975; Kitamura, 1978). In Table 2 no values are entered for inner circular muscle because of the uncertainty of the existence of this layer of muscle in the rabbit (Thuneberg, 1982).

*Isolated muscle.* Preparations of isolated longitudinal muscle removed from the cat ($n = 6$), rabbit ($n = 5$) and opossum ($n = 4$) were electrically and mechanically silent. The resting membrane potential recorded from these preparations was not significantly ($P > 0.05$) different from that recorded in longitudinal muscle cells of whole thickness preparations. Acetylcholine ($10^{-6}$ M) caused the occurrence of slow potentials, spikes and phasic contractions in all cells tested (cat, $n = 5$; rabbit, $n = 5$; opossum, $n = 4$).

In two experiments in each species, recordings were made in longitudinal muscle cells when a thin layer ($< 100\ \mu$m) of outer circular muscle was left attached. Spontaneous slow electrical activity was recorded from all longitudinal muscle cells tested. The configuration of slow waves (and spikes in the opossum) was not different from the configuration seen in intact preparations. In the same preparations, all spontaneous electrical activity was abolished when the adherent outer circular muscle was removed. When these preparations of isolated longitudinal muscle were exposed to acetylcholine ($10^{-5}$ M), slow potentials and spikes (all species) and phasic contractions were recorded.

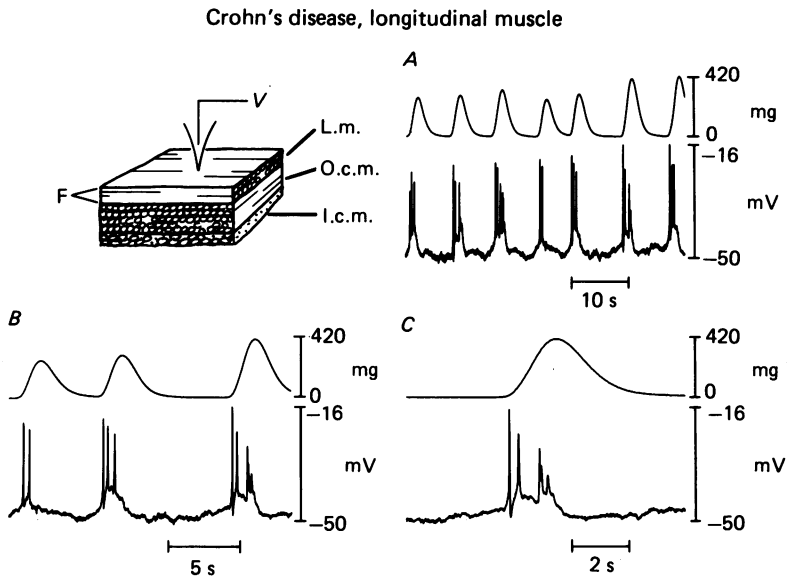


Fig. 9. Spontaneous mechanical and intracellular electrical activity in human ileum. Spontaneous activity recorded from a cell located in longitudinal muscle of a whole-thickness preparation of the human lower jejunum. Traces in panels *B* and *C* made at successively faster time bases compared to those in panel *A*. In each panel, top trace contraction, bottom trace electrical record. For additional details, see text.

*Electrophysiological recordings from human small intestine*

To date, recordings have been made from human small intestinal muscle obtained from four patients. All muscle biopsies were judged to be normal based on histological examination using hematoxylin–eosin and trichrome–Masson staining techniques.

Regularly occurring spontaneous slow electrical activity and spikes were recorded in cells located in the longitudinal muscle of intact preparations. An example is shown in Fig. 9. When spikes were absent, the amplitude of the slow wave was $9{\cdot}4 \pm 0{\cdot}4$ mV (mean $\pm$ S.E. of mean, $n = 12$). In one preparation, recordings were made from four cells in the longitudinal layer to which was attached 88 $\mu$m of outer circular muscle. In all four cells slow waves and spikes were recorded. However, when all adherent circular muscle was removed, spontaneous activity was absent. Treatment of this preparation with acetylcholione induced bursts of spikes superimposed on slow potentials. The frequency of occurrence of the bursts ranged from 5·9 to 7·0 cycles/min.

Recordings were made from four cells in the inner circular muscle of two intact preparations. All cells tested generated spontaneous electrical activity which consisted of slow waves and spikes (Fig. 10). In one preparation, recordings were made from cells located deep in the outer circular muscle of intact preparations. In nine of nine cells, there were spontaneous slow waves but no spikes. In one preparation which consisted of isolated outer circular muscle, recordings were made from four cells. In all four cells, spontaneous electrical or mechanical activity was absent. Some of the properties of electrical activity of muscle cells in different layers of the human

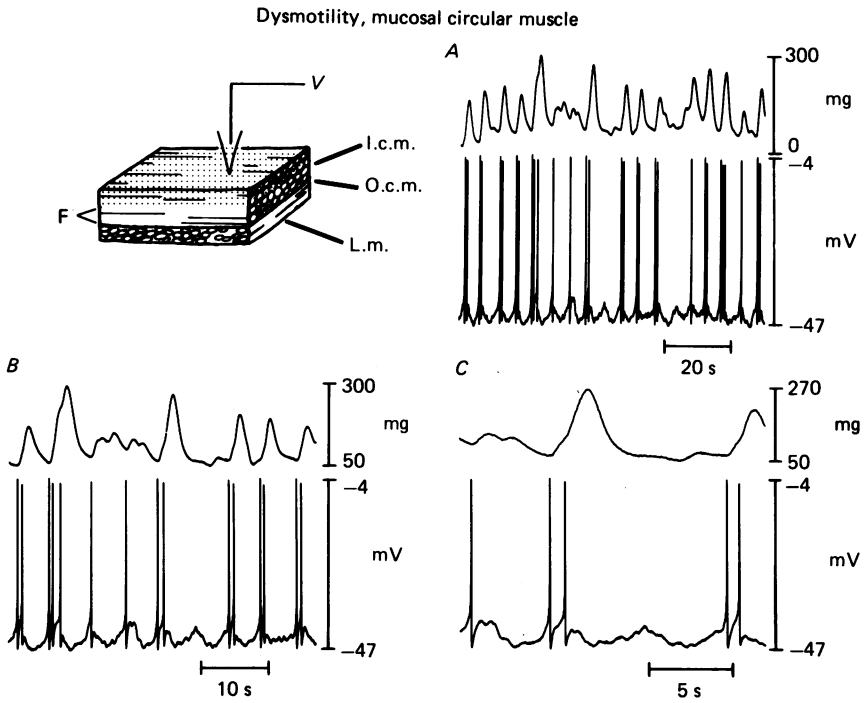


Fig. 10. Spontaneous activity recorded from a cell located in the inner circular muscle layer of a whole-thickness preparation of the human lower jejunum. Traces in panels *B* and *C* made at successively faster time bases compared to those in panel *A*. In each panel, top trace contraction, bottom trace electrical record. For additional details see text.

intestinal wall are summarized in Table 2. Although it appears that many of the properties of human intestinal smooth muscle are similar to those of the dog, rabbit and opossum intestine, it must be emphasized that too few data are available at this time. Considerably more data are required before human small intestinal muscle can be considered to be described adequately.

## DISCUSSION

Present day hypotheses regarding the mechanisms which regulate electrical and mechanical activity of the mammalian small intestine stem for the most part from studies done on the cat jejunum. Based on data obtained with extracellular and intracellular techniques, it has been suggested that slow waves in the mammalian small intestine originate in the longitudinal muscle (Bortoff, 1961, 1965; Bortoff *et al.* 1981) and spread passively to the circular muscle where they decay electrotonically (Bortoff, 1965; Bortoff & Sachs, 1970; Bortoff *et al.* 1981) or propagate by regenerative amplification (Connor *et al.* 1977). A number of observations, however, have made questionable the hypothesis that slow waves are generated in the longitudinal muscle. Close examination of previous reports of experiments done on isolated longitudinal

muscle reveals that only a small number ( $< 20\%$) of isolated longitudinal muscle preparations generated slow waves (Connor *et al.* 1974) and of those that did, only very localized regions produced slow waves (Kobayashi, Prosser & Nagai, 1967; Connor *et al.* 1974). It was reported that histological examination of some of these strips of isolated longitudinal muscle contained elements of Auerbach's plexus and of the underlying serosal side of the outer circular muscle (Bortoff, 1965; Bortoff *et al.* 1981). Thus, the occurrence of slow waves in a limited number of 'isolated' longitudinal muscle preparations could be explained by the fact that tissue adherent to the longitudinal muscle had some influence on the occurrence of slow waves. In fact, electrophysiological data obtained in these earlier studies on cat jejunum raised the possibility that slow waves may originate either in cells located between the longitudinal and circular muscle layers or in cells located on the serosal surface of the outer circular muscle (Kobayashi *et al.* 1967). Studies in the rabbit also suggested this possibility (Taylor *et al.* 1975; Cheung & Daniel, 1980).

The data obtained in the present study from the dog, cat, rabbit, opossum and human small intestine have led us to reject the notion that slow waves originate in longitudinal muscle cells for the following reasons. First, isolated longitudinal muscle cells in all species tested were electrically silent. It might be argued that the surgical procedures of dissection somehow damaged these preparations. However, the generation of slow waves and spikes by acetylcholine argues against this possibility. Our observations on isolated longitudinal muscle of the rabbit confirm previous observations (Cheung & Daniel, 1980). Secondly, in all species except the cat, the total amplitude and total rate of rise of slow waves were significantly larger and faster, respectively, in cells between the two muscle layers than in longitudinal muscle cells. Thirdly, slow waves were recorded from longitudinal muscle cells when a thin layer of outer circular muscle was left attached but were abolished upon complete removal of the thin layer of circular muscle. Fourthly, large amplitude slow waves with a rapid rate of rise were recorded from cells in the thin layer of circular muscle after careful removal of the overlying bundles of longitudinal muscle. In all species tested except the cat, slow waves in longitudinal muscle cells were of low amplitude. The low amplitude and slow rate of rise of slow waves in the longitudinal muscle cells suggests that, except for the cat, electrical coupling is poor between the site of origin of slow waves and longitudinal muscle cells. In the cat, slow waves recorded in longitudinal muscle and in cells between the two muscle layers were not different, suggesting strong electrical coupling. Indeed, anatomical studies have shown the presence of fibroblasts (Taylor, Kreulen & Prosser, 1977), interstitial cells (Thuneberg, 1982) and smooth muscle cells (Kobayashi *et al.* 1967) connecting the two muscle layers; this structural interconnexion may provide a functional interaction. Such interconnexions have not been observed in the canine gastrointestinal tract (Daniel & Sarna, 1978).

The data from the present study also suggest that the electrical properties of cells in the circular muscle were not uniform, especially in those species whose intestinal circular muscle consisted of a well defined inner and outer layer. Inner circular muscle cells generated slow potentials and spikes spontaneously and in the presence of acetylcholine. In contrast, outer circular muscle cells generated only slow waves. The positive correlation between the amplitude of slow waves and contractions and the absence of spikes in outer circular muscle suggest that excitation–contraction

coupling was spike-independent. These data confirm a previous observation which showed that when the inner circular muscle of the dog ileum was removed, the amplitude of circular muscle contraction was related to the amplitude of the slow wave recorded in circular muscle cells (Sanders, 1983).

Isolated inner circular muscle generated spontaneous slow potentials which carried spikes whereas isolated outer circular muscle was electrically silent. Although the frequency and amplitude of the slow potentials in the inner circular muscle were irregular in isolated preparations, they were regular in occurrence in intact preparations, suggesting coupling or synchronization between the two sites in the intestinal wall capable of generating spontaneous slow electrical activity. Although the nature of this coupling remains to be elucidated, it appears to occur through muscle cells which form the bulk of the outer circular muscle. Although cells in the bulk of the outer circular muscle did not generate spontaneous slow waves, they did transmit slow waves when the boundary between the longitudinal and circular muscle layers was intact or when the inner circular muscle layer was attached to the outer circular muscle layer. It has been well established that there are numerous gap junctions between cells within the outer circular muscle (Henderson, Duchon & Daniel, 1971; Gabella, 1974). Thus, there is a morphological basis to support coupling between the two sites in the wall of the intestine.

Although the ionic basis for slow waves was not addressed, slow waves in the bulk of the outer circular muscle were induced by acetylcholine. The mechanism may be similar to the one which is found in the taenia coli of the guinea-pig (Bolton, 1971). It remains for future experiments to determine if the ionic currents underlying slow waves are sensitive to other putative transmitters present in the outer circular muscle.

The type of cells responsible for generating spontaneous slow waves and slow potentials in the small intestine remains to be discussed. Common to both areas is the occurrence of an intrinsic plexus. Auerbach's plexus is found between the longitudinal and circular muscle and the plexus muscularis profundus between the inner and outer circular muscle layers (Cajal, 1893; Li, 1937, 1940). Since neither tetrodotoxin nor adrenergic or cholinergic antagonists affected spontaneously occurring activity, it seems reasonable to exclude the intrinsic nerves as the cause of the spontaneous activity. Recently it has been shown that the interstitial cells of Cajal in these two plexuses are morphologically similar and it has been suggested that these cells may play an important role in generating slow waves (Thuneberg, 1982). Although our data do not provide any direct confirmation of this notion, the data do provide circumstantial evidence in support of this hypothesis.

In summary, when the data of the present study are considered together with previously published data, there emerges a high degree of uniformity with regard to the site of origin of slow waves. In the dog, cat, rabbit, opossum and human, spontaneous slow waves are generated by non-neural cells located on the serosal side of the outer circular muscle. Non-neural cells located between the inner and outer circular muscle layers also generate spontaneous slow electrical activity. The way in which these two sites interact *in vivo* in the intact bowel remains to be elucidated. Finally, these data suggest that spike potentials from the circular muscle arise from muscle cells which form the inner circular muscle. The functional importance of the outer and inner circular muscle layers in the movement of intestinal content remains to be determined.

This work was supported by Research Grant AM 17238 from the National Institutes of Health. The authors express their appreciation to Jan Applequist and Jewel Garness for their assistance.

## REFERENCES

BOLTON, T. B. (1971). On the nature of the oscillations of the membrane potential (slow waves) produced by acetylcholine or carbachol in intestinal smooth muscle. *Journal of Physiology* **216**, 403–418.

BORTOFF, A. (1961). Slow potential variation of small intestine. *American Journal of Physiology* **201**, 203–208.

BORTOFF, A. (1965). Electrical transmission of slow waves from longitudinal to circular muscle. *American Journal of Physiology* **209**, 1254–1260.

BORTOFF, A., MICHAELS, D. & MISTRETTA, P. (1981). Dominance of longitudinal muscle in propagation of intestinal slow waves. *American Journal of Physiology* **240**, C135–147.

BORTOFF, A. & SACHS, F. (1970). Electrotonic spread of slow waves in circular muscle of cat. *American Journal of Physiology* **218**, 576–581.

CAJAL, S. R. (1893). Sur les ganglions et plexus nerveux de l'intestin. *Comptes rendus des séances de la Société de biologie et de ses Filiales* **45**, 217–223.

CHEUNG, D. W. & DANIEL, E. E. (1980). Comparative study of the smooth muscle layers of the rabbit duodenum. *Journal of Physiology* **309**, 13–27.

CODE, C. F. & SZURSZEWSKI, J. H. (1970). The effect of duodenal and mid small bowel transection on the frequency gradient of the pacesetter potential in the canine small intestine. *Journal of Physiology* **207**, 281–289.

CONNOR, J., KREULEN, D., PROSSER, C. L. & WEIGER, R. (1977). Interaction between longitudinal and circular muscle in intestine of cat. *Journal of Physiology* **273**, 665–689.

CONNOR, J., PROSSER, C. L. & WEEMS, W. A. (1974). A study of pace-maker activity in intestinal smooth muscle. *Journal of Physiology* **240**, 671–701.

DANIEL, E. E., HONOUR, A. J. & BOGOCH, A. (1960). Electrical activity of the longitudinal muscle of the dog small intestine studied *in vivo* using microelectrodes. *American Journal of Physiology* **198**, 113–118, 1960.

DANIEL, E. E. & SARNA, S. (1978). The generation and conduction of activity in smooth muscle. *Annual Review of Pharmacology and Toxicology* **18**, 145–166.

DUCHON, G., HENDERSON, R. & DANIEL, E. E. (1974). Circular muscle of the small intestine. In *Proceedings of the Fourth International Symposium on Gastrointestinal Motility*, Banff, Alberta, Canada, ed. DANIEL, E. E., pp. 635–646. Vancouver: Mitchell Press.

EL-SHARKAWY, T. Y. (1983). Electrical activities of the muscle layers of the canine colon. *Journal of Physiology* **342**, 67–83.

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

GABELLA, G. (1972). Intracellular junctions between circular and longitudinal intestinal muscle layers. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **125**, 191–199.

GABELLA, G. (1974). Special muscle cells and their innervation in the mammalian small intestine. *Cell and Tissue Research* **153**, 63–77.

HARA, Y. & SZURSZEWSKI, J. H. (1981). Mechanical and intracellular electrical activity of smooth muscle of canine jejunum. *Gastroenterology* **80**, 1169.

HARA, Y. & SZURSZEWSKI, J. H. (1986). Effect of potassium and acetylcholine on canine intestinal smooth muscle. *Journal of Physiology* **372**, 521–537.

HENDERSON, R. M., DUCHON, G. & DANIEL, E. E. (1971). Cell contacts in duodenal smooth muscle layers. *American Journal of Physiology* **221**, 564–573.

KAO, C. Y. & NISHIYAMA, A. (1964). Ovarian hormones and resting potential of rabbit uterine smooth muscle. *American Journal of Physiology* **207**, 793–799.

KITAMURA, K. (1978). Comparative aspects of membrane properties and innervation of longitudinal and circular muscle layers of rabbit jejunum. *Japanese Journal of Physiology* **28**, 583–601.

KOBAYASHI, M., NAGAI, T. & PROSSER, C. L. (1966). Electrical interaction between muscle layers of cat intestine. *American Journal of Physiology* **211**, 1281–1291.

KOBAYASHI, M., PROSSER, C. L. & NAGAI, T. (1967). Electrical properties of intestinal muscle as measured intracellularly and extracelluarly. *American Journal of Physiology* **213**, 275–286.

KURIYAMA, H., OSA, T. & TOIDA, N. (1967). Electrophysiological study of the intestinal smooth muscle of the guinea-pig. *Journal of Physiology* **191**, 239–255.

LI, P-L. (1937). Neue Beobachtungen über die Struktur des Zirkulärmuskels im Dümndarm bei Wirbeltieren. Eine vergleichende Studie. *Zeitschrift für Anatomie und Entwicklungsgeschichte* **107**, 212–222.

LI, P-L. (1940). The intramural nervous system of the small intestine with special reference to the innervation of the inner subdivision of its circular muscle. *Journal of Anatomy* **74**, 348–359.

MORGAN, K. G., MUIR, T. C. & SZURSZEWSKI, J. H. (1981). The electrical basis for contraction and relaxation in canine fundal muscle. *Journal of Physiology* **311**, 475–488.

MORGAN, K. G. & SZURSZEWSKI, J. H. (1980). Mechanisms of phasic and tonic actions of pentagastrin on canine gastric muscle. *Journal of Physiology* **301**, 229–242.

SANDERS, K. M. (1983). Excitation–contraction coupling without $Ca^{2+}$ action potentials in small intestine. *American Journal of Physiology* **224**, C356–361.

SZURSZEWSKI, J. H. (1981). Electrical basis for gastrointestinal motility. In *Physiology of the Gastrointestinal Tract*, ed. JOHNSON, L. R., vol. 2, pp. 1435–1466. New York: Raven Press.

TAYLOR, A. B., KREULEN, D. & PROSSER, C. L. (1977). Electron microscopy of the connective tissues between longitudinal and circular muscle of small intestine of cat. *American Journal of Anatomy* **150**, 427–442.

TAYLOR, G. S., DANIEL, E. E. & TOMITA, T. (1975). Origin and mechanism of intestinal slow waves. In *Proceedings of the Fifth International Symposium on Gastrointestinal Motility*, ed. Vantrappen, G. Belgium: Typoff Press.

THUNEBERG, L. (1982). Interstitial cells of Cajal: Intestinal pacemaker cells. *Advances in Anatomy, Embryology and Cell Biology* **71**, 1–130.