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INTERACTION BETWEEN LONGITUDINAL AND CIRCULAR MUSCLE IN INTESTINE OF CAT

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SUMMARY

1. Slow waves recorded from *isolated* longitudinal muscle averaged 13 mV and had slow rate of rise (0·04 V/sec) whereas when recorded from *intact* segments the amplitude averaged 27 mV and the rate of rise was more rapid (0·09 V/sec), often with a notch between the initial peak and the plateau. Membrane potentials of longitudinal muscle were similar in isolated and intact preparations (– 66 mV). Resting potentials of circular muscle averaged – 67 mV.

2. Small bundles of circular muscle tested in the double sucrose gap produced activity, either spontaneously or in response to stimulation, which fell into three categories: fast spikes (50–200 msec duration), slow spikes (1–5 sec duration), and small graded responses. The duration of fast spikes could be increased severalfold by the addition of TEA; the graded responses were converted to full-sized spikes by TEA.

3. Treatment of circular muscle with Ca-free Krebs solution eliminated spikes, and in intact preparations reduced the amplitude and rate of rise of slow waves and eliminated the notch on slow waves.

4. Current–voltage curves of longitudinal muscle show delayed rectification in the depolarizing quadrant; similar curves of circular muscle show anomalous rectification, i.e. a region where a very small current causes a large voltage change.

5. Non-polarized electrotonic coupling between longitudinal and circular layers indicates low-resistance pathways. Apparent space constants of longitudinal muscle are greater when attached to circular muscle than when isolated.

6. It is concluded that small slow potentials originate rhythmically in longitudinal muscle, that these spread passively to circular muscle where

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a regenerative amplification occurs which depends on Ca conductance and the amplified slow waves spread back to the longitudinal layer. In the intact intestine pacemaking is, therefore, separate from propagation and the circular muscle provides the bulk of depolarizing current for propagation.

INTRODUCTION

Slow electrical waves are generated in the longitudinal muscle layer of the small intestine of several mammalian species and are not found in the circular muscle in the absence of longitudinal muscle (Bortoff, 1965; Connor, Prosser & Weems, 1974; Kobayashi, Nagai & Prosser, 1966). When the two muscle layers are attached to one another as they are normally, slow waves can be recorded from both layers and propagation of this electrical activity occurs over distances of a few centimetres in the longitudinal direction (Bortoff, 1965; Kobayashi *et al.* 1966). In isolated longitudinal muscle slow waves are recorded only in localized regions, 2–3 mm in extent, and in intact intestine transection of the circular muscle layer for several millimetres interrupts propagation along the longitudinal axis of the gut (Kobayashi *et al.* 1966), i.e. the presence of circular muscle is necessary for propagation. The slow waves pace and coordinate the rhythmic contractions of both muscle layers but significant contractile force is not developed unless action potentials are generated during the crest of the waves. Both generation and propagation of slow waves occur in the presence of tetrodotoxin or atropine (Liu, Prosser & Job, 1969; Prosser, 1974), and they are generated by small strips of longitudinal muscle which are demonstrably nerve-free (Connor *et al.* 1974). Hence both processes are myogenic.

The present study describes the electrical activity of the two intestinal muscle layers and differentiates between activity which is characteristic of each layer in isolation and activity characteristic of intact intestinal musculature. Evidence is offered for reciprocal interaction between the longitudinal and circular layers. The observations indicate the importance of the circular muscle for synchronization and propagation of slow waves in the intact intestine and suggest a mechanism of this interaction. Preliminary reports of this study have been made (Kreulen, Prosser & Connor, 1976; Connor, Connor, Kreulin, Prosser & Weems 1975).

METHODS

Segments of small intestine were removed from cats anaesthetized with α-chloralose. The segments were immediately immersed in Krebs solution aerated with 95 % O_2–5 % CO_2. Those not used immediately were stored in Krebs solution at 4 °C and were warmed to 37 °C before use. The Krebs solution had the following composition

(mM): NaCl 120, KCl 4·7, $CaCl_2$ 2·5, $NaHCO_3$ 23·8, KH_2PO_4 1·2, $MgCl_2$ 1·2, glucose 5·5.

Several different muscle preparations were used: (1) cylindrical whole segments, (2) flat preparations containing both muscle layers and (3) isolated strips of each muscle layer. For recording from whole segments, a glass rod was inserted into the lumen of the intestine and was held in place by a thread tied at the ends. In some preparations a piece of longitudinal muscle approximately 3 cm long and 1 cm wide was separated under microscopic observation from the underlying circular muscle by sliding a fine glass rod between the two layers in the longitudinal direction for 3 cm. The resulting piece of separated longitudinal muscle remained attached at both ends to unseparated longitudinal muscle. Scanning electron micrographs of muscle separated in this way showed no circular muscle remaining attached to the longitudinal muscle. Flat preparations were prepared by separating both muscle layers from the underlying mucosa with a micro-spatula. Some of these preparations were dissected so that they contained portions of longitudinal muscle separated from circular muscle; others were dissected so that portions of the circular muscle were free of overlying longitudinal muscle. For recording, the flat preparations were firmly pinned to Sylgard at the bottom of the chamber.

Electrical recordings were made with glass micro-electrodes filled with 3 M-KCl. Signals were fed to a high-input impedance, capacity-compensated preamplifier and were displayed by a Grass polygraph. For preparations which had contractile activity which would prevent maintained impalements, 150 mM-sucrose was added to the Krebs solution to prevent movement. Successful impalements were those which maintained a steady voltage level for at least 30 s after a rapid change in potential which indicated penetration of a cell. The membrane potential was that voltage change observed when the electrode was pulled out of the cell. In all tabulations of data, *n* refers to the number of preparations. In each preparation two to fifteen successful impalements were recorded and values of resting potential, slow wave amplitude and rate of depolarization (dV/dt) were averaged. The final tabulation represents the mean of the means derived from *n* preparations.

Chambers of various sizes were used depending on the type of preparation. In all cases pre-warmed, oxygenated Krebs solution flowed in at controlled rates through a small tube submerged in the bath. Solution was removed by a vacuum tube at the other side of the bath; the level of solution was controlled by the level of the vacuum tube. The recording chambers were submerged in a water-bath kept at 37 °C. For whole-segment preparations a 25 ml. bath measuring 2·5 cm × 5 cm × 2 cm was used. At the flow rate which allowed change of solutions while maintaining an impalement (5–7 ml./min) the rate of solution turnover in the bath was measured colorimetrically. The bath solution was replaced approximately every 20 min, approaching that time asymptotically so that 90 % was replaced in 13–15 min. For flat preparations a 5 ml. bath measuring 2 cm × 5 cm × 0·5 cm was used. At the flow rate of 5 ml./min the bath solution was replaced every 5–8 min.

In addition to micro-electrode measurements, the double sucrose gap was also used to measure potential changes in small bundles of muscle. The apparatus has been described previously (Connor *et al.* 1974). For the present study, bundles 150–300 μm in diameter, were teased from either the circular or longitudinal layer and node widths were 100–125 μm.

To measure current spread in the intestinal muscle layers, flat preparations were used in which portions of longitudinal muscle were removed to expose an edge between the longitudinal and circular layers. Hyperpolarizing current pulses of 1 sec duration were injected by means of Krebs-agar filled pressure electrodes 500–800 μm in diameter which were shielded by several layers of silver foil connected to ground.

Electrotonic potentials were recorded differentially with two micro-electrodes, one in the bath above the tissue and one intracellularly at varying distances from the current-injecting pressure electrode. The amplitude of the recorded electrotonic potential was plotted on a logarithmic scale against distance. The data were fitted to a line by means of least-squares fit for an exponential function according to the equation: $y = ae^{-bx}$ where y is the ΔV at a distance x from the current-injecting electrode, a is the steady-state ΔV at $x = 0$ and $1/b$ is λ, the space constant. The correlation coefficient for all plots is $\geqslant 0{\cdot}95$. For comparison of curves obtained in different muscle fibre orientations, the data were normalized for the theoretical electrotonic potential amplitude at $x = 0$ (zero distance); this was obtained by extrapolating individual plots to zero.

RESULTS

Slow wave activity in longitudinal muscle

Spontaneous electrical activity was recorded from longitudinal muscle that was attached to the underlying circular layer (intact) and from longitudinal muscle that was separated from circular muscle (isolated). Results are summarized in Table 1. Typical slow waves are presented in Fig. 1. The rising phase was not a simple monotonic voltage change but rather it consisted of two or more portions differing from one another in rate of rise. This was especially true of longitudinal muscle in intact segments (Fig. 1 *A–C*). Slow waves in these preparations often had relatively rapid *initial* rates of rise separated from a slower rate of rise by a small negative-going notch. The rising phase was characterized by two rates; the 'total' rate of rise, measured from the baseline to the initial peak; and the 'maximum' rate of rise, measured from the fastest portion of the rising phase. The mean total rate of depolarization measured in fourteen whole-segment preparations was 0·08 V sec^{-1} and the mean maximum rate was 0·14 V $sec.^{-1}$.

For intact longitudinal muscle of cylindrical intestinal segments the mean resting membrane potential was -67 mV and the mean amplitude of slow waves was 30 mV. Membrane potential was not necessarily correlated with slow wave amplitude; that is, a preparation with a high membrane potential did not always have a higher amplitude of slow wave than a preparation with a smaller membrane potential. This held for both intact and isolated preparations.

In flat preparations of longitudinal muscle with circular layer attached, the mean slow wave amplitude of twenty-seven preparations was 27 mV and the mean resting membrane potential was -66 mV. The mean total rate of rise of slow waves in fifteen flat preparations was 0·09 V sec^{-1} and the mean maximum rate of rise was 0·19 V sec^{-1}. No significant differences ($P < 0{\cdot}05$) in resting potential, slow wave amplitude or rate of depolarization exist between longitudinal muscle of whole segments and longitudinal muscle of flat preparations.

Recordings from *isolated* longitudinal muscle strips showed that spontaneous electrical waves were not as uniformly distributed throughout the muscle layer as they were when recorded from intact longitudinal muscle. Rather in isolated longitudinal muscle, slow waves were localized to patches 3–5 mm in diameter which were separated by 2–5 mm from other active regions by quiescent areas. These quiescent regions had cells with

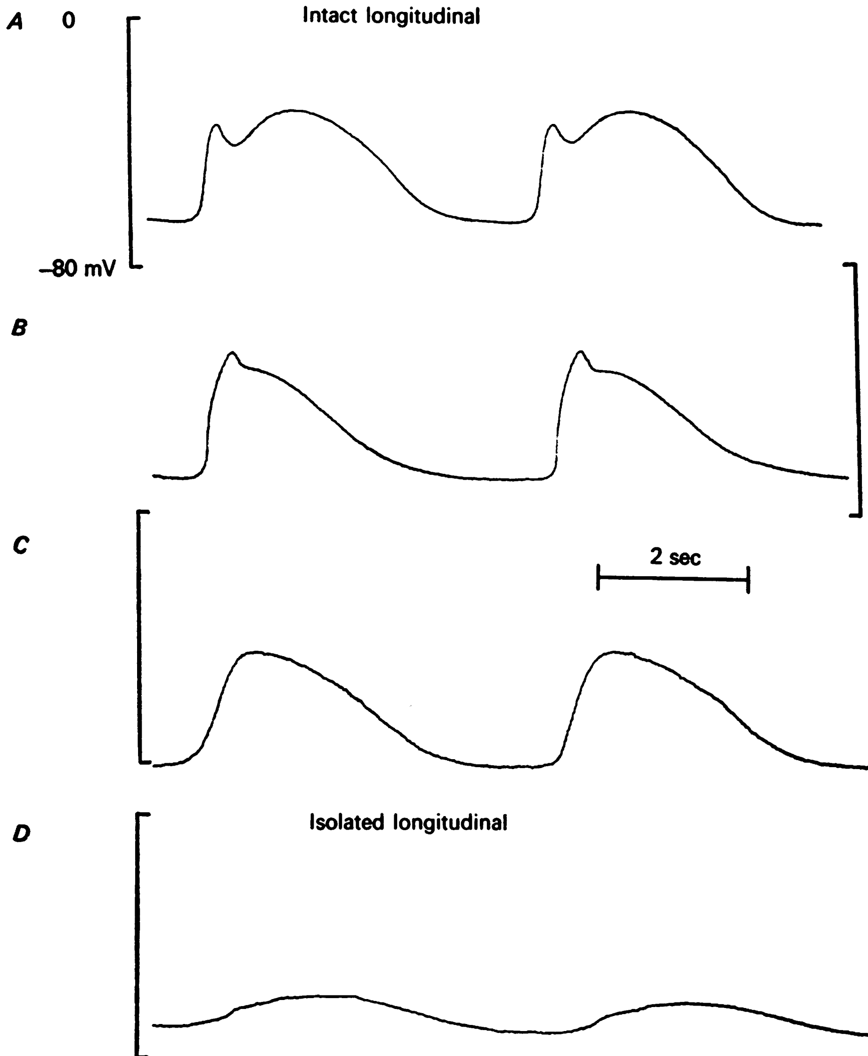

Fig. 1. Intracellular recordings of slow waves in longitudinal muscle layer. Different shapes are shown in recordings (*A–C*) from intact segments and (*D*) from isolated longitudinal muscle.

TABLE 1. Electrical parameters measured intracellularly in the small intestine of the cat

	Resting membrane potential				Amplitude of slow waves				Total rate of rise of slow wave			
	Mean (mV)		S.D. (mV)	n	Mean (mV)		S.D. (mV)	n	Mean (V/sec)		S.D. (V/sec)	n
Longitudinal muscle												
Intact segments	−67	±	7	35	30	±	5·6	35	0·08	±	0·03	14
	(range −50 to −80 mV				(range 16–40 mV)							
Flat preparation	−66	±	6·8	27	27	±	7·3	27	0·09	±	0·05	15
	(range −44 to − 88mV)				(range 15–44 mV)							
Intact												
Isolated	−66	±	7·0	19	13	±	7·3	10	0·04	±	0·0	7
					(range 3–26 mV)							
Circular muscle	−67	±	3·6	8								
	(range −56 to −80)											

membrane potentials similar to the active areas but they showed no slow wave activity. The location of active and inactive regions appeared to remain unchanged for several hours. Activity was never so localized in intact longitudinal muscle. These findings are consistent with earlier observations on isolated longitudinal muscle made in the double sucrose gap (Connor *et al.* 1974) where amplitude of slow waves ranged between 5 and 17 mV and only about 20 % of the preparations showed spontaneous activity. Presumably the non-active strips had been dissected from quiescent areas of the muscle.

The mean amplitude of slow waves in ten isolated longitudinal preparations was 13 mV (40 % of the value in the intact) and the mean resting membrane potential of 18 isolated preparations was -66 mV. The mean total dV/dt of slow waves from seven isolated preparations was 0·04 V sec^{-1} and the mean maximum rate of rise was 0·08 V sec^{-1}. These values are slower than the mean rates in intact longitudinal muscle by 66 and 53 % respectively. In addition to being slower, the depolarizing phase of slow waves in isolated longitudinal muscle was less complex than it was in intact muscle and usually did not have prominent notches or humps (Fig. 1*D*). Both the resting potentials and the frequency of slow waves were similar in isolated and intact preparations; also apparent space constants indicated intercellular coupling after isolation (see Fig. 12); these observations constitute evidence that the smaller slow wave amplitude and slower rate of rise in isolated longitudinal muscle were probably not due to injury.

The differences between slow waves recorded in intact and in isolated longitudinal muscle were very striking when the recordings were made from the same preparation. Fig. 2 is an example of recordings made in a flat preparation dissected so that it contained both layers: longitudinal muscle attached to as well as longitudional muscle free of underlying circular muscle. In the non-attached longitudinal muscle (Fig. 2*B*) the slow waves are of smaller amplitude and slower rate of depolarization than those recorded from longitudinal with circular attached; the resting membrane potentials are the same. Fig. 3 shows the differences in slow waves from attached and unattached longitudinal muscle on a cylindrical whole segment where a strip of longitudinal muscle was separated from the circular but left attached at the ends. In addition, a portion of the longitudinal was insulated from the circular muscle by a thin strip of plastic. In this preparation, as in most whole segments, the slow waves from the intact muscle had prominent notches on the rising phase (Fig. 3*A*); these notches were greatly diminished in undercut muscle (Fig. 3*B*) and absent from slow waves recorded from the insulated portion of the undercut longitudinal muscle (Fig. 3*C*). Slow wave amplitude in the

undercut muscle was 30% of that when the two layers were connected. The resting membrane potential was similar in each region.

The preceding observations indicate that the normal connexion of circular and longitudinal muscle is important for the slow wave activity observed in the intact intestine. Interestingly, contact apposition between the previously separated layers (Fig. 3*B*) resulted in slow waves with a faster rate of rise and slightly greater amplitude than the slow waves recorded from the separated longitudinal muscle which was insulated from circular muscle layer. The role of the circular layer is suggested by the differences in slow waves recorded in isolated longitudinal muscle: smaller amplitude, slower rate of depolarization and absence of a prominent negative-going notch on the rising phase.

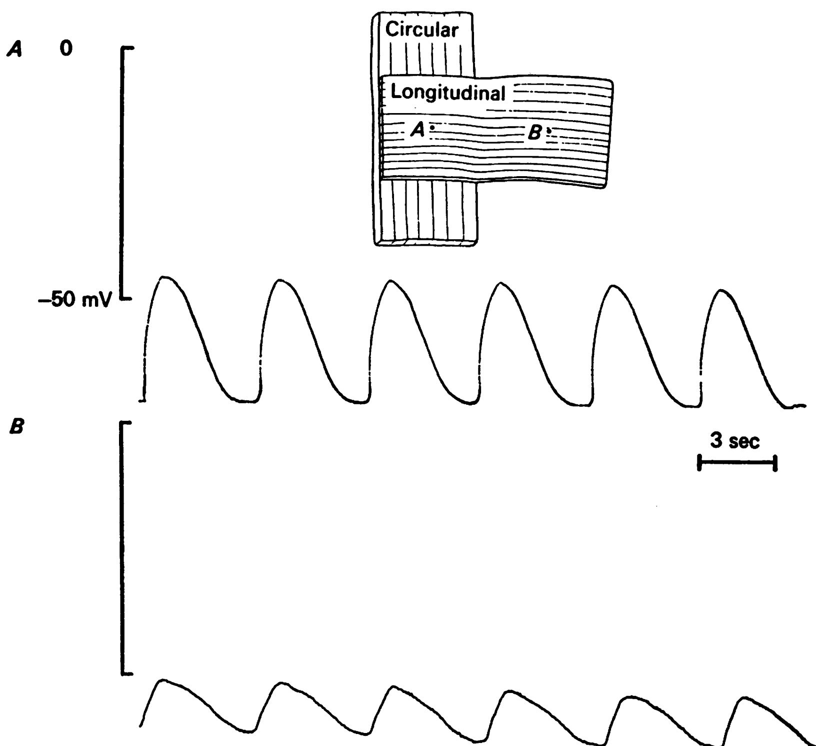

Fig. 2. Comparison of slow waves recorded intracellularly from longitudinal muscle in a flat preparation. *A*, intact portion of the preparation with two muscle layers attached; *B*, region from which circular muscle has been removed.

Effects of calcium; recordings from circular and longitudinal layers

Since regenerative activity in the circular layer is strongly dependent upon extracellular calcium ions (Liu *et al.* 1969; Connor & Prosser, 1974), decreasing the extracellular calcium concentration might be expected to mimic in some ways the effect on slow waves of separating the longitudinal from circular muscle. In whole segment preparations (Fig. 4) the rapid depolarization and notch were eliminated within 3 min

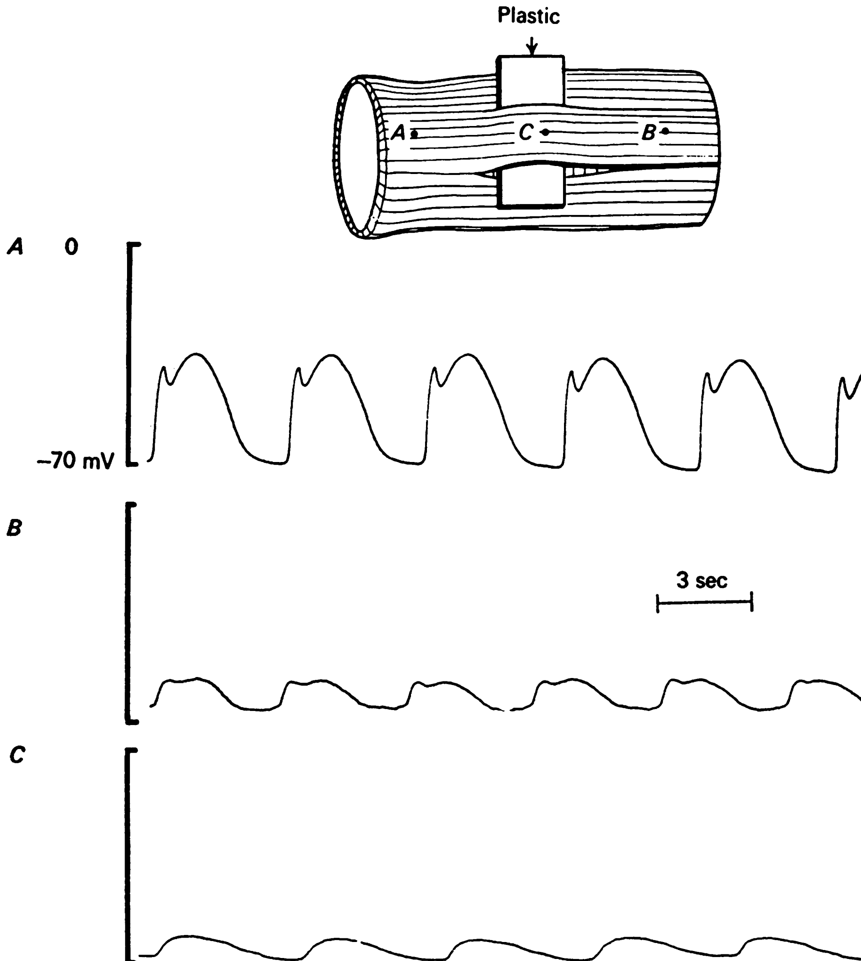

Fig. 3. Slow waves recorded intracellularly from longitudinal fibres in an intact segment where (*A*) both layers are connected, (*B*) longitudinal layer is separated from circular muscle by undercutting and (*C*) longitudinal layer is insulated from circular layer by a small plastic strip. Note differences in amplitude, rate of rise and shape of slow waves.

after changing the inflow to zero Ca Krebs. With longer periods of washout, changes occurred in two phases with a gradual decline in the rate of depolarization occurring throughout. In the first phase there was approximately a 20 % decrease in the duration of the slow waves (Fig. 4*B*, *C*). In the second phase, slow wave amplitude declined to approximately 50 % of control. These effects occurred with less than 10 mV depolarization. Decreased Ca also reduced slow wave frequency. After exposure to Ca-free Krebs for longer than 20–25 min, the membrane depolarized further and slow wave activity eventually stopped. If allowed to progress this far, the low Ca effects were only partially reversible.

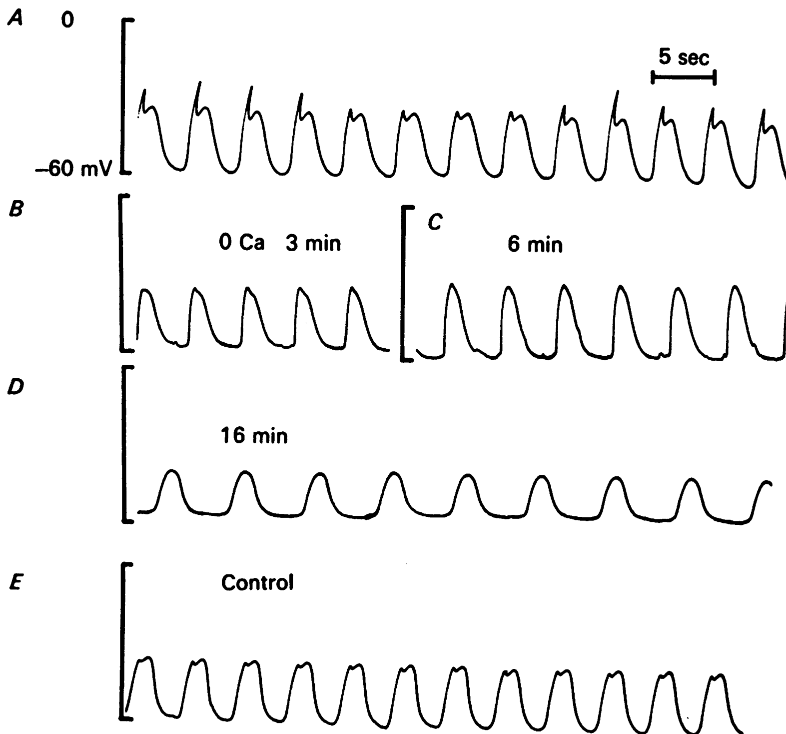

Fig. 4. Effects of superfusion of intact intestinal segment with zero Ca Krebs solution. All traces are from the same cell. Return to control (*E*) is after 3 min in normal Krebs solution.

In isolated longitudinal muscle the effects of diminished Ca were somewhat different. Within 3–5 min after changing the inflow solution to zero Ca Krebs, the slow wave amplitude increased by 10–80 % in different preparations and the rate of rise increased by an equivalent amount. Thus the initial action of low-Ca medium on amplitude was opposite in the

isolated and intact preparations. The amplitude increase was accompanied by a decrease in duration (Fig. 5). Longer exposure to low Ca led to decline in resting potential and when the depolarization exceeded about 15 mV, slow wave amplitude began to decline. In these preparations, frequency was reduced, much as in intact preparations. In both cases the effects of low Ca were reversible as long as the muscle was not allowed to deteriorate due to prolonged exposure.

A second type of experiment also illustrated the importance of Ca-dependent activity in the circular muscle. Starting with a flat preparation, a strip of longitudinal muscle several millimetres wide was removed from the underlying circular layer. This procedure left two sections of intact

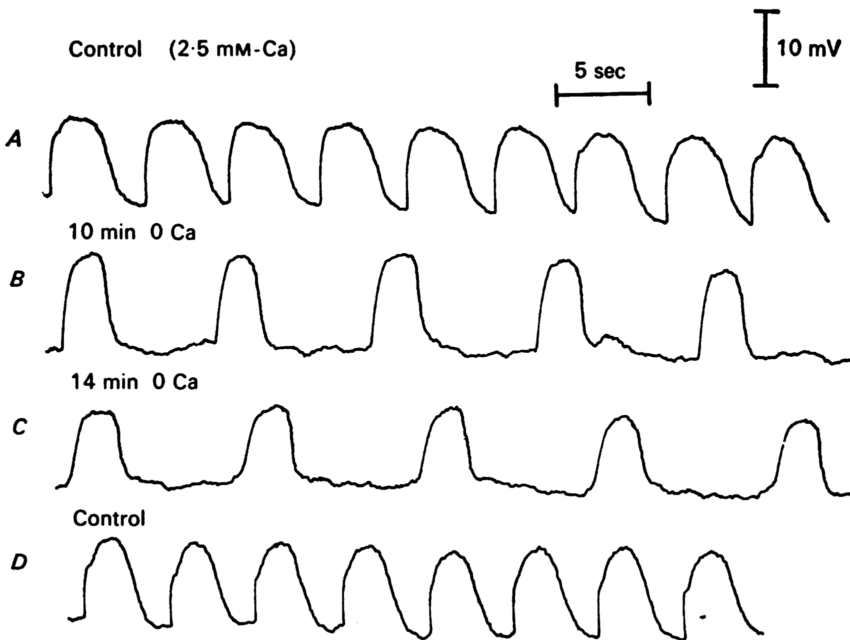

Fig. 5. Effects of zero Ca Krebs solution on slow waves in isolated longitudinal muscle. All traces are from the same cell.

longitudinal muscle connected by a bridge of circular muscle (Fig. 6). In normal Krebs solution typical slow waves were recorded in the circular muscle. After exposure to Ca-free Krebs, the activity recorded in the same circular muscle cell became arrythmic and appeared as though slow waves were coming from two sources which were slightly out of phase (12 and 16 min, Fig. 6). Simultaneously slow wave amplitude decreased. The maximum change in both rhythm and amplitude occurred after 5–7 min in Ca-free Krebs. Normal slow wave activity returned within 2–3 min

after restoration of Ca. The rapidity and reversibility of the Ca effect are more consistent with changes in membrane ionic current than with a destruction of intercellular coupling by reduced Ca.

Experiments to show the precise timing of activity in the two layers were difficult to execute and interpret because placement of an electrode

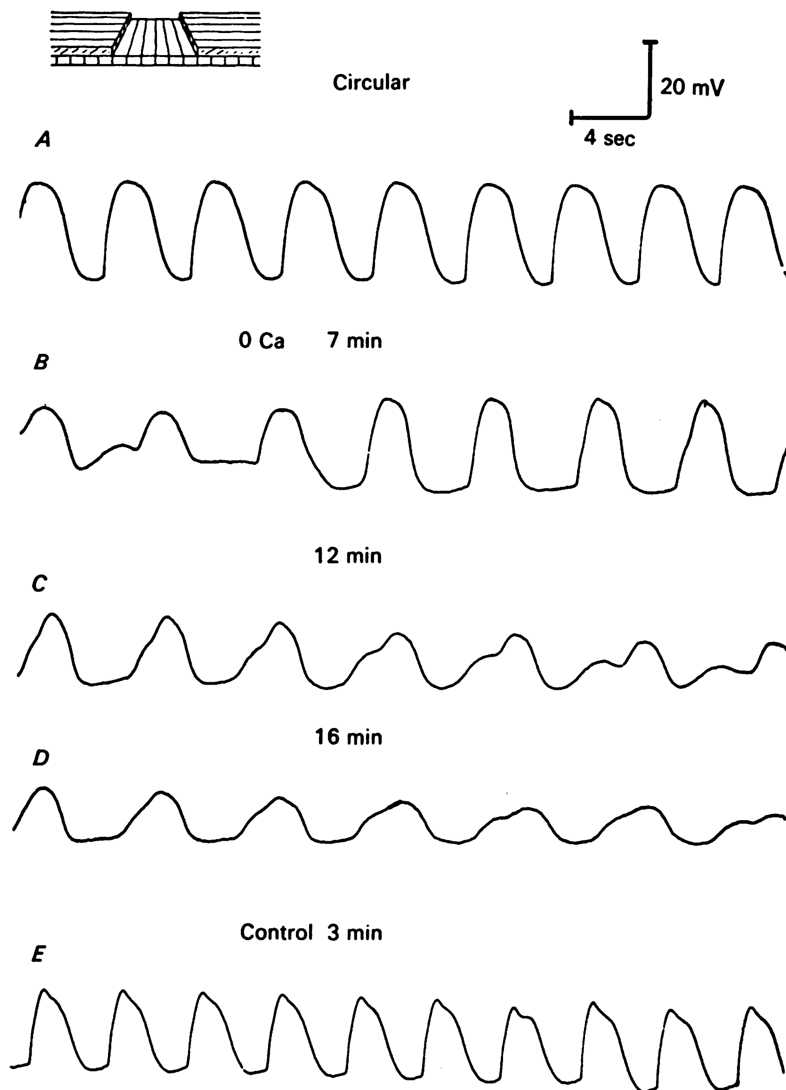

Fig. 6. Intracellular electrode recordings from a bridge of circular muscle between two intact regions as in diagram of preparation. Solution changed from normal Krebs to that containing 0·125 mM-Ca at end of record *A* and returned to normal (2·5 mM-Ca) at end of record *D*.

on the circular layer required some removal of the covering longitudinal muscle and the disruption of interlayer connexions. Slow waves were recorded in a number of preparations from the two layers simultaneously by two pressure electrodes, one on longitudinal muscle, the other inserted

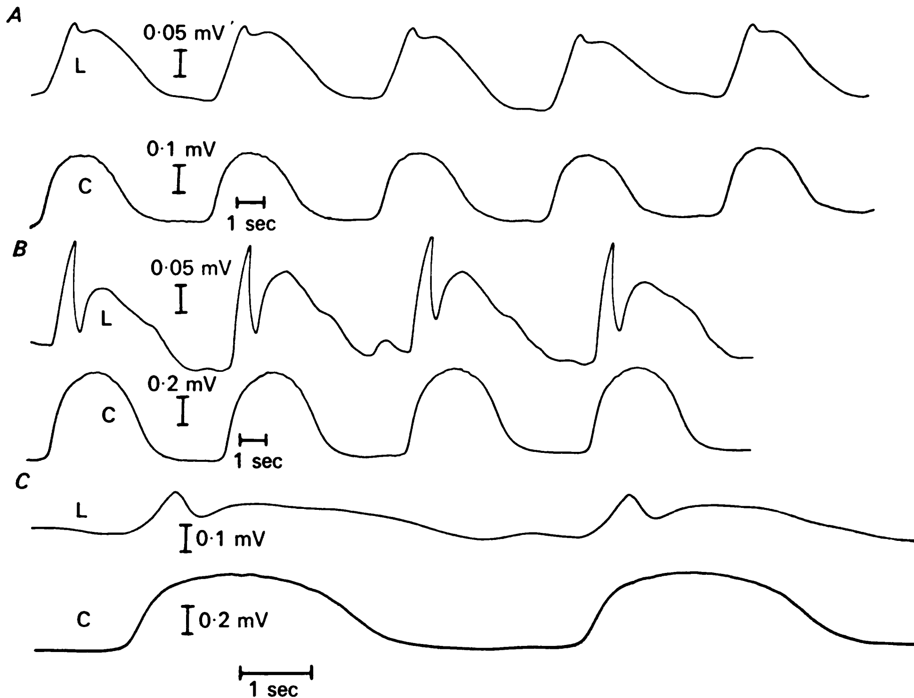

Fig. 7. Slow waves recorded by pressure electrodes from the two muscle layers, (L) longitudinal and (C) circular. Electrode on circular muscle was inserted through 1–2 mm slit in longitudinal layer; separations of electrode tips 1 mm. *A* and *B*, *C* were two preparations.

into the circular layer through an opening made by gently spreading longitudinal fibres apart; electrode separation was approximately 1 mm. The slow waves from longitudinal fibres were smaller in amplitude, usually had a rapid initial phase and deep notch while the waves from circular fibres were larger and showed monotonic rise and fall (Fig. 7). It was difficult to estimate time differences but frequently when the longitudinal electrode was in the transverse axis relative to that on circular muscle, the depolarizing phase of the circular wave coincided with or preceded the initial fast depolarization and notch of the longitudinal wave (Fig. 7).

Electrical activity in circular muscle

Micro-electrode recordings were made from strips of isolated circular muscle and from preparations which contained both muscle layers with a margin or window to expose circular fibres. The mean resting potential was approximately −67 mV in each type of preparation. Strips of isolated

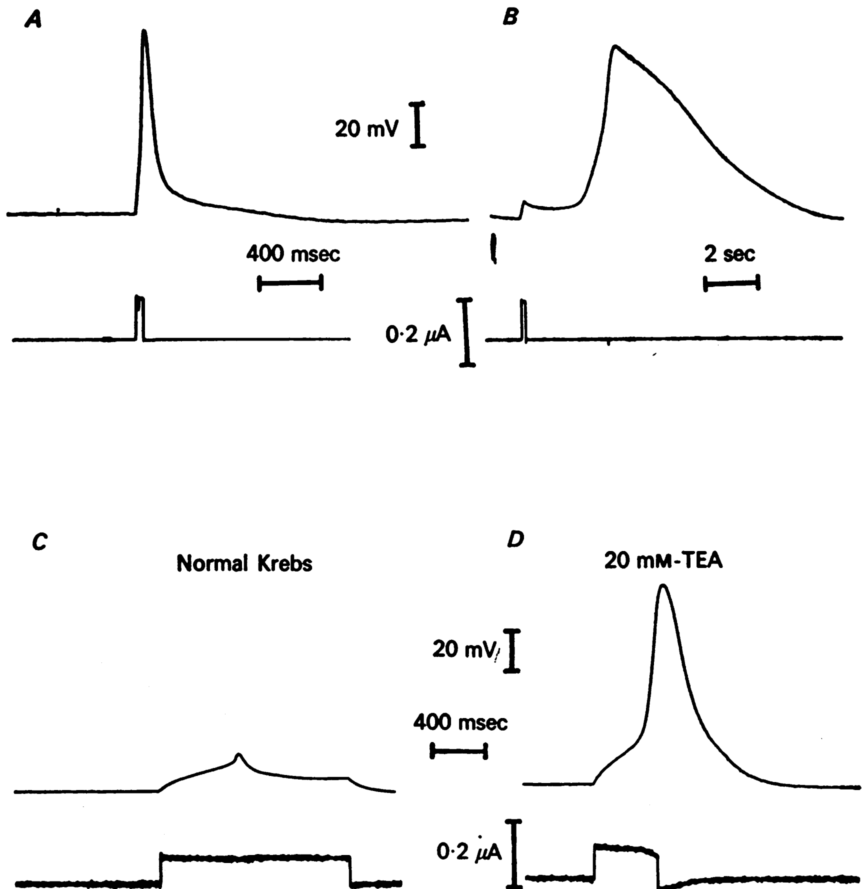

Fig. 8. Triggered action potentials in circular muscle bundles; double sucrose gap recordings. *A* and *B*, short and long action potentials in control saline. *C*, graded response in control saline. *D*, action potential from bundle of part *C* after exposure to 20 mM-TEA.

circular muscle in Krebs solution often showed some irregular spiking which causes contractions but these were eliminated by making the medium hypertonic with sucrose. Where longitudinal muscle was attached, the sucrose treatment had no evident effect on slow waves over a period

of 1 hr. In preparations that contained a region of both muscle layers plus a region of circular muscle from which longitudinal muscle had been removed, slow waves could be recorded from circular muscle but the amplitude of these waves decreased slightly with distance from an area of longitudinal muscle as previously reported (Kobayashi *et al.* 1966: Bortoff & Sachs, 1970).

In small bundles of circular muscle in the double sucrose gap, spontaneous spike activity was occasionally observed. More frequently activity was elicited by injection of small depolarizing currents. Responses ranged from no appreciable spiking to single spikes of up to 90 mV amplitude. Both single spikes and trains of spikes could be elicited. In preparations which generated action potentials of over 50 mV two types of spikes, characterized by their duration, were observed. Examples are given in Fig. 8. Short spikes, 50–200 msec duration (Fig. 8*A*), were the more common type and their properties have been described previously (Tamai & Prosser, 1966; Connor & Prosser, 1974). Longer spikes with duration of 1–5 sec were also observed in many preparations (Fig. 8*B*). They showed the same dependence on extracellular Ca previously found for short duration action potentials and thus appear to be due to changes in membrane conductance for Ca (Connor & Prosser, 1974). The long spikes were more labile than the short spikes and in some preparations the long action potentials converted to short ones over periods of 10–15 min. Threshold for triggering slow spikes was lower than threshold for the fast spikes and in the double gap was generally less than 20 mV from rest.

Only a fraction of the bundles tested in the sucrose gap gave action potentials of greater than 50 mV amplitude. The others showed a gradation from slightly less than this range to no appreciable spike. At first these differences were ascribed to injury. However, many preparations which generated only small action potentials showed normal membrane resistance when tested with small depolarizations. More importantly these fibres generated much larger spikes after addition of TEA. Fig. 8*C*, *D* shows an example of the conversion of a small fast spike to one with an amplitude of 80 mV and greater duration. We assume that the TEA is not repairing damaged membrane but is acting in its usual manner by blocking increases in membrane potassium conductance (Hille, 1970). In obviously damaged bundles, i.e. those with small membrane resistance and time constant, there was no effect of TEA. Similar enhancement of spikes in fibres of guinea-pig stomach has been reported by Ito, Kuriyama & Sakamoto (1970). This TEA effect suggests that the extreme differences in spiking characteristics between the preparation of Fig. 8*C*, *D* and those of Fig. 8*A*, *B* are due to differences in K conductance of different muscle bundles. Circular muscle bundles are considered to grade from low to high

with respect to membrane K conductances. Those bundles with slightly activated K conductance gave rise to prolonged action potentials while those with larger conductance generated shorter action potentials and finally, where the K conductance was still larger, the excitatory inward current might be largely balanced by outward K current with the result that only small spikes or graded responses were generated by the bundle.

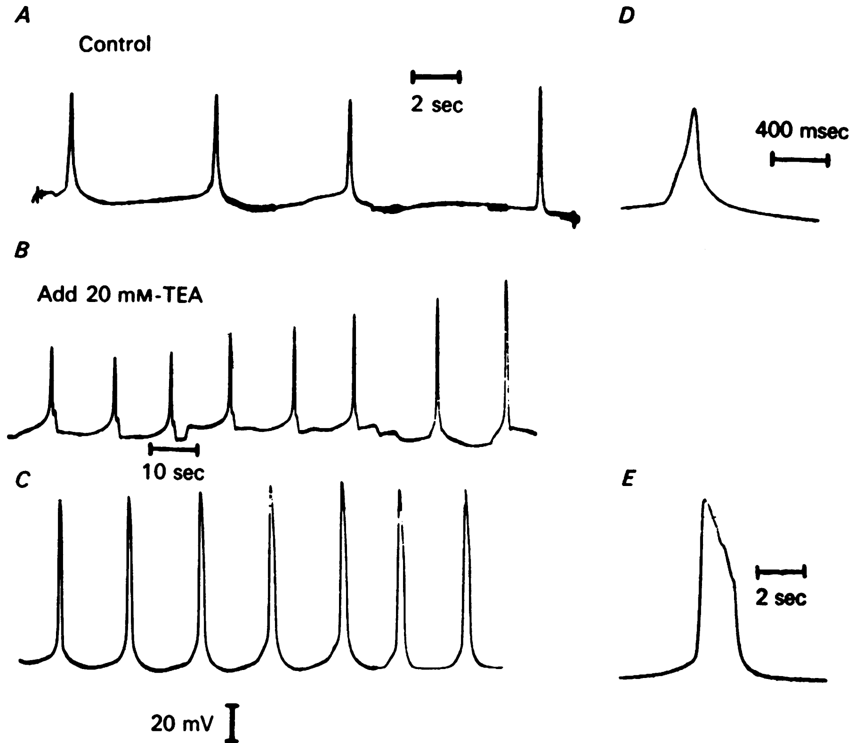

Fig. 9. Circular muscle spontaneous activity. *A*, action potentials are of relatively small amplitude and short duration. *B*, change in action potential time course brought about by the addition of TEA. *C*, spontaneous activity after the action of TEA was complete. *D* and *E*, detailed comparison of spikes before and after TEA action.

A similar mechanism for production of graded responses in crustacean leg muscle has been suggested by Mounier & Vassort (1975). Fig. 9 shows an example of a preparation which produced relatively small-amplitude spontaneous discharge in control saline (Fig. 9*A*). Upon addition of 20 mM-TEA to the superfusing solution (Fig. 9*B*) the spike amplitude doubled and the duration increased markedly (Fig. 9*C*, *E*). TEA also caused an increase in spontaneous firing rate which can be seen in the Figure.

Spontaneous activity occurred as either short or long spikes. Spontaneous action potentials of durations approximately 4 secs or tens of milliseconds are given in Fig. 10. The spontaneous firing of circular muscle is always associated with the pronounced 'diastolic depolarization' or pacemaker potentials shown in Fig. 10.

Voltage–current relations of each layer

Steady-state membrane characteristics of longitudinal and circular muscle strips were also measured by means of the double sucrose gap. Voltage–current curves for each type of muscle under voltage clamp are presented in Fig. 11. Two differences between the layers are apparent.

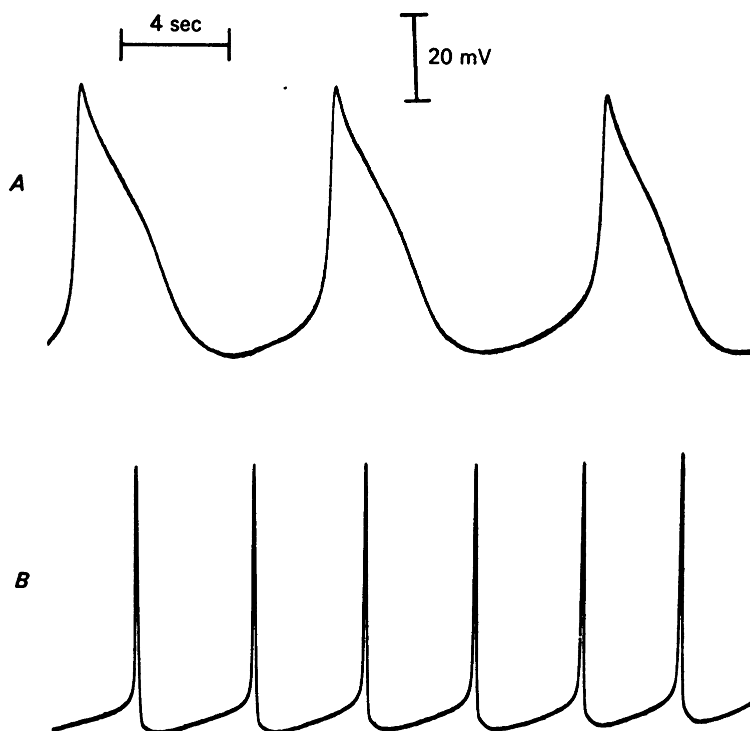

Fig. 10. Two types of spontaneous spikes in isolated circular muscle as recorded in the double sucrose gap.

First, circular muscle exhibits inward-going (anomalous) rectification at voltages near the resting potential whereas longitudinal muscle exhibits only delayed rectification in the depolarizing quadrant. That is, in circular muscle the ΔV produced by a depolarizing pulse is greater than that for an equivalent hyperpolarizing pulse; in longitudinal muscle the

opposite is true. Secondly, for preparations of similar size the slope of the V–I curve was never as steep at large depolarizations in circular as it was in longitudinal muscle. Similar V–I relations up to threshold were obtained under either voltage or current clamp.

The curves of Fig. 11. were taken after the voltage had been held at a given level for several seconds. Before steady-state conditions were reached, the depolarizing limb of the V–I curve in circular muscle was generally flatter than the steady-state curve; several seconds were required after the make of the voltage step before steady state was reached. Often a transient (lasting one or two seconds) negative resistance was observed in the V–I relationship during this time. There was also

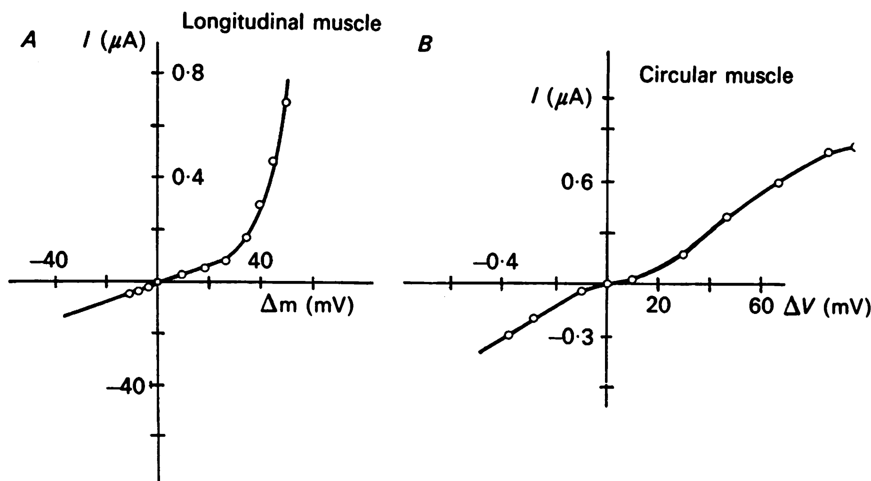

Fig. 11. Voltage–current relations for isclated longitudinal (A) and circular (B) muscle, measured in double sucrose gap under voltage clamp.

TABLE 2. Space constants in muscle layers of cat small intestine

Longitudinal muscle	λ (mm) number of preparations ± S.D.
Isolated	
Transverse to fibres	0·2 ± 0·07 (3)
Parallel to fibres	0·7 ± 0·22 (5)
Intact	
Transverse	1·6 ± 0·40 (4)
Parallel	1·1 (2)
Circular muscle	
Transverse	0·5 (1)
Parallel	3·5 ± 0·66 (3)
Circular muscle to longitudinal muscle	
Parallel to circular fibres	1·0 ± 0·22 (3)
Parallel to longitudinal fibres	1·2 ± 0·24 (2)

a transient negative resistance region in longitudinal muscle curves but it occurred at larger depolarizations (> 15 mV from rest) and the time course was much faster.

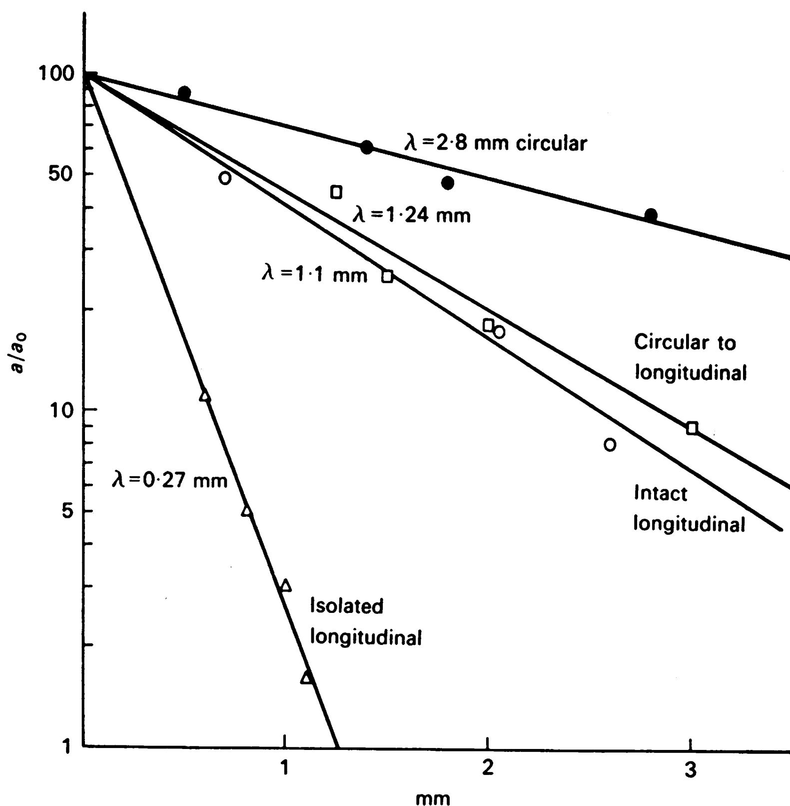

Fig. 12. Spatial decay of steady-state electrotonic potentials recorded in isolated and intact muscle layers. Amplitudes of potentials recorded by micro-electrodes (ordinate) are normalized for voltage at zero distance and are plotted on a logarithmic scale against distance (abscissa) from the current injecting pressure electrode. The apparent space constants (λ) for a particular muscle configuration is written next to the plot for each configuration. Values in circular muscle (●) were obtained from preparations which were composed of only circular muscle and electrodes were oriented in the long axis of the muscle fibres. Values in longitudinal muscle were obtained in preparations composed of either pure longitudinal muscle (isolated (△)) or longitudinal muscle attached to circular muscle layer (intact (○)) with the electrodes oriented perpendicular to the long axis of the longitudinal muscle fibres. Values for the line labelled circular to longitudinal (□) were obtained by placing the stimulating electrode on circular muscle and recording the potentials in the adjacent longitudinal muscle perpendicular to the long axis of the longitudinal fibres.

Electrotonic coupling between layers

To determine the magnitude and nature of the electrical coupling between the two muscle layers, electrotonic potentials were measured with a micro-electrode at various distances from a current injecting pressure electrode. This was done in flat preparations with isolated or intact muscle layers. Current spread from the circular to longitudinal layers is compared to current spread within the muscle layers in Fig. 12 and approximate space constants are tabulated in Table 2. The sequence of magnitudes of the space constants is as follows: circular (parallel to fibres) $>$ circular to longitudinal (parallel to circular fibres) $>$ intact longitudinal (transverse to longitudinal fibres $>$ intact longitudinal (parallel to longitudinal fibres) $>$ isolated longitudinal (parallel to fibres) $>$ isolated longitudinal (transverse to fibres). The space constant of longitudinal muscle when attached to circular muscle is longer than when isolated. Electrotonic spread from circular muscle to longitudinal muscle is similar to that from longitudinal muscle to circular muscle, as reported by Kobayashi *et al.* (1966). There seems to be no rectification in the pathway between layers.

DISCUSSION

Several findings presented in this paper and in preceding studies are central to an analysis of the origin of electrical activity and its propagation in the gut: (1) endogenous slow waves of isolated longitudinal muscle are of smaller amplitude than those recorded from this layer in intact intestine. Some strips of circular muscle, when isolated, are capable of producing voltage changes (spikes) 2–3 times the magnitude recorded from cells in either layer of the intact gut (compare Figs. 2 and 8 or 9). In other words, in the intact gut, some combination of factors is acting to augment the voltage changes originating in longitudinal cells while at the same time electrical activity in some circular muscle cells is being damped. (2) The voltage–current characteristics of longitudinal and circular muscle layers are very different. Circular muscle resembles in several ways such pace-making tissues as heart and certain neurons: in particular, it generates large action potentials, has high membrane resistance at voltages near resting potential (or inward-going rectification), and shows pace-making activity, either spontaneous or easily driven by small constant currents. Longitudinal muscle, on the other hand, shows only outward-going rectification, relatively small, brief action potentials and 'slow waves' (cf. Fig. 13, Connor *et al.* 1974). These slow waves of longitudinal muscle are not amenable to being driven in the ordinary sense of one supra-threshold stimulus eliciting a response, but over a narrow range of

frequencies, slow waves can be entrained by depolarizing pulses. It has also been shown (Connor *et al.* 1974) that within a certain range hyperpolarization reduces frequency and increases amplitude whereas depolarization increases frequency and decreases amplitude. (3) The fibres within each layer are electrically coupled and there is coupling between the two layers. Also, the integrity of both layers must be maintained for normal propagation to occur. There must be relatively low-resistance current paths *between* the two muscle layers. Electron microscopic observations reveal an abundance of interstitial cells and fibrocytes in the region between the muscle layers which make junctional contacts with muscle fibres of each layer and with each other (Taylor, Kreulen & Prosser, 1977). Since fibroblasts can serve as passive conductors between clusters of embryonic heart fibres, (Hyde, Blondel, Matter, Cheneval, Filloux & Girandier, 1969), it is probable that interstitial cells and fibrocytes provide low-resistance paths between the intestinal muscle layers.

It is possible to reconcile both the amplitude differences observed in various layer configurations and to account for propagation in a straightforward manner, although several important considerations remain unresolved. Since the two layers are electrically connected in a symmetrical manner, electrical activity in the intact gut must represent a complex summation of activity generated in the two layers. During excitation, current must flow from the layer with the higher amplitude activity (circular) to the other (longitudinal). Thus, the slow wave potentials seen in longitudinal muscle cells of intact segments represent the endogenous activity plus a component generated by the circular layer (see Figs. 3, 4, 8). Since slow waves in longitudinal fibres are larger throughout their time course when the two layers are in contact, it is probable that depolarizing current flows from the circular muscle during the entire portion of the electrical wave following initial excitation. Removal of circular layer input either surgically (Fig. 3) or by removing calcium ions (Fig. 4), lowers the slow wave amplitude of longitudinal muscle.

To initiate a slow wave from a given locus from which it is assumed to spread, excitatory currents normally arise in the longitudinal layer and spread to the circular layer. We view the pace-making activity of the longitudinal layer as the rate-setting event for segmental contractions in the intact gut even though both layers are capable of spontaneous activity. This is so because sheets of isolated circular muscle are generally quiescent and only irregularly show spontaneous pace-maker potentials and spikes. In sheets of longitudinal muscle, however, one invariably finds multiple loci in which spontaneous slow waves persist for hours. Data presented here (Fig. 1, Table 1) as well as earlier observations (Connor *et al.* 1974) show that *isolated* longitudinal muscle generates relatively small slow

waves, thus it is a weak pace-maker. However, the nature of the current–voltage relation of circular muscle is such that a large amount of current or large voltage change is not needed for excitation. For depolarization from rest levels up to approximately 20 mV the steady–state *V–I* curve is nearly flat (Fig. 11); this means that the circular muscle layer appears as a very high-impedance load to a depolarizing drive. This high impedance characteristic near rest was present even in the non-spiking preparations. This indicates that the circular muscle is very readily stimulated. The record of Fig. 10*B* must actually be an underestimate of the flatness of the *V–I* relation because the total current recorded contains a component of extracellular current flow. That is, a certain proportion of the current injected into the current-measuring pool of the sucrose gap will flow to the node through the extracellular volume because mixing of electrolytes and sucrose within that part of the bundle lying in the sucrose gap makes the sucrose an imperfect insulator (McGuigan, 1974).

The endogenous slow waves of the longitudinal layer are large enough to cause excitation in most of the bundles of circular muscle tested and because of the above current–voltage consideration, need supply only a small current. Once excitation is triggered in the circular layer the excitatory drive is amplified by Ca-requiring currents and fed back to the electrically coupled longitudinal layer and to contiguous circular muscle cells. According to this view, it is the circular muscle which provides most of the depolarizing current for propagation of the slow waves. Therefore, the pace-making mechanism is located separately from the propagating mechanism. The function of the primary pace-maker is no more than to provide the initial step in the excitation-propagation process. Thus the small intestine differs from neurons and cardiac muscle where the pace-maker propagation functions are apparently both part of the same mechanism.

There is a dramatic difference between the two layers in their mechanisms of generating spontaneous potentials. It has been ascertained in previous studies that the slow waves in longitudinal muscle represents rhythmic active transport of sodium, the efflux being maximal at the beginning of the hyperpolarizing phase (Connor *et al.* 1974). Slow waves are abolished by ouabain and by K-free medium and when the depolarization produced by these agents is compensated by applied current the waves do not recover (Connor *et al.* 1974). The slow waves are sensitive to O_2 in the medium and they mirror an oscillation in NAD:NADH (Connor, Kreulen & Prosser, 1976); when the muscle is voltage-clamped, current pulses can be measured of timing and sensitivities similar to the slow voltage waves. No resistance change is detected during a slow wave except where the slow wave crest carries over into the delayed rectification region. Pulses of

Na efflux have been measured during the hyperpolarizing phase (Job, 1969). The pacemaker potential (pre-potential) and action potential of the circular muscle on the other hand appear to reflect voltage dependent changes in Ca conductance (Liu & Prosser, 1969). These latter potentials may be driven electrically and are sensitive to changes in extracellular Ca^{2+} but are not reduced by the inhibitors which reduce the slow waves of longitudinal muscle.

Although the findings presented here implicate the circular layer as a current source throughout much of the time course of the slow wave, it has been difficult to show directly and independently that this layer is capable of supplying depolarizing current over such a time course. If all the circular muscle bundles tested showed the long action potentials illustrated in Fig. 8 and 10 there would be no problem because their duration overlaps that of the slow wave. However, the majority of bundles tested in isolation produced either brief action potentials (< 200 msec duration) or graded responses. The generation of a long excitatory response may be the result of combining the different types of fibres in an electrical network, i.e. an emergent property. In outline, the behaviour of an aggregate of circular muscle cells can be inferred from the data at hand. In any given sheet of circular muscle it is likely that a continuum of electrical types (Fig. 8) is present since in the examination of several hundreds of bundles of circular muscle, there has emerged no pattern indicating that different types of fibres segregate with respect to their spike characteristics into non-interacting regions. Because of the large delayed K current in the weakly or non-spiking bundles and the electrical continuity within the circular layer, the spiking bundles are faced with a situation which is roughly analogous to a voltage clamp. The spiking fibres, which would in isolation generate large voltage changes are limited (clamped) to smaller excursions because inward currents are drained through the delayed rectifier channels of neighbouring cells. When squid axon or other excitable tissue is voltage-clamped to levels moderately above threshold (-40 to -20 mV), the duration of inward current (excitatory) is several times longer than the action potential (cf. Hodgkin & Huxley, 1952). This property in the circular muscle could provide for excitatory currents of long duration. It would also account for the finding that slow waves recorded along the longitudinal axis of the gut from 'uncovered' circular muscle generally show decrement with increasing distance from the intact portion, but the fall-off is much less steep than that for passive spread and occasionally, no decrement is observed for several millimetres (Fig. 14 in Kobayashi *et al.* 1966).

The preceding account of reciprocal interaction between the two muscle layers is presented diagrammatically in Fig. 13. A region of isolated

longitudinal muscle is represented at the left, an intact segment at the right. The sequence described by the model is as follows: slow waves of approximately 10–20 mV are generated in some areas of the longitudinal muscle layer and current from these waves spreads electrotonically to the circular layer. This current initiates a Ca-dependent response in the extremely sensitive circular muscle layer. The particular response of a region of circular muscle depends upon the relative g_K of the fibres into which the current flows. This response has a much greater amplitude and a faster rate of depolarization than the slow wave which initiated it but may have a dissimilar time course. Depolarizing local currents flow from the activated region of circular muscle to the longitudinal muscle as well as to the adjacent regions of circular, thereby providing augmentation of slow waves in intact preparations and giving rise to local currents for propagation.

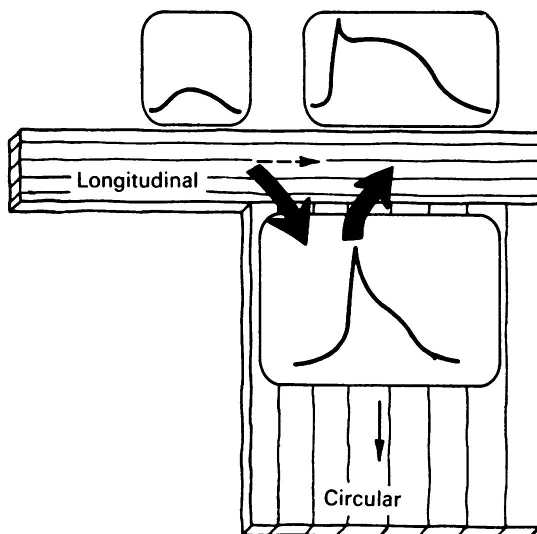

Fig. 13. Diagram of postulated interactions between intestinal muscle layers. Isolated longitudinal muscle represented at left, intact region at right. Electrical recordings from different areas are given in boxes. Arrows indicate spread of current. Slow waves are shown as originating in longitudinal muscle and being amplified in circular muscle to give large complex waves recorded from longitudinal fibres when attached to circular muscle.

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