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ON EXPLORING THE BASIS FOR SLOW POTENTIAL OSCILLATIONS IN THE MAMMALIAN STOMACH AND INTESTINE

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SUMMARY

The basic driving unit of oscillatory electrical activity in stomach and intestine is the slow wave, a propagating depolarization of myogenic origin. The slow wave controls contractile activity in the intestine by triggering action potential bursts, while in the stomach there is both action potential and spike-free slow wave activation. This review attempts to summarize recent characterizations of the slow wave and to explore in detail the evidence, which suggests that the mechanisms which generate the electrical oscillations are quite closely coupled to metabolic processes.

INTRODUCTION

Of the different types of preparations discussed in this volume, the state of knowledge regarding oscillatory electrical activity in smooth muscle is probably the most unsettled, a state of affairs not unexpected when considering the peculiarities of the tissue. Rather than risk running aground on issues of what really constitutes typical or overall characteristics in the generic category of smooth muscle, this discussion will be limited to observations on smooth muscle of the stomach and small intestine. The diversity found in these juxtaposed structures, in the view of this author, is sufficient for one review.

There are three major obstacles to quantitative study of smooth muscle electrical activity that have held back progress towards understanding the mechanisms involved in generating and propagating oscillatory activity: the tissue is composed of small, electrically interconnected cells, a feature shared by cardiac muscle and many neural preparations; an inhomogeneous population of muscle cells organized into layers of transverse orientation which in some cases cannot be physically separated; intrinsic nervous systems (plexuses) which can remain functional for the *in vitro* life of the muscle preparation. This complexity of structure reflects well the complex ensemble of functions normally performed by any given visceral organ in the best physiological tradition of form-function interrelation. Regions of the G.I. tract are commonly found in one of a number of different states, ranging from mechanical quiescence, through mild, segmental churning, to peristaltic contraction. Faced with such an array of behaviours, the matter of picking the most fundamental events has not been a matter of straightforward choice.

It has been known for some years (Alverez & Mahoney, 1921, 1922) that spontaneous, rhythmic electrical signals occur in both small intestine and stomach even in the absence of visible mechanical activity. Viewed with extracellular recording electrodes, even those suitable for chronic *in vivo* studies (Bass, 1968), two distinct types of electrical activity are seen, one of multisecond time course repeated at a slow rate, the other, spike potentials of about 100 ms duration or less which occur in bursts repeated at the same rate as the slow potentials. From analysing records of extracellular current flow Bozler (cf. Bozler, 1945, 1946) concluded that the slow signal was generated by a propagating wave of membrane depolarization lasting for several seconds. Subsequent intracellular measurements in both stomach and intestine have confirmed this conclusion. Various terms have been applied to the signal in the literature: slow waves, basic electrical rhythm, pacesetter potential, stomach action potential; this discussion will use the term slow wave.

In the intestine of mammals such as cat and dog the repetition frequency of the slow waves, *in vivo*, lies in the range of 7–20 cycle/min with a decrease in frequency existing as the intestine is traversed from duodenum to ileum. The decrease occurs in a nearly stepwise manner in the proximal portion with uniform frequency plateaus existing over distances of 10 or more cm. Small excised lengths of intestine studied *in vitro* show a steady decline in spontaneous frequency as the region from which they were taken is moved caudally (Alverez & Mahoney, 1921, 1922; Diamant & Bortoff, 1969; Nelsen & Becker, 1968; Sarna, Daniel & Kingma, 1971), indicating that, *in vivo*, a higher frequency, upstream pacemaker normally entrains a considerable downstream length. Transection and recoupling experiments in dog have shown that if high frequency input from the duodenum is removed, a frequency plateau extends over the rest of the intestine (Code & Szurszewski, 1970). The stomach operates at a single frequency which is much lower than intestine, generally there are only one to three slow waves per minute.

Many studies employing multisite *in vivo* recordings (Bass, 1968) have demonstrated that slow waves, not the spike potentials, are the propagating electrical signal in both stomach and intestine, spikes occurring intermittently in local regions of a frequency plateau with the slow waves traversing the distance. Normal propagation, of course, proceeds in the caudal direction. Propagation velocity increases on going from more cephalic to more caudal regions in the stomach, while in the intestine, velocity decreases from about 10 cm/s in canine duodenum to about 1 cm/s in the ileum (Armstrong, Milton & Smith, 1956; Bass, 1968; Code *et al.* 1968). Thus there are two possible mechanisms operative that would tend to break up the intestine into frequency plateau regions: declining spontaneous frequencies and declining propagation velocities.

In the intestine, contractions of sufficient magnitude to be detected by an implanted transducer are observed only when the slow waves trigger spikes in the vicinity, and the strength of contraction varies with the number of spikes per burst (Bass & Wiley, 1965). Both the slow waves and spikes are myogenic but significant neural modulation of the waveforms exists. In stomach, contractions are often observed without spike activity in the upper portions (Daniel & Irwin, 1968).

The relationship of this electrical activity to the important business of the small intestine musculature, mixing and propelling material, is not straightforward and

displays somewhat different characteristics between, for example, carnivores and ruminant herbivores. The basic element of motor activity is the contraction of a band of circular muscle fibres a few centimetres wide activated by spike potentials occurring during the slow wave crest. During a peristaltic wave, a ring of strong contraction travels down the intestine at the velocity of the slow wave. The peristaltic wave or rush has been reported to be a rare occurrence in dog (Code *et al.* 1968) but is common in other animals (Grivel & Ruckebusch, 1972). In the more normal mode of operation, rings of contraction occur nearly simultaneously at several stationary or slowly moving locations, dividing the active length of the intestine into segments (Cannon, 1902). The contractions recur at the slow wave frequency and it has been suggested that the wavelength of the periodic slow waves determines the segment lengths (Code *et al.* 1968). These so-called segmental contractions are usually thought to have primarily a mixing action, however, the region containing ongoing segmentation activity propagates slowly (a few cm/min) down the length of the intestine (Carlson, Bedi & Code, 1972; Grivel & Ruckebusch, 1972; Szurszewski, 1969) and has been shown to move intestinal contents.

Mechanical activity in the stomach is in most respects a more straightforward example of peristalsis, with a ring of contraction, launched in the upper corpus area, propagating with the slow wave at steadily increasing velocity toward the pylorus. In most waves the contraction of the terminal antrum and the pyloric canal is nearly simultaneous causing a portion of the contents which had been packed into the region by the initial part of the wave to be shot backward (retropelled) through a still narrowed ring in the low antrum. The net result is a thorough mixing of contents and incidently a very impressive cineradiographic demonstration. The relative quantities which are propelled into the duodenum and retropelled are determined, in part, by the length of the lower antrum involved in the nearly simultaneous contraction, the longer the portion, the larger the volume trapped and retropelled.

Thorough reviews on the mixing and propulsion of gastrointestinal contents are in the literature (Bass, 1968; Code *et al.* 1968; Daniel & Irwin, 1968) and the interested reader is referred there for a more proper treatment of this complex subject.

It is generally agreed that the basic coordinating signal for peristaltic and segmental contractions plus other more random types of activity in stomach and small intestine is the myogenic slow wave with strong, modulating neuronal and hormonal input. Discovering the mechanisms which underlie its generation is therefore an important step in understanding motility in these organs and this review will be concerned primarily with studies aimed toward this end. Several recent reviews in the same general area are in print (Bortoff, 1976; Daniel & Sarna, 1978; Prosser, 1974) and are suggested for the reader who wishes to obtain more diverse input on the subject.

DESCRIPTION OF THE SLOW WAVE

Electrical

The basic electrical characteristics of the slow wave in short lengths of intact small intestine or in preparations of the isolated muscle coat are fairly well agreed upon at this time, with quantitative revisions occurring over the past 10–15 years as recording techniques have been improved. Early microelectrode recordings from small intestine

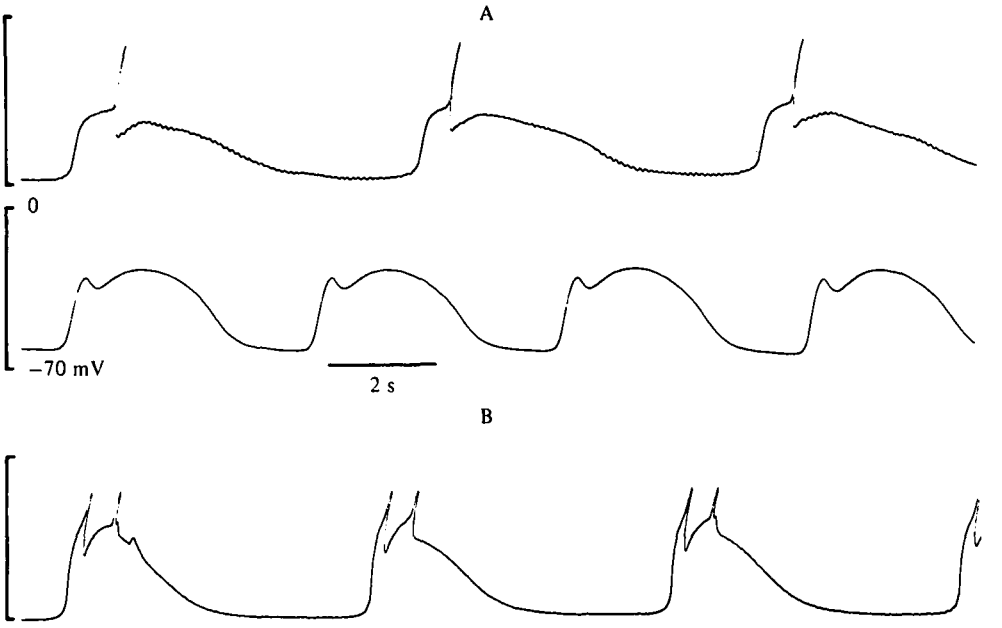

Fig. 1. Microelectrode recordings from the musculature of cat intestine. (A) Two examples of records taken from longitudinal layer cells. (B) Activity in circular muscle recorded from a 1 mm area from which the longitudinal layer had been dissected. Calibrations are the same in all records.

of cat, rabbit (Bortoff, 1961) and dog (Daniel, Honour & Bogoch, 1960) showed resting potentials of −40 to −50 mV and slow wave amplitudes of 10 to 15 mV excluding the small spike potentials which were sometimes superimposed. More recent studies on rabbit and cat have given larger values of both resting potential (V_r) and slow wave amplitude (ΔV_m): $V_r = -54{\cdot}8 \pm 0{\cdot}3$ mV, $\Delta V_m = 17{\cdot}9 \pm 0{\cdot}2$ mV for rabbit (El-Sharkawy & Daniel, 1975*a*); $V_r = -67 \pm 7$ mV, $\Delta V_m = 30 \pm 5$ mV for cat (Connor *et al.* 1977). Characteristic slow wave activity from cat intestine is illustrated in Fig. 1. The fast upstroke, notch, and plateau phase illustrated in the records from longitudinal muscle of intact segments are typical of both cat and rabbit preparations. In cat, the records from longitudinal or outer-most circular muscle cells are not markedly distinguished by differences in slow wave amplitude or duration as long as the structure of the layers is not too much interrupted. Spike potentials, when they occur, are most frequent during the plateau phase. Studies using extracellular recording electrodes (Kobayashi, Prosser & Nagai, 1967) and intracellular electrodes (Taylor, Daniel & Tomita, 1976) indicate that maximum slow wave amplitude occurs near the longitudinal–circular layer interface in both cat and rabbit. Slow waves as large as 40 mV have been reported in both cat (Connor *et al.* 1977) and rabbit (Taylor *et al.* 1976).

The activity pattern in guinea pig is somewhat different, with spike potentials being a more prominent feature of the total electrical complex and often achieving an overshoot of zero potential of 10–15 mV. Hidaka & Kuriyama (1969) and Kuriyama, Osa & Toida (1967) reported values for resting potential of −55 to −58 mV and slow wave amplitude of around 10 mV. Guinea-pig ileum becomes electrically quiescent

when exposed to saline solution made hypertonic by the addition of sucrose, while activity in cat and rabbit continues unabated following similar treatment. Hypertonic media have been used in a number of studies where microelectrodes were employed in order to reduce tissue movement. Bolton (1971) has reported enhancement of the slow wave amplitude by acetylcholine under normal tonicity conditions and restoration of spontaneous activity by this agent in hypertonic media.

A common feature of all the published records from cat, dog and rabbit is that membrane voltage seldom attains the zero potential, even during spike activity, with the peak voltage excursions usually well below zero. Though this stands in marked contrast to electrical behaviour in neural or cardiac preparations, enough evidence has accumulated to indicate that this non-overshooting electrical activity is the normal state of affairs in intestinal as well as several other types of smooth muscle.

Intracellular recordings from stomach muscle show an electrical complex consisting of a rapid initial depolarization, followed by a plateau upon which spikes are sometimes superimposed, similar in its gross respects to that of small intestine. The spontaneous frequency is lower and the slow wave duration greater. A recent, very thorough, characterization by El-Sharkawy, Morgan & Szurszewski (1978) of activity in dog stomach (see also Daniel, 1965) showed resting potentials averaging -69 to -74 mV as the recording location was moved from the orad side of the corpus to terminal antrum, and spontaneous slow waves (termed action potentials by these investigators) whose mean peak amplitude increased in the aboral direction from 29 to 71 mV, with a similar increase in mean amplitude of the plateau phase. The prevalence of spike potentials during the slow wave depolarization increased as the recording site moved from the corpus into the antrum. Similar results were obtained from a sampling of human tissue. Resting potentials of guinea-pig stomach, as reported by Kuriyama, Osa & Tasaki (1970), varied from -58 to -61 mV, sampling from fundus to the pylorus, and slow wave amplitude ranged from a reported 'few millivolts' to 40 mV. As in the guinea-pig intestine, spike potentials overshot zero potential by 5–10 mV. A report by Golenhofen & Lammel (1972) shows data in substantial agreement with these. Intracellular data from cat have not been published.

General pharmacological effects on intact musculature

It was reported early on (Daniel *et al.* 1960) that rhythmic activity in the small intestine of dog was very sensitive to anoxia, temperature, metabolic inhibitors or uncouplers, and ouabain at doses which inhibit the Na–K transport system (pump) in mammalian tissue. At the time, these findings led Daniel (1965*a*) to suggest that the slow waves could be generated by an electrogenic Na–K pump which underwent cyclic variation in its rate of transport. More recent *in vitro* studies have been done on tissue from cat and rabbit and have generally confirmed the original findings. Treatments which presumably affect cell metabolism, e.g. anoxia or dinitrophenol, require relatively extended periods of exposure ($>$ 30 min) for their effect to be expressed on slow wave activity. Ouabain (10^{-6} to 10^{-4} M) and exposure to zero potassium saline act much more quickly in abolishing activity. For the extremely small preparations used in the double sucrose gap, which also facilitates rapid extracellular fluid exchange, ouabain (10^{-5} M) or zero K eliminates slow waves within 3–4 min (Connor, Prosser & Weems, 1974), while in more bulky preparations the action requires somewhat longer

times (Liu, Prosser & Job, 1969). Flux studies on taenia coli (Brading & Widdicombe, 1974) indicate that 10^{-6} M ouabain is required for 50% inhibition of the Na–K pump. The two laboratories that have studied the action of ouabain in detail have reported dose-dependent action of the drug, i.e. at low concentrations, 10^{-7} to 10^{-6} M, the slow waves often vanish but the membrane resting potential becomes only 5–10 mV more positive than the slow wave trough (or diastolic) voltage (Connor & Prosser, 1974; El-Sharkawy & Daniel, 1975*c*; Liu *et al.* 1969). A concentration of 10^{-5} M abolishes the waves and depolarizes the membrane from 10 to 15 mV (Connor *et al.* 1974). Similar results have been obtained with cat and rabbit. In a recent study on rabbit (Taylor *et al.* 1976) it was reported that slow waves, blocked with ouabain (10^{-7} M), quickly recover with membrane hyperpolarization. The data presented, however, show incomplete blockage of slow wave activity at the normal polarization. Zero external potassium eliminates slow waves and gives a depolarization of 10–15 mV. The zero potassium effects were completely reversible in all cases; the effects of ouabain at the low concentrations were sometimes reversible. The complex effect of ouabain at different dosages has been cited as an argument against the direct involvement of the Na–K pump in slow wave generation (El-Sharkawy & Daniel, 1974) but such conclusions are not really justified since most oscillatory systems will assume one stable state or another as a critical parameter is varied.

Rhythmic activity in stomach, compared to intestine, is relatively resistant to either ouabain or zero K, a finding established in dog (Szurszewski, 1975), cat (Papasova, Nagai & Prosser, 1968), and guinea-pig (Ohba, Sakamoto & Tomita, 1977) preparations by both microelectrode and sucrose gap techniques. Ouabain (10^{-6} to 10^{-5} M) and zero K did produce a rapid 5–10 mV depolarization in both dog and guinea-pig preparations, but slow wave activity persisted at diminished amplitude for 20–30 min even in the rapidly equilibrating double sucrose gap (Ohba *et al.* 1977; Szurszewski, 1975). It would seem likely, then, that the ultimate cessation of activity when it was observed was due to changes in internal ionic content rather than a direct effect on membrane transport.

The slow wave complex in stomach and intestine of the larger mammals is unaffected by ganglion-blocking agents such as atropine and tetrodotoxin (Daniel, 1965; Liu *et al.* 1969; Papasova *et al.* 1968; Szurszewski, 1975) reinforcing other data that the important electrical events are myogenic. Guinea-pig appears to be an exception, although reports are somewhat confusing. Kuriyama *et al.* (1970) reported that the slow wave amplitude in stomach was reduced by TTX (10^{-7} g/ml) and abolished at 10^{-6} g/ml, while intestinal waves vanished at 10^{-7} g/ml. The ability of the muscle cells to generate spikes when stimulated was unaffected. Atropine (5×10^{-6} g/ml) also blocked slow wave generation in intestine and, at 10^{-6} g/ml, inhibited and sometimes blocked activity in stomach. Magaribuchi *et al.* (1972) reported inhibitory action of both TTX (10^{-6} to 10^{-5} g/ml) and atropine (10^{-6} g/ml) on slow waves in some experiments, but either no effect or a transient blockage in others. Ohba, Sakamoto & Tomita (1975) report that neither TTX (10^{-6} g/ml) nor atropine (10^{-6} g/ml) blocked rhythmicity in small strips of either longitudinal or circular muscle from guinea-pig stomach. Therefore, the possible importance of neurogenic control of rhythmicity, which would be suggested by uniform inhibitory action of these agents, is left in doubt by the conflicting observations.

Pacemaker activity site of origin

Beyond the 'organ level' data summarized above, which, in the opinion of this reviewer, are fairly consistent among published studies, there is considerable disagreement between the experimental findings of various investigators from studies designed to simplify the experimental preparation in order to determine the *cellular* basis for spontaneous oscillations or for the propagation of these oscillations. Unfortunately for the development of the area, there are relatively few laboratories engaged in cellular electrophysiological studies, and those that are often utilize different animals. For small intestine the most notable differences have arisen between studies on cat and rabbit. While the variant findings may indicate shortcomings in experimental design and techniques, as has been suggested previously (Bortoff, 1976; Daniel & Sarna, 1978), or some true mechanism difference between carnivores and herbivores, it is quite possible that much of the variation is due to architectural differences in the preparations. In both stomach and intestine there are a number of different ways used to prepare 'sub'-organ preparations, usually strips or sheets of the muscle coat freed of the mucosa. One procedure is simply to cut strips, 0·3–2 mm wide, parallel to the long axis of the cells in one of the two layers. These cut preparations are generally termed longitudinal or circular muscle preparations, but because the strip is wider than the muscle cells are long (*ca.* 100 μm), it must also contain nearly a full complement of transversely orientated cells as well as the intrinsic neural plexuses. Some experimenters report doing controls where one or the other layer is dissected away (Ohba *et al.* 1975; Szurszewski, 1975), but this is not always the case (Mills & Taylor, 1971). Sheet preparations have also presented a problem in terminology, and in intestine there has been some confusion as to what tissue a given preparation actually contains. In cat the longitudinal and circular muscle layers separate quite readily, as confirmed by light- and electron-microscope studies (Connor *et al.* 1974; Prosser 1974; Taylor, Kreulen & Prosser, 1977). In rabbit, the dissection techniques employed in phsyiological experiments separate the musculature within the circular muscle layer. These procedures leave the longitudinal layer, Auerbach's plexus, and the outer circular layer as a unit (Daniel & Sarna, 1978). In at least one series of experiments (El-Sharkawy & Daniel, 1975*a–c*) this complex has been referred to as a longitudinal muscle preparation.

Studies in which the longitudinal and circular muscle from cat intestine are separated have shown that the circular muscle preparation is generally quiescent, although spontaneous spike activity is sometimes noted, while the longitudinal layer continues to generate slow wave activity. Detailed analyses of the isolated longitudinal muscle preparation by microelectrode recordings (Connor *et al.* 1977; Kobayashi, Nagai & Prosser, 1966) have shown that waveforms and spontaneous frequency observed in isolated longitudinal muscle are similar to that of intact musculature, but that the slow waves are smaller, mean 13 ± 7 mV, compared to $27 \pm 7{\cdot}3$ mV, and found only in localized regions a few millimetres square, separated by quiescent areas in which resting potentials were normal.

These findings have been contrasted to data taken from rabbit intestine by El-Sharkawy and Daniel (Daniel & Sarna, 1978; El-Sharkawy & Daniel, 1974, 1975*c*). Here *propagating* slow waves were observed over the whole area of the preparation

with amplitudes significantly larger than those of cat isolated longitudinal muscle. Direct comparison between the two sets of findings is probably inappropriate because of the mixed nature of the rabbit preparation. In fact, the data from the rabbit preparations are very similar to data from the intact musculature of cat.

The differences of electrical activity in the separated and intact musculature of cat intestine serve to introduce one of the key problems in analysing intestinal spontaneous activity: is the pacemaking mechanism an integral part of the slow wave recorded in the intact organ, or is the signal recorded in the intact organ a complex made up of a pacemaker component summed with secondary (driven) components? Certainly the latter seems to be true with the slow wave-spike complex, because the spike components can be dropped out without greatly affecting the integrity of the slow waves. The data from the separable layers of cat intestinal muscle have long suggested that the pacemaking depolarization originates in the longitudinal muscle (cf. Bortoff, 1965) and is itself rather small. To generate the large slow waves observed in the intact musculature would therefore require an amplifying excitatory input from the circular layer. The electrical coupling between the longitudinal and circular muscle layers which would be required in such a model has been demonstrated directly by passing current into cells of one layer and measuring electrotonic potential spread in the other (Connor *et al.* 1977; Kobayashi *et al.* 1966). A recent electron microscopy study of the muscle coat shows (Taylor *et al.* 1977) numerous fibrocyte-type cells bridging the layers and forming gap-junctions with the muscle cells in either layer, thereby providing an anatomical route for current flow. Gap junctions between muscle cells in both layers were also demonstrated in this study, whereas before there had been some doubt cast upon their existence in longitudinal muscle (cf. Daniel & Sarna, 1978). Studies from our laboratory (Connor *et al.* 1977) have given considerable support for the possibility of reinforcing excitation from the circular layer. It was demonstrated that a percentage ($\sim 50\%$) of the small isolated bundles of circular muscle tested were capable of generating large overshooting action potentials (i.e. larger than waveforms observed in the intact muscle coat). In a small percentage of these, action potential duration was as great as 2–4 s, while for the majority it lay between 100 and 200 ms. The remaining bundles of the total studied normally gave small graded responses to current stimulus; however, when the potassium conductance blocker, tetraethylammonium ion (TEA) was applied (5–10 mM) these bundles generated overshooting action potentials. A very similar action of TEA on graded spikes had been reported previously by Ito, Kuriyama & Sakamoto (1970) for circular muscle from guinea-pig stomach. Although it has not been directly shown at this time, the difference in excitability of the circular muscle bundles of intestine is probably due to the relative levels of both resting g_K, and g_K activated during depolarization. In turn, both of these quantities may be controlled by intracellular Ca^{2+} levels (Mironneau, Savinean & Rahéty, 1977; Vassort, 1975). The circular muscle action potentials are dependent upon the presence of Ca^{2+} in the external medium and are blocked by the standard g_{Ca} blockers (Connor & Prosser, 1974; Liu *et al.* 1969). Since the circular layer forms part of a distributed electrical network with the longitudinal layer, this calcium dependent activity should provide excitation there.

One of the principal effects of bathing medium low in calcium on the intact gut is reduction of slow wave amplitude. This effect, studied by microelectrode recording

Connor *et al.* 1977), is most prominent where the slow waves are of large amplitude (> 25 mV) and occurs before a significant loss of resting potential is observed. Exposures longer than 15–20 min. generally lead to a loss in resting potential. In preparations where slow wave amplitude is smaller, the change in low calcium is much less severe. A reduction in frequency also occurs rapidly and will be discussed at a later point. Prolonged exposure to low calcium, 5% of normal or less, reversibly abolishes the waves. Similar findings have been reported for rabbit (El-Sharkawy & Daniel, 1975*c*). By contrast, the intrinsically smaller slow wave of *isolated* longitudinal muscle does not undergo an amplitude reduction in low calcium; in fact, an amplitude increase of 10–80% was sometimes noted (Connor *et al.* 1977). Thus, a calcium-dependent reinforcement of slow wave amplitude appears to be operative in the small intestine.

In stomach there is difficulty establishing whether the normal spontaneous drive resides in one or both of the muscle layers, and again, much of the problem arises from species differences and scarcity of data. Papasova, Nagai & Prosser (1968) concluded from their observations on cat stomach that spontaneous activity originated in the longitudinal layer. In the antrum of guinea-pig stomach slow waves are recorded from circular muscle whether or not the longitudinal layer is present (Ohba *et al.* 1975). *In vitro* studies on dog stomach have used small longitudinally cut strips of intact muscle coat or longitudinal muscle with most of the circular removed for both sucrose gap and microelectrode recordings (El-Sharkaway *et al.* 1978; Szurszewski, 1975), but apparently the isolated circular muscle has not been examined. Despite the uncertainty of origin, which may or may not be important, it has been generally concluded that the slow wave in stomach is composed of two components (excluding spike potentials): a driven 'second component' (Daniel, 1965; Ohba *et al.* 1975; Papasova *et al.* 1968; Szurszewski, 1975) accounting for most of the slow wave plateau and which is associated with contractile activity, and a smaller pacemaker, or 'first component'. In guinea-pig this first component has been studied in some detail and will be further discussed.

Investigations of the oscillator mechanism

In a field where there is confusion over basic experimental data, it should come as no great surprise that questions regarding the oscillator mechanism are being asked at a very simple level. Transmembrane voltages in biological systems arise from two sources: ion gradients with semipermeable membranes (diffusion potentials), and unbalanced active transport of ions (electrogenic transport). Since active transport maintains the ion gradients in living cells and is usually electrogenic, a finding well documented in smooth muscle (cf. Thomas, 1972), both mechanisms are usually operative, and cyclic changes involving either, or both, could give rise to voltage oscillations.

A study of slow wave activity in isolated longitudinal muscle of cat using the double sucrose gap (Connor *et al.* 1974) confirmed many of the findings of earlier microelectrode studies: in particular, the absence of pacemaker-type depolarization between waves, relatively small amplitudes (2–15 mV), sensitivity to ouabain and zero $[K]_o$, and a rather low percentage of tissue which generated slow waves. Electrogenic Na pumping was demonstrated in strips even where there was no spontaneous activity.

In the spontaneous preparations it was also found that ouabain (10^{-5} M) or zero [K] depolarized the node membrane by an amount which always exceeded the slow wave amplitude. This depolarization was of course smaller than the slow wave amplitude of the intact gut. Repolarization of the node membrane by applied current did not restore the slow waves. Frequency and amplitude were altered somewhat by membrane polarization, depolarization increasing frequency and decreasing amplitude, hyperpolarization having the opposite effect. The slow waves could be abolished by sufficient hyperpolarization (20–30 mV) but the amount required was generally far in excess of the amplitude of the waves themselves. In spontaneous preparations the waves could be entrained to higher frequencies of pulsatile stimulus. This behaviour had previously been demonstrated in longitudinally cut strips from rabbit intestine by Mills & Taylor (1971) and from cat intestine by Specht & Bortoff (1972). However, in the isolated longitudinal preparation, current stimulus could not produce slow wave activity where none existed spontaneously even though spike activity could be driven in these preparations. The term driven might therefore be avoided in favour of entrainment when dealing with slow waves, because the basic spontaneity seems to be required.

In a part of this study that has proved to be rather controversial, a voltage-clamp circuit was used to hold V_m constant in spontaneous preparations. Following execution of voltage control, periodic inward currents were observed. These spontaneous currents had the same voltage and pharmacological sensitivities as the free-running slow waves. It was shown, through numerical convolution, that the slow wave voltage time course could be accurately reconstructed from the voltage-clamp current time course and nodal membrane resistance and capacitance. The convolution procedure is equivalent to playing back a recorded membrane current through a parallel circuit composed of R_M and C_M. These results were first criticized because of the small percentage of preparations which showed spontaneity (El-Sharkawy & Daniel, 1975*c*), but this finding has become less of an issue as other studies since have also shown that pacemaking activity is generated only at certain locations (Taylor *et al.* 1976). A much more serious question, and one which cannot be directly attacked in a multicellular preparation, is that of membrane voltage inhomogeneity within the experimental node. It is quite possible as Anderson (1977) has shown with the mesotubarium preparation, that a certain population of nodal cells is electrically isolated from the population which is monitored from the voltage pool of the double sucrose gap. This situation would be realized if the two populations were separated by a relatively high internal resistance. The internal resistance would partially decouple the two populations by acting as one element of a voltage divider and attenuating the voltage changes produced in one region by activity in the other. Since coupling between smooth muscle cells is bilateral, this would mean that a spontaneous, isolated population would be affected very little, whether the voltage of the monitored population were clamped to various levels or free running, since most of the V_m difference between the two regions is dropped across the internal resistance. However, it is an unavoidable corollary of this type of model that the driving activity is many times larger than what is driven in the monitored population; the greater the isolation, the greater the difference. To generate the correspondence between clamp current and slow wave voltage observed would have required the existence of uncontrolled voltage changes many times larger

than anything observed in isolated longitudinal muscle either in the sucrose gap or with microelectrodes. In the case of Anderson's records, the spontaneous currents under voltage clamp would be capable of generating action potentials less than 10% of the amplitude actually observed in that preparation.

A third line of criticism more recently in vogue (Daniel & Sarna, 1978) involves the possible effects of external series resistance (R_s). As documented in a number of experimental and theoretical studies (Attwell & Cohen, 1977; Cole & Moore, 1960; Connor, Barr & Jakobsson, 1975; Jakobsson, Barr & Connor, 1975; Johnson & Lieberman, 1971), this factor becomes extremely important during periods of low membrane impedance such as the transient period immediately following a voltage-clamp step or when large membrane conductances are activated, as in squid axon or heart muscle. In the longitudinal muscle preparations, however, conductance changes, if they occurred at all, were too small to measure and the currents observed under voltage clamp would have generated less than 0·2 mV across the external series resistance. It is not predicted from simple theory that voltage drops of this magnitude would artifactually generate spontaneous activity.

A description of pacemaking activity in guinea-pig stomach, which is in many ways similar to that in intestine, has been developed by Tomita and his colleagues (Ohba *et al.* 1975, 1976, 1977). Their studies have employed strips of circular and longitudinal muscle in the double sucrose gap with simultaneous voltage monitoring by microelectrodes. They have reported that the presence or absence of longitudinal muscle makes no difference to the results. Hyperpolarization of the node membrane beyond a certain point gives a large reduction in the amplitude of spontaneous slow waves, and it was shown that the wave is made up of two components: a 'first component' which is small ($\sim$ 10 mV amplitude) and relatively insensitive to membrane voltage, and a 'second component' which rides on the first, presumably triggered by it, and accounts for most of the voltage excursion during the normal slow wave. The second component is dropped out by membrane hyperpolarization and a sizeable conductance change could be demonstrated during its time course. The spontaneous frequency showed a voltage dependence which was less steep than that of intestine. No conductance changes could be detected during the first component and when voltage-clamp circuitry was activated, rhythmic inward currents were generated as in cat intestine studies. Exploration of different areas of the node by microelectrodes failed to demonstrate any appreciable voltage inhomogeneities, although such a finding, by itself, has not, and should not, deflect determined critics of the technique.

These two series of studies, which have attempted to carry the analysis of slow waves furthest toward a cellular mechanism, are then in agreement that the basic pacemaking event is a small potential change which is affected by membrane voltage, but is not directly controlled by it as the pacemaker activity in heart or many neurones appears to be. The agreement extends no further, however, due to very clear differences in the behaviour of the slow waves in the two preparations. The pronounced differences in response to ouabain and potassium withdrawal have been described. The stomach preparation is even less dependent upon external sodium than the intestine. The effects of calcium ion and calcium conductance blockers on spontaneous frequency are opposite in the two preparations, although in cat stomach the effect on frequency is the same as in intestine (Papasova *et al.* 1968). Thus, while Connor *et al.*

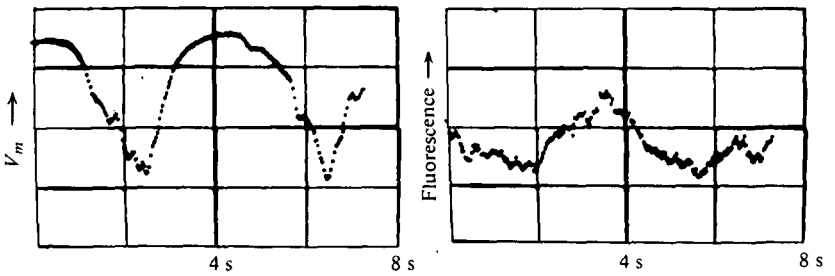

Fig. 2. Slow wave voltage (volume recording) and fluorescence (λ = 460 nm) recorded at the surface of longitudinal muscle. Records shown are the average of 20 waves. The signal averager was triggered at the slow wave crest, displaying the following wave in the central portion of the sweep.

(1974) concluded that their findings were best interpreted in terms of 'rhythmic modulation of current from an electrogenic ion transport system, most probably the sodium-potassium pump' (see also Prosser, Weems & Connor, 1976), Ohba *et al.* have taken a more discrete approach, viewing the pacemaker process as a metabolic process other than the Na-K pump (Ohba *et al.* 1977).

With regard to particular mechanisms, it is worth noting several recent studies of sodium and potassium fluxes in vascular smooth muscle by Siegel *et al.* (Siegel, Ehehalt & Koepchen, 1978; Siegel, Koepchen & Roedel, 1972; Siegel, Roedel & Hofer, 1976). These investigators have also suggested that the activity of the electrogenic sodium pump determines the membrane potential oscillations.

The possible direct involvement of metabolic input has been indicated by a different type of experimental approach. Measurements of NADH fluorescence from cat intestinal muscle showed the presence of a small oscillation having the same frequency as the slow waves (Connor, Kreulen & Prosser, 1976). The signal as measured was barely resolvable against the noise level. The apparatus has now been modified considerably to improve the signal-to-noise ratio and also to measure an external voltage signal within the area from which the fluorescence signal is taken. Records are illustrated in Fig. 2, showing the voltage and fluorescence traces in low calcium saline. Preparations studied in this apparatus are selected for absence of measurable mechanical activity; the low calcium control was included as a further protection against mechanical artifact.

Despite the data indicating relative insensitivity of the pacemaker to membrane voltage, the success of the Hodgkin–Huxley model (Hodgkin & Huxley, 1952) in explaining the nerve action potential in terms of regenerative feedback through voltage-controlled ion conductances, and the success that modifications in the basic model have had explaining repetitive and pacemaker activity in neurones and other excitable cells (Connor & Stevens, 1971; Connor, Walter & McKown, 1977; Frankenhaeuser & Vallbo, 1965; Kernell & Sjöholm, 1973; McAllister, Noble & Tsien, 1975), makes this type of model the logical first choice for explaining smooth-muscle behaviour. Indeed, there is general agreement that the spike potentials of G.I. smooth muscle are generated by sequential, voltage-dependent conductance changes to calcium and potassium ions. Also, it is probable that a component of the slow wave is generated by conductanc

changes as described above. It is important to re-emphasize that the spikes in smooth muscle which are causally related to large phasic contraction in G.I. muscle are dissociable from pacemaker or slow wave activity. *In vivo* studies tend to show that any given section of gut spends much of its time in a state where very little mechanical activity is measurable but with ongoing slow wave activity (cf. Code *et al.* 1968). Leaving aside the problem of how these two very different types of potentials can co-exist in tissue with syncitial properties, it can be said that the data which have been used to bolster voltage-controlled conductance models for the slow wave pacemaker are not compelling and in some instances are not even suggestive. Here the distinction must be clearly drawn between the axon-type conductances, which are functions only of voltage and time (i.e. voltage-controlled) and a second class of conductance mechanism which is regulated by a messenger substance (i.e. the post-synaptic receptor complex or the potassium conductance which is activated by internal calcium (see Meech, 1972)).

The slow waves of both stomach and intestine are remarkably insensititive to reduction in $[Na]_o$. In the guinea-pig stomach slow wave amplitude is reduced very little when $[Na]_o$ is reduced from the normal 137 mM to 14 mM either by choline or DDA substitution (Obha *et al.* 1977). In the rabbit intestine replacement of 76 mM of $[Na]_o$ (50%) with sucrose or lithium produced no effect on slow wave amplitude or rate of rise (El-Sharkawy & Daniel, 1975*c*). Using the value of 40 mM for $[Na]_i$ determined for the tissue in this same series of experiments (El-Sharkawy & Daniel, 1975*b*) one would calculate a change in E_{Na} from 34 to 16 mV. With a mean resting potential of -55 mV this would amount to a 20% change in the driving force for Na, the effects of which possibly could have been detected in the slow wave rate of rise. Reduction in $[Na]_o$ to around 20 mM (13% of normal) abolished slow wave activity but only after extended exposures (roughly 20 min). Similar observations were made on cat intestine using both the intact muscle coat and small bundles of isolated longitudinal muscle (Connor *et al.* 1974; Liu *et al.* 1969). Again, low concentrations of sodium led to the loss of slow wave activity but the times required were 20–30 min, more on the order of intracellular than extracellular exchange times (Casteels, Droogmans & Hendricks, 1973). The lack of immediate effect of Na replacement was most apparent in the double sucrose gap where membrane potential could be monitored continuously and extracellular washout times were brief. Stomach muscle activity has proved to be even more resistant to low sodium saline. While activity is eliminated by exposure to 0 Na for 30 min or so (Ohba *et al.* 1977) an external concentration of 10 mM sodium is sufficient to restore near normal activity.

It has been suggested in at least one study (El-Sharkawy & Daniel, 1975*c*) that chloride ions, which, if actively transported (Casteels, 1969) could have an equilibrium potential more positive than V_r and generate the slow wave plateau phase in rabbit intestine. Evidence given was that Cl replacement with a variety of large anions gave a small transient increase in the plateau phase in some preparations, which was followed in all cases by a great reduction of the plateau phase. It was suggested that the reduction resulted from depletion of internal Cl, but most of the Cl substitutes used in the study were also calcium chelators (Cristofferson & Skibsted, 1975) making it probable that the effects might just as well be due to low Ca as low Cl (see also Prosser *et al.* 1976). The possibility that [Ca] was considerably lower than normal during Cl

substitution is also suggested by the experimental records, since contractile activity was eliminated and the slow wave rate of rise greatly reduced. Although a model was proposed whereby the slow wave was generated by sequential conductance changes to Na and Cl, the evidence must be regarded as marginal.

The clearest demonstration of conductance-driven oscillations has been given by Bolton (1971, 1975) for ACh-induced oscillations in guinea-pig intestine. Sizeable conductance variations were demonstrated at various times throughout the course of the waveform and there was a rapidly equilibrating sensitivity to $[Na]_o$. It is uncertain, however, that the generating mechanism for these oscillations is the mechanism which normally generates slow waves in the guinea pig, since the induced wave frequency is quite different from the native (Bolton, 1971). Moreover, in cat intestine, ACh (10^{-6} w/v) has been applied after the normal slow waves were blocked with either ouabain or K-free saline with the result that voltage oscillations reappeared which had the general appearance of the normal slow waves, but had the rapidly equilibrating sensitivity to $[Na]_o$ observed in guinea-pig but not in the untreated cat preparation (Prosser, 1974). ACh induction would appear to be an alternate or supplementary mode of generating slow potentials, and one which probably plays some part in normal function since ACh releasing neurones are found in the gut, but is probably not the fundamental driving mechanism.

Alterations in external calcium concentration, the only other ion capable of giving substantial depolarizing drive, have a marked effect on activity in both stomach and intestinal muscle, but the effects are compound in a given preparation and do not clearly point to one central mechanism of action. The Ca-dependent electrogenesis of spike potentials and the Ca effects on slow waves have different pharmacological sensitivities and are affected at different rates by low calcium. Smooth muscle spikes are blocked by agents such as verapamil or D-600, and at 10^{-8}–10^{-6} M concentrations of cobalt or manganese, while slow wave amplitude is changed relatively little (Golenhofen & Lammel, 1972; Liu *et al.* 1969). Reduction of $[Ca]_o$ at normal $[Na]_o$ produces a marked slow wave amplitude reduction in both stomach (Szurszewski, 1975) and intestine when the layers are intact. In dog stomach, Szurszewski has demonstrated an enhancement of amplitude in twice normal calcium saline and, more significantly, increased size of phasic contractions in the absence of spikes. Thus, the calcium effect on amplitude may be due to calcium dependence of reinforcement activity as shown for cat intestine or, in the terminology of stomach muscle, upon a dependency of the 'second potential' amplitude on calcium. Saline prepared without the addition of calcium (referred to as zero Ca saline) commonly abolishes slow wave activity within 30–40 min. Realistically, the [Ca] to which the tissues are exposed in these situations is probably anywhere from 5 to 100 μM, depending on solution preparation and the rate of superfusion. In dog stomach, spontaneous activity is abolished in 0·6 mM Ca (Szurszewski, 1975). The slow waves of intestine can also be blocked at 0·2–0·4 mM $[Ca]_o$ if $[Mg]_o$ is raised sufficiently (Connor & Mangel, unpublished).

EGTA addition (2–5 mM) to zero Ca saline rapidly and reversibly abolishes the normal slow wave pattern in guinea-pig stomach (Ohba *et al.* 1977) and cat intestine (Prosser *et al.* 1977). In the cat intestine the normal slow wave pattern is replaced by yet another type of slow potential oscillation after exposure times to EGTA or EDTA saline longer than 10 min. These oscillations had a period of around 20 s, were

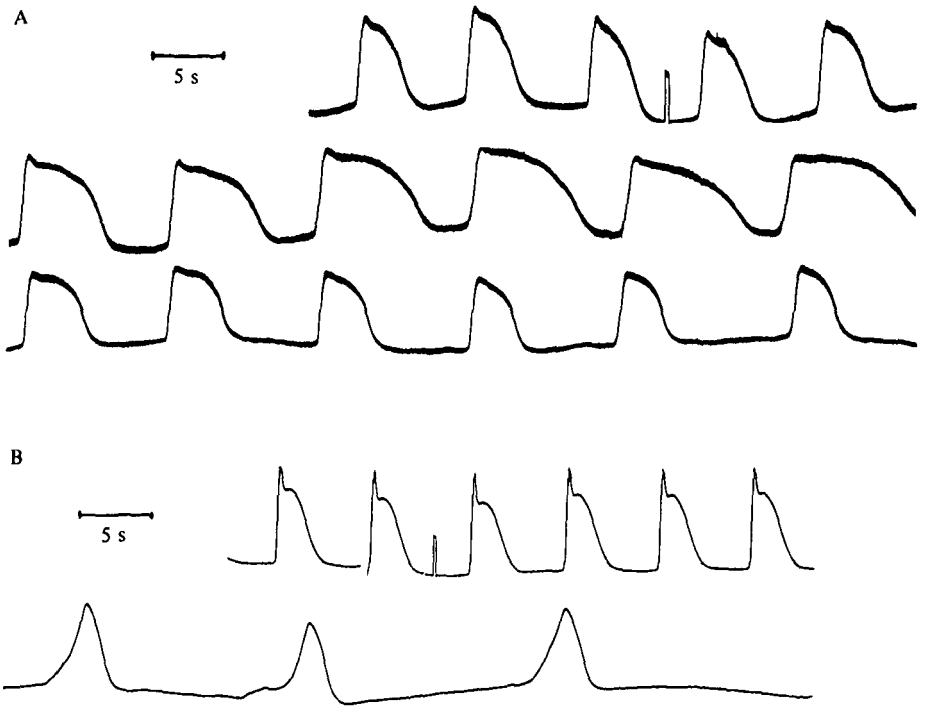

Fig. 3. Recordings from circumferentially cut strips of intestinal muscle made in the double sucrose gap. This preparation contains both longitudinal and circular muscle. (A) Top trace: control records. Middle: 20 mM Ca (8 × normal). Bottom: 0·25 mM Ca (0·1 × normal), 9·6 mM Mg (8 × normal). Slow wave activity became extremely slow and irregular 2 min subsequent to the record shown. Effects were quickly reversed in normal saline. (B) Top trace: control records. Bottom: termination of slow wave activity 3 min after exposure to 5 mM cobalt saline with normal calcium. A 10 mV calibration pulse is given in the top trace of part A and B.

generated by Na influx, and sometimes depolarized the cell membrane to near 0 mV. They were blocked by standard calcium channel blockers. Similar observations were made in stomach muscle from skate, toad and frog (Prosser *et al.* 1976). It is probably best to regard these Ca-depleted oscillations, again, as a form of alternate activity produced by mechanisms not directly related to the normal slow wave. It is worth noting, though, that intercellular coupling was not lost during the prolonged exposures to very low calcium although measurements to determine what fractional loss, if any did occur, were not made.

The final point to be made regarding regenerative conductance mechanisms is that, in most activity records, there is a conspicuous lack of slow depolarization leading into the slow wave upstroke, analogous to diastolic depolarization in heart, although exceptions are observed. In a large preparation where there is propagation of the slow wave, this is not unexpected, as most regions would be driven, but the flat inter-wave baseline is the rule, not the exception, in double sucrose gap records where propagated input does not exist (Connor *et al.* 1974; Mills & Taylor, 1971; Ohba *et al.* 1975). The models which have proved successful in generating rhythmic activity through voltage-controlled conductance changes inherently generate a 'diastolic depolarization' (cf. Connor & Stevens, 1971; Connor *et al.* 1977; Frankenhaeuser &

Vallbo, 1965; Kobayashi *et al.* 1966; McAllister *et al.* 1975). By contrast, the isolated circular muscle of intestine, when it does exhibit spontaneous activity, shows a 'diastolic depolarization' accompanied by a conductance decrease (Connor, Connor, Kreulen, Prosser & Weems, 1975; Connor *et al.* 1977).

In the search to find a consistent explanation for slow wave *amplitude* dependence on various factors, the dependency of spontaneous frequency often has been overlooked or given only minimal attention, a reasonable approach since frequency is a function of oscillation amplitude in most models of excitability and one tends to concentrate on what is commonly regarded as most fundamental. The observation that low calcium decreases frequency while leaving amplitude unaffected or increasing it in isolated longitudinal muscle of intestine (Connor *et al.* 1977) has led us to begin a careful examination of the effects on frequency of this ion. Cobalt, manganese (*ca.* 1 mM) with normal $[Ca]_o$, or high magnesium (10–15 mM) produce effects similar to those of low calcium saline. The effects on frequency are similar whether experimental strips contain both longitudinal muscle and circular muscle or isolated longitudinal muscle; slow wave amplitude is decreased in the mixed strips, however. Examples of high Mg – low Ca and Co saline action are shown in Fig. 3. Prolonged exposure to Co, Mn, or high Mg-low Ca saline causes spontaneous activity to cease in many preparations, and as illustrated in Fig. 3; the cessation is generally accomplished by an extreme reduction of frequency without an appreciable loss of amplitude beyond the rapid drop noted early in the exposure period. That is, the slow waves often die, leaving V_m near the resting potential. Solutions containing high calcium lengthen the slow wave and decrease the diastolic interval as shown in Fig. 3A. For $[Ca]_o$ greater than 25 mM, slow wave activity is sometimes blocked. It is uncertain at this time whether the effects of calcium on frequency and waveform are mediated by internal level changes or by changes in surface-bound calcium. For example, cobalt and manganese are generally considered to be Ca-channel blockers and therefore could lower $[Ca]_i$ by reducing influx; however, at the concentrations necessary to modify slow wave activity, these ions might also displace calcium from surface binding sites. The action of low calcium saline differs from the well known action of low calcium on nerve axon where the effect is thought to be primarily due to surface phenomena (Frankenhaeuser & Hodgkin, 1957; Hille, 1968). In the axon, low calcium leads to repetitive discharge or hyperexcitability; that is, the membrane responds somewhat as if it were partially depolarized. In the longitudinal muscle, low calcium gives an effect similar to that of hyperpolarization, i.e. the spontaneous frequency decreases or, in the extreme, rhythmicity is abolished. Results of preliminary studies in our laboratory indicate that treatments which reduce/increase slow wave frequency also reduce/increase tonic levels of tension in the longitudinal muscle and presumably intracellular calcium levels. Because the individual muscle cells are so small, it has not been possible yet to address the problem of intracellular calcium level changes directly through the use of techniques such as the very promising indicator dye method currently in use for giant cells (Ahmed & Connor, 1979; Brown *et al.* 1975; Dipolo *et al.* 1976).

As pointed out in a number of places (cf. Connor *et al.* 1974; Daniel & Sarna, 1978). the most troublesome aspect of oscillatory schemes which are directly coupled to metabolism, whether by means of the Na–K pump or otherwise, comes in providing a mechanism for synchronizing, or at least phase-locking, the large population of cells

encountered in experimental preparations where such coordinated activity is observed. Because of the speed of intercellular communication required, an electrical signalling mechanism is the most reasonable of the choices known at the present time. Therefore synchronization, phase-locking, and entrainment by electrical stimulus probably should be regarded as different aspects of the same problem, the voltage dependency of frequency. If the calcium effect on frequency is an intracellular one, i.e. if the significant action of changes in external calcium or of calcium conductance blockers is to alter the mean level of calcium in some cellular compartment, then one could account for the coupling of membrane potential to metabolic activity by postulating a membrane conductance to calcium which increases with positive voltage displacement. The calcium flux carried by such a mechanism need not be big enough to produce a significant voltage change in order to have the capability of significantly changing the level of intracellular *free* calcium.*

To generate oscillation it could be supposed that net calcium influx normally exceeds the combined uptake and extrusion resulting in a build-up of $[Ca^{2+}]_i$. This build-up in turn modifies intracellular processes or quantities (unpsecified here) which cause the membrane potential to change and restores the initial $[Ca]_i$. An applied depolarization would accelerate the steady calcium influx, giving a faster rate of accumulation and a decreased cycle time while a hyperpolarization would do the opposite. Low $[Ca]_o$ and calcium conductance blockers should reduce calcium influx giving a frequency reduction. With regard to the unspecified intracellular processes several types of mechanisms have been discussed in the literature which may have applicability to gut smooth muscle. Rapp & Berridge (1977) have analysed closed-loop systems with cAMP levels and adenylate cyclase activities being the quantities related to intracellular calcium and composing the primary elements in oscillatory regulating systems. As another possibility, it is well documented that intracellular calcium levels control a certain fraction membrane potassium conductance (cf. Connor, 1979; Meech, 1972; Vassort, 1975; Whittam, 1968), conductance rising as $[Ca]_i$ increases. Tomita & Watanabe (1973) have suggested that this mechanism coupled with calcium extrusion by the membrane could account for oscillatory activity. Because of the direct inhibitory effect of calcium on Na-K ATPase activity (Dunham & Glynn, 1961; Epstein & Whittam, 1966) it is also possible that increases in $[Ca]_i$ could inhibit the Na-K pump directly and with appropriately phased calcium uptake would generate voltage oscillations.

* To illustrate this, one can estimate the charge movements in a small sphere 5 μm in diameter bounded by a membrane having the specific resistance of taenia coli, 50000 Ω cm^2 (Abe & Tomita, 1968). Since the membrane time constant of intestinal muscle measured under space-clamped conditions is roughly the same as that of taenia coli this is a reasonable value to choose. The resting value of *free* internal calcium is conservatively estimated at 10^{-7} M (Dipolo *et al.* 1976). To cause a steady transmembrane voltage change of 1 mV, an insignificant quantity with respect to normal measurement capabilities, would require a current of $1{\cdot}6 \times 10^{-14}$ A, which if carried by Ca^{2+} would be an influx of $0{\cdot}83 \times 10^{-19}$ mol/sec. This influx would increase the *total* calcium concentration of the sphere by $1{\cdot}3 \times 10^{-6}$ M per second or over 10 $\times$ the free calcium level. Of course only a fraction of the influx would appear as the free ion since intracellular calcium is strongly buffered but since the membrane is the site of entry, the free calcium concentration would be greatest near the membrane inner surface. The characteristics of calcium buffering, transport and distribution are not well enough understood at this time to extend this illustration further than the point that small calcium fluxes and changes in the fluxes, while being without much direct effect on electrogenesis may have significant effects on internal concentration of the free ion.

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