COMPARATIVE STUDY OF THE MEMBRANE PROPERTIES AND INNERVATION OF THE LONGITUDINAL AND CIRCULAR MUSCLE LAYERS OF THE RABBIT DUODENUM

By

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Abstract

The studies presented in this thesis are the first attempts to compare in a comprehensive manner the basic electrical and mechanical properties of the two muscle layers of the small intestine of the rabbit, a species that showed electrical control activity (ECA). The activities of the two muscle layers were distinctly different. Cells in the longitudinal muscle layer (LM) were spontaneously active with action potentials occurring on every control potential (CP). Similarly, muscle strips dissected along the long axis of LM (LS) contracted spontaneously at the same frequencies as the ECA. Cells of the circular muscle layer (CM) usually did not exhibit spontaneous spiking activity although ECA was also present.

The characteristics of the ECA of the two muscle layers from the same muscle strips were similar in terms of amplitude, frequency, and their response to temperature change and external electrical stimuli. How the ECAs of the two muscle layers interact was investigated in light of the hypothesis that LM is the site of origin of ECA and that the ECA in CM is the result of electrotonic spread from LM (Bortoff, 1961, 1976; Connor, Kreulen, Prosser & Weigel, 1977). This hypothesis was tested directly in this study by measuring electrotonic coupling between the two muscle layers. It was found the there was little electrotonic interaction between muscle layers. Therefore, the result of this study is not consistent with the existing model in regard to the origin of the ECA.

Study of the control of muscle function by the intrinsic nerves also showed drastic differences between the two muscle layers. LM was innervated by cholinergic excitatory nerves and possibly by an inhibitory neural system. In CM, three types of neural excitatory events were identified in addition to the powerful non-adrenergic inhibitory nerves. Besides the familiar cholinergic excitatory nerves, a tetrodotoxin-resistant component and an excitatory response that emerged only after prolonged repetitve stimulation was also observed. The neurotransmitters for these two excitatory neural systems remain to be identified.

The results of this study indicate that the properties of the two muscle layers of the small intestine are very different. Nonetheless, normal physiological function of the intestine requires good coordination of the two muscle layers. The exact role of the individual layers in motility is not well defined. How these two muscle layers each with its separate neural, hormonal and local control mechanisms interact to produce the final intestinal motility pattern will be a challenging problem in the future.

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General Introduction

The muscularis externa of the small intestine carries out the essential motor functions of mixing the intestinal contents and the transport of these contents aborally in a well synchronized manner. Motility of the small intestine is difficult to study, and the statement that "on no subject in physiology do we meet with so many discrepancies of fact and opinion as in that of the physiology of intestinal movements" (Bayliss & Starling, 1899) still holds true to-day.

The problems in the study of intestinal motility are many. As will be dealt with in more detail in the sections which follow, the main reasons include: 1) the variation between animal species; e.g. guinea-pig, a popular experimental animal, is different from most other animal species in not having the basic rhythmic oscillation of the muscle cells, the electrical control activity (ECA); 2) the small size of the muscle cells and the orientation of the muscle layers make it difficult to study with many of the techniques commonly used in other systems; 3) the complexity of the motor activity of the intestine which is modulated by neural, hormonal and other local factors such as prostaglandins.

In this study, I attempt to define the basic properties of the two muscle layers of the muscularis externa, and the role of intrinsic nerves in influencing their activities. Using intracellular recording techniques, the activities of the cells of each of the two layers can be directly compared. To eliminate as many variables as

possible, *in vitro* recordings from intestinal strips were made. Such preparations are devoid of extrinsic neural influences and probably not greatly affected by gastrointestinal (GI) hormones. After first establishing the basic properties of the muscle layers in isolation, the action of different modulating factors can be studied. In this project, only the role of the intrinsic nerves was studied. In the future studies with prostaglandins and GI hormones using a similar approach will yield valuable information as to the mode of action of these agents on the muscle layers. Finally, the interaction of all these factors, as encountered under *in vivo* conditions, in modulating intestinal motility may then be more clearly defined.

I

The Anatomical Features of the Intestinal Smooth Muscle

The smooth muscle cells of visceral organs are small. They are about 150μ in length and 2 to 3.5μ in diameter at the relaxed state (Lane, 1965; Prosser, Burnstock & Kahn, 1960). Their nuclear and cytoplasmic contours are smooth. As the cells contract, they become ellipsoid and invaginations are observed at points of myofilament attachment to the plasma membrane; these alternate with membrane vesicle-containing projections of the intervening membranes. The nuclei of the contracted cells are shortened to 70μ in length and the diameter increased to 6μ (Lane, 1965).

There are three layers of smooth muscle cells in the small intestine. The outermost layer of muscle cells is oriented longitudinally, and the underlying layer is oriented circularly. These two layers make up the muscularis externa which is primarily responsible for motor function of the gut. The third muscle layer, the muscularis mucosae, lies closest to the lumen. This muscle layer is not directly involved in the propulsive function of the intestine.

The smooth muscle cells are aggregrated in bundles or sheets that are surrounded by connective tissues (Schofield, 1968). The circular muscle coat is thicker than the longitudinal muscle. In the rabbit duodenum, the circular muscle is 65μ thick compared to 32μ in the longitudinal muscle (Prosser, 1973). The circular coat is composed of encircling bundles of muscle cells, these bundles vary from 100 to 400μ in diameter and are connected to each other by regions of anastomosis; i.e. by smaller bundles which leave one large bundle

and join another. The longitudinal layer is relatively homogenous and is sheet-like in appearance.

Within the circular muscle coat, a distinct innermost layer is also present. The cells in this layer are more electron dense than the main muscle layer. The inner layer is separated from the main layer by a plexus formed from communicating nerves from the myenteric plexus and the Meissner's plexus (Duchon, Henderson & Daniel, 1973; Gabella, 1974).

Neighbouring muscle cells are generally separated from each other by 60 to 80nm. Basement membrane material and collagen filaments fill the intercellular space. Each cell is surrounded by 10 to 12 others in a staggered arrangement (Merrilles, 1968; Bennett & Rogers, 1967) and rarely are they positioned end-to-end.

Various types of cell-to-cell contacts have been described in the smooth muscle system. In the circular muscle layer of the intestine, nexuses are frequently found (Henderson, Duchon & Daniel, 1971). Very few nexuses are detected in the longitudinal muscle, and if found, are quite small compared to those in the circular layer (Henderson, et al., 1971; Gabella, 1972a; Gonella et al., 1975). In the longitudinal muscle, a small number of close appositions, which are regions in which plasma membranes of adjacent cells run parallel and separated by a space of about 10nm, and many intermediate contacts, characterized by a separation of about 50nm between adjacent membranes, were found (Henderson et al., 1971). In areas where the longitudinal layer and the circular layer are not separated by the nerve plexus, the smooth muscle cells of the two layers may form close appositions and

intermediate contacts.

SUMMARY - Anatomy of the Muscle Coats

1. The muscle coats responsible for motility lie on the outside of the intestine and consist of an inner circular layer and an outer longitudinal.
2. The smooth muscle cells are small, about 150μ in length and 2-5μ in diameter.
3. In the circular layer, the cells are frequently connected by nexuses. Nexuses are rare in the longitudinal layer and cells form close appositions or intermediate contacts instead.

II
Electrophysiology of Intestinal Smooth Muscle - Passive Membrane Properties

1. The Syncytial Nature of the Smooth Muscle

The muscle coats of the viscera are made up of small individual smooth muscle cells. In 1938, Bozler reasoned that:

> "uncoordinated activity of the small muscle cells, therefore, could never produce the regular movements which are observed in these organs. In the absence of external stimuli, rhythmic contractions of smooth muscle can only be understood by postulating some mechanism of conduction which coordinates the activity of numerous elements" (Bozler, 1938).

The idea that smooth muscle cells are well coordinated in their mechanical activity and behave like a syncytium as a result of electrical coupling between smooth muscle cells is now well established. With intracellular recording, Tomita (1966a, b) first demonstrated convincingly that the cells in taeni coli of guinea-pig are electrically connected. That smooth muscle cells are electrically coupled by some kind of

low-resistant pathway is supported by studies of electrotonic spread between muscle cells using extracellular and intracellular polarization techniques. Electrotonic potential generated by applying a square pulse through a pair of large external electrodes can be detected from cells at distances many times the length of a single cell from the stimulating electrodes. Thus a propagating action potential can be initiated in distant smooth muscle cells if a sufficient depolarizing current is passed between the stimulating electrodes. When current is injected into single cells through microelectrodes, the spatial decay and the time course of the electrotonic potential is very different from that obtained by extracellular polarization, being much shorter. For example, a time constant of 10ms was obtained for the guinea-pig taeni coli using intracellular polarization (Kuriyama & Tomita, 1965) as compared to about 100ms from experiments using external electrodes (Tomita, 1966a). The spatial decay is also much sharper with intracellular polarization and electrotonic spread can hardly be detected in adjacent cells (Sperelakis & Tarr, 1965). Depolarizing pulses applied intracellularly also failed to evoke action potentials although spikes could be easily triggered by external electrodes (Kuriyama & Tomita, 1965).

These apparent discrepancies with extracellular and intracellular polarization can be resolved by a model of smooth muscle in which cells are electrically interconnected through low resistant pathways. Thus current applied with an intracellular electrode into one cell will be able to escape into its neighbouring cells in all three dimensions via low-resistance connections. Therefore, the time constant is brief and

the spatial decay of this potential change is short. With external polarization, current is injected into a large number of cells simultaneously and the time course of potential change in these cells is much slower because current cannot rapidly escape into adjacent cells which are equally charged with the same current intensity. Therefore, the current in such case can only escape across the cell membrane and not through the cell coupling. The potential develops slowly as the charge can only accumulate over a small area of membrane (Bennett, 1972). Since the potential change due to the applied current is similar for all the cells in the radial directions through the muscle, the potential only decreases markedly in the longitudinal direction away from the stimulating electrodes. The space constant is therefore much longer than that obtained with intracellular polarization.

The inability to generate action potential by intracellular applied current can also be explained by this model. The active inward current generated by the depolarizing membrane of a single cell will have to supply a large local circuit current to the surrounding cells in order to depolarize their membranes to threshold. At the same time, these surrounding cells will supply the outward current that readily compensates for the discharge of the membrane capacity of the cell, thereby aborting the regenerative response (Noble & Stein, 1966).

Another indication of the syncytial nature of smooth muscle is the spontaneous oscillation of the membrane potential. ECA of the small intestine is tightly coupled with little phase differences between cells around the circumference (Sarna,

Daniel, & Kingma, 1971; Daniel & Chapman, 1963; Kobayashi, Nagai & Prosser, 1966; see following sections on ECA).

2. Morphological Correlate of the Electrically Coupled Smooth Muscle Cell

Electrophysiological studies have indicated that smooth muscle cells are electrically coupled to each other through some kind of low-resistance pathway. That the nexuses is the morphological basis for electrical transmission has been claimed in many physiological systems (see Bennett, M.V.L., 1972) including smooth muscle systems (Dewey & Barr, 1962, 1968). However, in spite of the fact that nexuses are abundant in some smooth muscles, e.g. the circular coat of the intestine, they are rarely found in others, e.g. the longitudinal coat of the intestine (Henderson et al., 1971; Daniel, Daniel, Duchon, Garfield, Nichols, Malhotra & Oki, 1976). In such cases, probably the other types of contacts, namely the close apposition and intermediate contacts, are sufficient to serve as low resistance intercellular contacts. Another possible explanation is that the nexuses in these muscles are so small or labile that they defy detection with standard techniques. In any case, the role of the nexuses as the sole structural basis for electrical coupling is not well established in smooth muscle and no direct evidence indicates that it provides a low resistant contact between smooth muscle cells (Daniel et al., 1976).

3. Cable-like Properties of Intestinal Smooth Muscle

Using an external polarization method, Tomita (1966a; Abe &

Tomita, 1968) first demonstrated cable-like properties in smooth muscle. Smooth muscle cells can be represented electrically by a series of independent cables formed by end-to-end connexions between cells. Using external polarization, the interconnexions in the transverse direction can be disregarded because the tissue is at equi-potential in this direction (Abe & Tomita, 1968; Tomita, 1970). Under such conditions, it was found that many smooth muscles satisfy the criteria required to demonstrate cable properties (for more detailed reviews, see Tomita, 1970 and Bennett, M.R., 1972). As would be expected for current injection in a cable (Hodgkin & Rushton, 1946), the amplitude of the steady electrotonic potentials decays exponentially with distance from the stimulating electrode and increases with time in a manner described by an error function. The time to reach the half-amplitude of the electrotonic potential increases linearly with distance along the cable. The slope of this is expressed by $\tau_m/2\lambda$, where τ_m is the time constant of the membrane and λ is the space constant. In accordance with the cable equation, the initial depolarization of the action potential rises exponentially as demonstrated in nerve and skeletal muscle membranes. The cable-like character in smooth muscle is verified by examining the relationship between the frequency of alternating currents and the potential change in the membrane with the modified cable-equation developed by Tasaki & Hagiwara (1957). Again, the experimental observation fits very well with theoretical prediction (Tomita, 1966b).

SUMMARY - The Electrical Nature of Smooth Muscle Cells

1. Electrical and mechanical activities are well-coordinated in the

small intestine. This requires synchronization of many individual smooth muscle cells and is achieved by good electrical coupling between muscle cells. Thus the smooth muscle behaves like a syncytium.

2. Nexuses have been suggested as the structure providing the low-resistant pathways for electrical coupling between cells. However, this may not always be true, especially for the longitudinal muscle where such close contacts between cells are rarely found.
3. Using the partition stimulation method, cable-like properties have been demonstrated in a variety of smooth muscle.

III
The Spontaneous Electrical Activities of the Intestine

1. The Electrical Control Activity (ECA)

Monitoring of the electrical activity of the intestine was pioneered by Alvarez in the 1920's. Using external electrodes and rather crude electronic equipment, he reported the presence of spontaneous rhythmic electrical potentials in the small intestines of rabbits, cats and dogs (Alvarez & Mahoney, 1922a). It was also noted that the frequency of rhythmic contractions at different segments of the intestine corresponds to the frequency of these electrical oscillations. Furthermore, this electrical activity was omnipresent and persisted even in the absence of mechanical activity (Alvarez & Mahoney, 1922b). With more sensitive equipment, Bozler (1938b) showed that there was a fast component consisting of action potentials superimposed on the slow oscillation. It was further established that contraction is associated with the spike activities and not with the

slow potential change (Bozler, 1938b, 1946). Electrical activities composed of a slow oscillating component and a fast spike component were confirmed when intracellular recordings was introduced. The slow component results from periodic depolarization of the membrane lasting for several seconds, and the action potentials, when they are present, occur at the peak depolarization (Daniel, Wachter, Hanour & Bogoch, 1961; Gonella, 1965). It is now clear that the slow oscillation, the spike activity and the mechanical activity have a direct relationship to each other (Bass, Code & Lambert, 1961). The slow oscillation, commonly known as the slow wave, has been termed electrical control activity (ECA) because it controls in time and space the appearance of action potentials, and hence, the contraction (Sarna, 1975). The action potentials are referred to as electrical response activity (ERA) because they are associated with contractile responses (Sarna, 1975a).

2. Synchronization and Frequency of ECA

Intestinal motility is a complex action that requires coordinated activity of many muscle cells. The basic underlying control of contraction is the ECA since spikes normally occur only on the depolarized phase of the ECA, the control potential (CP). Using extracellular electrodes under *in vivo* (Daniel & Chapman, 1963) and *in vitro* (Kobayashi *et al.*, 1966) conditions it is well established that the ECA occurs with no discernable phase lag around the circumference of the intestine.

There is a decreasing ECA frequency gradient along the small intestine (Alvarez & Mahoney, 1922a; Sarna, Daniel & Kingma,

1971). The frequency gradient is an inherent property of the different parts of the small intestine, which decreases in rate from duodenum to the terminal ileum. Isolated segements from duodenum contract and show a higher ECA frequency than those from ileum. For example, the intrinsic frequency of ECA for isolated duodenal segments from the dog is about 19/min and the rate decreases exponentially to about 11/min in the ileum (Sarna et al., 1971). In vivo recordings also show such a gradient except 1) the frequency at any site is higher than that if the segment is isolated and 2) there is a frequency plateau at the duodenum, and a linear decrease in ECA frequency in the aboral direction rather than the exponential decrease observed in isolated segments. Thus the in vivo frequency at the duodenum is approximated 20/min and at the ileum 14/min (Sarna et al., 1971).

The ECA of the small intestine behaves with characteristics similar to those of relaxation oscillators (Nelsen & Becker, 1968; Diament & Bortoff, 1969a, b) as described by Van der Pol (1940). The ECA gradient (both observed and intrinsic frequencies) can be simulated most successfully by a chain of bidirectionally coupled relaxation oscillators (Sarna et al., 1971). In this model, each segment (with no phase lag around the circumference) represents an independent oscillator having its intrinsic frequency. When these oscillators are coupled in a chain-like manner, output of each oscillator feeds into its neighbor as when the intestine is intact, the higher frequency oscillators (i.e. the segments from the upper small intestine) tend to pull the frequency of the lower frequency oscillators (segments from the lower small intestine) up to that of their own. This frequency pulling is a fundamental

characteristic of relaxation oscillators. Using models based on these bidirectionally coupled relaxation oscillators, the essential characteristics of the ECA of the small intestine and the stomach can be simulated, e.g. the in vivo and intrinsic frequencies of different segments of the intestine, and the phase lag patterns, (see Sarna, 1975b for a more detailed description of the model).

The frequency of the intestine ECA can be altered by electrical stimulation, under both in vivo (Sarna & Daniel, 1975) and in vitro (Mills & Taylor, 1971) conditions. The maximum driven frequency at a particular site is the same for intact and isolated segments, although the latter shows a lower intrinsic frequency. Hyperpolarization of the cell membrane depresses the ECA frequency and increases the amplitude while depolarization increases frequency and decreases amplitude in the cat small intestine (Connor, Prosser & Weems, 1974). However, Taylor, Daniel & Tomita (1975) reported that in the rabbit jejunum, depolarizing pulses reduced control potential amplitude and frequency whereas hyperpolarization increased control potential amplitude and frequency.

3. The Origin of the ECA

The current evidence suggests that the ECA originates from the smooth muscle cells, although it was speculated that they were of neural origin in the early days (Berkson, 1933; Ambache, 1947). Isolated segments of intestine in the absence of extrinsic innervation continue to show rhythmic contraction and electrical activity (Bortoff, 1961). Vagatomy also has no effect (Daniel et al., 1960). ECA activity persists in the presence of a wide variety of nerve blocking agents

such as procaine, a local anaesthetic (Holaday, Volk & Mandell, 1958), tetrodotoxin, a potent neurotoxin which selectively blocks the sodium permeability increase of an action potential (Liu, Prosser & Job, 1969), ganglionic blocking drugs (Daniel et al., 1960), cholinergic and adrenergic antagonists (Daniel et al., 1960). Direct recordings from the myenteric plexus also show no relationship between neural activities and the ECA (Ohkawa & Prosser, 1972). Procedures that lead to the degeneration of ganglion cells such as cold storage or hypoxia also do not abolish the rhythmic activity (Hukuhara, Kotani & Sato, 1962).

Although the myogenic origin of the ECA is well accepted, the mechanism and its loci of origin are not as clear. The problem can be attributed to the complex anatomical arrangement of the preparation. Given the small size of the smooth muscle cells and the constant rhythmic movement of the preparation, it is not suprising that many studies of the electrical activity of the smooth muscle used extracellular recording methods. In almost every instance when intracellular recordings were made, the tissues were treated with agents such as hypertonic sucrose to eliminate the mechanical response. Extracellular electrodes record electrical events from a large number of cells and give only a qualitative overall picture. The events at the cellular level remain unobserved. Also the interpretation of many extracellular records is uncertain. For example, depolarization of the ECA gives a positive wave in the electromyogram if recorded with an external monopolar electrode. However, the depolarization of an action potential, which is much faster and larger in amplitude, gives predominantly a negative deflection. Sucrose gap recording or intracellular recordings

in hypertonic sucrose solution also creates uncertainty because of the effects of sucrose, which is known to hyperpolarize the membrane potential and alter the membrane properties (Coburn, Ohba & Tomita, 1975 a, b). Voltage clamp techniques using a double sucrose gap is further complicated by the series resistance, and the errors and artefacts introduced in such a complex multicellular tissue is difficult to assess (Coburn et al., 1975).

There are presently two hypotheses for the site of origin of the ECA and both of these were based on studies of the cat small intestine. The first was put forward by Bortoff (1961, 1965, 1976) in which he concluded that ECA originates only from the longitudinal muscle and spreads electrotonically to the circular muscle. A more recent proposal was that of Connor, Kreulen, Prosser & Weigel (1977) in which the ECA originates also in the longitudinal muscle but after spreading into the circular layer passively, a "regenerative amplification" takes place in the circular muscle. Then the waveform modified by amplification spreads back to the longitudinal layer. These hypotheses were derived from experiments involving attempts to separate the two muscle layers so that the electrical activities of each layer could be studied independently. However, this procedure may lead to damage to either or both layers, and certainly a "clean" layer without any contamination of the other is difficult to obtain. This is especially the case with isolated "longitudinal muscle" which usually contains a small layer of circular muscle along with the myenteric plexus. More detailed discussion of these problems has recently been presented by Daniel & Sarna (1978) and the evidence for the two models will be more critically analyzed in the following chapters. Suffice it to say that the data necessary to determine

the site of origin and mechanism of spread ECA are very scanty. All the models are very speculative and no critical tests have been applied to evaluate these models. Furthermore, contradicting evidence that the ECA originates from the circular layer rather than the longitudinal muscle layers had been reported in the rabbit small intestine (Taylor, Daniel, & Tomita, 1975) and in the cat colon (Christensen & Hauser, 1971a, b).

4. Ionic Mechanism of ECA

The mechanism for the generation of ECA is also controversial. The involvement of the sodium pump has often been proposed. Thus the turning off or on of an electrogenic pump would produce depolarization and repolarization respectively. The initial implication of the sodium pump in the ECA was based on *in vivo* observations that factors known to affect the pump also depressed the ECA. The dog's small intestine was found to be sensitive to temperature and hypoxia (Daniel *et al.*, 1960). Low sodium or substitution of sodium with lithium, which is not transported by the pump, and application of ouabain, a cardiac glycoside known to inhibit the ATPase, depressed ECA under *in vivo* conditions (Daniel, 1962, 1965; El-Sharkaway & Daniel, 1975b). Job (1969) reported a maximal rate of efflux of radioactive sodium early in the repolarizing phase of the ECA, consistent with extrusion by the sodium pump. Metabolic inhibitors also disrupt the rhythmic activity (Daniel, 1965). In the cat small intestine, ouabain (10^{-7}M) or a potassium-free solution abolished the ECA (Connor *et al.*, 1974). However, neither ouabain (10^{-3}M) nor a potassium-free solution could eliminate the ECA in rat small intestine, (Mangel & Nelson, 1978). In the rabbit small intestine, ECA, abolished by ouabain or lithium-Krebs, could be restored by repolarization of the depolarized membrane (Taylor *et al.*, 1975).

An alternate explanation is that membrane conductance changes underlie the membrane oscillation (El-Sharkaway & Daniel, 1975c). Thus on this hypothesis the rising phase of the ECA is due to an increase in sodium permeability and the plateau phase to an increase in chloride permeability. However, there are problems with both the pump model and the conductance change model. For example, large ECA up to 40mv has been recorded in rabbit (Taylor et al., 1975) and cat (Kreulen, Prosser & Connor, 1975) intestinal cells. This is beyond the theoretical limit of the contribution of the sodium pump to the membrane potential (El-Sharkaway & Daniel, 1975c). As pointed out by Daniel & Sarna (1978), the strongest point in favor of the conductance change model rather than the sodium pump model in that ECA can be electrically driven in both the stomach and small intestine (Mills & Taylor, 1971; Specht & Bortoff, 1972; Connor et al., 1974; Sarna & Daniel, 1975). Using the double sucrose gap technique, Connor et al., (1974) reported that no changes in membrane conductance were observed during the course of a slow wave in the cat small intestine although Mills & Taylor (1971) using similar techniques reported a decrease in electrotonic potential on the plateau of the ECA of rabbit small intestine.

The mechanism for ECA probably will not be elucidated in the near future. There are two main obstacles 1) the most direct method of looking at ionic currents and conductance changes is the voltage-clamp method. Smooth muscles, because of their geometrical complexities, are not ideal for voltage-clamping (McGuigan, 1974; Coburn et al., 1975b). Attempts at such clamping are far from satisfactory in meeting criteria for voltage control at all times and throughout the clamped

node. They do contribute to our knowledge of some of the properties of the smooth muscle (Anderson, 1969; Mironneau, 1976; Inomoto & Kao, 1976). 2) ECA may be initiated in only a small group of cells (the pacemakers). Therefore, any ionic or other changes may take place only in these cells and not in the others. Unless such pacemaker cells have been located or proven to be non-existent within a preparation, the approaches usually applied will not likely yield relevant information and may add to the confusion.

5. The Action Potential

Spontaneous firing of action potentials appear to be common in the smooth muscle cells of the intestine, especially in the longitudinal layer, e.g. guinea-pig taeni coli (Bulbring, 1957); guinea-pig small intestine (Suzuki & Kuriyama, 1975), cat small intestine (Tamai & Prosser, 1966); rabbit colon (Gillespie, 1962a), and rabbit duodenum (Gonella, 1964). There are several distinctive characteristics of the action potentials in the smooth muscle cells:

1) They are of longer duration than those of nerves or skeletal muscle. Thus in the rabbit jejunum (El-Sharkaway & Daniel, 1975a) and guinea-pig taeni coli (Bulbring, Burnstock & Holman, 1958), the duration is about 15msec.

2) The spikes are not random but regulated by the ECA. Presumably the depolarized phase of the ECA, the control potential, represents the most excitable period of the cycle. Guinea-pig is exceptional in not having regular ECA, but whenever slow depolarizations are present, the spikes are always associated with them (Kuriyama, Osa & Toida, 1967;

Suzuki & Kuriyama, 1975). In the rabbit intestine, the spikes are preceded by a small depolarization, the prepotential (Gónella, 1954; El-Sharkaway & Daniel, 1975a). Although the spikes and the ECA are closely associated, they are produced by different ionic mechanisms (see below). The spikes which are more sensitive to the ionic environment, can be dissociated from the ECA (Tamai & Prosser, 1966). For example, verapamil can abolish the action potentials at concentrations which do not affect the frequency or amplitude of the ECA (El-Sharkaway & Daniel, 1975b).

3) The action potentials in intestinal muscles are less dependent upon the external sodium than those of nerves and skeletal muscles. Like other excitable membranes, the action potential of smooth muscle also show time and voltage dependent changes in ionic conductance of the cell membrane (Kumamoto & Horn, 1970; Inomata & Kao, 1976). It is now generally accepted that the inward current is carried mainly by the calcium ion (Mirronneau, 1976; Inomata & Kao, 1976) although there may also be a small Na^+ component in the uterine muscle (Mironneau, 1976). The evidence against Na^+ as the principal carrier of the inward ionic current is strong from studies in several smooth muscles. Action potentials can be evoked in low or sodium-free solutions in guinea-pig taeni coli (Kuriyama & Tomita, 1965), guinea-pig ileum (Suzuki & Kuriyama, 1975), cat uterine muscle (Daniel & Singh, 1958). Tetrodotoxin, a neurotoxin which specifically blocks the sodium channels of nerves and skeletal muscles, has no effect on the action potential of smooth muscles (Kuriyama et al., 1967; Bulbring & Tomita, 1967) or the amplitude of the inward current in voltage-clamped tissues (Anderson, Ramon &

Snyder, 1971; Kunamoto & Horn, 1970).

All the available evidence in the study of the ionic mechanism underlying the action potential in smooth muscle indicates that calcium is essential. Reducing the external calcium invariably abolished the action potential. In sodium-free solutions, the spike amplitude of taeni coli is a function of the external Ca++ concentration (Holman, 1957, 1958). Voltage clamp studies also support that the inward current is carried essentially by calcium (Kunamoto & Horn, 1970; Anderson et al., 1971; Inomata & Kao, 1976; Mironneau, 1976). The action potential and the inward calcium current using voltage clamp techniques can be blocked by manganese ions, a selective calcium antagonist (Bulbring & Tomita, 1969; Kunamoto & Horn, 1970; Anderson et al., 1971; Mironneau, 1976). Other calcium antagonists, such as cobalt and lanthanum (Anderson et al., 1971), D-600 (Mironneau, 1976) and verapamil (El-Sharkaway & Daniel, 1975b) also abolish the action potential. Furthermore, action potentials can be maintained by solutions in which calcium has been substituted by barium or strontium, which are similar in ionic size to calcium (Sakamoto, 1971; Nonomura, Hotta & Ohashi, 1966).

The repolarization phase of the action potential is presumably due to the increase in potassium conductance, as in other excitable systems. Voltage clamp studies showed an outward potassium-dependent current with the reversal potential similar to that of the potassium equilibrium potential (Anderson, 1969; Inomata & Kao, 1976). Tetraethylammonium (TEA), a selective blocker of potassium conductance increase, reduced this current and prolonged the action potential

(Inomata & Kao, 1976).

6. Myogenic Control of Motility - Role of ECA

Intestinal contraction is initiated by action potentials which are coordinated by myogenic ECA. There are three important features of the ECA that are intimately related to the motor activity and the propulsive function of the smooth muscle of the small intestine.

1) Rhythmicity - The periodic depolarization of the membrane of the ECA provides the underlying excitability cycle of the muscle. Thus action potentials are normally found only on the control potentials when the membrane potential is lowest.

2) The Frequency Gradient - The oral-to-caudal pattern of propulsion is maintained by the higher ECA frequencies in the upper intestine (Szurszewski & Code, 1970). The higher frequency of contraction at the oral end helps to propel the contents of the intestine downwards and impedes transport in the reverse direction.

3) Synchronization - The control potential of the individual muscle cells is closely synchronized with little phase difference around the circumference of the intestine (Kobayashi et al., 1966). The synchronization of ECA results in simultaneous firing of action potentials around the circumference. Consequently, all the muscle cells at that location contract simultaneously. At the same time, there is a different phase relationship of the control potentials of the muscle cells along the length of the intestine. In the proximal intestine, the control potentials are phase-locked. The distal sites away from the proximal intestine show phase lag to those occurring more proximally (Sarna, Daniel & Kingma, 1971). Therefore, the action potentials also fire sequentially along the intestine in time intervals according to the phase lag.

The resultant mechanical activity is then a ring-like contraction around the intestine travelling down the intestine sequentially, thereby propelling the luminal contents in the same direction in a well synchronized manner.

SUMMARY - Myogenic Activities of the Small Intestine

1. Spontaneous periodic oscillation of the muscle membrane potential, collectively known as the ECA, occurs continuously in the smooth muscle cells of the intestine in most mammalian species.
2. There is a decreasing frequency gradient of the ECA along the small intestine.
3. The ECA has been suggested to originate from the longitudinal layer and to spread into the circular layer.
4. The sodium-pump is intimately related to the ECA and has been proposed as the mechanism for its generation. An ionic mechanism involving premeability changes has also been proposed.
5. Action potentials in smooth muscles are dependent upon external Ca++.
6. The action potentials are normally regulated by the ECA and occur on the depolarized phase of the ECA. Therefore, contraction of the intestine is also rhythmic in accordance with the frequency of the ECA.

IV
The Innervation of the Small Intestine - Anatomical Features

1. Extrinsic Innervation

The gastrointestinal tract is controlled by the autonomic nervous system. The parasympathetic nerves of the autonomic nervous system are carried chiefly by the vagus nerve, which supplies the esophagus, stomach, small intestine, the first portion of the colon. The pelvic nerve supplies parasympathetic input to the remainder of the colon and the rectum. The efferent fibres of the vagus and pelvic nerves are preganglionic, and they form synapses with neurons of the intrinsic plexuses of the stomach and intestines (Kuntz, 1955; Pick, 1970). The preganglionic sympathetic fibers innervating the small intestine originate mainly from ganglia at T_9 and T_{10} and synapse in the celiac (duodenum) and superior mesenteric (jejunum and ileum) prevertebral ganglia. Postganglionic fibers pass from the celiac ganglion to the stomach and proximal part of the duodenum along the branches of the celiac artery. Fibres from the superior mesenteric ganglion pass along the branches of the superior mesenteric artery to the remainder of the small intestine.

Using fluorescence histochemical techniques, Norberg (1968) and Jacobowitz (1965) traced the noradrenergic axons and the majority of varicose terminal axons were found in close apposition with enteric ganglion cells or blood vessels, suggesting that postganglionic sympathetic fibres terminate close to the nerve cell bodies. Adrenergic nerves were very rarely found in the musculature itself.

2. Intrinsic Innervation

Aside from the extrinsic nerves, which originate from the CNS, whole sets of nerve networks which are anatomically and functionally distinct can be found within the GI tract. The outermost plexus, the myenteric (or Auerbach's) plexus, lies between the two outer muscle coats of the muscularis externa. The ganglia and connecting strands of nerve fibres are isolated from blood vessels and connective tissues by a basal lamina. They show a compact structure and are characterized by a dense neuropil, resembling the CNS (Gabella, 1972b). Within this network, primary, secondary and tertiary plexuses, defined according to the size and position of the nerve bundles, can be distinguished (Auerbach, 1864; Richardson, 1958). The primary plexus is made up of large nerve bundles and forms a wide meshwork containing multipolar ganglion cells. Unipolar and bipolar ganglion cells are also found within this plexus. The interstices of this network are traversed by thinner secondary nerve bundles and by the tertiary plexus. The fine nerve bundles from the tertiary plexus, which are unmyelinated, enter into the muscle coats at intervals, accompanied by blood capillaries and interstitial cells. They then run parallel to the main axis of the muscle fibres, branching into smaller bundles and finally terminating in restricted areas as single fibres free of Schwann cells (Richardson, 1958; Bennett & Rogers, 1967).

Another major intramural nerve network is the submucous plexus, which is interconnected with the myenteric plexus by fine nerve bundles. Two separate plexuses can be physically separated within this network (Gunn, 1968). The outer plexus (Henle's) contains multipolar neurons very similar to those found in the myenteric plexus. This plexus is

open-meshed and very irregular. The neurons are loosely arranged in ganglia similar to those of the myenteric plexus. A distinctive feature of Henles' plexus is the variable degree of development along the intestinal tract. In most regions of the small intestine, it is thin and poorly developed. However, in the ileo-colic sphincter region and the internal anal sphincter region it is well developed. The inner plexus (Meissner's) of the submucous network is composed of small unipolar or bipolar neurons (Gunn, 1968). The meshwork of this plexus is more regular. It is estimated that the Meissner's plexus contains 2-3 times as many cells as the myenteric plexus (Leaming & Cauna, 1961).

3. The Innervation of the Muscle Coats

Longitudinal Muscle: In the guinea-pig ileum (Gabella, 1973) and the small intestine of rats (Lane & Rhodin, 1964; Taxi, 1965), the longitudinal layer is innervated by varicose, vesicle-containing axons from the secondary and tertiary plexus of the myenteric plexus. There are no nerve bundles running within the longitudinal muscle coat in either of these animals. In the rabbit longitudinal coat, nerve bundles are also rare. In areas where the longitudinal coat is thicker, axons arising from the tertiary plexus of the myenteric plexus penetrate the muscle layer (Richardson, 1958). They then break up into smaller bundles and continue for considerable distance before terminating.

The nerve and the muscle cells do not come into close contacts. Close appositions of about 20mn as in the skeletal neuromuscular junctions are rare (Richardson, 1958; Lane & Rhodin, 1964). The separation

of the peripheral nerve bundles of the plexus and the longitudinal cells is rarely less than 80 nm apart (Taxi, 1965; Gabella, 1977b). The nerve fibres are varicose, forming bead-like expansions every 1-3μ and containing synaptic vesicles and mitochondria. The varicose nature of the nerves and the lack of single axon-muscle type of junctions suggests the "en passage" type of transmitter influence (Bennett & Rogers, 1967). The varicosities are free of Schwann cell processes and are believed to be the sites of transmitter release as the inter-varicosities contain only neurotubules and/or neurofilaments. Stimulation of a nerve would theoretically cause transmitter release from all its varicosities, thus affecting a great number of muscle cells. The cells not directly influenced by the transmitter could be affected through electrotonic coupling. Because of the wide gap between the varicosities and the muscle cells, transmitters must have to diffuse through distances of at least 100nm to reach the muscle cells. Bennett & Rogers (1967) estimated that for a transmitter to produce an effect on the muscle cell, the varicosities have to be within 300 n m of the muscle membrane. The scarcity of nerves running within the longitudinal muscle coat implies that the main source of neural transmitter comes from the myenteric plexus itself. This has been supported by physiological experiments using plexus-free longitudinal muscle strips of guinea-pig ileum (Paton & Zar, 1968). In contrast to the control strip, the plexus-free strip is not responsive to transmural neural stimulation.

Circular Muscle: As pointed out by Burnstock (1970), most electronmicroscopic studies of the innervation of the intestine have

been confined to the longitudinal layer. It has been generally assumed that the innervation of the circular muscle follows a similar pattern as that of the longitudinal muscle. Studies of the innervation of the circular coat especially in mammals are few and sketchy. However, these studies reveal that the nerve-muscle relationship is very different from that of the longitudinal layer. The innervation is denser and there are more close (20nm) neuromuscular contacts. In the toad, the density of such close neuromuscular junctions is very high and is comparable to that of the vas deferens (Rogers & Burnstock, 1967). In mammals however, such close contacts probably do not occur as frequently (Gabella, 1972).

An interesting observation in the circular muscle is the close relationship between the inner circular muscle layer and the large number of extrinsic and intrinsic nerve fibres. In the space between these two circular layers, there are numerous nerve bundles oriented parallel to the circular axis. No ganglion cells were found in this plexus, the plexus muscularis profundus (Duchon, et al., 1973). The functional role of this plexus and that of this special inner circular layer remains to be determined.

SUMMARY - The Anatomical Features of the Innervation of the Intestine

1. The small intestine is innervated by autonomic nerves of both extrinsic and intrinsic origins.
2. Extrinsic nerves - both parasympathetic and sympathetic divisions of the autonomic system innervate the intestine. These extrinsic nerves terminate mostly on the neurons of the enteric plexuses and rarely on the muscle cells.
3. Intrinsic nerves - There are two major nerve plexuses within the intestine - the myenteric or Auerbach's plexus between the muscle coats and the submucous plexus close to the lumen.
4. The innervation of the intestinal smooth muscle cells is very different from those of the neuromuscular junctions in skeletal muscle. They rarely form close neuromuscular junctions.

V
Neural Control of Intestine Motility

1. Extrinsic Nerves

As discussed in the preceding section, virtually all of the extrinsic nerves terminate at the myenteric plexus, making synaptic connections with the ganglia cells. Therefore, any influence from these nerves on the smooth muscle cells has to be mediated chiefly through the intrinsic nerves. Indeed, the extrinsic nerves can be described as a modulator of intestinal activity for the intrinsic plexus is capable of maintaining all the integrative functions by itself.

The extrinsic nerves modulate the electrical and mechanical activities of small intestine without any direct effect on the ECA itself. Stimulation of sympathetic or parasympathetic nerves does not markedly alter the ECA frequency (Van Harn, 1963; Gonella, 1965). Vagotomy and pharamacological agents in doses sufficient to block the cardiovascular effects of vagal stimulation usually has negligible permanent effect on normal intestinal function (Daniel & Chapman, 1963).

The effect of stimulation of the extrinsic nerves on the excitability and mechanical activities is variable. There are two possible explanations for the variability. Firstly, the extrinsic nerves are usually mixed, i.e. they consist of nerves of both parasympathetic (excitatory) and sympathetic (inhibitory) origin. Therefore stimulation would activate these two opposing systems at the same time (Youman, 1968; Hirst & McKirdy, 1974a). The second possible explanation is that these nerves exert their action not directly on the muscle cells, but mostly

through the mediation of the enteric ganglion cells, some of which may be excitatory and some inhibitory. Therefore, the response to extrinsic nerve stimulation is not consistent. For example, Van Harn (1963) found that in the cat, whether the response is stimulatory or inhibitory also depends upon the state of activity of the intestine itself. Thus although vagal (mostly parasympathetic) stimulation produced excitation of the intestine 79% of the time, inhibition was also observed 13% of the time when the intestine was active. There was no response to vagal stimulation 18% of the time. Similarly, stimulation of the splanchnic nerve (mostly sympathetic), inhibition was observed 74% of the time, excitation 24% and no response 12% of the time. Kewenter (1965) in studying the vagal control of the jejunal and ileal motility also found that vagal stimulation can induce both excitatory and inhibitory effects.

Gillespie (1962b) recorded the electrical event of stimulation of the pelvic nerve (parasympathetic) to the rabbit colon with microelectrodes. With single pulses, excitatory junction potentials (EJP) leading to action potentials and mechanical contraction was observed. Higher frequency stimulation led to facilitation of the junction potentials until these junction potentials summated. With continued stimulation, the muscle cells remained depolarized and contracted without the presence of action potentials, indicating that depolarization itself was able to sustain the increase in tension. Furness (1969a) also reported the finding of EJPs in both the longitudinal and circular muscle layers of the guinea-pig and rabbit colons by stimulating the pelvic nerves. A direct increase in acetylcholine output due to parasympathetic stimulation was measured in other experiments (Beani, Bianchi & Crema,

1969).

The sympathetic innervation inhibits the activity of the intestine. Rather than acting on the smooth muscle cells directly, it acts primarily to control synaptic transmission within the entire plexus. The sympathetic nerves terminate close to the enteric neurons and very few adrenergic nerves can be found within the musculature (Norberg, 1964, Jacobowitz, 1965). Inhibition induced by sympathetic nerve stimulation can take place without any detectable change of the muscle membrane potential (Bennett, Burnstock & Holman, 1966; Gillespie, 1962a). This may be explained by the indirect action of sympathetic nerves in the intestine muscles. Although the muscle can be directly affected with high frequency stimulation, this can be accounted for by the overflow of neurotransmitters from the nerve plexus perhaps as a non-physiological response. This direct action on the smooth muscle can be pharmacologically differentiated from its effect on the nerve plexus as different classes of adrenoceptors are activated. Thus in the smooth muscle membrane, the adrenergic receptors are mostly beta receptors whereas at the enteric plexus they are mostly alpha receptors (Kosterlitz, Lydon & Watt, 1970; Gillespie & Khoyi, 1977).

The physiological consequence of sympathetic nerve stimulation is the reduction in motor activity and the ACh output at both the resting and stimulated state (Beani, Bianchi & Crema, 1969). The site of inhibition appers to be preganglionic (of the enteric neurons) and presynaptic. Direct recording from the myenteric neurons showed that sympathetic stimulation reduced or abolished the EPSP without changing the electrical properties of the neurons (Hirst & McKirdy, 1974a). The

inhibition is prevented by phenoxybenzamine - an alpha-adrenergic antagonist (Nishi & North, 1973b). Moreover, noradrenaline does not have any effect on iontophoretically applied ACh on the neurons. This, together with the finding of the greater efficiency of the sympathetic nerves in inhibiting the parasympathetic stimulation than exogenously applied ACh (Gillespie & Khoyi, 1977) indicates that the site of action is on the parasympathetic nerve itself before it synapses with the enteric neuron. The inhibition is therefore presynaptic with the reduction of the amount of ACh transmitter release from the excitatory terminal.

2. Intrinsic Nerves

In intestinal smooth muscle cells, close neuromuscular junctions are rare. The nerves contain varicosities and transmitters are released from these sites. The transmitters then diffuse over long distances to the smooth muscle cells. Thus a large number of cells are activated simultaneously. In addition, responses due to the transmitters can spread to cells distant from the release sites through low resistance pathways.

Two types of electrical events in the smooth muscle membrane have been described due to stimulation of the intramural nerves - an excitatory event that leads to contraction and is represented by a depolarization of the muscle membrane - the excitatory junction potential (EJP), and an inhibitory event - that leads to the hyperpolarization of the muscle membrane and inhibits contractile activities - the inhibitory junction potential (IJP). The nerves responsible for both the EJP and the IJP probably originate from the myenteric plexus

for either response can be evoked in the absence of the submucous plexus (Daniel & Taylor, 1975) or in preparations which do not contain the circular muscle layer and the submucous plexus such as taeni coli (Bennett, 1966; Burnstock, Campbell, Bennett & Holman, 1964).

There are several distinctive features of the junction potentials of the intestinal smooth muscle: 1) the long latency; 2) the long duration; 3) the grading of the response according to the strength of stimulation, and 4) the dual innervation of muscle cells by excitatory and inhibitory nerves. These features will be discussed in detail in the following sections.

3. The Excitatory Junction Potential (EJP)

The excitatory event due to nerve stimulation in the intestine was first studied by Gillespie (1962b) in the rabbit colon. Stimulation of the parasympathetic nerve led to a depolarization of the smooth muscle cell; an EJP. If the EJP was large enough, an action potential was initiated and contraction resulted (Gillespie, 1962b). He also found that brief electrical stimulation across the wall of the gut caused junction potentials and spike potentials very similar to those produced by stimulation of the extrinsic nerve (Gillespie, 1964). This effect was due to the stimulation of the intrinsic nerves. The latency of the EJP was about 220msec. The guinea-pig taeni coli also demonstrated EJPs with a latency of 100-200msec after stimulation of the intramural nerve. The EJPs lasted from 500msec to close to a second in duration (Table 1).

The size of the EJP increased with the strength of stimulation

TABLE I. Characteristics of JPs recorded from vertebrate smooth muscles (from Holman, 1970)

Preparation	Nerve stimulated	Recording method**	Latency (msec)	Estimated junctional delay (msec)	Rise time (msec)	½ Decay time (msec)	Total duration (msec)	Reference
A. Excitatory Junctions								
Cat nictitating membrane	Post-g	External electrodes	20	10–15	50 80		≈ 500	Eccles & Magladery, 1937*a*
Rabbit detrusor muscle	Post-g	CME	35–100	--	< 100		≈ 1000	Ursillo, 1961
Guinea-pig small mesenteric arteries	Pre-g	CME	145–175	---	≤ 100		< 1000	Speden, 1964
Rabbit arteries (ear, mesenteric)	Post-g (field)	CME	12–40	< 12	min 70 variable ≈ 100		500 1000	Speden, 1967
Chick oesophagus	Post-g (field)	CME	90–160	--	150–250		700 950	Ohashi & Ohga, 1967
Guinea-pig taenia coli	?Post-g (field)	CME	100–200	—	200–400		500–800	Bennett, 1966*b*
Rabbit colon	?Pre-g Post-g	CME + pressure electrodes	400	--	---	--	600	Gillespie, 1962*b*
Rabbit colon	Pre-g (field)	CME	220	—	≈ 250	--		Gillespie, 1964
Guinea-pig vas deferens	Pre-g	CME	20 (minimum)	10	[½ rise time 15–20]	≈ 150	< 1000	Burnstock & Holman, 1961
Guinea-pig vas deferens	Post-g (field)	CME	6 (minimum)	—	≈ 40		≈ 500	Kuriyama, 1963*b*
Guinea-pig vas deferens	Pre-g	CME			45 100	135 300		Tomita, 1967*a*
Guinea-pig vas deferens	Post-g (field)	CME	-	--	45 100	≈ 200	—	Tomita, 1967*a*
Mouse vas deferens	Post-g (field)	CME	10–20 (minimum < 10)	—	10 20		100 250	This laboratory unpublished work
Rat vas deferens	Post-g (field)	CME	10–20	—	10 20		≈ 150	This laboratory unpublished work
Dog retractor penis	Post-g	CME	—	--			300 350*	Orlov, 1962
B. Inhibitory Junctions								
Guinea-pig taenia coli	Post-g? (field)	CME	80		200 500		< 1·0	Bennett, Burnstock & Holman, 1966*b*
Guinea-pig taenia coli	Post-g? (field)	CME	140	-	200–300	260 590		Bülbring & Tomita, 1967
Guinea-pig jejunum	Post-g? (field)	CME	50–80	--	120 280		< 1·0	Kuriyama, Osa & Toida, 1967*c*

*Orlov's (1962) published records suggest that duration may be greater than this.
**CME: capillary microelectrode

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either intramurally or by way of the extrinsic nerves (Gillespie, 1964). Successive responses to stimulation at low frequencies showed facilitation, i.e. the amplitude of the EJP became bigger with successive stimuli (Burnstock, Campbell, Bennett & Holman, 1964; Gillespie, 1962b, 1968). Higher frequency stimulation (greater than 2HZ) led to an overall depolarization, which sometimes reached 25-30mv. At such levels, the spikes disappeared although the tension of the muscle remained at maximum (Gillespie, 1962b, 1968; Burnstock et al., 1964). Thus the contraction can be maintained by depolarization in the absence of action potentials. In longitudinal muscle of guinea-pig ileum, Kuriyama, Osa & Toida (1967) also showed enhanced depolarization and frequency of spiking at increased stimulus frequency. In the guinea-pig taeni coli, some cells showed maintained depolarization while others showed an initial depolarization followed by repolarization with continual stimulation (Bennett, 1966b). However, these muscles were influenced by both EJP and IJP. In such cases it is suggested that the IJP may cancel out the effect of the EJP.

EJPs can apparently be more easily elicited in muscle cells by extrinsic parasympathetic stimulation than by intramural stimulation. In the guinea-pig colon, only 5% of the cells in the longitudinal coat respond with an EJP if stimulated transmurally. However, 80% of the cells show EJP with pelvic nerve stimulation. Swamping of EJPs by IJPs when both are elicited simultaneously by field stimulation may explain this result. In guinea-pig taeni coli, Bennett (1966b) also reported that only 10 out of 80 cells give EJP by transmural stimulation. Cells which showed EJPs were grouped together. In

exploring along a 1.5cm length of taeni coli, only a small area of about $1mm^2$ contained cells from which EJPs were recorded. The rest produced only IJPs (Bennett, 1966b).

Although most of the above studies have been done on the longitudinal muscle coats, EJPs have also been reported in the circular layer of guinea-pig caecum (Ito & Kuriyama, 1973). Pelvic nerve stimulation produced EJPs in 20% of the cells in the circular layer of the guinea-pig colon and in 10% of such-cells of the rabbit colon (Furness, 1969a). Spontaneous EJPs have been reported in several tissues (Bennett, 1966b; Furness, 1969a).

The EJPs in most tissues appeared to be the result of ACh release from the nerve terminal acting on the muscarinic receptors of the muscle membrane. Thus atropine blocked the EJPs elicited by intramural stimulation in the circular muscle of guinea-pig caecum (Ito & Kuriyama, 1973), guinea-pig taeni coli (Burnstock et al., 1964), guinea-pig ileum (Kuriyama, Osa & Toida, 1967; Hirst, Holman & McKirdy, 1975), and rabbit jejunum (Daniel & Taylor, 1975). Mechanically, the contraction due to intramural nerve stimulation was also abolished by atropine (Campbell, 1966). In the rabbit colon, however, it was difficult to block the excitation with atropine at concentrations high enough to block the effect of exogenous ACh (Gillespie, 1968).

The EJP is probably due to a permeability change of the smooth muscle membrane similar to that which occurs at the motor endplate. Effective membrane resistance was reduced during the EJP and the size of the EJP increased with hyperpolarization of the membrane (Hidaka & Kuriyama, 1969). The estimated reversal potential was 0mv. The EJP

was blocked by low external potassium and calcium as was the effect of exogenous ACh. The ACh induced depolarization was not affected by chloride ions but was potentiated by high calcium and sodium. Thus the effect of ACh seems to be to stimulate a permeability increase for all cations non-selectively (Bulbring & Kuriyama, 1963).

4. The Inhibitory Junction Potential (IJP)

The inhibitory effect of sympathetic nerve stimulation on intestinal motility is well known. There is good evidence to indicate that the inhibition is not usually indicated by a direct effect on the musculature, but rather by an action on the synaptic transmission of the enteric plexus (see Background Section V). However, at high frequencies of stimulation, the overflow of transmitter permits a direct effect on the muscle.

In the mid-1960's, it became apparent that the gastrointestinal tract is widely innervated by another population of powerful inhibitory nerves which arises from the enteric plexus. Stimulation of these nerves led to abolition of spontaneous spiking activity, hyperpolarization of the muscle membrane, and reduction of tension of the muscle (Burnstock, Campbell, Bennett & Holman, 1963, 1964). This intrinsic inhibitory nerve was distinguished from the sympathetic nerves in the following ways: -

1) The intrinsic inhibitory nerve action persisted after sympathetic denervation (Furness, 1969b).

2) Single pulse stimulation of the intrinsic nerves elicited hyperpolarization of up to 25mv. Maximum hyperpolarization of up to 50mv could be reached with repetitive stimulation at 10 HZ. Single

pulses applied to the sympathetic nerve never produced any membrane response, and high frequency stimulation of up to 80 HZ was required to produce the maximum hyperpolarization of only 16mv (Burnstock, et al., 1964; Bennett, Burnstock & Holman, 1966a, b; Furness, 1969a).

3) When the intramural nerves were stimulated at frequencies above 5 HZ, the membrane potential did not remain at its maximum initial hyperpolarization, but slowly repolarized towards the resting value. On cessation of the stimuli, the membrane potential returned to the resting level, sometimes with a rebound depolarization which initiated action potentials at frequencies higher than the normal (Bennet , et al., 1966b ; Bennett, 1966a). The hyperpolarization induced by sympathetic stimulation remained constant during the course of stimulation and the effect persisted for several seconds after cessation of stimulation. After the complete recovery of the membrane potential, the action potentials fired at their previous rate (Bennett et al., 1966a).

4) When relaxation responses became fatigued by continual stimulation of one type of nerve, the response to the other remained unaffected (Burnstock et al., 1964).

5) The two types of nerve responses could be differentiated pharmacologically. In contractility studies, sympathetic-induced relaxation were blocked by guanethidine, bretylium and DMPP (Burnstock, Campbell & Rand, 1966; Holman & Hughes, 1965). Relaxation due to the effects of intrinsic nerves were not affected by these agents. In fact there is no known specific blocking agent for the IJP. Both types of nerves were blocked by procaine. Similar electrophysiological studies

also showed that the IJP was not affected by bretylium and guanethidine, unlike the hyperpolarization induced by sympathetic stimulation (Burnstock, et al., 1964; Bennett, Burnstock & Holman, 1966a, b; Furness, 1969a).

6) Prolonged cooling abolished the effect of sympathetic nerves before the effect of intrinsic nerves (Holman & Hughes, 1965).

7) Hyperpolarization due to sympathetic nerve stimulation or exogenous noradrenaline was the result of an increase in both potassium and chloride conductance while the IJP was apparently due to a specific increase in potassium only (Tomita & Watanabe, 1973; Tomita, 1972).

5. Characteristics of IJP

Intramural stimulation with single pulses produces a transient hyperpolarization, the IJP, in most cells of the gastrointestinal tract. Facilitation was sometimes observed for the first 2 to 3 IJPs in the guinea-pig colon (Furness, 1969a). The amplitude of the IJP was graded up to a high of about 25mv depending on the strength of stimulation (Bulbring & Tomita, 1967; Bennett et al., 1966b; Furness, 1969a). Repetitive stimulation sometimes led to a hyperpolarization of about 50mv. However, this was not sustained (Bennett, Burnstock & Holman, 1966b). Spontaneous IJPs have also been reported (Bennett, 1966b; Bennett et al., 1966b; Furness, 1969a).

The latency of the IJP to stimuli was from 45 to 100msec (see Table I). In the taeni coli, the conduction velocity of the inhibitory nerve was estimated to be 10-20cm/sec from studies of the relationship between latency and the distance from the stimulating electrode (Bulbring & Tomita, 1967; Ito & Kuriyama, 1973). This is well below that of the value of about 500 cm/sec for the somatic motor nerve (Katz & Miledi, 1965).

The absolute refractory period of the inhibitory nerve was 3-4msec (Bulbring & Tomita, 1967).

The total duration of an IJP is usually close to one sec, reaching its maximum amplitude at about 200 msec and decaying almost exponentially. There are two possible explanations for this long duration: 1) slow inactivation of the transmitter or 2) continuous release of transmitter during the course of IJP. Furness (1969b) is of the opinion that the conductance change must continue almost throughout the IJP (thus implying a continuous release of transmitter) to account for its effectiveness in obliterating EJPs due to pelvic nerve stimulation for almost the whole duration of the IJP.

IJPs could be elicited at a distance 2-3cm away by electrical stimulation or by distension of the intestinal segment oral to the recording site (Hirst & McKirdy, 1974b; Hirst, Holman & McKirdy, 1975). IJPs resulting from stimulation at such distant sites were blocked by curare but not those at sites within 5-6mm of the stimulating electrode. These results suggest that the inhibitory pathway is polysynaptic with at least some cholinergic connections (Hirst & McKirdy, 1974b; Daniel & Taylor, 1975). Similar observations were reported using hexamethonium instead of curare (Burnstock, Campbell & Rand, 1966; Ito & Kuriyama, 1971).

6. Ionic Mechanism of IJP

Studies of the effect of changing the external ionic concentration on the IJP indicate that the hyperpolarization is most likely due to the increase in permeability to potassium ions (Bennett, Burnstock & Holman,

1966b; Bennett, 1966c; Hidaka & Kuriyama, 1969; Tomita, 1972). Reduction of the external potassium concentration increased the size of the IJP. Chloride appeared to be not involved because changes in the amplitude of the IJP due to variation of the external chloride ion occurred as would be expected from the effect of changing the membrane potential (Tomita, 1972).

Direct demonstration of conductance change estimated by reduction of the electrotonic potential in response to intracellular current injection was unsuccessful (Bennett & Rogers, 1967; Hidaka & Kuriyama, 1969; Creed & Gillespie, 1977). However, if current was applied by large external electrodes during stimulation of inhibitory nerves, a reduction in the size of the electrotonic potentials was observed in some preparations (Creed & Gillespie, 1977). By comparing the size of the electrotonic potential before and during nerve stimulation, the equilibrium potential was estimated and was in the region of 85-95mv. By varying the membrane potential with applied current, it was found that the size of IJP varies with the membrane potential. Depolarization of the membrane increased while hyperpolarization decreased the size of IJP, and the junction potential finally reversed its polarity at around 85mv (Ito & Kuriyama, 1975). The estimated value of the reversal potential for the IJP in guinea-pig taeni coli was similar (Tomita, 1972). The reversal potential of the IJP is similar to the equilibrium potential for potassium ions. Thus an increase in potassium permeability by the inhibitory transmitter is a very likely explanation for the hyperpolarization.

7. Putative Transmitter for IJP

The gastrointestinal tract contains a whole variety of as yet unidentified neural elements. The intrinsic inhibitory nerve is a good example. The transmitter responsible for the IJP is distinct from the other better known inhibitory transmitters such as adrenaline or noradrenaline (Burnstock et al., 1964), serotonin or gamma-aminobutyric acid (Hidaka & Kuriyama, 1969) of the nervous system. Others such as histamine, prostaglandins, various amino acids, substance P and bradykinin were also rejected as likely candidates because they did not mimic the nerve-mediated response (Burnstock, 1972, 1975). The possibility that the inhibitory mediator stimulates an electrogenic sodium pump had also been eliminated (Hidaka & Kuriyama, 1969).

Currently, the most widely held hypothesis is that purines such as ATP or related compounds may be the transmitters (Burnstock, 1972, 1975). As in all other cells, ATP is synthesized and stored in nerve cells. It was demonstrated that labelled ATP release is increased 8-20 fold by stimulation of the enteric nerves (Su, Bevan & Burnstock, 1971; Kuchii, Miyahaya & Shibata, 1973). This is nerve mediated and reduced by TTX. Also exogenously appled ATP mimics the action of the inhibitory nerves in some preparations (Burnstock, Campbell, Satchell & Smythe, 1970; Tomita & Watanabe, 1973). For example, Tomita & Watanabe (1973) found that ATP at 10^{-6} to 10^{-5}M, suppressed the spikes and relaxed the guinea-pig taeni coli. ATP at 10^{-4} hyperpolarized the membrane, reducing the membrane resistance and the size of the IJP. Like the IJP, the conductance change due to ATP was also the result of an increase in potassium permeability. On morphological grounds, Burnstock (1970)

claimed that the varicosities releasing the putative transmitter can be identified by their content of large opaque vesicles (80-200nm).

However, the purinergic nerve hypothesis is far from satisfactory and has not been tested vigorously. Some of the problems with this hypothesis have recently been discussed by Daniel (1978). For example, many nerves (e.g. sympathetic) or smooth muscle cells (Kuchii et al., 1973) also released purine compounds upon electrical stimulation. In some tissues, the amount of ATP required to induce a physiological response was very high (Daniel, Sarna & Crankshaw, 1977), while in others, ATP could not mimic the effect of the intrinsic nerves (Ohga & Taneika, 1977). Desensitization of some smooth muscle to high doses of ATP did not affect the responses to nerve stimulation, as would be expected (Ohga & Taneika, 1977). The claim that large opaque vesicles represent purinergic vesicles has also been questioned (Daniel, Taylor, Daniel & Holman, 1977).

Recently, the possiblity that coenzyme A (CoA), released along with ACh during neural stimulation, may serve an inhibitory function has been suggested (Cook, Hamilton & Okwuasaba, 1978). Other agents, such as the recently discovered vasoactive intestine peptide (VIP) have been suggested as putative inhibitory transmitters. VIP has a relaxant effect on smooth muscles, and VIP nerves within the GI tract have been identified (Alumets, Edvinson, Hakanson & Sundler, 1978). In conclusion, the mediator for the intrinsic inhibitory nerve remains to be identified and much more experimental evidence is required to substantiate the "purinergic nerve" hypothesis.

8. Post-Inhibitory Rebound Excitation

In preparations that were normally spontaneously active and had relatively low resting membrane potentials, e.g. guinea-pig taeni coli, IJPs in some cases were followed by a depolarization beyond the resting potential. During this period, a single action potential or a burst of action potentials was initiated (Bennett, 1966a; Furness, 1970). This "rebound" excitation occurred in the presence of atropine (Bennett, 1966a), hyoscine, guanethidine, and hexamethonium, but was blocked by TTX (Furness, 1970).

The frequency of action potential firing initiated after the IJP increased with the frequency of stimulation of the intramural nerves. If the mechanical responses of these tissues were measured, a contraction following the relaxation due to the IJPs was observed (Campbell, 1966).

The mechanism of "rebound" excitation is not known. Hyperpolarization of some smooth muscle membranes by externally applied pulses can initiate similar "rebound" depolarization or action potentials (Furness, 1970; Tomita, 1966a; Ito & Kuriyama, 1971; Suzuki & Kuriyama, 1975). Rebound excitation by hyperpolarization is well-known in nerve fibres and denervated skeletal muscle (Marshall & Ward, 1974). However, in contradiction to this explanation, the "rebound" excitation may not be related to the degree of hyperpolarization, thus posing the possibility of the release of yet another unknown excitatory agent (see Holman & Hirst, 1977).

9. EJP-IJP Interaction

With intramural stimulation an IJP was commonly observed in many

preparations. EJP was rare occurring in 5% of the cells in taeni coli (Bennett, 1966b). Mixed responses with both EJP and IJP components were recorded consistently (Bennett, 1966b; Ito & Kuriyama, 1973), indicating that the same cell would be influenced by both excitatory and inhibitory transmitters. Furness (1969a) reported that 15% of the cells of the guinea-pig colon showed such a mixed response. He reasoned that the rare occurrence of EJP was because of the powerful and over-riding inhibitory effect of IJP. During intramural stimulation, both types of nerves would be activated so that the excitatory response would be masked by the IJP. With stimulation of the pelvic nerve, which does not simultaneously activate the intrinsic inhibitory nerves, EJPs were readily detected in 80% of the longitudinal cells of the guinea-pig colon compared to only 5% with intramural stimulation. By studying the interaction of EJP elicited by pelvic nerve stimulation and the IJP from intramural stimulation, it was found that IJP had a dominating effect on the EJP, i.e. when both types of nerves were activated at the same time, only the IJP was observed.

10. Other Responses to Electrical Stimulation

Electrical and mechanical responses that are not associated with the familiar cholinergic, adrenergic or the non-adrenergic inhibitory systems are common in the gastrointestinal tract. This is not suprising in light of the occurrence of a rich variety of active agents such as serotonin substance P, dopamine, prostaglandins, VIP, etc. with functions as yet unclear. Thus two atropine-resistant excitatory components of nervous origin had been observed in the guinea-pig plexus-longitudinal

muscle preparation (Ambache & Freeman, 1968; Ambache, Verney & Zar, 1970). Non-cholinergic excitation of the circular muscle of the guinea-pig ileum had also been demonstrated (Kottegoda, 1968, 1970). Gillespie (1968) reported than an exceptionally high dose of atropine (10^{-4}M) was required to block excitation elicited by parasympathetic stimulation when only 10^{-7}M was required to block the effect of exogenously applied ACh. A non-cholinergic EJP had also been reported in the chicken rectum (Takewaki & Ohashi, 1977).

In the guinea-pig colon, spontaneous potential changes that resemble depolarizing junction potentials were observed, and some of these were big enough to initiate action potentials. Similar responses were elicited by field stiumulation of short duration in the presence of TTX and hyoscine (Furness, 1970). Wood & Perkins (1970) also found that the circular muscle of the cat was stimulated by pulses as short as 0.25 msec to give phasic contractions in the presence of TTX, atropine, hexamethonium and pentolinium. Interestingly, Ito & Kuriyama (1971) observed that the spontaneous firing of action potentials in the guinea-pig rectal muscle was suppressed in the presence of TTX following one second current pulses of either polarity.

VI
Role of the Enteric Plexuses in Controlling Motility

Peristalsis results in the propulsion of food particles from the oral end toward the anal end of the intestine and depends upon the coordinated movements of its two muscle layers. In 1899, Bayliss & Starling noted that peristalsis was caused by a coordinated reflex independent of extrinsic nerves. This reflex could be evoked by electrical

or local mechanical stimulation, the most effective being a bolus. They concluded that:

> "since the whole act is evoked by the presence of the bolus in the gut, we must say that the irritation of the mucous membrane and the stretching of the walls of the gut at any point set up impulses which are transmitted both up and down the intestine, and cause excitation above, inhibition below".

The observation that the bolus induces excitation above and inhibition below is now known as the "law of the intestine".

It is now well established that the enteric plexuses of the gastrointestinal tract are capable of integrating all the functions of peristalsis. Denervation of the extrinsic nerves did not impair peristalic responses (Langley & Magnus,1905; Bulbring, Lin & Schofield, 1958). Early studies by Bulbring, et al., (1958) showed that the peristalic reflex required the integrity of both the submucous and the myenteric plexus. Asphyxiation of the mucous membrane and application of local anaesthetics to the mucous membrane abolished the reflex. Cocaine also abolished the response preseumably by disconnecting the sensory input to the motor output of the circuit (Feldberg & Lin, 1949; Bulbring et al., 1958).

Recently, more ambitious attempts to map out the neuronal pathway of the reflex circuitry have been made with direct electrophysiological recordings from the enteric plexus. Intracellular recording of the myenteric plexus of the guinea-pig showed two types of neurons with different characteristics (Nishi & North, 1974a; Hirst, Holman & Spence, 1974). One type, the S cell, received an extensive cholinergic input and could be excited repeatedly at high frequencies. The action

potentials in this type of cells were abolished by TTX. The other type was the AH cells characterized by the lack of synaptic inputs. Action potentials in this type of cell were resistant to TTX and showed a prolonged afterpotential lasting for 5 to 20 secs. Therefore, the AH cells could not sustain repetitive stimulation (Hirst & Spence, 1973; Hirst & McKirdy, 1974).

If the guinea-pig small intestine was stimulated electrically or by distension oral to the recording site, synaptic potentials could be recorded from S cells 2 to 3 cm aborally. No synaptic potentials, however were observed in the AH cells (Hirst & McKirdy, 1974; Hirst, Holman & McKirdy, 1975). By analysis of the synaptic input on S cells due to distension of the intestine, two populations of S cells were distinguished. In one group, the excitatory synaptic potentials were recorded within about one second of the onset of stimulation and did not persist for longer than two seconds. S cells of the other population did not show synaptic potentials until 2 to 8 secs after the onset of distension, and the discharge persisted for 3 to 8 secs.

If the recordings were made from the muscle layers instead of the neurons, the distension of the intestine led to first an IJP in the circular layer with a latency of 1 to 1.5 secs, followed by an EJP in both the longitudinal and the circular layers with a latency of 3 to 8 secs (Hirst, Holman & McKirdy, 1975). The coincidence of timing of the IJP with the first group of S cells and the EJP with the other group of S cells, suggested that distension of the intestine at the oral end stimulates two long descending pathways (Hirst, Holman & McKirdy, 1975). The faster pathway mediates inhibition of the circular muscle and the slow pathway mediates excitation of both muscle layers. Thus, inhibition

followed by excitation of the smooth muscle is proposed as the electrophysiological basis for peristalsis. The AH cells, which do not receive synaptic input, have been proposed as sensory neurons involved with the initiation of descending inhibition. These electrical events are directional and cannot be recorded from sites oral to the site of stimulation.

The neurons of the submucous plexus were also investigated with intracellular electrodes. In contrast to the myenteric plexus, no long neural pathways and no cells similar to the AH cells were found (Hirst & McKirdy, 1975). Almost all cells received an extensive excitatory synaptic input similar to those of the S cells. About one-third of these cells also received an inhibitory input activated by transmural stimulation, giving a prolonged hyperpolarization of up to 20mv in amplitude and 5 secs in duration. The exact connection of the submucous plexus to the myenteric plexus in the peristalic reflex was not clear. However, it is generally accepted that the submucous plexus is also required for peristalsis (Bulbring, et al., 1958; Frigo & Lechini, 1970). In experiments in which the submucous plexus was removed, EJPs could not be generated in response to distension although the IJPs were not affected (Hirst, Holman & McKirdy, 1975). Therefore, the submucous plexus is essential for the descending excitatory pathway. It was speculated that the inhibitory potentials in the submucous neurones are responsible for the long latency of the EJP in respect to the IJP (Hirst & McKirdy, 1975; Holman & Hirst, 1977).

These studies provide the first analysis of the neuronal basis of the peristalic reflex. However, it is quite clear that the understanding of the neuronal circuitry is incomplete. Other types of neurons within the myenteric plexus, e.g. burst type neurons detected by extracellular

recordings (Wood, 1975) have yet to be identified using intracellular electrodes and their function is not clear.

1. An Ascending Excitatory Pathway

Bayliss & Starling (1898) originally observed that excitation occured above and inhibition below the site of stimulation. Studies of the neuronal pathway described above demonstrated the descending inhibition. However "excitation above" has not been observed. An ascending excitatory reflex with a latency of about 2 sec at close distance to the site of distension was found by Costa & Furness (1976) by measuring tension changes of the circular muscle of the guinea-pig small intestine. The amplitude of contraction was greatest 5 mm oral to the stretch. This reflex was abolished by d-tubocurarine and consisted of two separate components sensitive to either hyoscine or methysergide. The pathway of this reflex lay within the myenteric plexus and was not modified by the removal of the mucosa and submucosa.

A descending inhibitory reflex most prominent within 1 cm of the site of distension was also observed by these authors. The onset of relaxation was rapid and the muscle remained relaxed during the maintainance of the distension. At greater distance, this relaxation was not maintained. When the stimulus was removed, the circular muscle quickly regained tension and frequently gave a transient contraction, which was not consistently related to the amplitude and duration of the preceding relaxation. TTX abolished the relaxation and the after-contraction. d-Tubocurarine and pentolinium antagonized the relaxation depending on the distance from the stimuli; at distances 10 mm or more anal to the point of distension, relaxation was completely abolished. The inhibition

occurred in the absence of the mucosa and submucosa, but required the presence of the myenteric plexus. Thus this descending inhibitory pathway resembles that derived from electrophysiological studies of Hirst & McKirdy (1974).

Similar observations were reported with the guinea-pig and cat colons. Frigo & Lecchini (1970) found that a bolus was propelled only when there was simultaneous ascending contraction and descending inhibition of the circular musculature. The peristalic reflex was not elicited from areas devoid of the mucosal and submucosal layers. TTX and ganglionic blocking agents also abolished the peristaltic reflex in the cat and guinea-pig colons (Crema, Frigo & Lecchini, 1970).

VII
Other Factors Involved in Controlling Intestinal Motility

I have reviewed the myogenic and neural factors in controlling motility in the preceding sections. They are probably the two main determinants in *in vitro* experiments with isolated preparations. A third and equally important control system, especially in *in vivo* conditions, is that provided by the GI hormones. The study of the mechanisms and functions of the GI hormones is very recent and very little is known about them. Since this thesis is concerned only with the myogenic and neural aspects of intestinal motility, the action of the GI hormones on intestinal motility will be mentioned very briefly. For example, Cholecystokinin (CCK) stimulates motor activities of human duodenum, jejunum and the sigmoid colon while secretin has the opposite effect (Gutierrez, Chey & Dinoso, 1974; Dinoso *et al.*, 1973). Vasoactive Intestinal Polypeptide (VIP) has a biphasic effect on the small

intestine comprised of an early relaxation followed by contraction (Kachelhoffer et al., 1976). Glucagon is also inhibitory on intestinal motility (Dotevall & Kock, 1963). Motilin stimulates motility only in the fasting state (Itoh et al., 1975) while pentagastrin stimulates motor activity (Weisbrodt et al., 1974).

Aside from the recognized hormones, other natural occurring substances in the GI system may also affect motility. Such agents include peptides like angiotension, bradykinin and Substance P. Lately, the lipid-soluble acids classified under prostaglandins have been suggested to serve a very important local regulatory role in intestinal tone (Bennett, A., 1976). In general, PGEs and PGFs are stimulatory for the longitudinal muscle of the intestines. For the circular muscle, PGEs are inhibitory and PGFs are stimulatory in the small intestine (Bennett, 1976). The functional role of many of the other naturally occurring agents remains to be determined.

SUMMARY - Neural Control of Motility

1. The extrinsic nerves function mainly to modulate the excitability of the intestine via the enteric plexuses.
2. The intrinsic nerves are capable of controlling all the basic motor functions of the intestine. The motor nerves to the smooth muscle cells originate from the enteric plexuses.
3. Two main types of electrical events in intestinal muscle are produced by stimulation of the intrinsic nerves: the EJP (a depolarizing event) and the IJP (a hyperpolarizing event).
4. The EJP is usually cholinergic and may lead to action potentials

and contraction.

5. The IJP inhibits spiking activities and leads to relaxation. The transmitter for the IJP is unknown although ATP has been suggested.
6. Both the myenteric plexus and the submucous plexus are essential for the peristaltic reflex. Intracellular recordings from the enteric neurons have helped in mapping out the neural circuitry for the reflex.
7. At present, an ascending excitatory neural pathway, a descending excitatory neural pathway and a descending inhibitory neural pathway have been proposed to be involved in controlling the events of peristalsis.
8. In addition to the myogenic and neural factors considered here, hormonal and local factors may also be very important in the control of intestinal motility.

Objectives of the Present Study

In this project, I attempt to define the basic electrical and mechanical properties of the two muscle layers and their interaction with the intrinsic motor nerves. Specifically, the following questions are investigated:

PART I

1) Are there any differences in the electrical and mechanical characteristics of the two muscle layers?

The electrical and mechanical activities of the two muscle layers have not been studied until very recently. Previously, it was assumed that they had very similar properties. In 1975, Suzuki & Kuriyama reported large differences both in their electrical and mechanical activities, and their responses to a variety of pharmacological agents in the two muscle layers of the guinea-pig small intestine. Differences in reponses to the two muscle layers also were observed in studies of the effect of the GI hormones (Anuras & Cooke, 1978), the prostaglandins (Bennett, A., 1976), and the stimulation of the intrinsic nerves (Hirst, Holman & McKirdy, 1975; Anuras, Christensen & Cook, 1977).

However, no serious attempt had been made to compare the activity of the muscle layers of the intestine of those species with ECA. Most electrophysiological studies in such cases involved extracellular recording which cannot yield quantitative and reliable information about the cellular activity. So far, no direct comparison was made of the two

muscle layers using intracellular recordings. The only comparative study of the two layers was made on preparations in which the two layers had been physically separated (Kobayashi, Nagai & Prosser, 1966; Connor et al., 1977). As discussed in Background and will be further elaborated in Discussion, these preparations probably cannot provide an accurate description of the activities of the individual layers because of the unavoidable damage and the difficulty in separating the two layers cleanly. In this study, the cellular electrical activities of the two layers will be compared directly using intracellular electrodes. Recordings were made from the cells of one layer first, and then the electrode was inserted deeper to record from the cells of the other layer. Thus, the electrical activities of cells at similar sites from each of the two layers can be compared. Gross physical damage and other problems associated with physically separating the two layers were therefore avoided.

2) What is the relationship of the ECAs in the two layers?

It is well established that ECA occurs in both layers of the small intestine (Kobayashi, Nagai & Prosser, 1966; Daniel & Taylor, 1975; Connor et al., 1977). From studies with isolated muscle layers, it had been concluded that ECA could only originate from the longitudinal muscle (LM) because circular muscle (CM) by itself was not capable of initiating ECA (Bortoff, 1961, 1965; Kobayashi, Nagai & Prosser, 1966). Furthermore, it was postulated that the ECA in CM arises from the electrotonic spread from [redacted] These conclusions have never been substantiated with direct recording from the unseparated layers. In this study, I directly compare the ECA of the two layers

at a cellular level. I also examine the effect of different parameters such as temperature and external electrical stimulation on the ECAs of the two layers. From these, the characteristics of the ECAs of the two layers can be directly compared.

3) What kind of interactions exist between the two muscle layers?

Cells of the two muscle layers are oriented at right angles to each other and physically separated by the myenteric plexus at most places. Yet intestinal motility depends on the coordination of the two layers. The basis for such coordination can depend on one or more factors: mechanical, electrical and neural.

In the first part of this study, the electrical interaction between the layers was investigated. This is of particular interest in light of the hypothesis that ECA spreads electrotonically from LM into CM (Bortoff, 1961, 1965, 1976; Connor et al., 1977). Therefore electrotonic coupling between the cells of the two layers will be studied. In the second part of this project, the role of the intrinsic nerves in controlling mechanical activities of the individual layers will be investigated.

PART II

What roles do the intrinsic nerves play in the control of motor activities of the muscle layers of the small intestine?

Preliminary studies of the opossum and guinea-pig small intestines indicate that the intrinsic innervation of the two muscle layers were different. The longitudinal muscle was predominantly under the influence of

the cholinergic excitatory nerves while the circular muscle was dominated by the non-adrenergic inhibitory nerves (Anuras, Christensen & Cooke, 1977; Hirst, Holman & McKirdy, 1975). Wood (1975) further proposed that the circular muscle was under "tonic inhibition" by the inhibitory nerves because high concentrations of neural blocking agents such as tetrodotoxin and procaine induced spontaneous mechanical activities in the circular muscle of the guinea-pig. In the second part of this study, the cellular response of the smooth muscle cells from the two layers to field stimulation of the intrinsic nerves will be studied. Parallel studies of the mechanical activities under similar experimental conditions will also be made in order to see if the electrical and mechanical activities can be correlated.

METHODS

Tissue Preparation

New Zealand rabbits (either sex) were killed either by cervical dislocation or by i.v. injection of pentobarbital. Segments of duodenum were taken from 2 inches below the pyloric region and kept at room temperature in oxygenated physiological solutions. Muscle strips, with or without the mucosa, about 0.5cm in width and 1 to 1.5 cm in length were obtained from these segments.

Since the two muscle layers were geometrically arranged at right angles to each other (see Background, Section I), two types of muscle strips were dissected in relation to the orientation of the long-axis of the muscle cells from the respective layers. Strips dissected along the long-axis of the muscle cells of the longitudinal layer will be termed longitudinal strips (LS) and similarly, those dissected along the long-axis of circular muscle cells will be termed circular strips (CS).

Electrophysiological Recordings

Glass micropipettes filled with 3M KCl were used for intracellular recordings. The length of the tip portion of these microelectrodes was between 0.7 to 1.0cm. The resistance of the electrodes used was between 40 to 70 megohms. The tip potential of these electrodes was less than 7mv.

The microelectrodes were connected to a WPI M4A electrometer

and the electrical signals were displayed on a Tektronix D13 storage oscilloscope (Fig. 1). Records were made by photographing directly off the oscilloscope with a Nihon-Kohden oscilloscope camera. The electrical recordings were also stored in magnetic tapes with a Hewlett-Packard 3968A instrumentation recorder. A Grass S88 stimulator with SIU5 stimulus isolation units was used for stimulation.

The set-up of the organ bath was similar to the original design by Abe & Tomita (1968). The muscle chamber was separated into two compartments (a stimulating compartment and a recording compartment) by one of the stimulating electrodes. Stimulation of the tissue was achieved by two large chloride-plated silver electrodes 1cm. apart. The side of the stimulating electrode facing the recording compartment was coated with Araldite for insulation to eliminate stimulus artefacts. Also a hole big enough for the placement of the muscle strip was present on this partition plate. A muscle strip was pulled through this hole so that at least 5mm of the strip was inside the stimulating compartment. The muscle strips were also pinned to the Sylgard bottom to facilitate penetration. Recordings of electrical activities were made at different distances from the partition electrode. The muscle strips were bathed with physiological or other solutions by constant perfusion. The temperature of the bathing solutions were kept constant by being circulated first through the water jacket of the organ bath. The fluid level of the bath was kept constant by suction.

To monitor the amount of current being passed between the stimulating plates, two platinum wires 2mm apart were placed in the middle of the stimulating compartment and the potential difference of

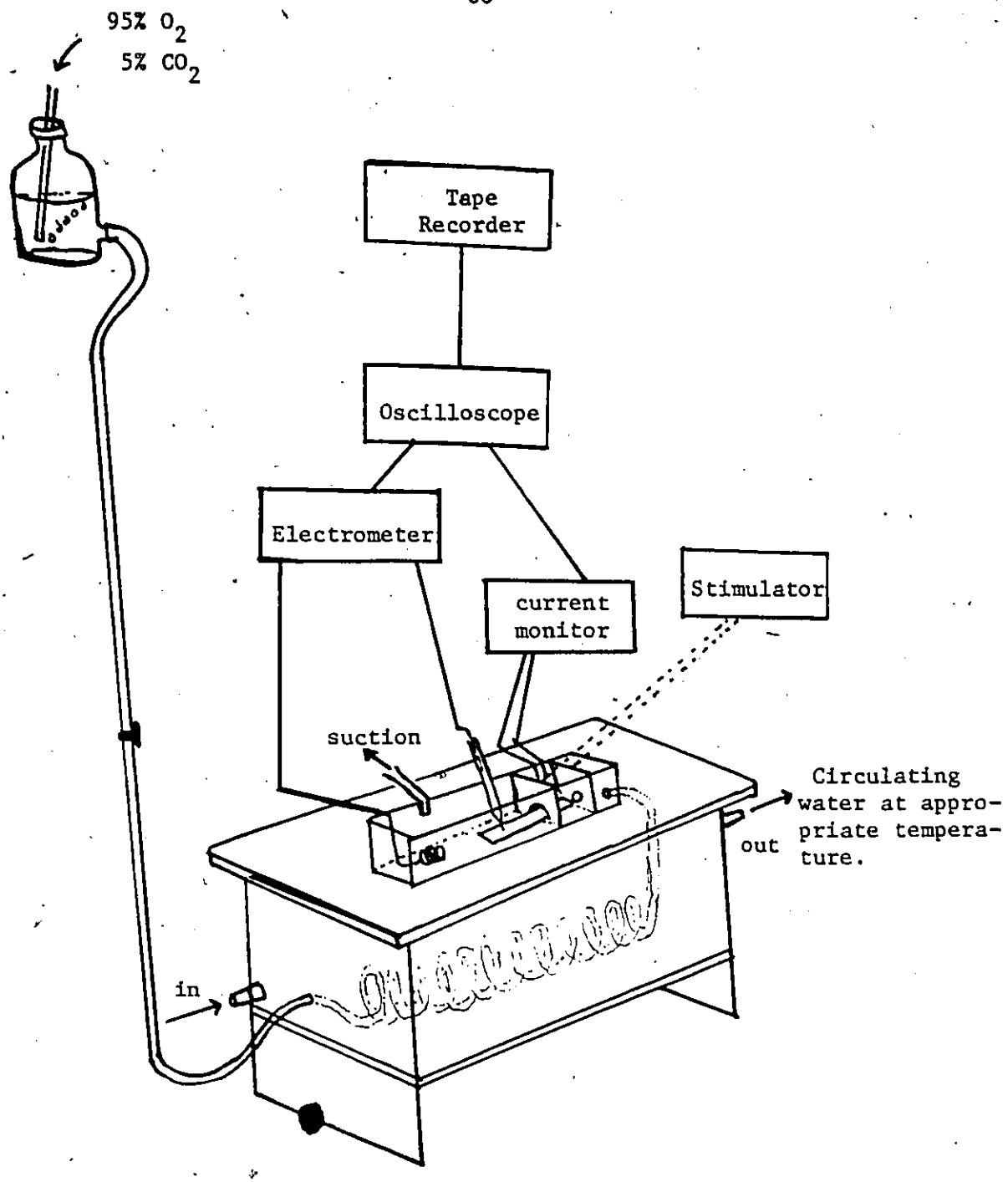


Fig. 1. Schematic representation of the muscle bath and the arrangement of the electronic equipment.

the two wires gave an indication of the current density generated by the stimulating electrodes.

The criteria for successful impalement were:

1) rapid change of voltage as the electrode penetrated the cell;
2) steady voltage baseline throughout the recording period;
3) for LM, the spontaneous action potentials exhibited fast rate of rise and peaks close to or beyond the zero membrane potential.

In all the experiments using LS, the strips were oriented so that the orad end was inside the stimulation chamber. Recordings were made from cells anatomically aborad to the part of the muscle strip in the stimulation chamber. This directional arrangement may be critical in studying the neural responses because the neural pathways had been shown to be very assymetric in oral and aboral directions (see Background, Section VI).

Mechanical Recordings

In order to relate the electrical activites with mechanical activities, isometric tension recordings of muscle strips were made under similar experimental conditions as those in electrophysiological studies. The design of the set-up is represented in Figure 2. Platinum ring electrodes 1cm apart and concentrically placed around the strips were used for stimulation. The tension change of the muscle strips were recorded on a Beckman R611 dynograph via Grass FT-03C force displacement transducers.

Data Analysis

All values were expressed as the mean and standard deviation.

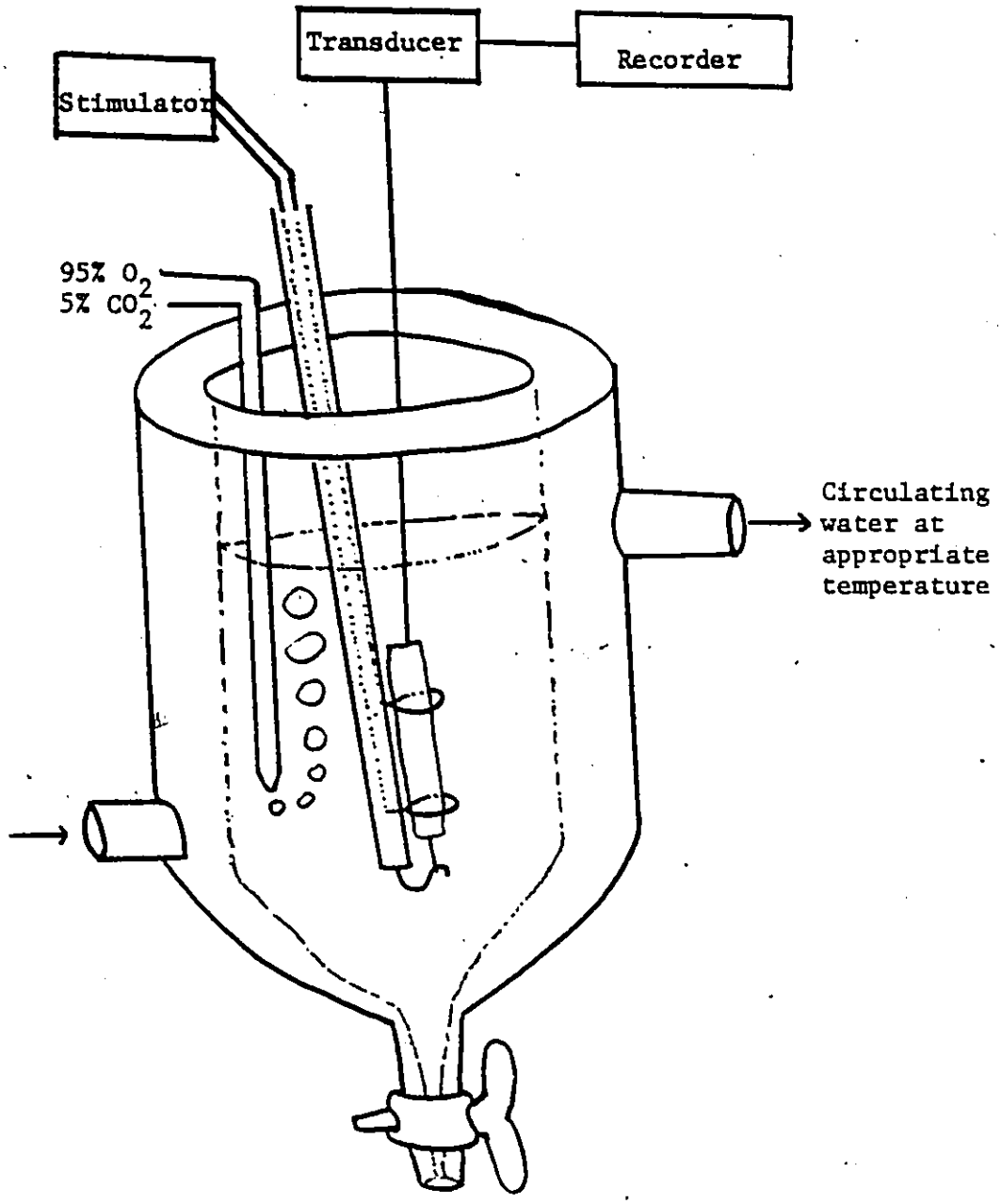


Fig. 2. Set-up for recording isometric tension of muscle strips.

Linear regression, paired and unpaired t-tests were performed using a Wang calculator. For the determination of the membrane potential and the size of control potentials, only those cells that satisfied the following criteria (in addition to those listed above) were included:

1) the zero membrane potential did not change after the penetration, and 2) the membrane potential remained steady for at least one minute. These recordings therefore represented the most reliable estimation of the membrane potential.

TABLE 2

Composition of the Physiological Solution

NaCl	133.19mM
KCl	4.70mM
$CaCl_2$	1.92mM
$MgSO_4$	0.78mM
NaH_2PO_4	1.17mM
$NaHCO_3$	18.57mM
Glucose	11.50mM

The solutions were equilibrated with 95% O_2-5% CO_2 gas mixture and had a pH of 7.4.

Drugs

Tetrodotoxin (TTX)	Sankyo
Atropine sulphate	Glaxo Laboratories
Hexamethonium Bromide	Sigma
Verapamil	Knoll

PART I

Spontaneous Electrical Activities of the Muscle Layers

A. Longitudinal Strips (LS)

The longitudinal muscle (LM): LM showed spontaneous electrical activities (Fig. 3) which were composed of two components - the electrical control activities (ECA) and the electrical response activities (ERA). ECA is the periodic oscillation of the membrane potential. From a base-line potential of about 52mv (52.5 ± 5.3mv, n=35), the membrane potential of the cells underwent phases of depolarization, the control potential (CP), at a frequency of 17 to 20 cycles/min at 36°C. The size of the CP varied between preparations and ranged from 8mv to 18mv (12.14 ± 3.4mv). This value was similar to that reported by Gonella (1965) on the same preparation. The size of the CP was directly correlated to the membrane potential, with the amplitude of the CP increasing with that of the MP (Fig. 4a).

The duration of the CP as a fraction of the total period between oscillations varied with different preparations, ranging from 50 to 65% of the total duration. On top of every CP in LM were spontaneous action potentials. The number of spikes per CP varied with preparations, ranging from three to a maximum of nine (Figure 3). However, the number of spikes per CP was similar between cells within the same preparation.

The interval between spikes of a CP were not constant; usually it was shortest in the beginning and increased subsequently with every spike (Fig. 3). The spikes were preceded by prepotentials and followed

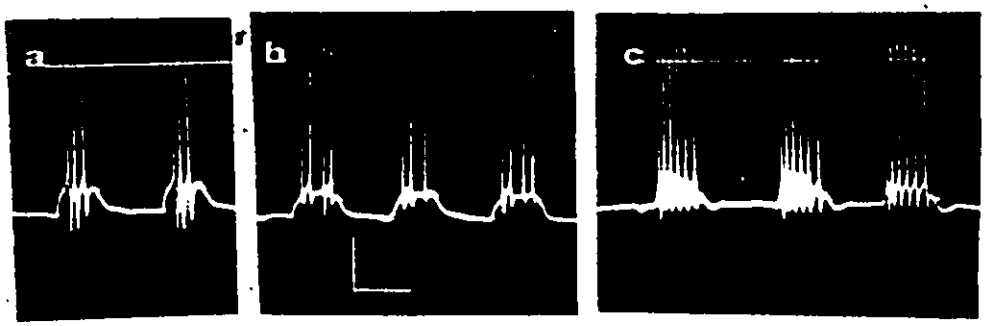


Fig. 3. Spontaneous electrical activity of LM cells. Notice the variation in the number of spikes per CP between preparations and the variation in spike amplitudes within bursts. Calib. vert: 20mv; horiz: 2sec.

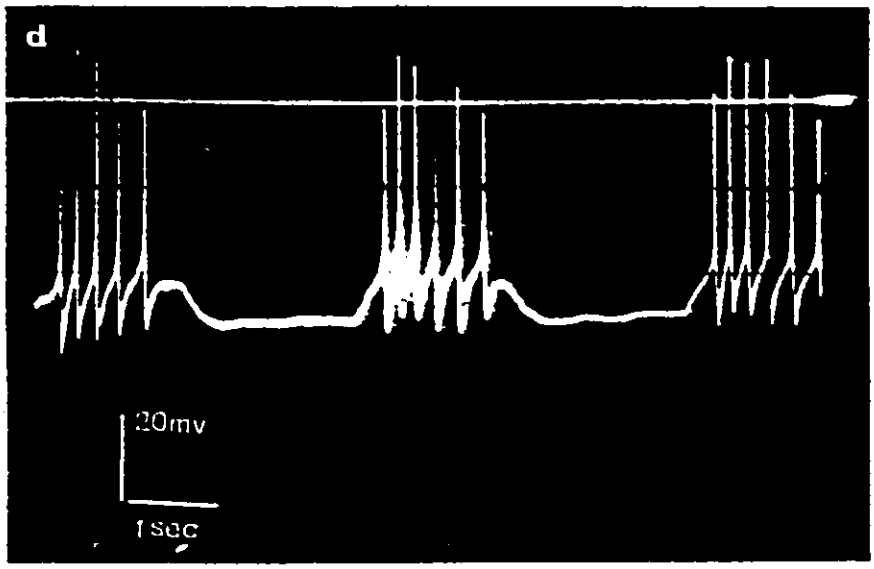


Fig. 3d. The interval between spikes usually increases progressively along the CP. It is most conspicous in cells where the number of spikes per CP is high.

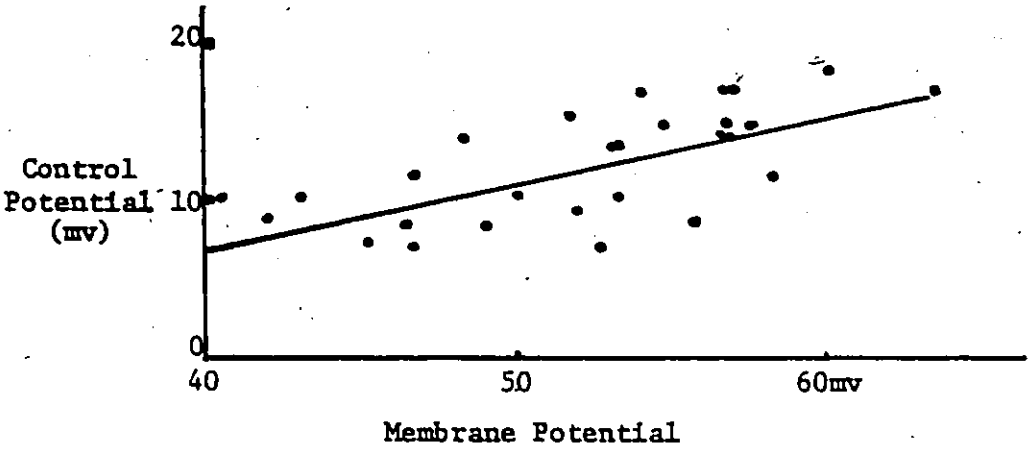


Fig. 4a. The relationship between the MP and the CP at 36 C. The points can be fitted by a line with the equation $y = -9.2 + 0.406x$. The change of CP amplitude was correlated with the change of the MP ($p<0.05$).

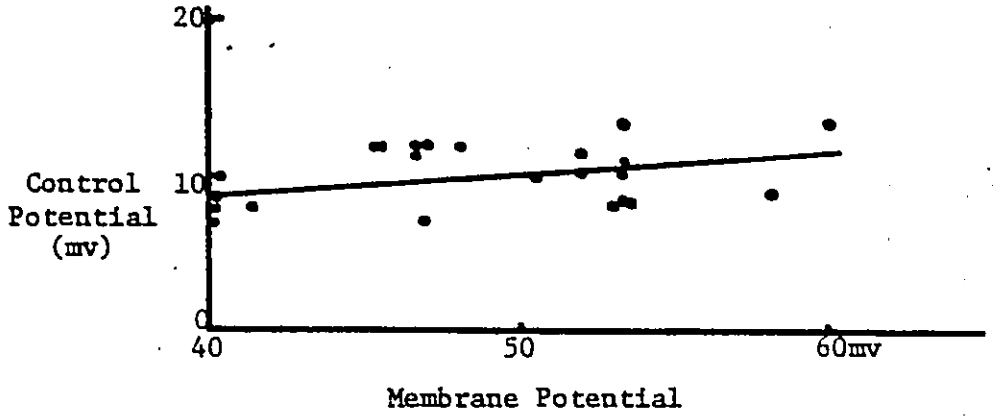


Fig. 4b. The relationship between the MP and the CP at 30 C. The points can be fitted by a line with the equation $y = 3.39 + 0.1352x$. The change of CP amplitude was also correlated with the change of the MP ($p<0.05$).

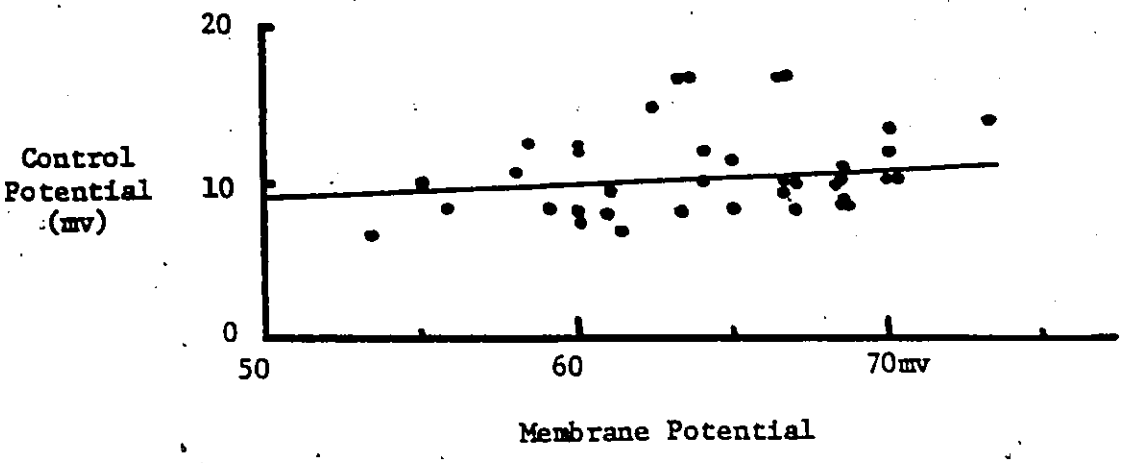


Fig. 4c. The relationship between the MP and the CP of CM at 36°C. The points can be fitted with the equation: $y = 4.1072 + 0.098x$. Unlike the LM cells, the increase in CP amplitude was not significantly correlated with the increase in MP ($p>0.1$).

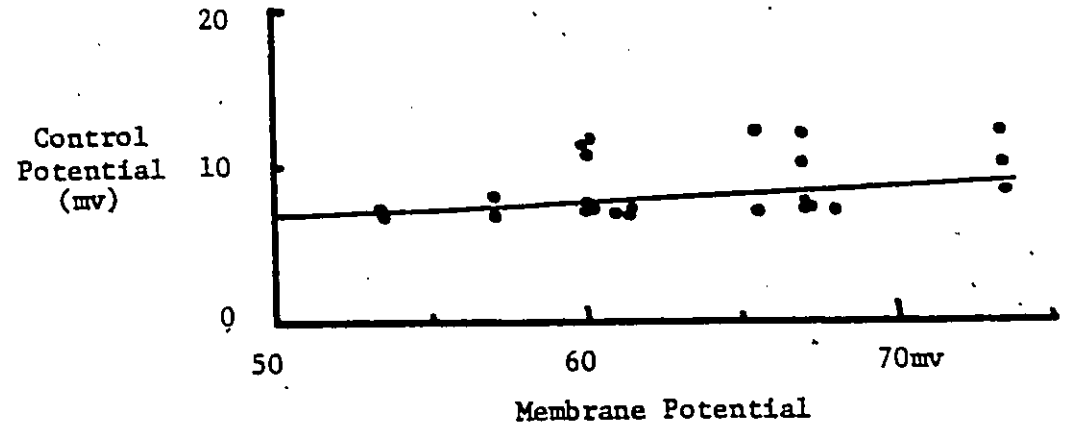


Fig. 4d. The relationship between the MP and the CP of CM at 30°C. The points can be fitted with the equation: $y = 1.67 + 0.103x$. The increase in CP amplitude also was not significantly correlated with the increase in MP ($0.1>p>0.05$).

by afterpotentials which in some cases hyperpolarized beyond their baseline membrane potentials (Fig. 3). The heights of spikes were not constant, varying even within the same burst. Most spikes overshot the zero membrane potential and the overshoot potentials were as high as 15-18mv.

The Circular Muscle (CM): The longitudinal muscle layer of rabbit duodenum is only 32mm in thickness (Prosser, 1973) and the circular muscle layer underneath can be reached by deeper penetration of the microelectrode. The smooth muscle cells of CM could easily be distinguished from LM for their electrical activities were drastically different. Fig. 5a was a recording from LM, showing the typical electrical activities described above. As the electrode was inserted deeper, a sudden change of activity was observed, indicating that a CM cell was penetrated (Fig. 5b). The MP was higher by about 10mv and there were no spontaneous action potentials. Cells recorded under these conditions were CM because cells with electrical characteristics similar to those described as CM cells above were encountered in strips with the mucosa removed and the muscle strips placed so that the circular muscle layer was now at the top. No cells with electrical activities similar to those of LM were seen with the usual depth of penetration because the circular layer is much thicker (about 70μ) than the longitudinal layer. Also cells in the LM Layer and the CM layer could be distinguished by their electrotonic coupling (Section E below) and neural responses (Results, Part II).

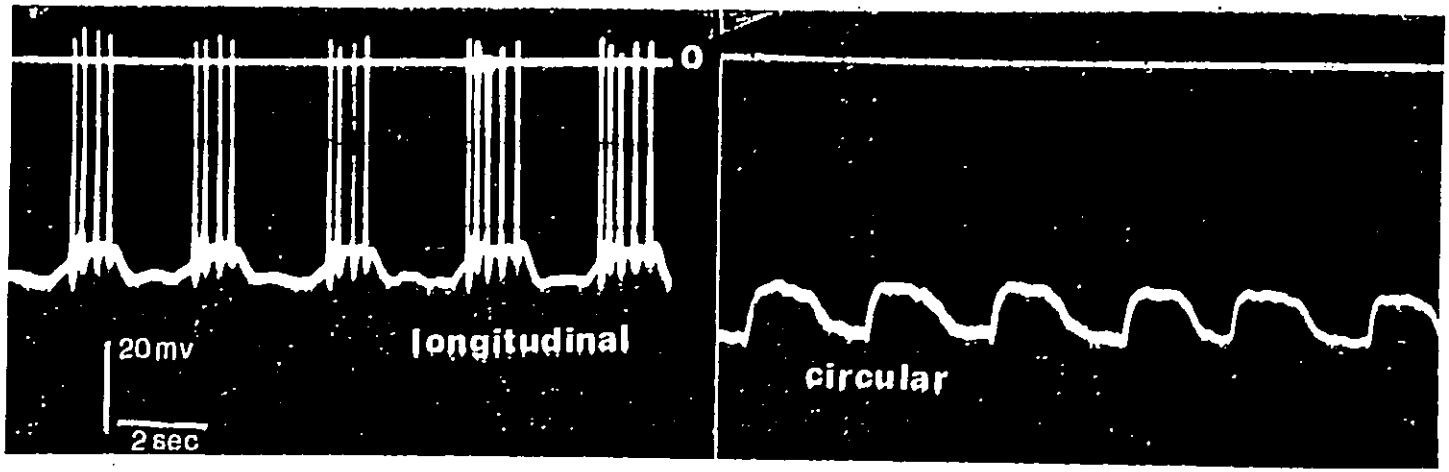


Fig. 5. The spontaneous electrical activity of a LM cell and a CM cell immediately underneath. The electrical activity of the CM is very different with the absence of the spontaneous action potentials and the membrane potential of the CM is also higher.

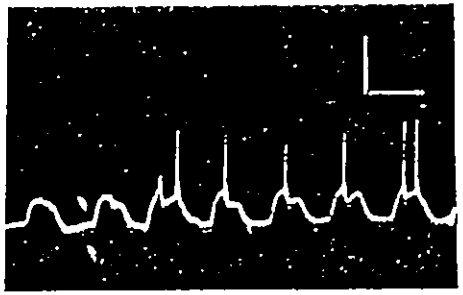


Fig. 6. Spontaneous spiking activity recorded from a CM cell.
Calib. vert: 20mv; horiz: 2sec.

On rare occasions spontaneous action potentials were observed in a few CM cells. However, they were not regular and did not occur in every CP or from every cell (Fig. 6). Spikes with an overshoot could be triggered by depolarizing pulses in all the CM cells (Fig. 7). Therefore, the cells in the circular layer were electrically excitable. On the average the MP of CM (64.2 ± 5.2mv, n=40) was significantly higher than that of LM ($P<0.05$). The size of CP was 10.39 ± 2.9mv at 36°C .

Whether the lack of spontaneous spiking activity in CM was due to the higher MP as compared to the LM was also investigated. In 18 CM cells studied, the average threshold for the action potentials initiated by depolarizing pulses was 37.65 ± 4.75mv. Thus with a MP of 64mv and the depolarization of 10mv during the CP, the CM cells would still be far away from the threshold for firing. Alternatively, the MP of LM was only 52mv and with a threshold at 30.6 ± 3.6mv (n=8), the LM cells were well within the threshold of firing of the action potentials on top of the CPs. Unlike the LM, the amplitude of the CP was not correlated to the MP in CM (Fig. 4c & d).

1. Mechanical Activities of Muscle Strips

Mechanical recordings of longitudinal strips (LS) showed spontaneous rhythmic contractions at frequencies similar to the ECA (Fig. 8). Most circular strips (CS) were mechanically quiescent, in agreement with electrophysiological observations (see Results, Part II). Atropine and tetrodotoxin (TTX) did not affect the frequency of the ECA or the spiking and contractile activities (Fig. 9). Therefore, these

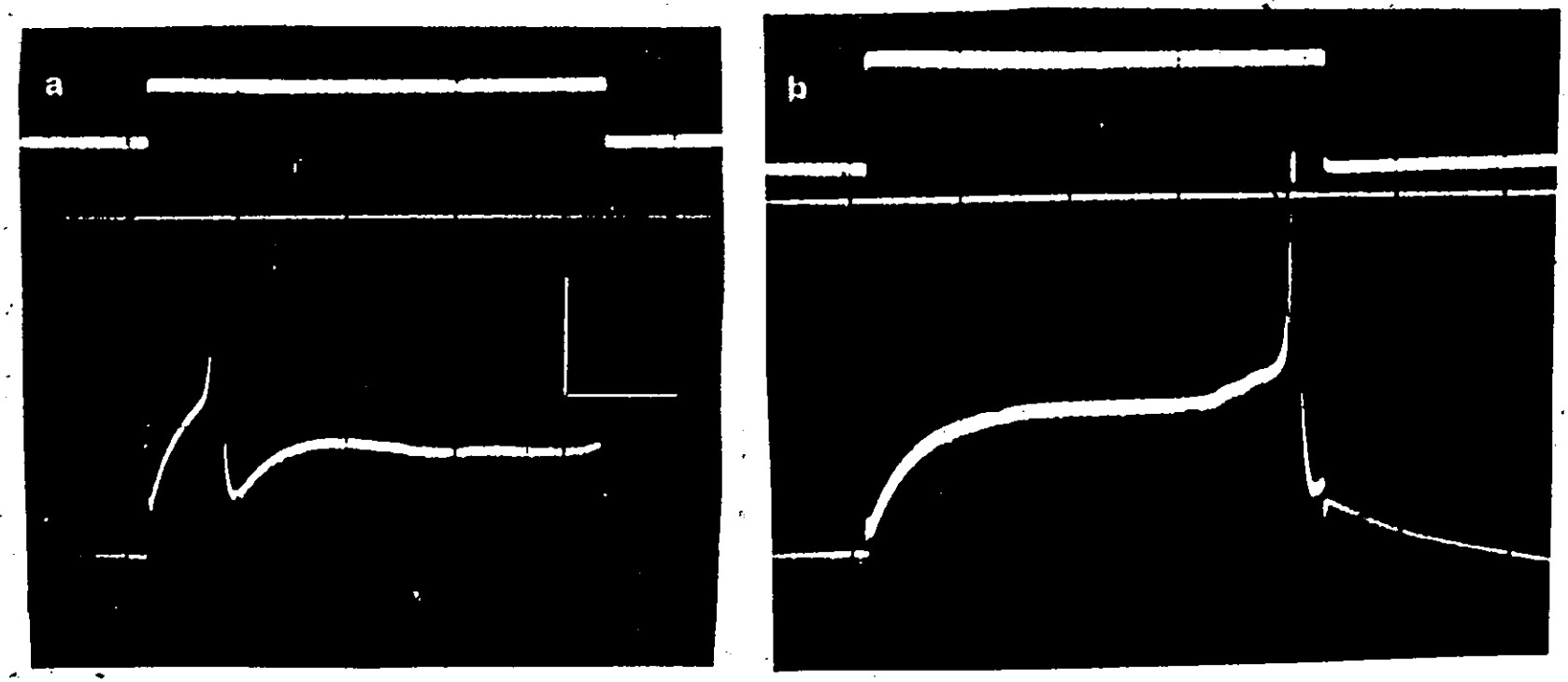


Fig. 7. Action potentials in two CM cells triggered by long depolarizing pulses showing overshoot and afterpotentials. Calib: vert. 20mv; horiz. 100msec.

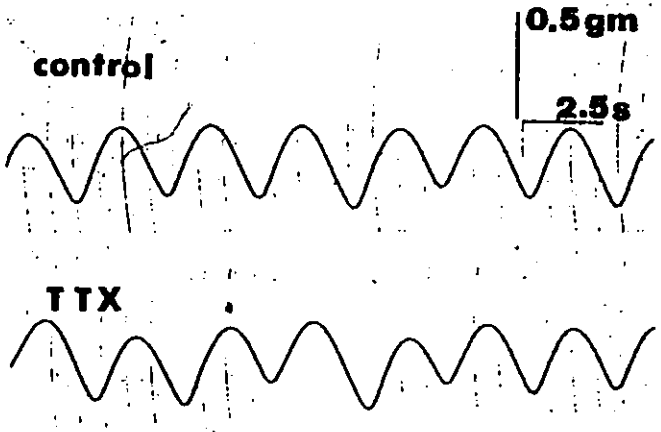
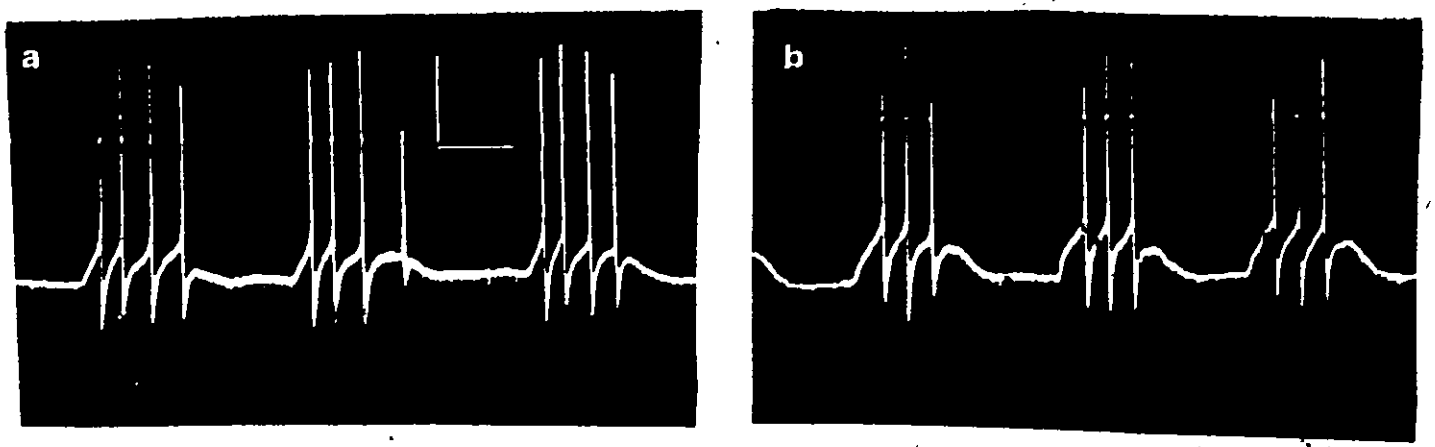


Fig. 8. Mechanical recording of a LS, showing spontaneous contraction at the frequency of ECA. This contraction was not affected by TTX (3×10^{-6}g/ml).

Fig. 9. a. Spontaneous electrical activity of a LMcell. b. The electrical activity was not affected by atropine (6×10^{-7}g/ml). Calib: vert. 20mv; horiz. 2sec. temp. 30°C.



spontaneous activities were probably not of neural origin. Verapamil, a calcium-antagonist, abolished the spontaneous rhythmic contractions and induced relaxation of the muscle strips. At low concentration of verapamil (10^{-7} g/ml), the spontaneous spikes of LM were abolished without any significant effect on the CP in agreement with the observations by El-Sharkaway & Daniel (1975).

2. Effect of Temperature on Spontaneous Activities

Lowering the temperature reduced the frequency of ECA. Thus at 30°C, the frequency was reduced to 9/min from 17-20 cycles/min displayed at 36°C. The number of spikes per CP of LM was 3 to 4, with the total absence of the higher frequencies seen at 36°C (Fig. 10). For the LM, the temperature decrease caused a significant reduction ($P<0.05$) of the MP and the size of the CP. At 30°C , the MP for LM was 47.3 $\pm$ 5.2mv (n=26) and the size of the CP was 9.85 $\pm$ 2.33mv. The size of the CP was also correlated with the MP at 30°C (Fig. 4b).

Unlike the LM, the MP of CM was less sensitive to temperature change. At 30°C, the MP of CM was 63.4 $\pm$ 5.8mv (n=21). This was not significantly different from that at 36°C ($P>0.05$). The CP however, decreased significantly from an average of 10.38mv at 36°C to 8.54 $\pm$ 2.1mv at 30°C ($P<0.05$).

The effect of temperature on the ECA of the two layers were similar. Not only were the frequencies of the two layers at different temperatures the same, but the duration of the CP of the two layers were also identical. For example, the mean duration of CP taken from

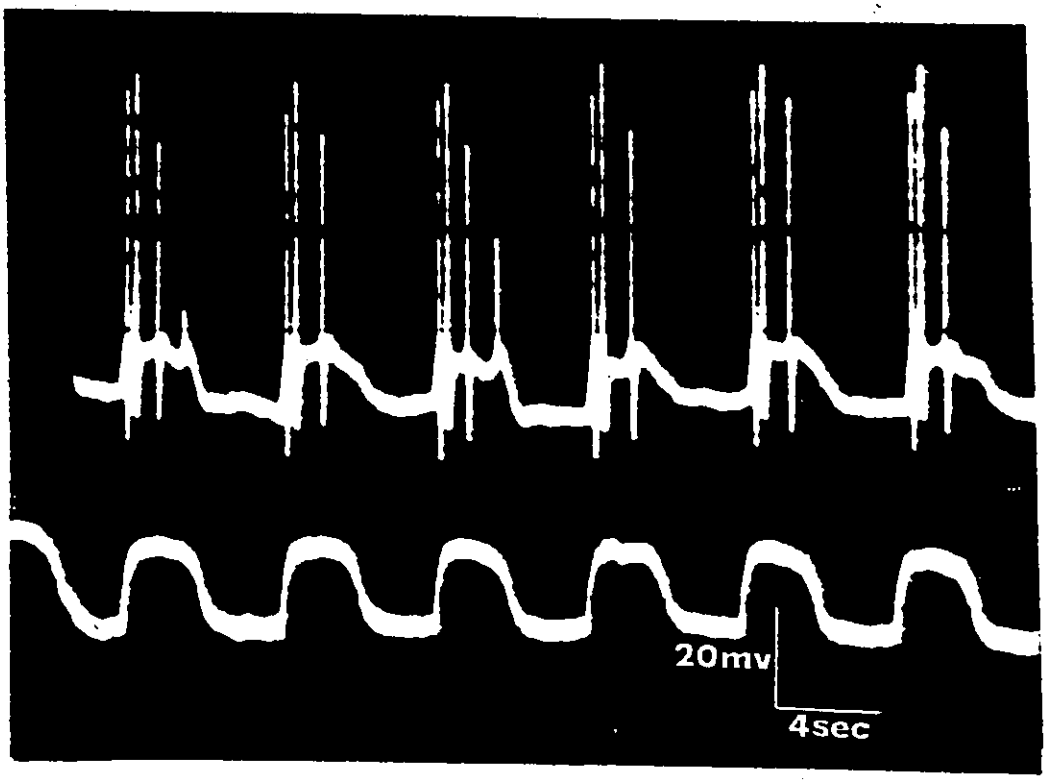


Fig. 10. Electrical activity of a LM cell (above) and a CM cell immediately underneath. Temp. 30°C.

10 consecutive cycles of LM of Fig. 5a was 1.93 ± 0.08 sec. The corresponding values for the CM cell immediately beneath it (Fig. 5b) was 2.02 ± 0.08 sec. Similarly, the example of Fig. 10 when the temperature was at 30°C showed that the duration of the CP of LM was 3.67 ± 0.2 sec. This value was not significantly different from the corresponding value of 3.69 ± 0.44sec for the CM below.

3. Irregular Activity of the LM

Although the MP and spiking activities of the two muscle layers were different, characteristics of the ECA of the two layers in terms of frequency, duration of the CP and temperature sensitivity were similar. However, in five out of a total of 39 LS examined, there was a drastic dissimilarity in electrical activities of the two layers. In all cases, the CM showed regular ECA activities. At the same time, LM displayed electrical patterns in which the regular ECA became difficult to distinguish. Fig. 11 shows an example of the electrical activities of a LM cell and the CM cell immediately below.

B. Circular Strips (CS)

The electrical activities of strips dissected along the circular axis differed from the LS in several respects. Cells in each muscle layer usually showed a significantly lower MP than those from LS. Thus the MP for LM and CM were 40.2 ± 3.9mv (n=34) and 54 ± 5.5 (n=97) respectively. ECAs in CSs were not well maintained, especially in preparations where the MPs were low. Some strips showed waxing and waning of the ECA (Fig. 12). It was interesting to note that if present, ECA occurred in

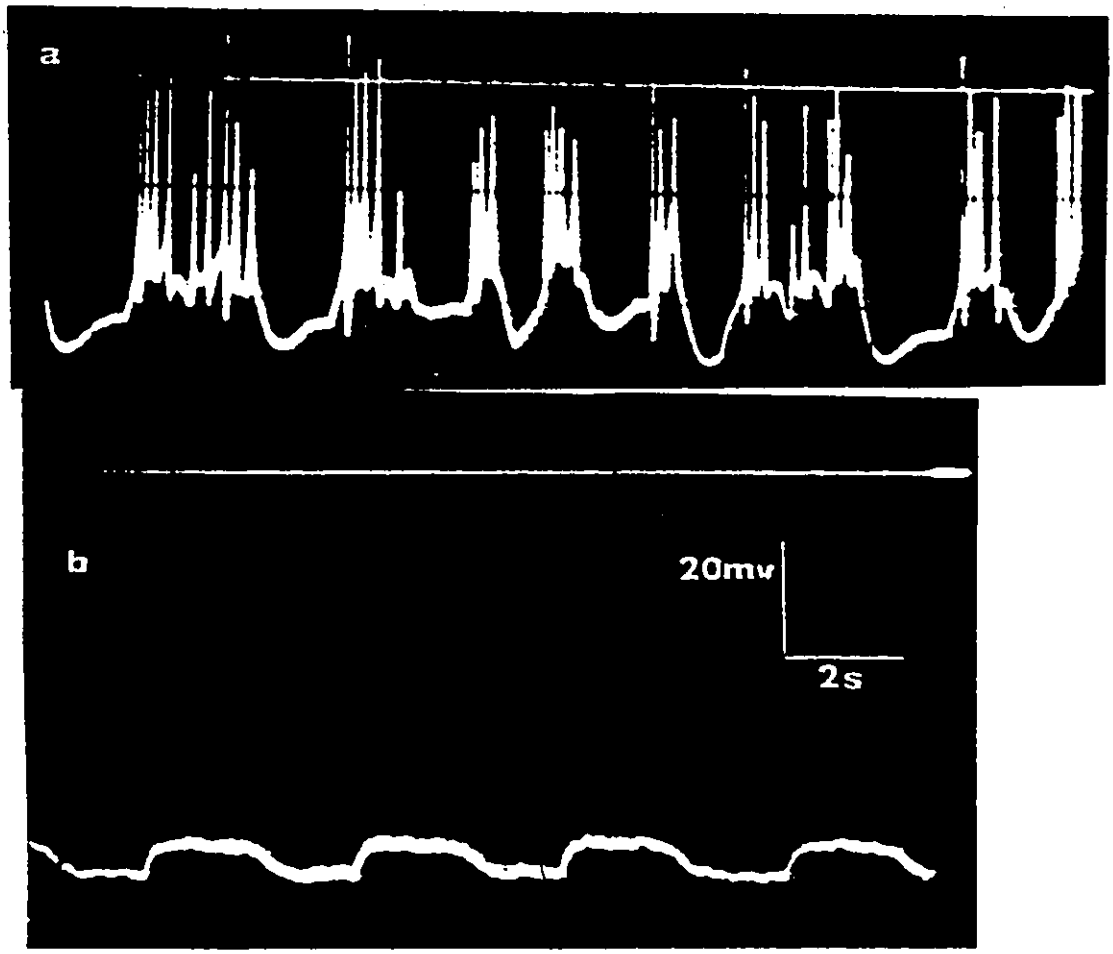


Fig. 11. a. Irregular electrical activity of a LM cell in which the regular ECA was not easily distinguished.

b. The cell in CM directly underneath a. showed very regular ECA unlike that of a.

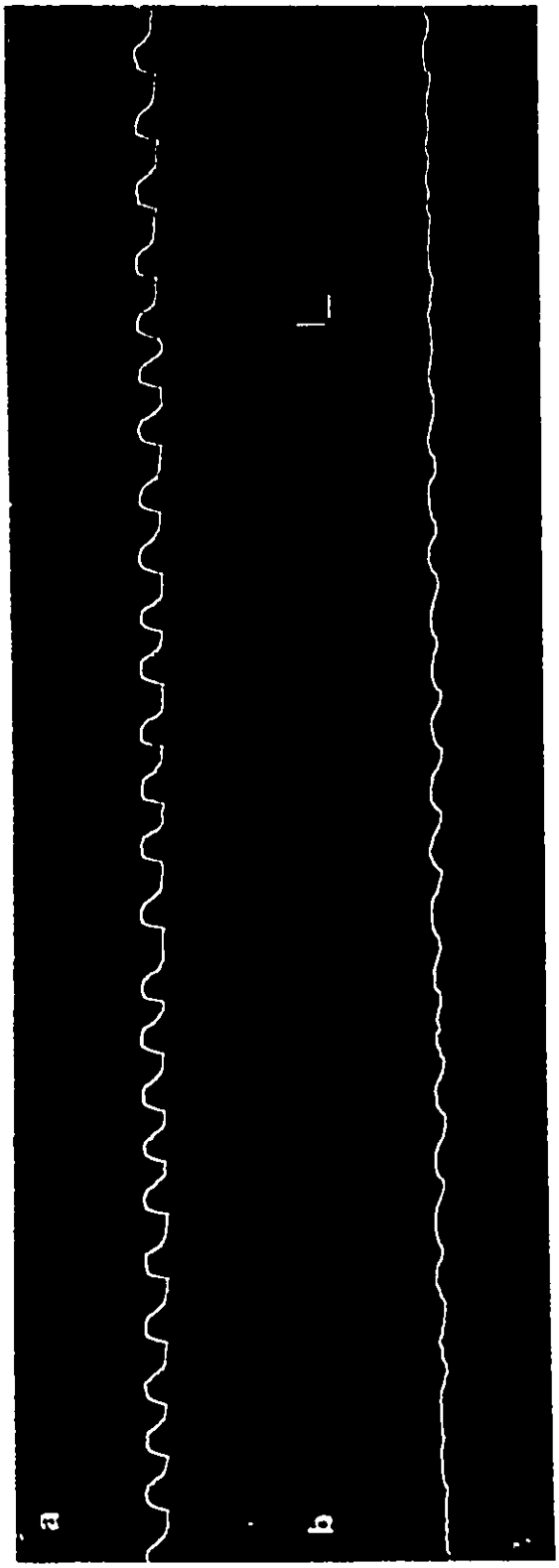


Fig. 12. Representative CP activities of the circular muscle cells of LS (top) and CS (bottom). CP activity was much better maintained by LSs. Calib: vert. 10mv; horiz. 1sec.

both layers.

C. Effect of Strong Electrical Stimulation on ECA

Strong electrical stimulation at 0.5 - 5msec duration can alter the ECA cycle of the muscle layers in both LS and CS, advancing the subsequent CP. This effect could be initiated in the presence of TTX and/or atropine. Stimuli applied about the middle of the cycle was most effective in advancing the subsequent CP (A cycle defined here refers to the period between the beginning of two consecutive CP or the period between the lowest mechanical tension of the rhythmic contractions in the mechanical records, see Fig. 13). By applying pulses at different phases of the ECA cycle, a phase-response curve (PRC) can be obtained by plotting the time at which a stimulus was applied within a certain cycle against the duration of that cycle. Thus the period of a cycle is shortened if the next CP is advanced. As was evident from Figs. 14, 15, 16 and 17) the stimulation was most effective in triggering the next CP when applied at the time when the cycle reached 55-60% of the total cycle. Stimuli applied before this period was not very effective in inducing the phase advance. Conversely, stimuli applied after the 55% mark would immediately trigger the subsequent CP. The effect of electrical stimulation was not influenced by the polarity of the pulses.

The ECAs of both muscle layers were affected equally by strong field stimulation whether LS or CS were used. Fig. 16 demonstrates the effect of electrical stimulation on a LM cell and a CM cell from another preparation. The PRC for both cells were identical. The PRC was not

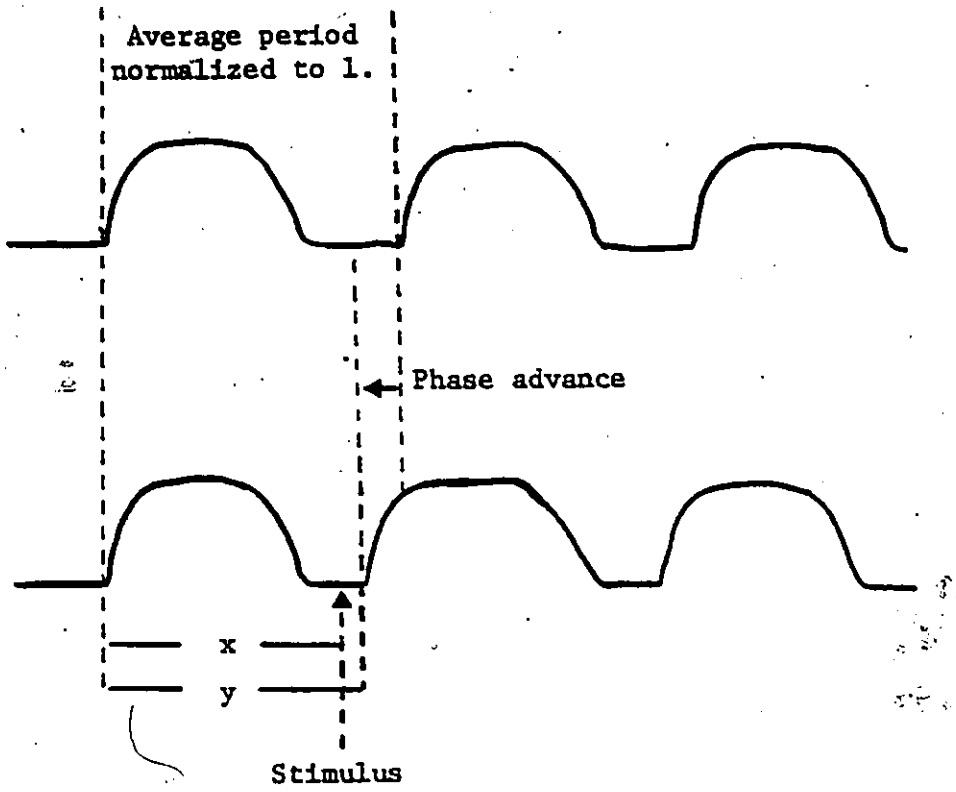


Fig. 13. Diagrammatic representation of the effect of electrical stimulation on the phase relationship of the ECA. The phase-response curve (PRC) is obtained by plotting x, the time of the normalized period when the stimulus was applied, against y, the duration of that period.

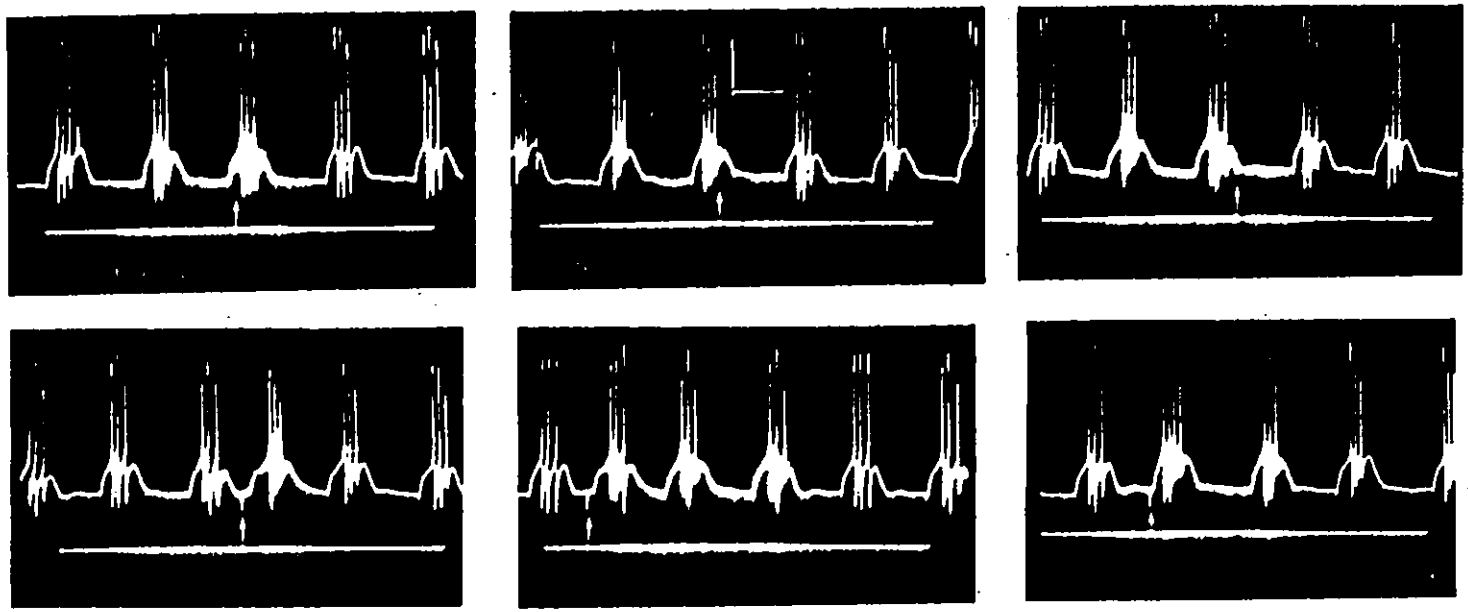
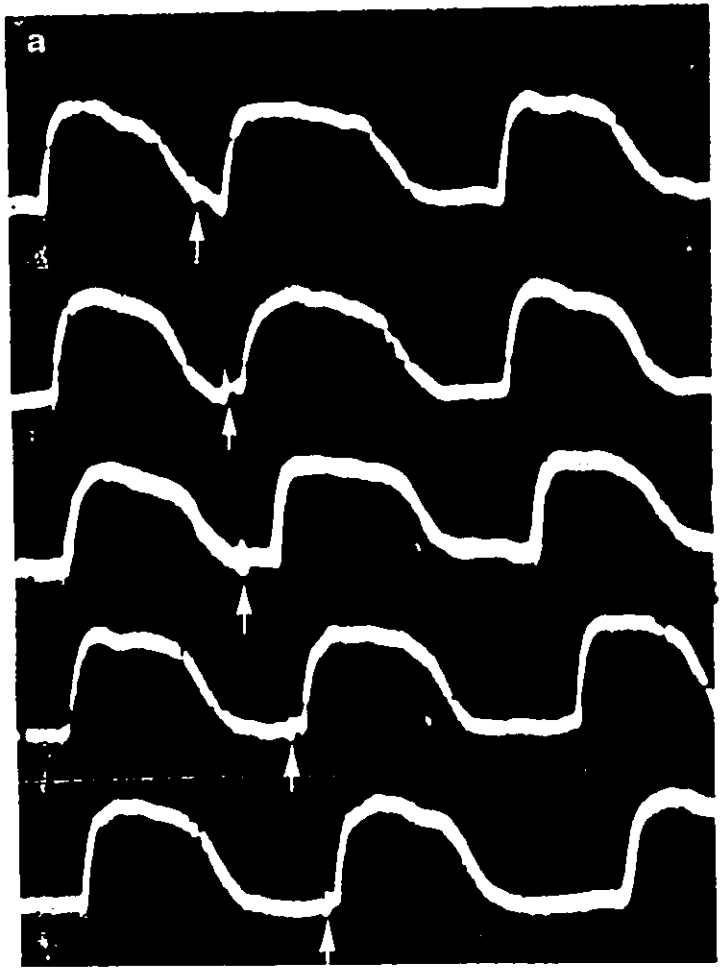


Fig. 14. The effect of electrical stimulation (arrow) on the phase relationship of a LM cell. The most effective phase-advancing occurred when the stimuli were applied during the period between CPs. Calib: vert. 20mv; horiz. 2sec.



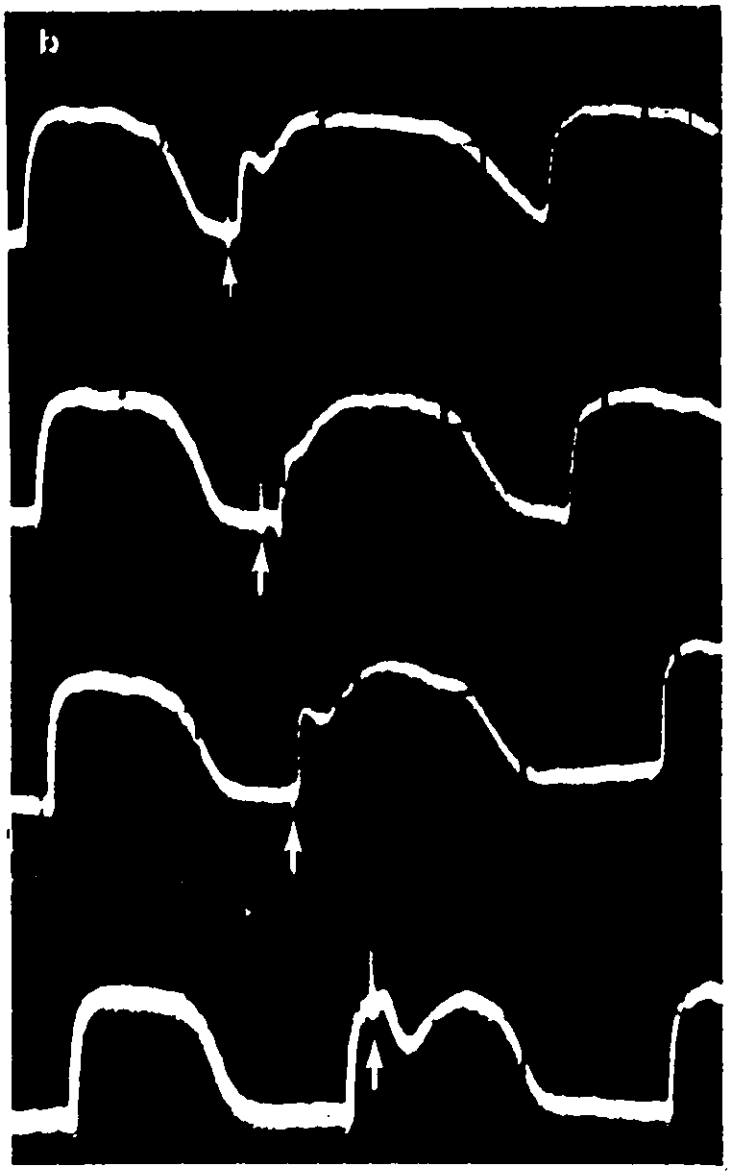


Fig. 15 a,b. Effect of electrical stimulation on the phase relationship of two CM cells. Notice that the phase shift was retained in the next cycle and that the duration of the CPs of the advanced cycles was increased. In b. the stimuli also evoked IJPs.

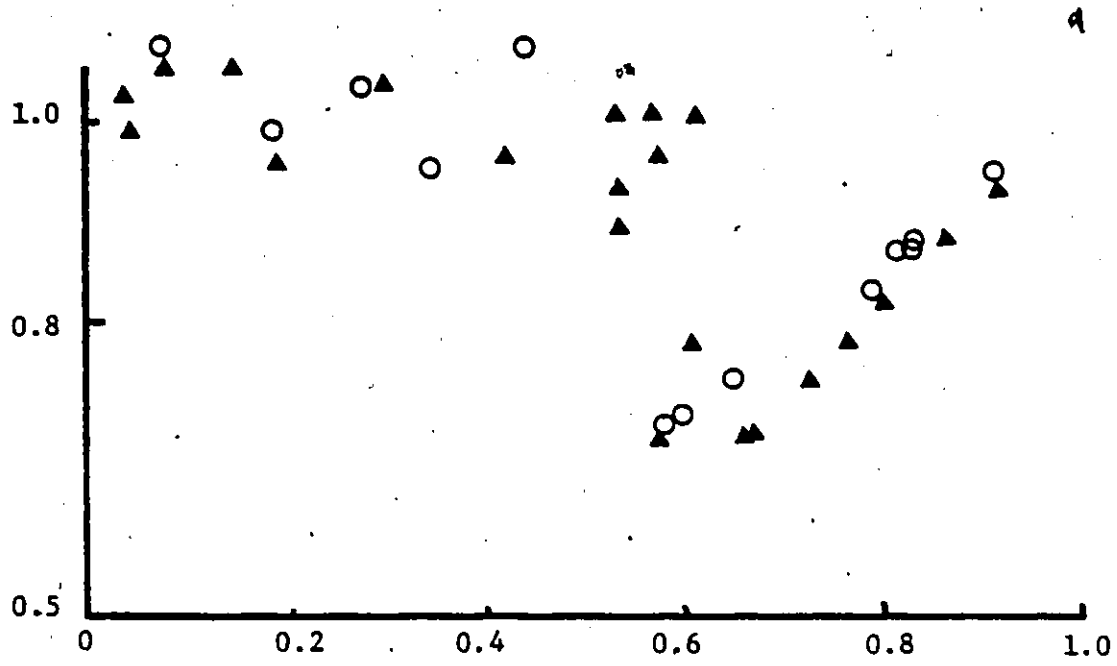


Fig. 16. PRC of a LM cell (O) and a CM cell (▲) from another preparation at 36°C. Note that the PRC for the CM cell is very similar to that of the LM cell, with the phase-advancing most effective when the stimuli were applied at 0.6 of the ECA cycle (see text).

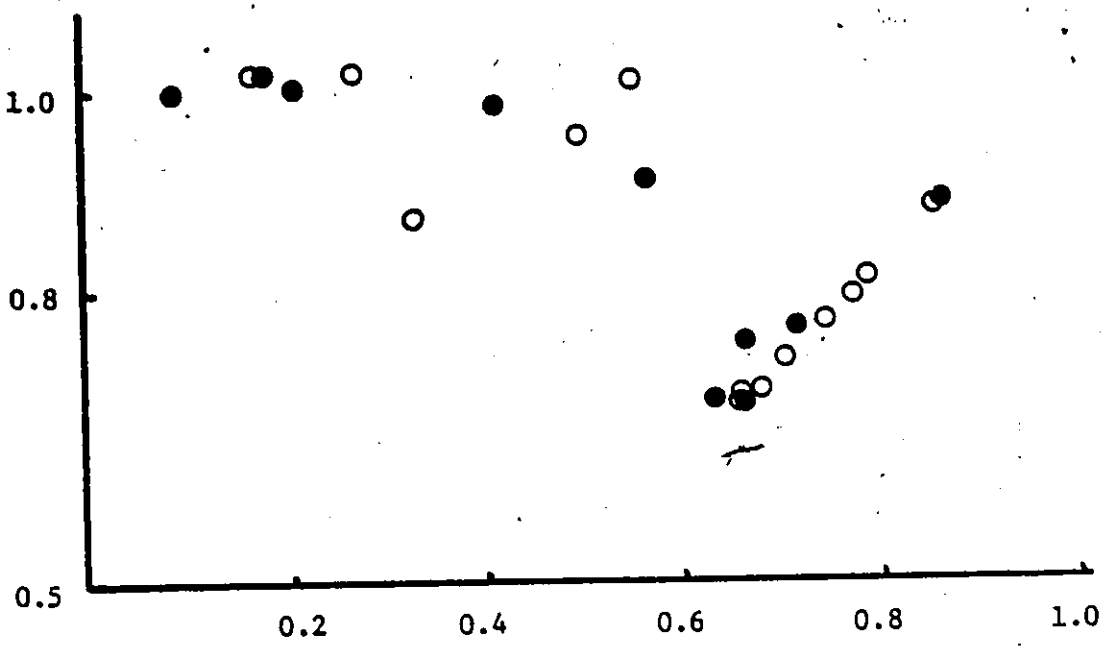


Fig. 17. PRC of the ECAs from a LM cell (○) and a CM cell (●) immediately underneath at 30°C. The effect of electrical stimulation on the phase relationship was the same for both cells.

affected by temperature. Fig. 17 shows the effect of electrical stimulation on a LM cell and a CM cell immediately beneath at 30°C. It can be seen that the responses of the two cells were identical. The PRC at 30°C was also identical to that at 36°C (cf. Fig. 16 & 17).

There were two salient features of the effect of phase-advancing by electrical stimulation. 1) The electrical stimuli reset the rhythm of the subsequent ECA. There was no obvious compensation to readjust the ECA immediately to the former rhythm (see Fig. 14 for example of electrical recordings and Fig. 18 for mechanical recordings). After an electrical pulse, the whole ECA sequence was advanced. The period of each cycle of the advanced sequence was similar to that of control.

2) The duration of the CP immediately following the stimuli was lengthened, especially for CM cells (Fig. 15). The size of the increase in duration was dependent upon the timing of the stimulus. The bigger the phase-advance, the bigger was the increase in duration of the CP. Since the total duration of the advanced cycle was not changed significantly, the increase in duration of the CP resulted in a shortening of the inter-CP period. For example, in Fig. 15b, the durations of the advanced cycles were similar to the controls (Table 3). However, the CP duration increased dramatically according to the degree of phase advance. The duration of the average CP (from 10 cycles) was 2.03 $\pm$ 0.14 sec, representing 62% of the duration of an average cycle (3.29 $\pm$ 0.18 sec). In the examples shown in Table 3, the CP took up 100% of the ECA cycle; in ii) 80%, and in iii) 67%. In iv) there was no phase advance and the CP was similar to the control and occupied only 63% of the cycle.

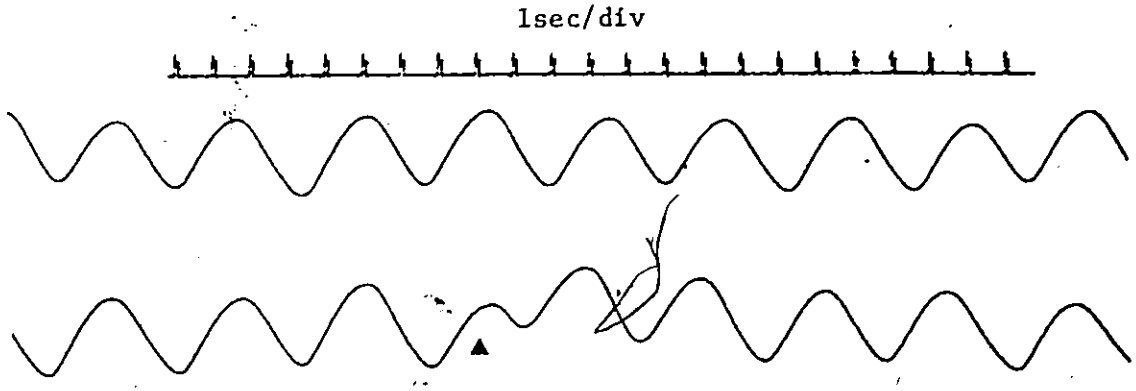


Fig. 18. Effect of electrical stimulation on the rhythmic contraction of a LS. Top: control; bottom: an electrical pulse applied in the middle of a cycle caused phase advancing of the following cycle. Note that the phase shift was retained on subsequent cycle following the stimulus.

Fig. 15b	Duration of advanced cycle	Duration of shortened cycle	Duration of CP	% of ECA cycle
i	3.29 sec	2.07 sec	3.29 sec	100
ii	3.00 sec	2.40 sec	2,40 sec	80
iii	3.47 sec	2.53 sec	2.33 sec	67
iv	3.27 sec	2.97 sec	2.07 sec	63

Table 3. Relationship of the duration of the CP of the advanced cycle with the phase advance (as determined by the duration of the shortened cycle). The duration of the advanced cycles were similar to the controls (3.29 $\pm$ 0.19 sec) but the duration of the CPs were much longer than the controls (2.03 $\pm$ 0.14 sec, representing 62% of an ECA cycle), depending upon the degree of phase advance.

The effect of electrical stimulation on ECA was supported by mechanical recordings from LS. The PRC of the mechanical studies was similar to that from electrical recordings except that the whole curve was shifted to the left by a few percent (Fig. 19). This was expected as the electrical activity leads the mechanical activity by this amount of time (Fig. 20).

D. Electrical Parameters of the Muscle Layers

If rectangular pulses of 400 msec were applied through the external electrodes, electrotonic potentials (EP) could be detected from muscle cells away from the insulated partition plates. The observed EP is due to current spread within the muscle cells. However, electrical coupling is usually limited to muscle cells within the same layer and only when the muscle strip is aligned according to the long-axis of the cells (see Section E). An example of electrotonic coupling from a circular strip is presented in Fig. 21. Good electrotonic potentials could be detected from the CM cells away from the stimulating electrode. The size of EPs decreased exponentially with distance from the electrode. By plotting the logarithm of the size of the EP against distance from the partition plate, a straight line was obtained from which the space constant (the distance at which the potential dropped to 1/e of that at zero distance) could be determined (Fig. 22). The space constant for CM was about 1.0 mm (range 0.7mm-1.1mm, n=6). From the space constant, the membrane time constant could be calculated according to Hodgkin & Rushton (1946) by plotting the time to reach half amplitudes of the EP against distance from the partition plate. The slope of the resulting line is expressed as $\tau m/2\lambda$. The time constant for CM obtained by this method was 100 msec (range 80-110 msec, n=6).

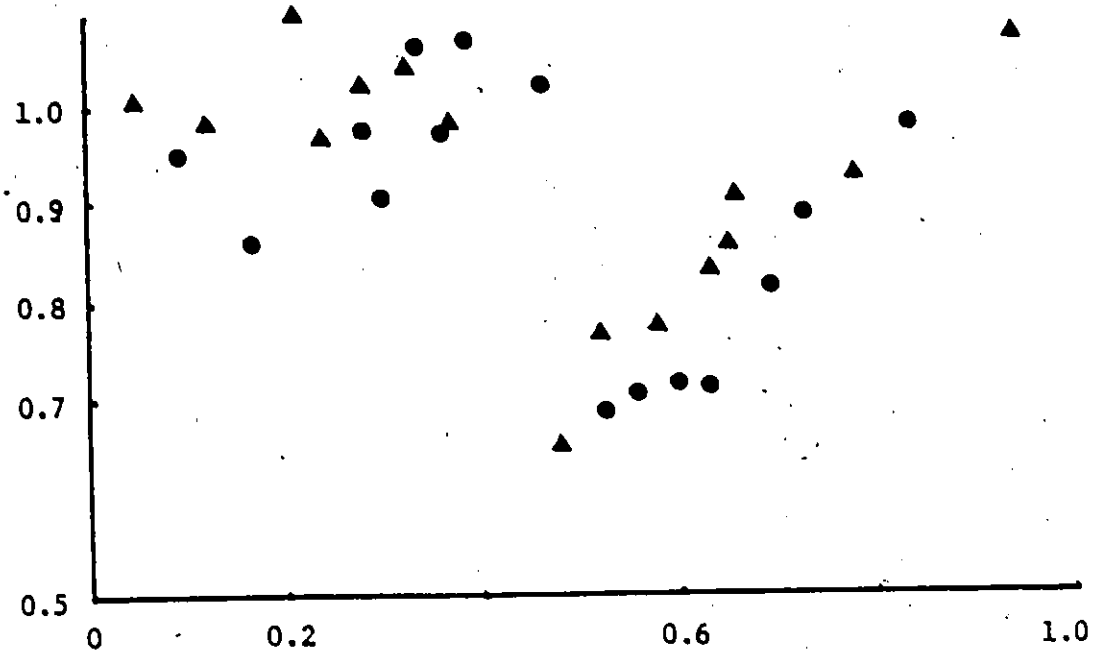


Fig. 19. PRC of the mechanic responses from two separate LSs. Note that the curves are shifted to the left with the best phase shift at about 0.5 instead of 0.6 of the cycle as in the electrical records (Fig. 16). This is in agreement with the electrical and mechanical relationship demonstrated in Fig. 20.

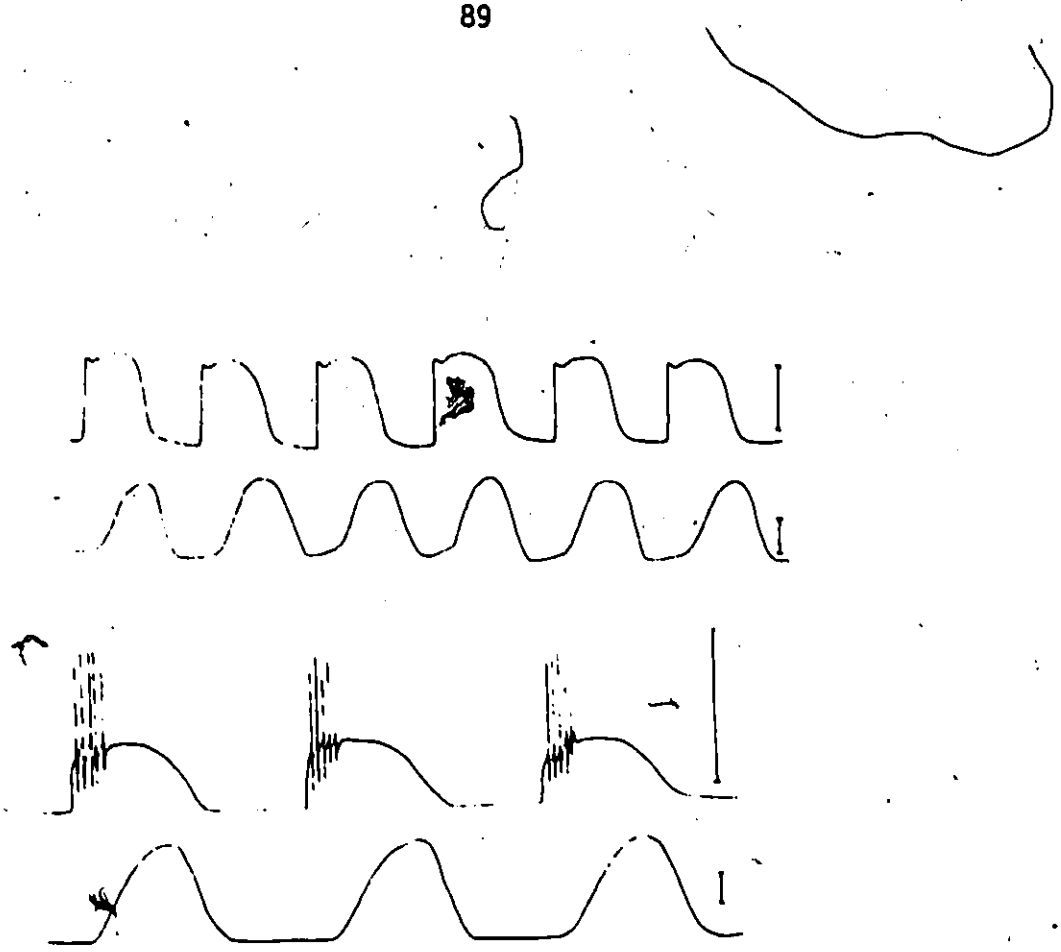


Fig. 20. The relationship between ECA (top tracings) and contractile activity (bottom tracings). The CPs always lead the contractile response. (from El-Sharkaway & Daniel, 1975a).

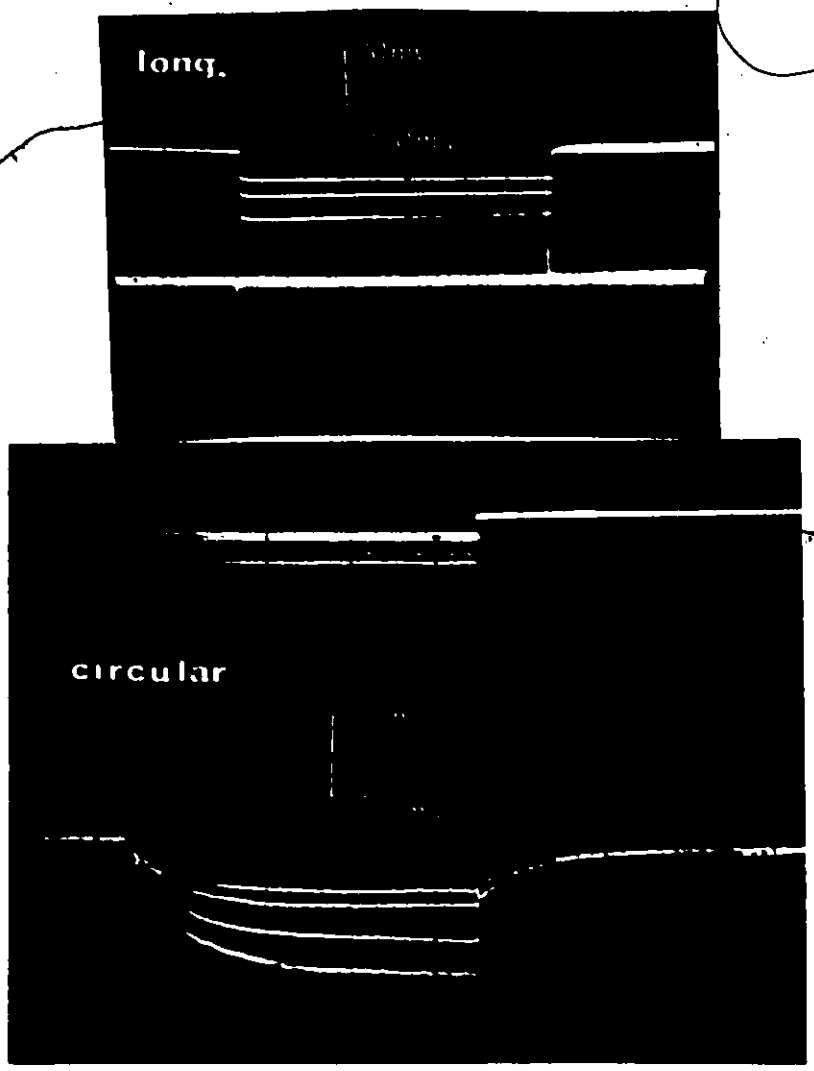


Fig. 21. When the muscle strip is aligned along the axis of the circular muscle and rectangular pulses are applied by external electrodes, electrotonic potentials can be recorded from the circular muscle cells (bottom) but rarely in the longitudinal cells. The converse is true if the muscle strip is oriented along the axis of the longitudinal muscle.

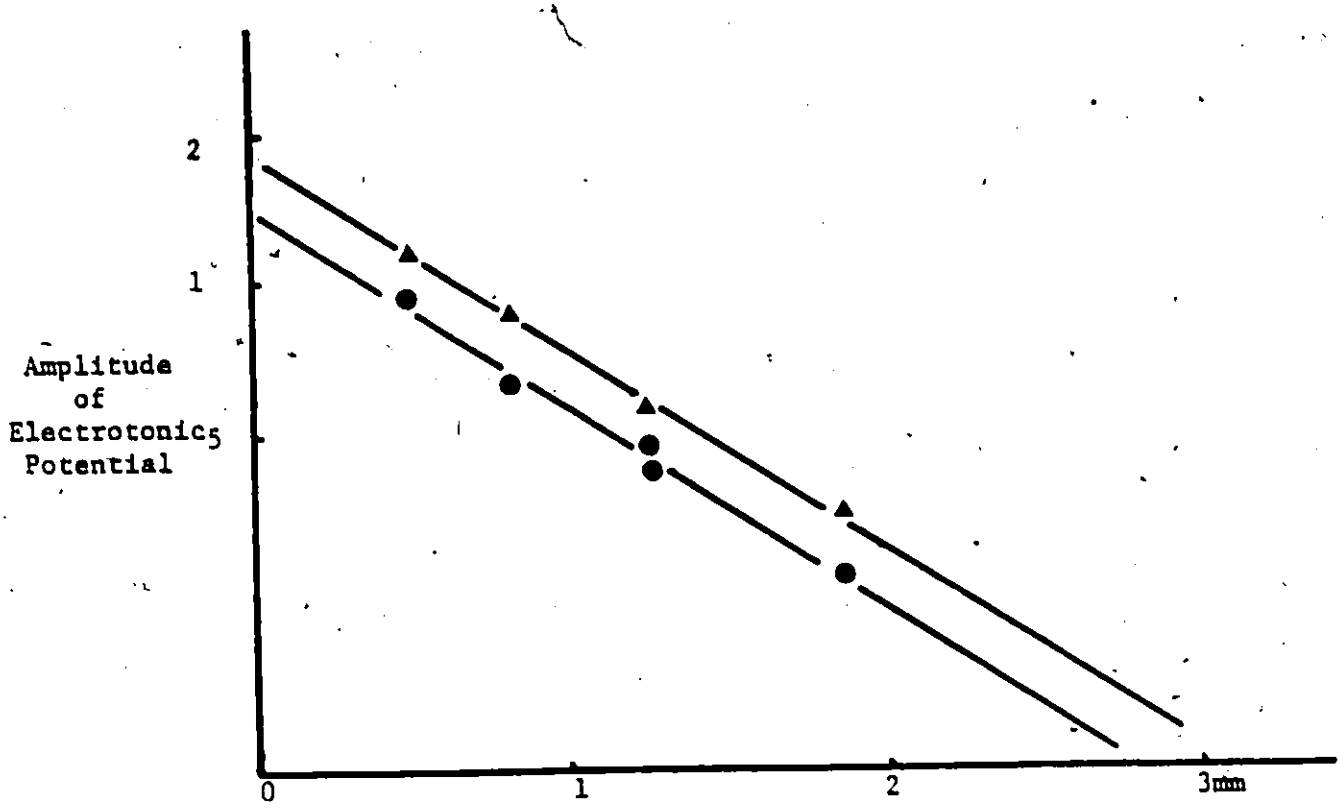


Fig. 22. The semi-log plot of the decay of electrotonic potentials of CM cells at various distances from the current electrode - two current intensities.

The electrical parameters for LM from LS were determine in the same manner. However, such long hyperpolarizing pulses tended to trigger CP more easily in LM than in CM, making it difficult to measure the EP (Fig. 23). The space constant estimated from LM was slightly less than 1.0mm (range 0.72-0.96mm, n=6) and the time constant was 92 msec.

E. Interaction between Muscle Layers

The preceding results demonstrated that the control waves of the two muscle layers were closely related in terms of frequency, duration of the CP and responses to temperature change and to external stimulation. Direct comparison of the size of CPs of LM/CM pairs (i.e. that from the longitudinal cell and the CM cell immediately below) showed that there was no significant difference between the two muscle layers at both 36°C and 30°C. Thus at 36°C, the mean size of CP for LM was 9.93 ± 2.3mv (n=12) and the corresponding value for the paired CM was 10.51 ± 1.66mv. The MP from these LM/CM pairs was significantly different ($P<0.05$), with the MP for LM being 51.48 ± 5.0mv and the MP for CM at 61.8 ± 6.21 mv. Similarly, the amplitude of the CPs for the paired LM/CM was not significantly different at 30°C. The amplitude was 9.0 ± 2mv (n=8) and 9.82 ± 1.36mv for LM and CM respectively. The MP for such pairs was also significantly different ($P<0.05$), being 48.4 ± 6.4mv for LM and 68.42 ± 6.36mv for CM.

In light of the proposal that CP originates from LM and that there is good electrotonic coupling between the muscle layers allowing electrical interaction between the muscle layers (Bortoff, 1961, 1976;

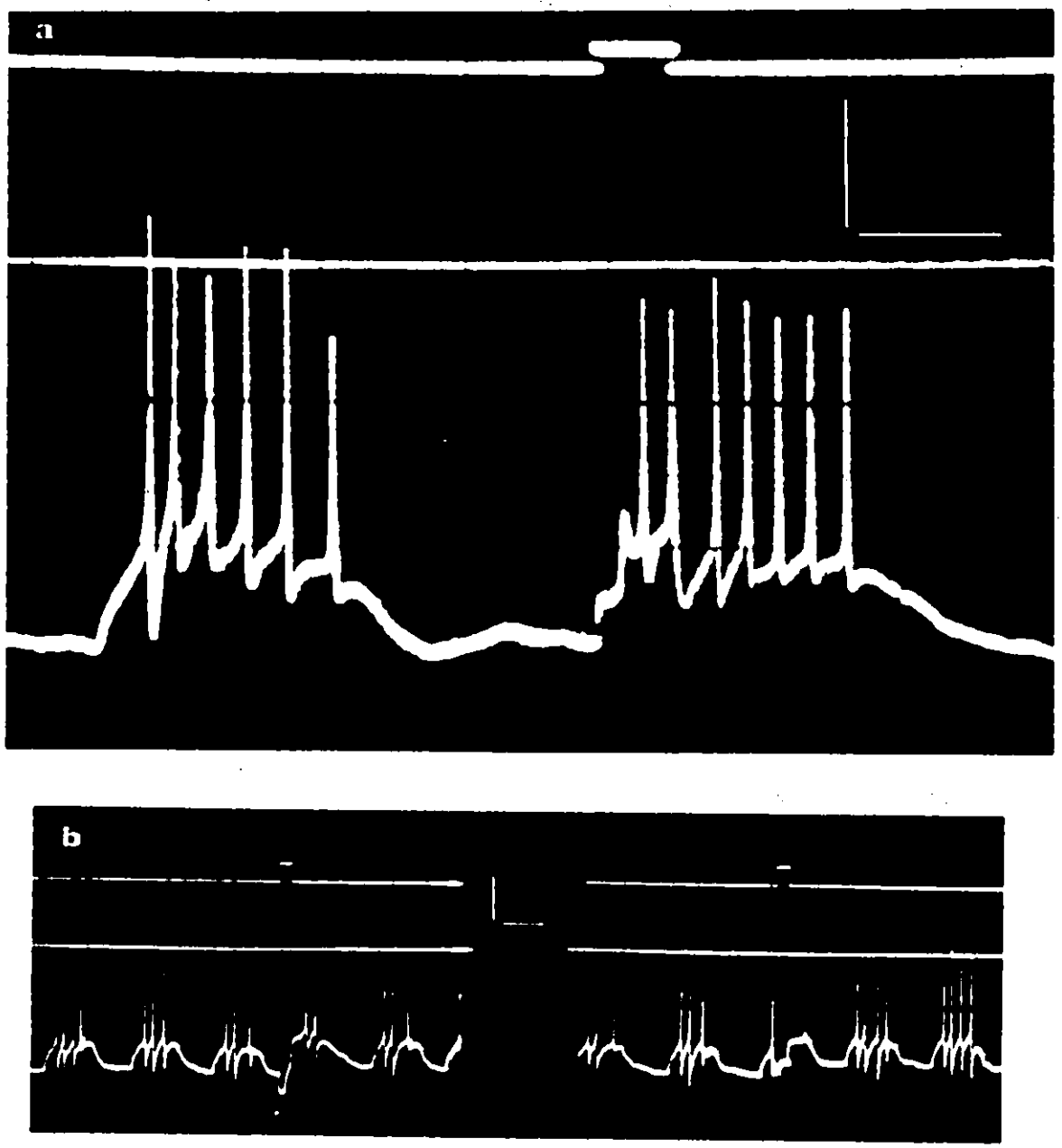


Fig. 23. Effect of long (400msec) depolarization pulses (a) and hyperpolarization pulses (b) on LM activity. Calib: vert. 20mv; horiz. 2sec.

Connor et al., 1977), electrotonic coupling between muscle layers was also examined by comparing the size of EPs from similar LM/CM pairs aligned along the long axis of either layer. In 16 LM-aligned preparations (LS) studied, no significant change of MP was detected in CM cells although all the LM cells within the distance tested showed electrotonic coupling. Similarly, in each of over 20 CS tested, EP was found only in CM cells (Fig. 21).

The problem of electrotonic coupling between muscle layers was also studied with circular strips treated with verapamil (3×10^{-7} g/ml). These preparations have several advantages in this kind of study compared to those using normal physiological solutions: 1) The spontaneous spiking activities of LM are abolished, making it easier for recording because of the elimination of the mechanical movements. 2) With circular strips that do not show ECA, the possible changes of EP with the ECA cycle (Mills & Taylor, 1971) and the possible effect on electrotonic coupling between the layers are avoided and 3) Verapamil is reported to increase the membrane resistance (therefore the size of EP and the space constant) of smooth muscle cells in guinea-pig taeni coli (Riemer, Kolling & Mayer, 1976). The presence of verapamil should therefore maximize electrotonic coupling between cells. However, it must be stressed that the effect of verapamil on the membrane properties of the rabbit duodenum is not known. In any case, this experimental condition should be optimal for the study of electrotonic coupling between cells. In 18 LM/CM pairs studied in circular strips under these conditions, electrotonic coupling between muscle layers was observed to various extent (Fig. 24). The biggest EP detected

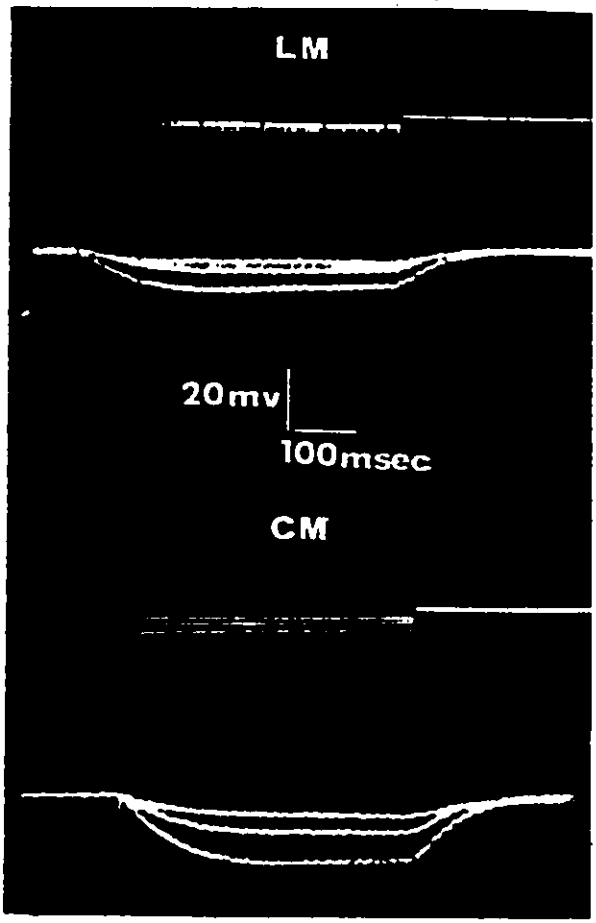


Fig. 24. In verapamil treated CS, electrotonic coupling between muscle layers could be observed. The CM cell was directly underneath LM.

in LM cells from CSs was 46% of that of the underlying CM. In two other cases, there was no sign of electrotonic coupling in LM. The mean value of the EP in all 18 LM cells was 34.2 $\pm$ 8.9% of that of the CM. These data indicated that there were pathways for electrotonic coupling between the muscle layers under these conditions. However, the coupling was poor (considering the whole muscle coat was only about 100μ thick) and not present in every cell.

DISCUSSION

A. The Spontaneous Activity of the Muscle Layers

The electrical activities of the smooth muscle cells from the two muscle layers were very different. The muscle cells of the two layers can be differentiated readily by the following characteristics:

1) Spontaneous spiking activities - LM was always spontaneously active with action potentials occurring on every CP in every cell. Consequently LM was also always active mechanically. Thus a LS will contract rhythmically at the same frequency as the ECA. The fact that neither TTX nor atropine had any effect on the spiking activities made it unlikely that they were of neural origin. CM was usually quiescent in terms of spiking and mechanical activities.

2) MP - The MP of LM was significantly lower than that of CM. LM was also more susceptible to temperature change. Lowering the temperature from 36°C to 30°C reduced the MP of LM without any noticeable effect on CM.

3) Electrotonic coupling - Electrotonic coupling of the muscle cells was dependent upon the orientation of the muscle layers under normal conditions. EP could only be detected in the muscle layer where the long axis of the cells were aligned in the same direction as the stimulating current.

These differences between the two muscle layers in terms of spontaneous spiking activities and MP are in agreement with that of the guinea-pig small intestine (Suzuki & Kuriyama, 1975). In addition, the responses of the two muscle layers to stimulation of the intramural nerves

are also very different. LM is innervated by cholinergic excitatory nerves whereas CM is innervated by a non-adrenergic inhibitory nerve (Hirst, Holman & McKirdy, 1975) and by three very distinctly different neural excitatory systems (see Part II of Results).

B. Electrical Control Activity

ECA was always present in both muscle layers of LS. It regulated the excitability of the smooth muscle cells cyclically. Spontaneous action potentials occurred only on top of the depolarized phases of the ECA, the CPs. ECA was not affected by TTX or atropine, indicating its myogenic origin.

In general, the ECA of the two layers from the same preparation were very similar in terms of frequency, amplitude and duration of the CP. Reducing the temperature decreased the frequency of both layers. ECA and rhythmic contraction could be maintained for long periods of time (over 10 hrs) in the organ bath if LSs were used. However, ECA was not well maintained in CS. The reason for this discrepancy is not easily explained. It is noteworthy that in CS, when present the electrical control activities of the two layers were well coordinated. When the ECA disappeared in one layer, it could not be found in the other layer.

The phase relationship of the ECA can be altered by external electrical input. The stimuli were most effective on the repolarized period between CPs. This effect was probably independent of nerve mediation as TTX and atropine did not alter the response. The effect of electrical stimulation was not dependent upon the orientation

of the muscle layers, unlike electrotonic coupling, and was identical for the two muscle layers. Altering the temperature did not alter the phase-response relationship.

In all the preparations used for electrical and mechanical studies, short duration pulses of 0.5 - 5msec were sufficient to produce the phase shift. The effect was not dependent upon the polarity of the pulses. This was in contrast to studies with external recordings that only depolarization pulses of long duration (several hundred msec) were required to trigger CPs (Sarna & Daniel, 1975; Mills & Taylor, 1971). The duration of the advanced CP was prolonged. Therefore not only can electrical stimulation trigger CPs, it can also affect the CP as a whole through mechanisms as yet unknown.

The good correlation in electrical control activities between the two layers did not hold true for all preparations. In five cases, the ECA of the two layers were very dissimilar. While CM still displayed the regular ECA, a more complex pattern of electrical activities was observed in LM. The cause for this deviation from the regular activities is not known. The MP in these preparations were not differnt from the others.

C. Electrotonic Coupling of the Muscle Layers

Electrotonic coupling of muscle cells within each layer was observed. The EP decayed exponentially with distance from the stimulating plates. It has been proposed that gap-junctions are the structures providing the pathways for coupling between cells (Dewey & Barr, 1963, 1968). Although gap-junctions are present in CM, they are rarely found in the LM of rabbits (Gonella et al., 1975), dogs (Henderson et

al., 1971) and guinea-pig small intestine (Gabella, 1972). However electrotonic coupling between LM cells could be demonstrated despite the lack of gap-junctions (Suzuki & Kuriyama, 1975).

It has been assumed in general that the two muscle layers are very well coupled electrotonically. In animal species that exhibit ECA it has been suggested that ECA originates only from LM and then spreads electrotonically into CM, which by itself is not capable of initiating ECA (Bortoff, 1961, 1965, 1976). A modified version of this theme is that the signal, after spreading electrotonically into CM from LM, is further amplified by CM (Connor, et al., 1977). Since the electrical activities of CM are passively dependent upon LM, the electrical and mechanical activities of the two layers should be synchronous, as pointed out by Bortoff (1976). It also follows that any potential change in one muscle layer should pass into the other as the coupling is non-rectifying (Connor, et al., 1977).

Unfortunately, these inferences were based on studies with extracellular recordings (Bortoff, 1961, 1965, 1976). In all cases, quantitative evidence at a cellular level was lacking. There was no attempts to compare the electrical activities and electrotonic coupling of the two layers directly. The recent report by Connor et al., (1977) suffered from the same drawback. No direct quantitative comparisons were made of the electrical activities of the two layers. In addition, hypertonic sucrose solutions were used to inhibit mechanical activities. This added an unknown complication since hypertonic sucrose solutions were known to increase the MP, disturb the ionic balance of the cells, and alter the electrical activities and electrical coupling of the

smooth muscle cells (Tomita, 1972; Coburn, Ohba & Tomita, 1975a, b).

The experimental approach in this study was designed to compare directly within the same preparations:

1) the basic electrical activities (and the neural responses) of the two muscle layers
2) the electrotonic coupling between and within muscle layers

The basic assumption that the two layers were well coupled electrotonically used in support of the idea that ECA originated from LM and spread into CM could not be substantiated in the present study. If the layers were well coupled electrotonically, the results of this study would be difficult to explain:

1) A clear difference in MP and electrical activities of the two muscle layers was observed in this study. Similar differences in the electrical activities and MP of the LM and CM was found in guinea-pig small intestine (Suzuki & Kuriyama, 1975).

2) Inhibitory junction potentials (IJPs), which have a duration of about 1sec and an amplitude of 15-20mv, were frequently observed in CM (see Results, Part II, Section B). No sign of such events were observed in LM, as would be expected if the two layers were coupled electrotonically. Similar observations have been presented by Hirst & McKirdy (1974) for the guinea-pig small intestine.

3) The spread of electrotonic currents readily occured within muscle layers but rarely between layers in normal physiological solutions. Although good electrotonic coupling between layers was difficult to demonstrate in normal physiological solution, electrotonic coupling

was observed in some cells in the presence of verapamil. This may be a result of an increase in membrane resistance by verapamil thereby facilitating current spread between cells through low-resistant pathways. However, even under these favourable conditions, electrotonic coupling between muscle layers was not uniform, ranging from no coupling to a maximal of 46%. In light of the fact that CPs from similarly paired LM/CM cells were identical in amplitude, it was clear that ECA could not have originated from one layer and spread electrotonically in a passive manner into the other.

The poor electrotonic interaction between muscle layers was not unexpected on anatomical ground because the two muscle layers rarely come close together. In the guinea-pig small intestine, more than half of the space between the muscle layers is occupied by the myenteric plexus and blood vessels. The remainder of the space is essentially taken up by collagen fibres, leaving only a very small portion of the space to allow smooth muscle cells from the two layers to come into close contact. In the guinea-pig, nexuses between muscle cells from the two layers were observed, though infrequently (Gabella, 1972).

From this study, it is clear that ECA of the two layers are closely related. Changes of temperature affected the ECA of the two layers equally. Strong electrical stimulation altered the phase relationships of the ECA of the muscle cells irrespective of the muscle layer or electrotonic coupling. In cases where the ECA was fading or absent, both layers displayed the same result. Therefore there is little doubt that the ECAs of the two layers are intimately linked to

each other. There are two possible alternate models to account for the good coordination of ECA of the two muscle layers with limited electrotonic coupling. Sarna, Daniel & Kingma (1971) proposed that the circular muscle may act as an relaxation oscillator that can be triggered by the activity of the longitudinal muscle layer. Another possible explanation would invoke the existence of a group of specialized cells - the "driver" cells - that triggers activity in both layers. The muscle cells in both layers are therefore "follower" cells in this case. Under such conditions, the ECA of the two layers will be synchronous without the need of electrotonic interaction. Parameters that change the activity of the "driver" cells would be reflected also in the two layers. However, there is little experimental evidence to support either of the two models at this point.

Earlier studies by Kobayashi et al., (1966) on the cat intestine indicated that CP was biggest on the LM closest to the myenteric plexus. For the rabbit small intestine, Daniel & Taylor (1975) suggested that CP was biggest in CM cells closest to the plexus. Although these results are conflicting, it does indicate that ECA may be most active in the region where the two muscle layers are closest to the plexus and to each other. A "pacemaker" region close to the myenteric plexus is implicated in both cases. In experiments in which the layers had been separated physically, damage to these regions was most likely. The main support for the proposal that ECA originates only from LM came from studies in which the two layers were physically separated. Under these conditions, ECA was observed in the strips containing LM but not those containing CM (Bortoff, 1961, 1965; Connor et al., 1977). How-

ever, it is very difficult to separate the two layers cleanly. Strips from the LM layer usually contained part of the CM layer (Bortoff, 1961; El-Sharkaway & Daniel, 1975). If the parts that are responsible for initiating ECA lay at the regions closest to the plexus (in light of the size of the CP) they are most likely to be stripped away or damaged when separating the two layers. Therefore, it would not be suprising to find that the isolated circular layer does not show ECA. Daniel & Taylor (1975) reported that in strips containing only LM cells, as determined by electronmicroscopy, there were no ECA. The underlying CM layers retained ECA. Again the reason could be due to the damage of the essential ECA pacemaker region. In any case, the origin of ECA and the interaction of ECA between muscle layers cannot be resolved by the existing approaches and remains speculative at best. However, the present study did rule out the kind of electrotonic interaction between muscle layers essential for the models of ECA origin proposed by Bortoff (1961, 1976) and Connors et al., (1977).

PART II

The Innervation of the Muscle Layers

A. The Longitudinal Muscle Layer

1. Spontaneous Neural Activities of LM

Of a total of 107 LM cells from 34 preparations surveyed, spontaneous excitatory junction potentials (SEJPs) were detected in only 16 cells from 9 preparations. SEJP appeared to be a very localized event as even within the same preparation, SEJP could be found in one cell and yet be absent in other cells in the immediate vicinity. SEJPs occurred at all phases of the ECA cycle (Fig. 25). They were most conspicous in between CPs when they were free from the interferences of the spontaneous spiking activities on the CP. Some SEJPs occurring at this time were large enough to initiate action potentials. The action potentials generated usually did not reach full height and afterpotentials were rarely observed.

Analysis of the time course of the SEJPs showed a fast and a slow component similar to those reported in guinea-pig vas deferens (Bennett, M.R., 1972). The SEJPs may be single or occur in multiples (Fig. 26 and 27). The majority of these responses were "mixed" showing initially a fast component followed by a slow component similar to those in the guinea-pig vas deferens (cf. Figs. 26, 27 with the examples given by Bennett, 1972 reproduced in Fig. 56). The time to peak for both the fast and slow components was approximately 100 msec. Both components decayed exponentially (Fig. 28). The time constant of decay for the fast components was 20-40msec and that for the mixed and slow

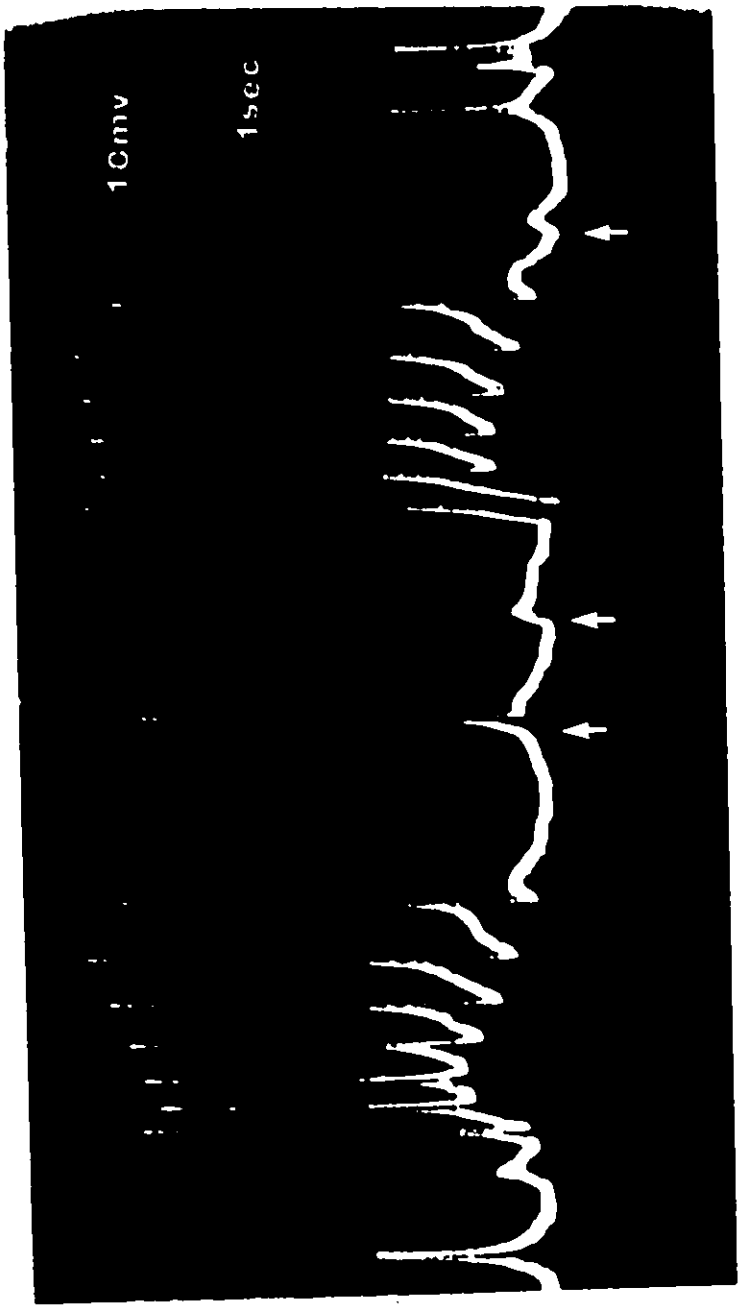


Fig. 25a. Spontaneous EJPs in LM, some big enough to initiate spikes.

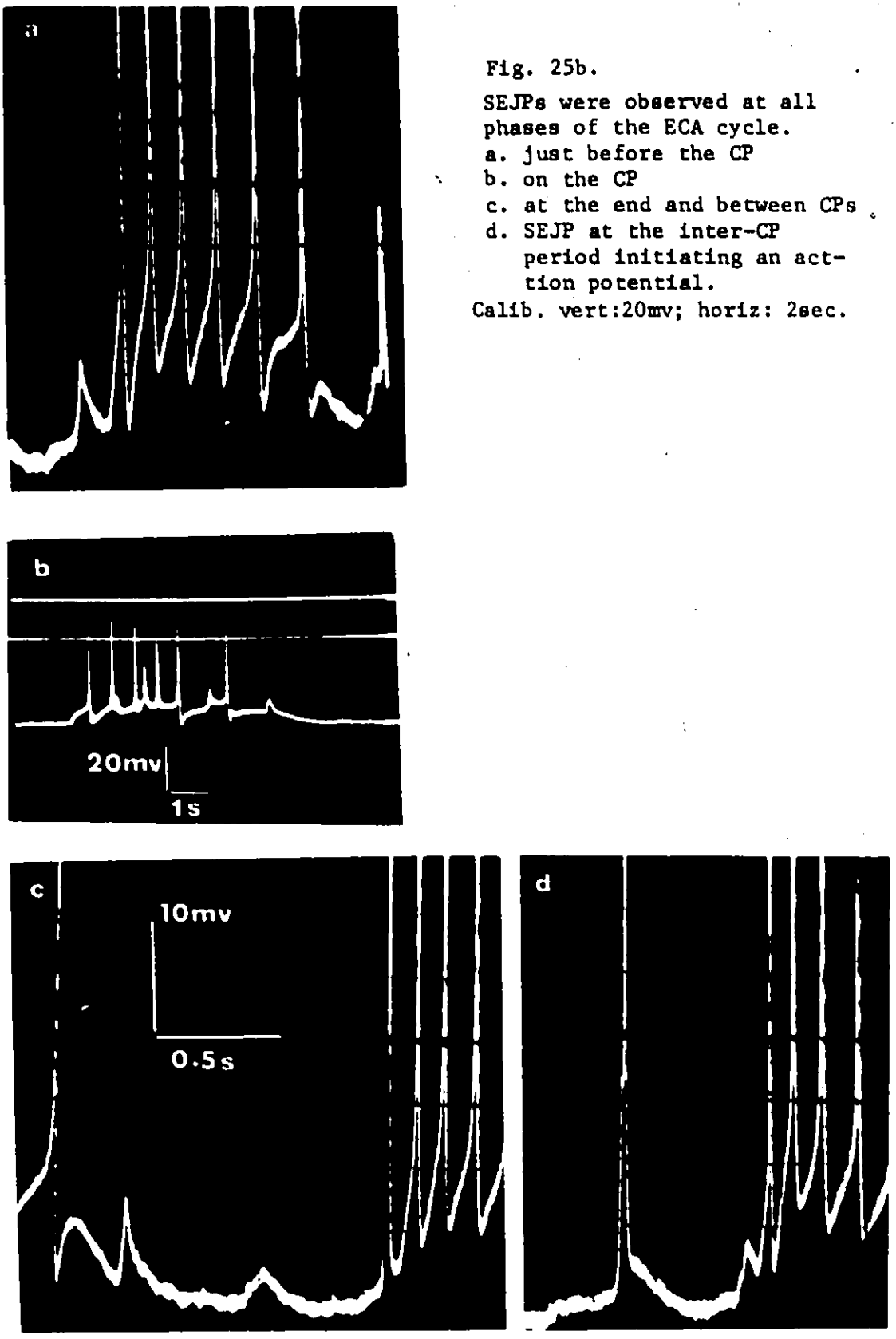
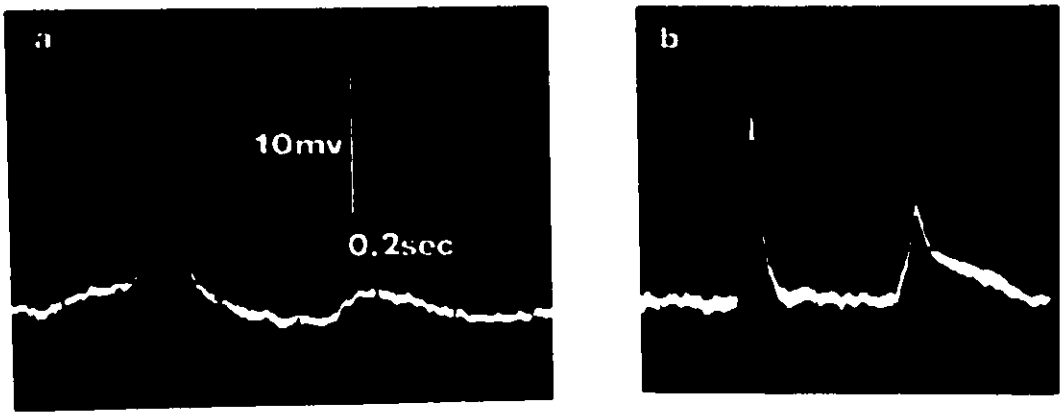


Fig. 25b.

SEJPs were observed at all phases of the ECA cycle.
a. just before the CP
b. on the CP
c. at the end and between CPs
d. SEJP at the inter-CP period initiating an action potential.

Calib. vert:20mv; horiz: 2sec.



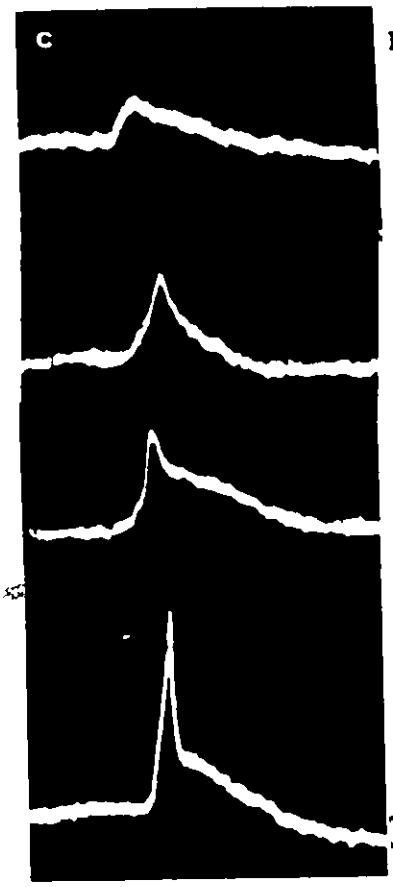
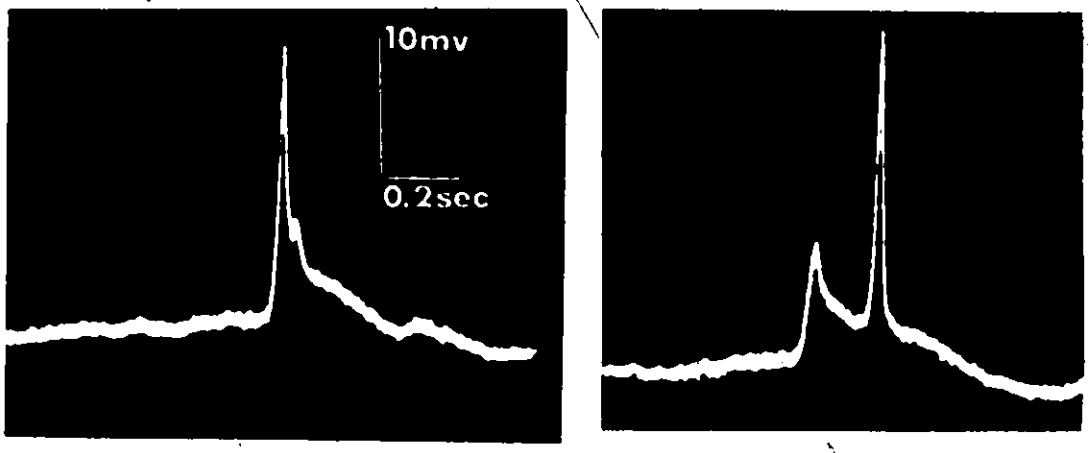
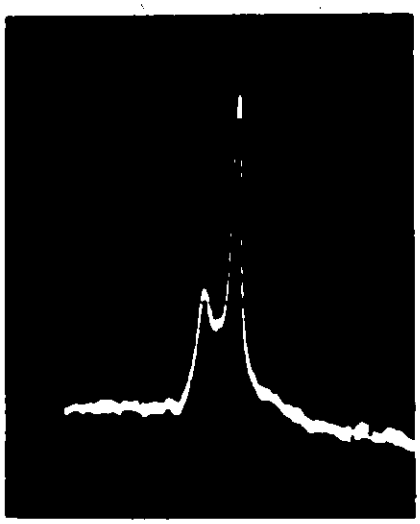


Fig. 26. Two types of SEJPs (fast and slow) were observed. They occurred singly (a) and as mixtures (b). In (c), various combinations of the two components are shown. This is very similar to the SEJPs in guinea-pig vas deferens (see Fig. 56).





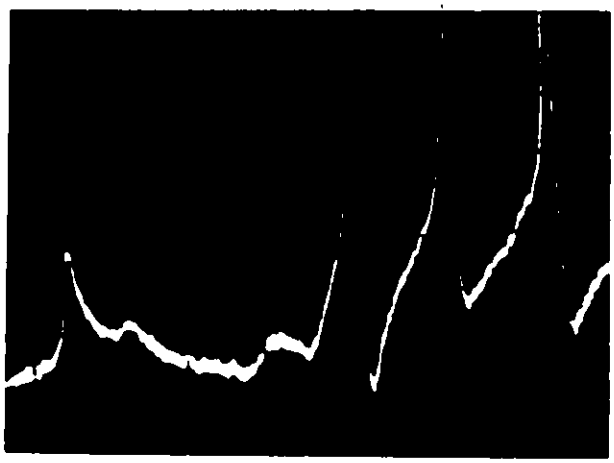
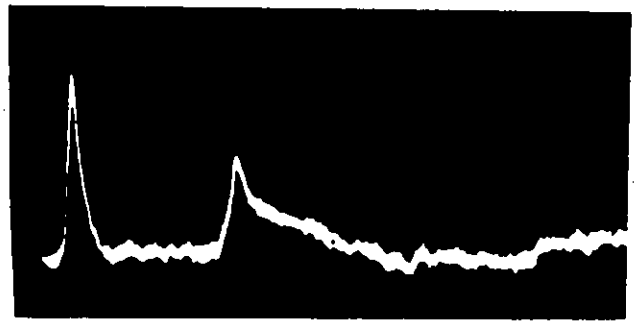
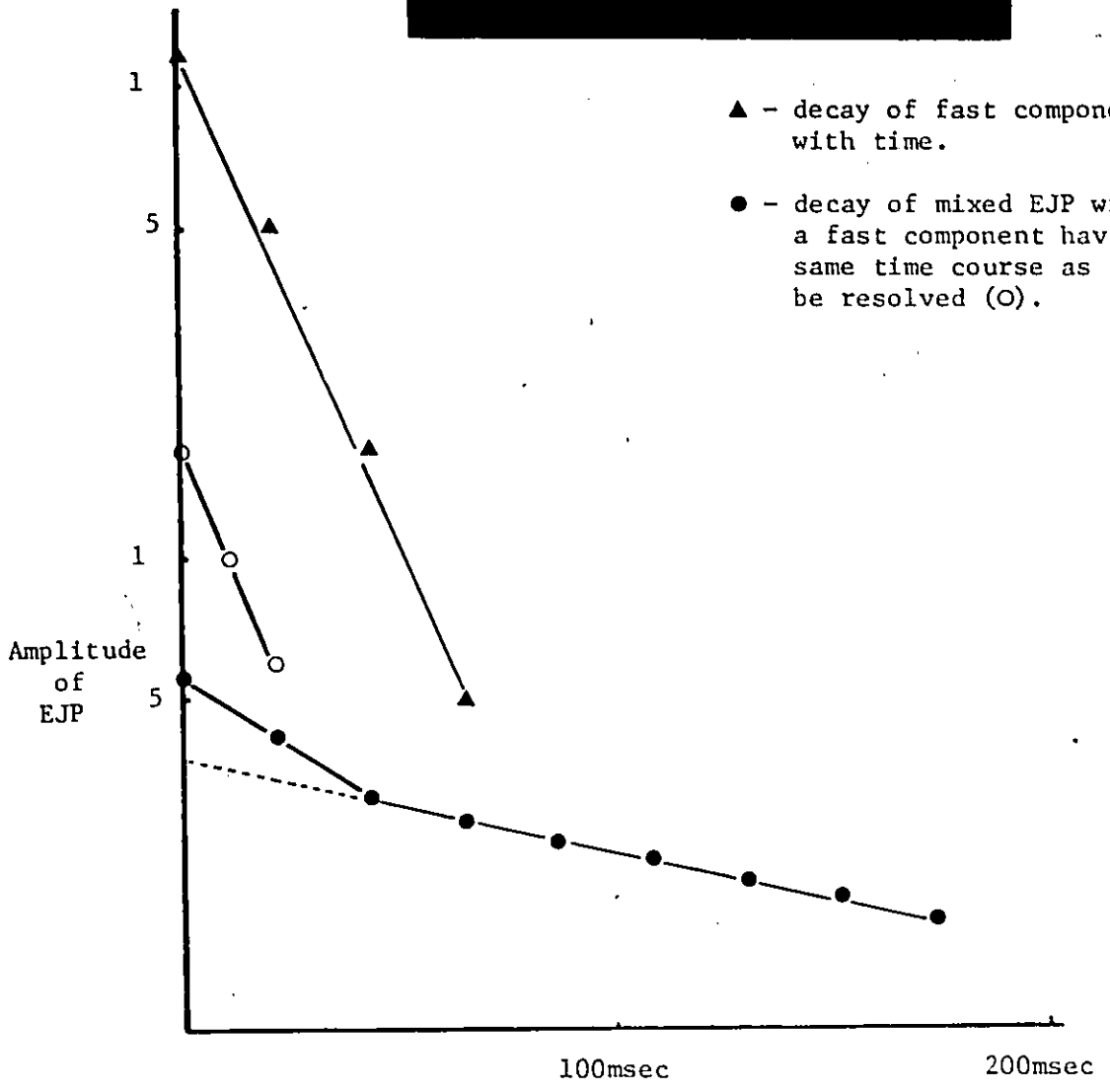


Fig. 27.

Examples of multiple SEJPs that summated at different times giving rise to various shapes of the SEJP complexes.





▲ - decay of fast component (left) with time.

● - decay of mixed EJP with time, a fast component having the same time course as (▲) could be resolved (O).

Fig. 28. The amplitude of SEJPs (semi-log) decayed exponentially with time. The fast components decayed with a time constant of 20msec and the slow component of 218msec.

component was 90-250msec. SEJPs were never observed in the presence of atropine.

2. Effect of Field Stimulation of the Intrinsic Nerves on LM

Stimulation of the intramural nerves with 0.5msec duration pulses produced excitatory junction potentials (EJPs) in the muscle cells of LM. Atropine (3×10^{-7}g/ml) blocked EJPs indicating that muscarinic receptors were activated. The excitatory response to a single stimulus can be manifested in several ways:

1) If the stimulus was applied just before the onset of the CP, the interval between spikes on the CP became shortened (Fig. 29). This "crowding" effect was especially true of the interval between the first and second spikes. The total number of spikes on the CP might not increase, depending upon the cell and the size of the EJP (Figs. 30, 35). In most cells (90%), field stimulation with single 0.5msec pulses did not produce any detectable change of membrane potential. However, the crowding response could almost always be produced. The activation of the cholinergic excitatory nerves was implicated because atropine could completely abolish it (Fig. 29).

2) In response to a single stimulus, the EJP could be seen as a clear depolarization (Fig. 29, 32, 35). The latency of the response was almost about 100msec.

3) Single pulses may also produce multiple responses which often led to firing of action potentials (Figs. 31, 32, 35). In such cases, there appeared to be temporal summation of several EJPs. Action potentials were elicited if the summed depolarization was high enough. Similar to those initiated by SEJPs, the action potentials due to EJPs

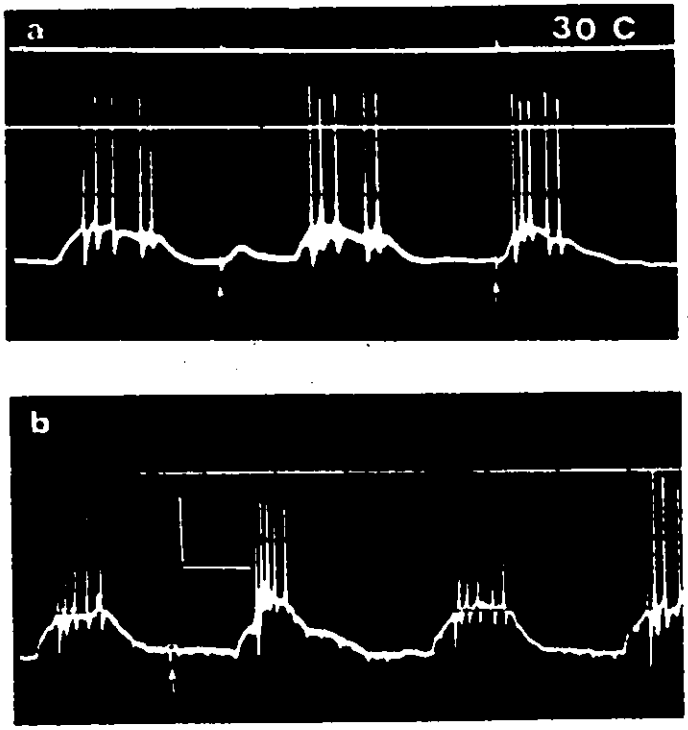


Fig. 29.

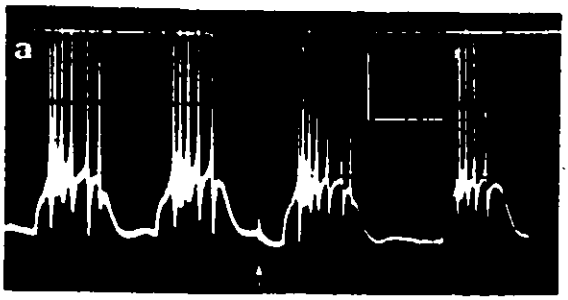
Stimulation with 0.5msec pulses may cause "crowding" of the spikes on the CP without increasing the number of spikes.

a. a clear EJP was elicited in the middle of the inter-CP period. A pulse applied just before the CP caused crowding of the spikes.

b. & c. were from the same cell. Single (b) and repetitive (c) stimulation induced crowding.

d. The crowding of spikes could not be produced in the presence of atropine (10^{-7}g/ml). d. was from the same preparation as b. & c.

Calib. vert: 20mv; horiz: 2sec.



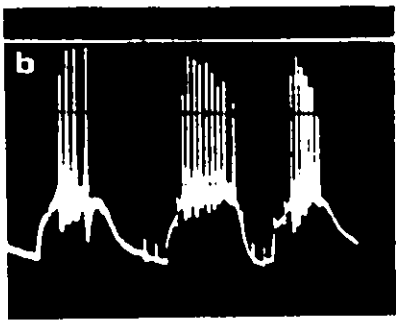


Fig. 30.

Single (a) and repetitive (b) stimulation resulted in an increase in the number of spikes on the CP.

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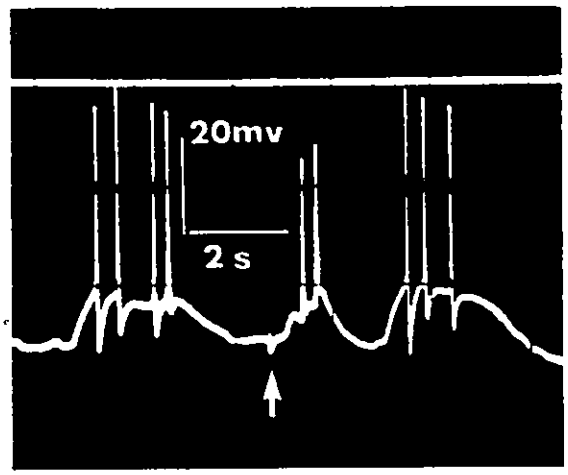


Fig. 31.

In response to single stimulation, an "excitatory complex" made up of more than one EJP may also appear.

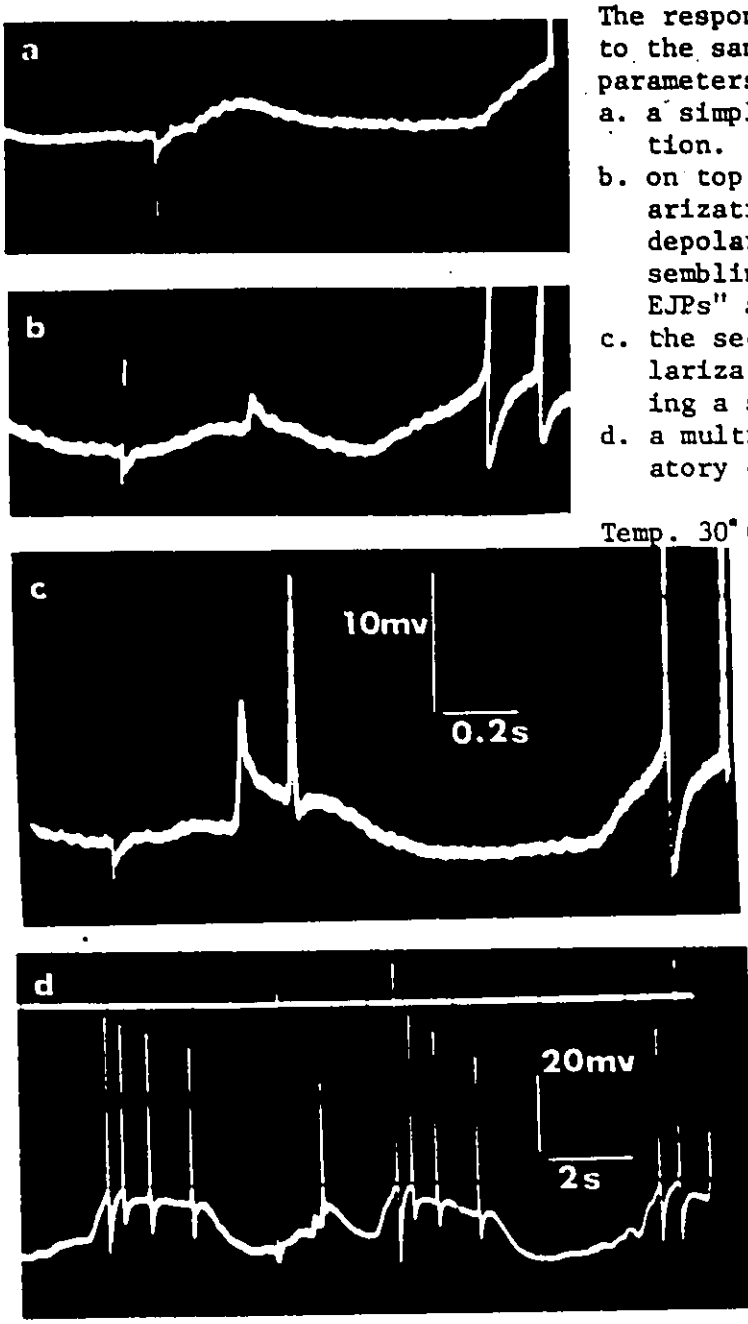


Fig. 32.

The response of a cell to the same simulation parameters is variable:
a. a simple depolarization.
b. on top of the depolarization, a sharp depolarization resembling the "mixed EJPs" appeared.
c. the secondary depolarization initiating a spike.
d. a multiple "excitatory complex".

Temp. 30° C.

usually did not reach the full amplitude and the afterpotentials were either absent or very small (cf. the action potentials related to neural stimulation with the spontaneous spikes on top of CPs in Figs. 21, 22, 33, 35).

4) The response to neural stimulation was variable, even in the same cell and with the same stimulus parameters (Fig. 32). Fast components similar to that of SEJP were also observed (Fig. 32b, c).

5) In all cases, repetitive stimulation of the preparation at 5 to 10 HZ was more effective that a single pulse. In cells where an EJP was not elicited by single pulses, repetitive stimulation often brought about conspicous changes in activity. Fig. 33 shows an example of a cell where single pulses of supramaximal strength did not produce any change of basal electrical activities, but repetitive stimulation at 5 HZ produced a dramatic change in activity showing significant increase in spiking frequency. These increases in excitability could not be produced in the presence of atropine (Fig. 29a). In some preparations, repetitive stimulation also produced EJP "excitatory complexes" in between CPs in addition to increasing the frequency of spikes on the CP (Fig. 34). Sustained depolarization as reported in rabbit colon (Gillespie, 1962b) was not observed during repetitive stimulation under our experimental conditions.

3. Interaction of the EJP with ECA

EJP did not have any significant effect on the frequency or the phase of the ECA. The stimulus strength required to elicit neural responses was less than those needed to produce phase advance electrically. Therefore, junction potentials could be produced without any significant

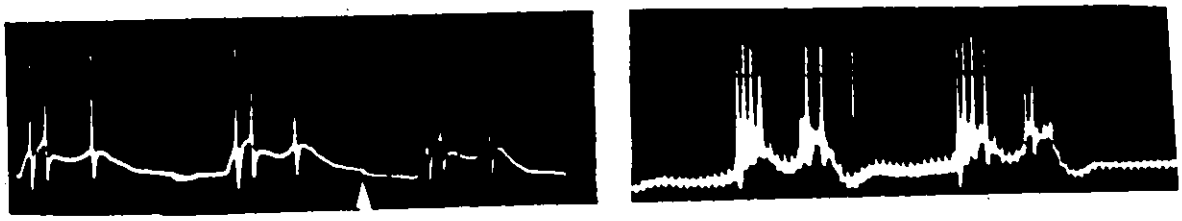
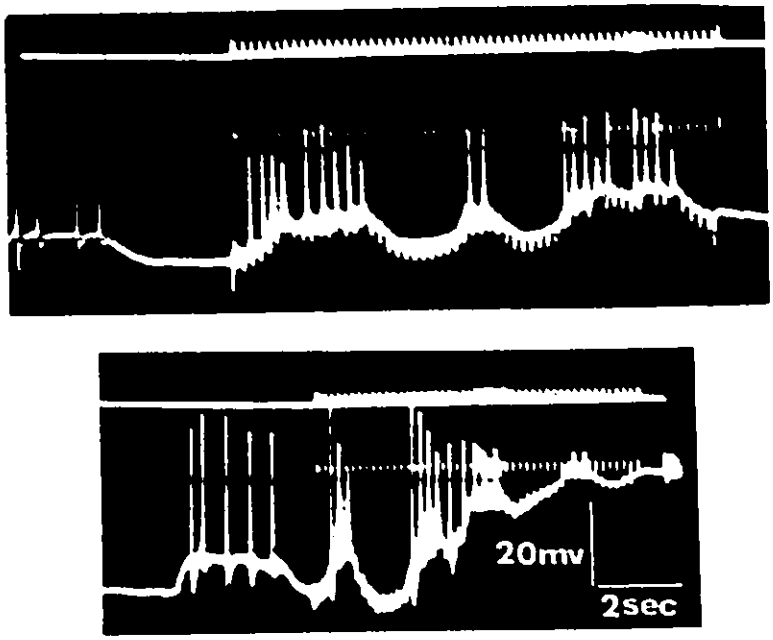


Fig. 33a. A cell not responsive to single pulse stimulation showed increased spiking activities with repetitive stimulation. Temp. 30°C. Calib: vert. 20mv; horiz. 2sec.



b. Repetitive stimulation did not produce an overall depolarization. In cells where the EJPs were largest, excitatory complexes appeared in between CPs, in addition to the increase in the number of spikes on CPs. Temp. 30°C.

change on the phase of ECA. Conversely, the stimulation parameters required to produce phase changes were also large enough to elicit neural responses. Although the cellular response to a constant stimulus was variable, even within the same cell, EJP was most visible between CPs. Fig. 35 shows the effect of stimulation at various phases of the ECA in one cell. As the stimulus was applied closer and closer to the CP, the EJP complex eventually merged with the succeeding CP. In such cases, the total depolarized assemblage made up of the EJPs and the CP was much longer in duration than a single CP. The total number of spikes in this instance was eight as compared to only four on a normal CP. If stimulation was applied late near the end of the CP, EJP became much less conspicous (Fig. 35d).

4. Mechanical Responses of LS

Mechanical responses from LS to field stimulation under similar experimental conditions were compared to the observations from electrophysiological studies. Single pulses of 0.5msec rarely produced noticeable responses. Repetitive stimulation at 5 to 10 HZ produced an increase in tension (Fig. 35). The frequency of rhythmic activities was not significantly changed. Carbachol or ACH also produced contractions in these strips (Fig. 37). The excitatory responses to field stimulation and cholinergic agents were abolished by atropine (3×10^{-7} g/ml). In the presence of atropine, relaxation in response to field stimulation was observed (Fig. 36). However, since no inhibitory response was detected electrophysiologically in LM, even in the presence of atropine (Fig. 38), the relaxation in these LSs can not be fully accounted for.

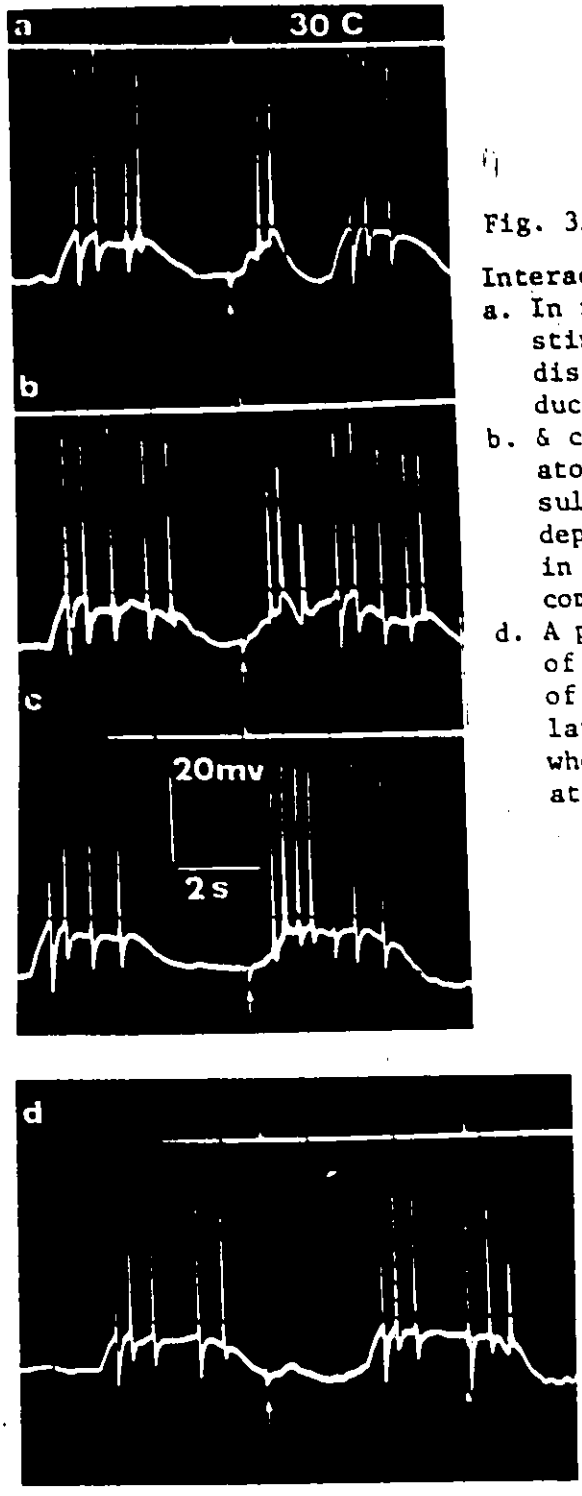
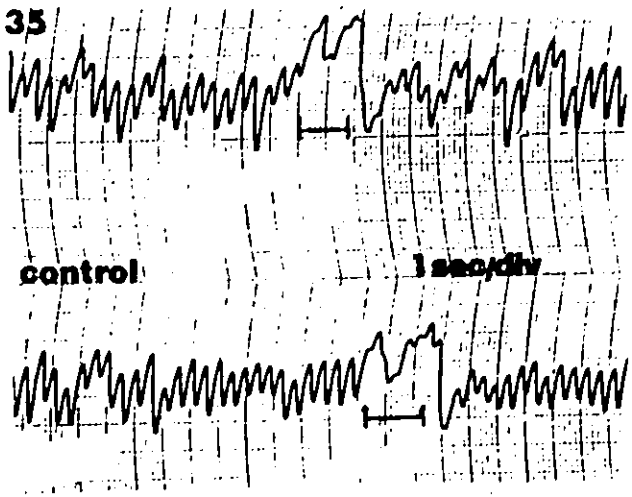


Fig. 35.

Interaction of EJPs with the ECA:
a. In response to a single pulse stimulus, an excitatory complex distinct from the CPs was produced.
b. & c. The merging of the excitatory complex with the CPs resulted in a longer period of depolarization and an increase in the number of spikes when compared to a normal CP.
d. A pulse applied in the middle of the CP and right at the end of the CP did not produce the large EJPs seen in a. to c. when the stimuli were applied at the inter-CP period.



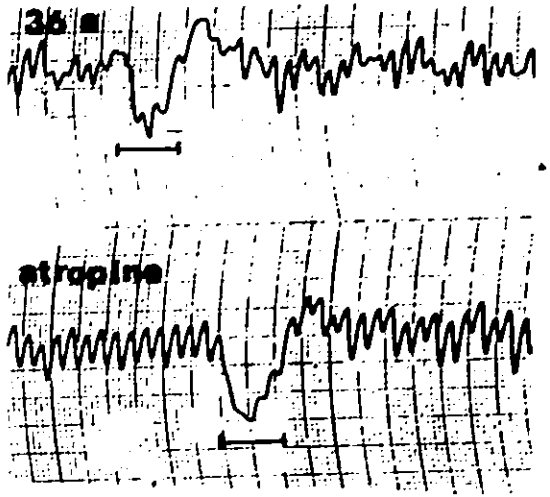
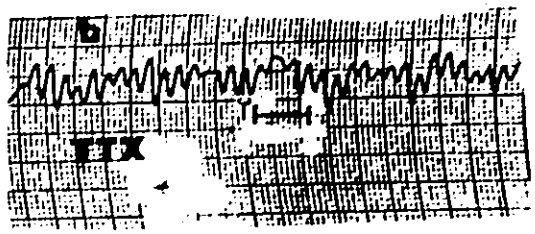


Fig. 35. Increase of tension of LSs with repetitive stimulation at 2HZ/0.5msec.

Fig. 36. Atropine (3 x 10^{-7}g/ml) abolished the contraction and unmasked relaxation (a). Both contraction and relaxation were abolished by TTX at 3 x 10^{-7}g/ml (b).



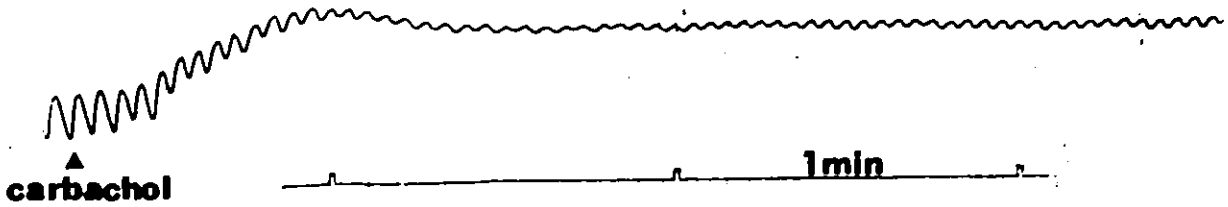


Fig. 36. Carbachol (1 x 10^{-6}g/ml) produced gradual and sustained contraction in longitudianl strips.

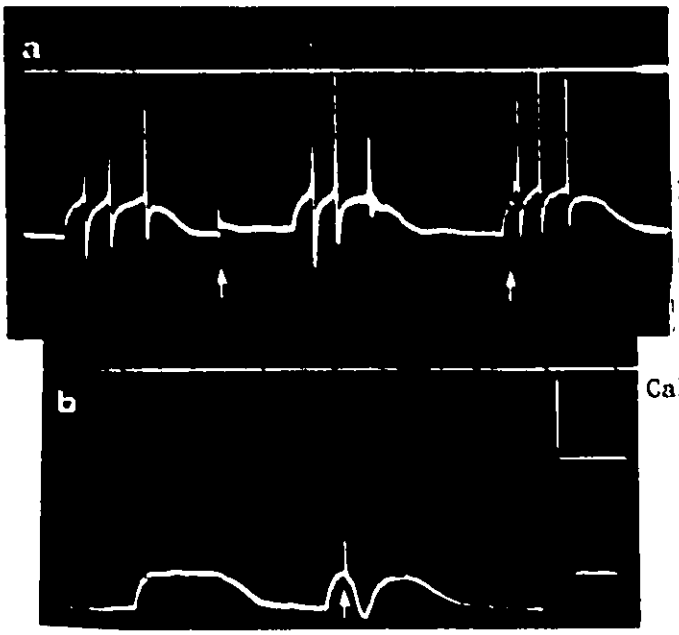


Fig. 38.
In the presence of atropine (3×10^{-7}g/ml), IJP of big amplitude was observed in CM (b) but not in LM (a). (a) was directly above (b) in the same preparation.
Calib. vert: 20mv; horiz: 2sec.

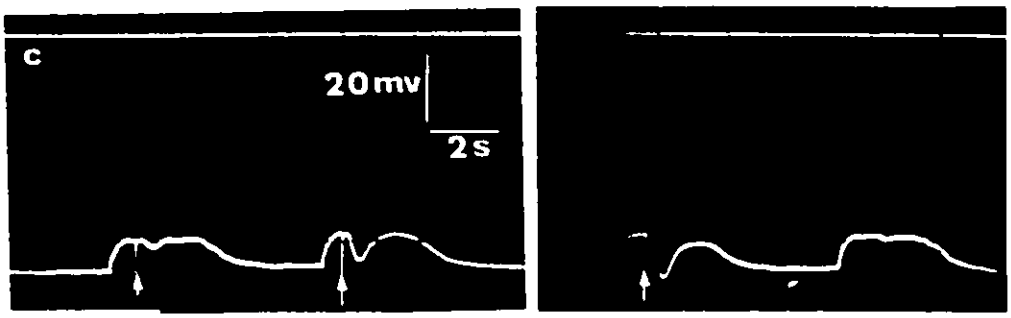


Fig. 39.
The size of IJP was dependent upon the strength of stimulation.

The possible mechanisms were described in detail in the Discussion.

B. The Circular Muscle Layer

1. Inhibitory Innervation

The predominant response to intramural nerve stimulation in CM was the inhibitory junction potential (IJP) similar to that in the guinea-pig intestine (Bennett, Burnstock & Holman, 1966; Hirst & McKirdy, 1974; Hidaka & Kuriyama, 1969). IJP could be detected in 74 of 100 cells. The latency of the IJP was 120 $\pm$ 6.24msec (range 84-142, n=42) and the duration 786 $\pm$ 10.4msec. The size of IJP varied with the strength of stimulation (Fig. 39) and could reach a maximum of 15-18mv. However, much variation in size occurred between cells even within the same preparation. Repetitive stimulation at 5 to 10 HZ led to a sustained hyperpolarization larger than that of an individual IJP (Fig. 40). Atropine increased the size of IJPs, suggesting that a cholinergic component was also present. In five preparations, the size of IJPs before and after atropine (3-6 X 10^{-7}g/ml) were compared. Before atropine treatment, the mean value of the amplitude of IJPs from 15 cells at the plateau phase of the CPs was 3.85 $\pm$ 2.1mv. The corresponding value after atropine treatment was 12.14 $\pm$ 3.28 (n=14).

2. Interaction of IJP with ECA

IJPs could be elicited at any point of the ECA cycle (Fig. 41). The size of IJPs may or may not vary with the phase of the ECA. The IJP often appeared most prominent at the plateau of CPs, and appeared to be diminished just before and during the rising of the CPs. In most instances, these apparent discrepancies could be fully accounted for by the algebraic differences of the potential change of CP and the IJP. If

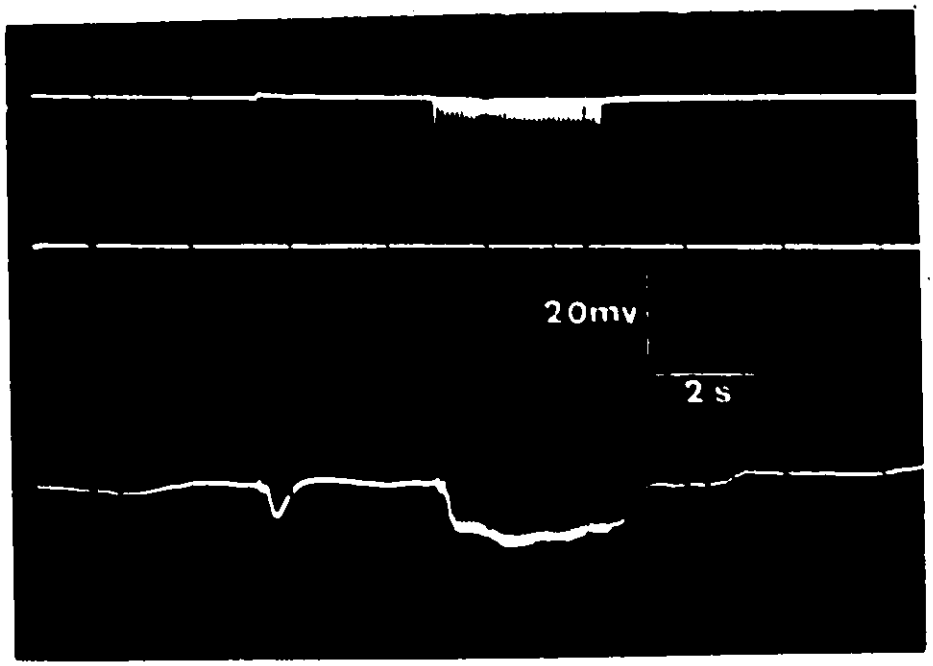


Fig. 40. Repetitive stimulation leads to summation of IJPs and the resulting hyperpolarization is much bigger than that of an individual IJP.

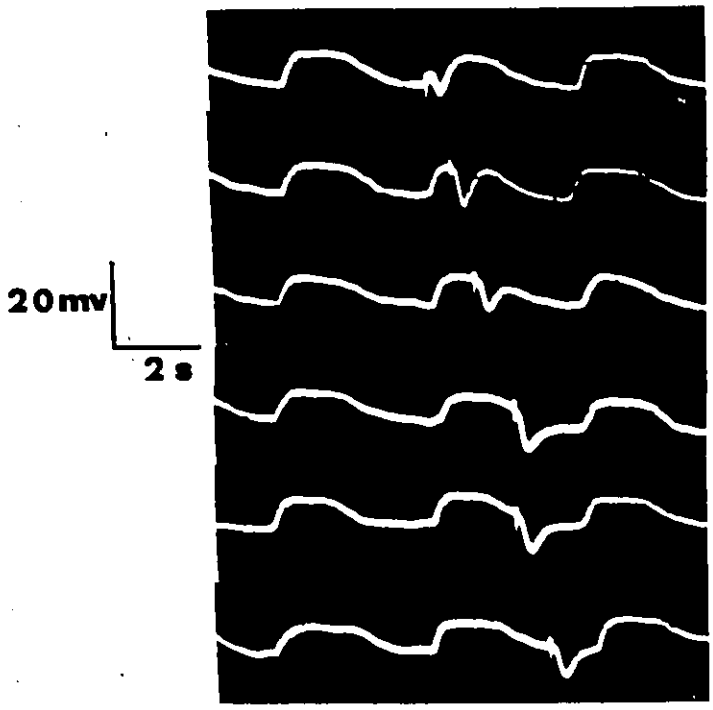
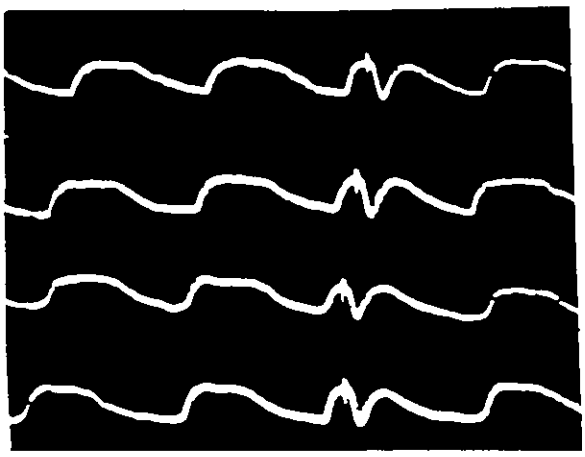


Fig. 41. a. IJP can be elicited at any phase of the ECA.



b. The size of IJP does not alter appreciably given the same stimulation parameters within the same cell.

IJPs resulted from a specific conductance change, then the size of IJPs should vary with the difference between the membrane potential and the equilibrium potential of the IJP, all else being constant. Thus IJPs occurring at the top of CPs should be bigger than those at the higher membrane potential level at the inter-CP periods. In Fig. 42 the IJP at the top of the CP was 12mv. If MP was the only determining factor of the size of IJP, then the theoretical value for IJP at the inter-CP period should be 8.01mv (see Appendix for calculation). However, there were IJPs of different sizes occurring at this MP. In Fig. 42a, the IJP was 8.5mv, in good agreement with the predicted value of 8.2mv. In Fig. 42, the amplitude of the hyperpolarization was at least 13mv (because of the rising phase of the CP), which was even bigger than the control value. This variation of the IJPs at the inter-CP period was constantly observed. The relationship of the size of IJP with the ECA is then difficult to predict, especially at the inter-CP period.

3. Excitatory Responses of CM to Field Stimulation

Excitatory responses to field stimulation with single pulses at 0.5msec duration could be detected from CM cells. These responses were difficult to study electrophysiologically with microelectrodes because:

1) Cells that showed excitatory responses were rarely found (9 out of a sampling from 103 cells). The low incidence of the excitatory responses may not reflect the true density of excitatory innervation. In the rabbit and guinea-pig colons where there is dual innervation by both excitatory and inhibitory nerves, the inhibitory effect always completely overcomes the excitatory response when both nerves are activated (Furness,

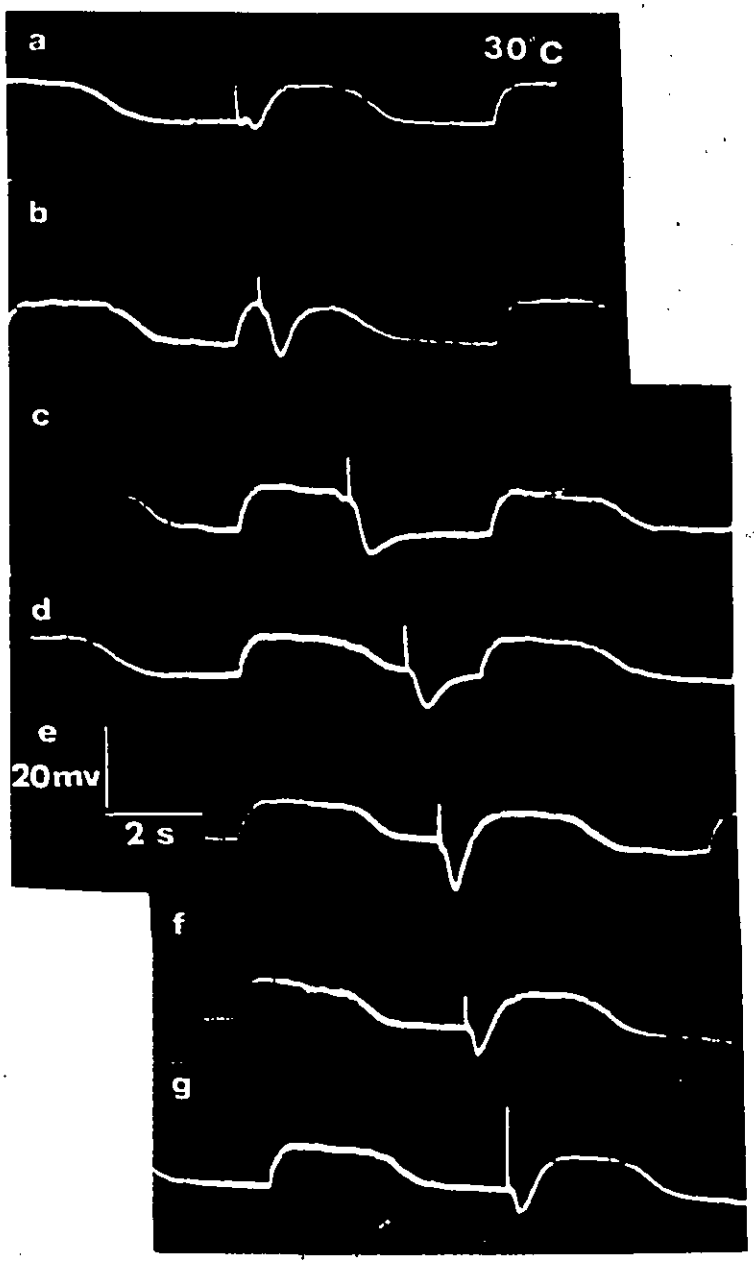


Fig. 42. IJP at various phases of ECA. The apparent small size in (a) could be accounted for by the algebraic sum of the rise of CP and the IJP. In (d), the small size of IJP was in accordance with the change of MP as expected because it was closest to the equilibrium potential of the IJP. However in (e), while still at that MP, the IJP was comparable to that at (b), in contradiction to theoretical considerations if the size of IJP was solely dependent upon the MP. The size of IJPs in f, g, and probably e did not fully develop because of the rising phase of the CPs. In e, f, and g, there was also phase advance due to the stimulation.

1969b). With field stimulation, all nerves are stimulated indiscriminately. If the inhibitory effects were much more powerful than the excitatory effect, the majority of cells would respond only with an IJP. That atropine increased the size of IJPs gives strong support to this interpretation.

2) The elicited excitatory responses often leads to firing of action potentials and contractions (Fig. 43). The action potentials produced by field stimulation differ from those produced by long depolarizing pulses (Fig. 7) in that a conspicous hump is observed at the end of the spike. No afterpotential as seen with depolarization induced spikes is apparent in these cases. The contractile action resulting from the action potential was very sharp and strong so that the electrode was invariably dislodged. Because of the paucity of these cells, and the difficulty in keeping the electrode in the cells, more detailed electrophysiological study was not possible. Therefore, mechanical studies of circular strips (CS) were also undertaken.

4. Mechanical Studies of CS

CSs were often not spontaneously active. In 8 out of 20 strips, there were periodic bursts of contractile activity mostly occurring in the first two hours of the experiments (Fig. 44). The origin and mechanism of this activity is not known. It was not blocked by TTX (10^{-6}g/ml), atropine (10^{-6}g/ml) or hexamethonium (10^{-5}g/ml). In all the strips studied, carbachol initiated similar bursting activity (Fig. 45). Carbachol did not produce any change of tone. The carbachol induced contractile activity was readily abolished by atropine.

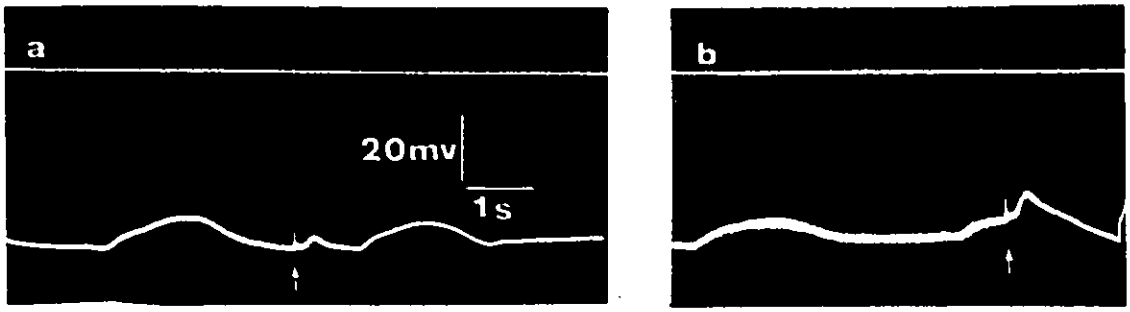
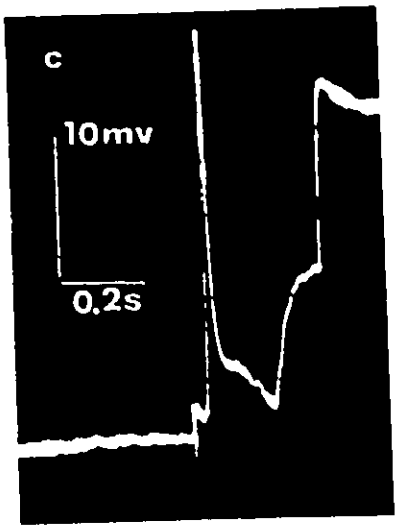
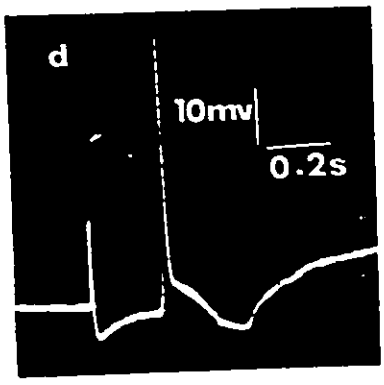


Fig. 43.

EJPs recorded from CM cells; they may be large enough to initiate action potentials (c, d.)





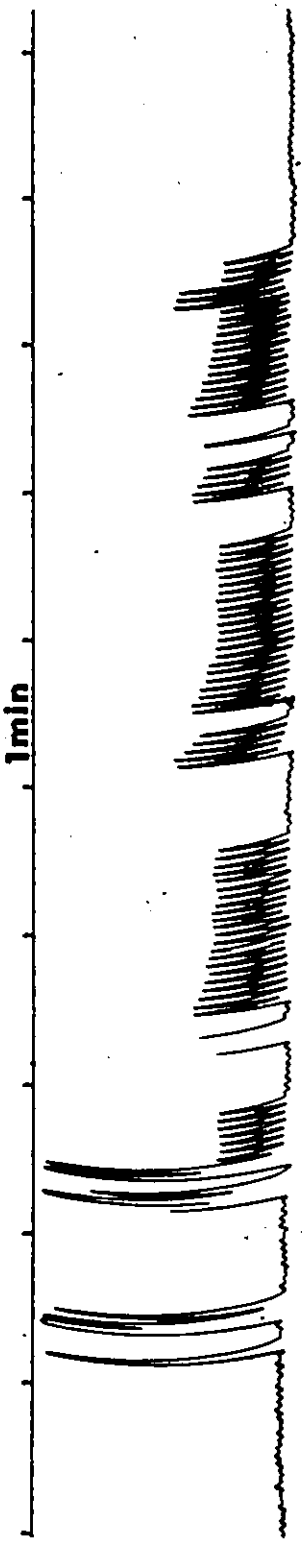


Fig. 44. Spontaneous contractile activity of a circular strip.

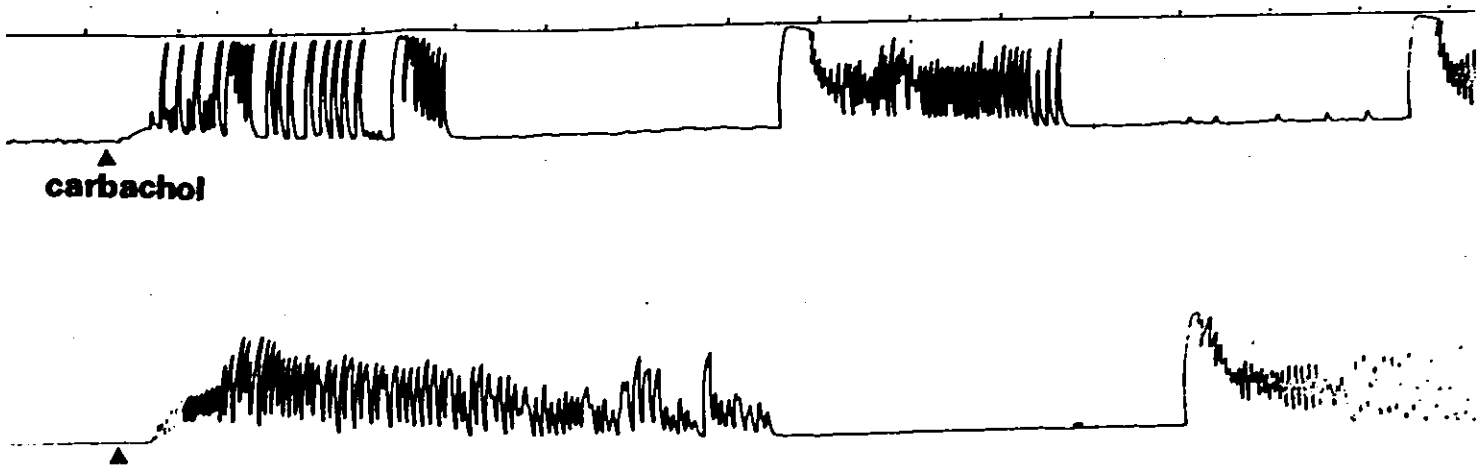


Fig. 45. Carbachol (1×10^{-6} g/ml) induced periodic contraction in CSs similar to those observed spontaneously.

The response of CSs to field stimulation is extremely complex. At least three different types of neural excitatory responses could be distinguished. The final electrophysiological and mechanical manifestations therefore reflect the interaction of all these different excitatory systems with that of the inhibitory system, making the responses difficult to analyze.

I have classified the neural excitatory responses into three categories (Table 4):

1) Cholinergic "On" Excitation - i.e. contraction in response to field stimulation that were sensitive to atropine (3-6 X 10^{-7}g/ml). This type of contraction could be seen in 75% of the CSs with a single 0.5msec pulse (Fig. 46). Atropine completely abolished this response if the contraction was not mixed with the second type of excitatory response that was not sensitive to atropine (Fig. 47). The other 25% did not respond to single stimulation of 0.5msec duration. With repetitive stimulation, 80% of CSs showed sustained contraction during the stimulation period. Fig. 48 shows an example of a CS that did not respond to single pulse stimulation but exhibited a marked "on" contraction with repetitive stimulation (5-10 HZ), followed by an "off" contraction after the stimulation period. Atropine reduced the "on" contraction to various degrees, depending upon the strips (e.g. in Fig. 46, the reduction was 50% and in 48, 70%). The residual "on" contraction was probably due to direct stimulation of the muscle cells (see below).

2) TTX-tolerant Excitation - In response to single stimulation of 0.5msec duration, not all the contractions were completely abolished by atropine. In 65% of the CSs, there was a component that could be

Table 4

Stimulus	Excitatory Response %	Effect of Atropine	TTX
Single (0.5msec)	75	10% abolished 65% decreased	abolished
Repetitive "on" contraction	80	all reduced with residual contraction	
"off" excitation	100	little or no effect	abolished
Repetitive stimulation with 5msec pulses	100	all reduced with prominent residual contraction	

100% represents 20CSs tested.

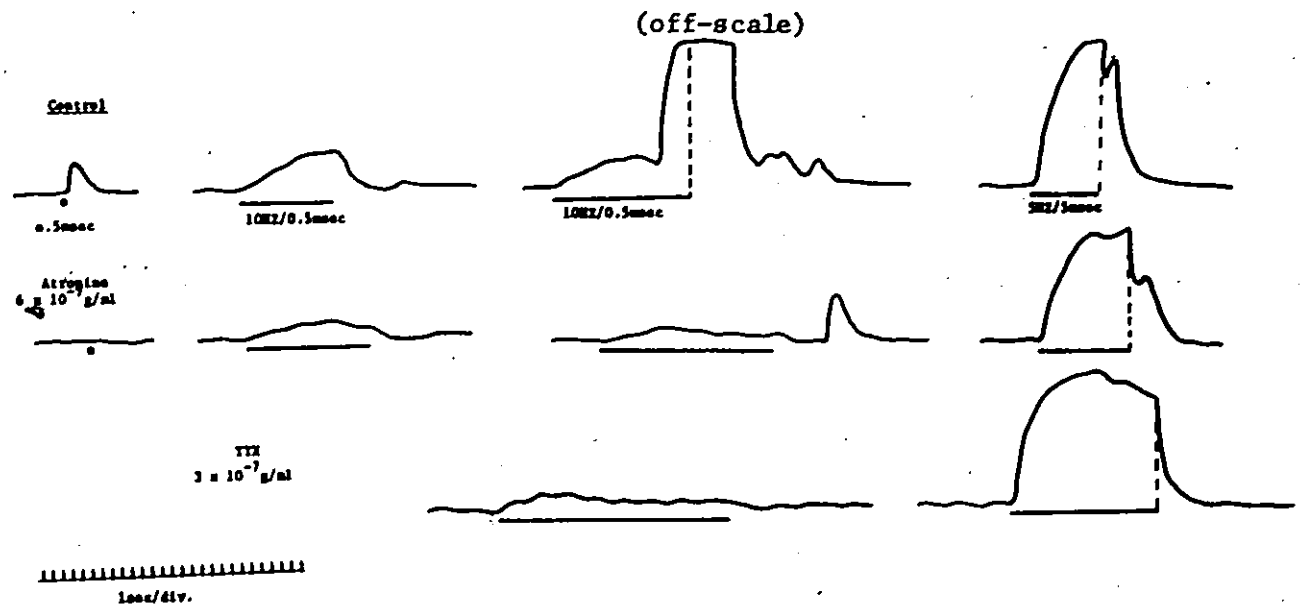


Fig. 46. The response of a CS to single and repetitive stimulation. Atropine abolished the contraction elicited by single pulse stimulation and reduced the "on" contraction due to repetitive stimulation. With longer stimulation periods, an "off" contraction was also produced which was not abolished by atropine.

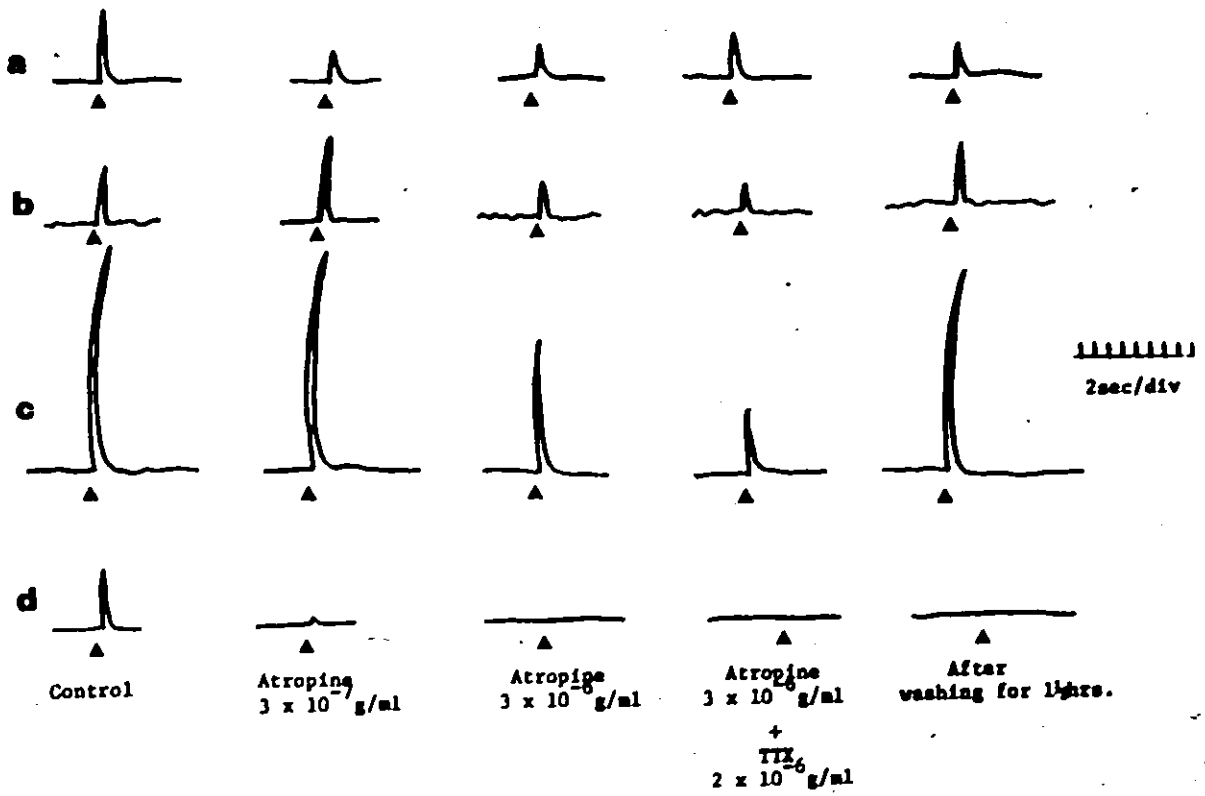


Fig. 47. The effect of atropine and TTX on the response of 4 CSs taken from the same intestinal segment to single pulse stimulation. Atropine abolished the contraction in d. but was not as effective in a, b, and c. The atropine-resistant contraction also was not abolished by TTX at 2 x 10^{-6}g/ml. Higher concentration of TTX was required to block this component (see Fig. 49 and text).

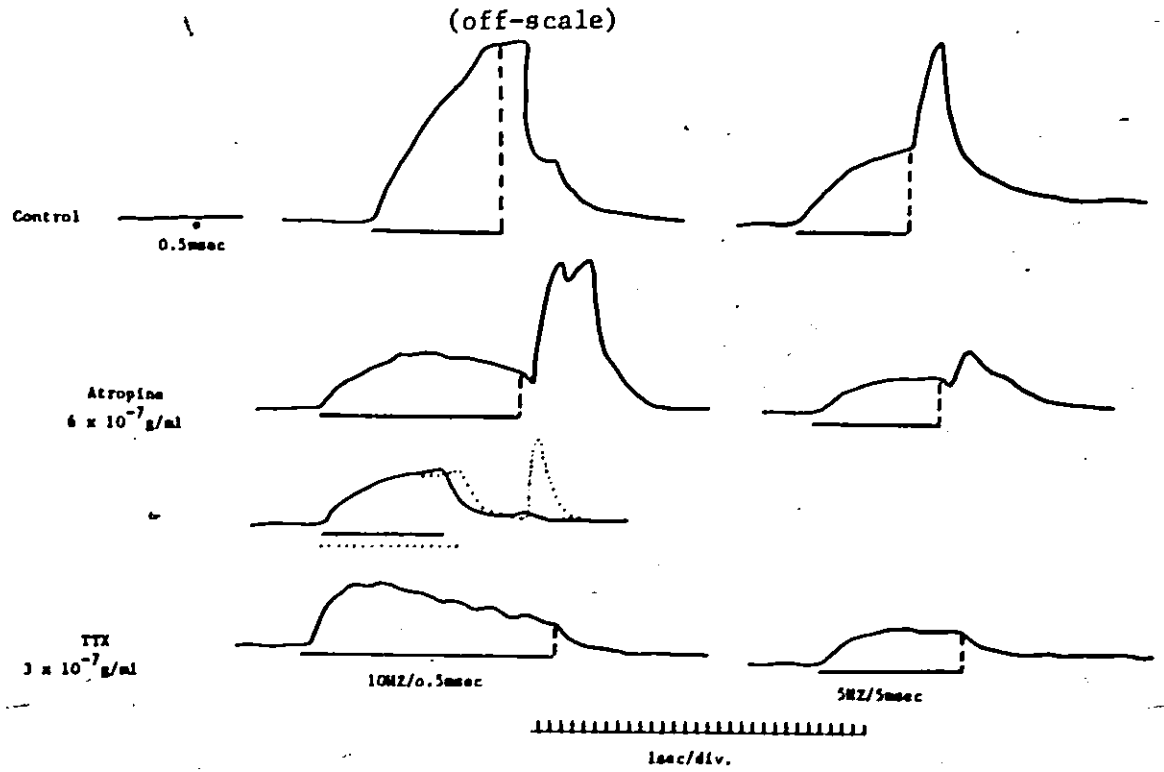


Fig. 48. 25% of the CSs did not respond to single pulse stimulation. Upon repetitive stimulation, a marked "on" contraction followed by an "off" contraction was observed in most of these preparations.

abolished only by high concentration of TTX (8×10^{-6}g/ml). Fig. 47 shows examples of CSs taken from areas adjacent to each other. In d, the contraction was readily abolished by atropine, but in a, b and c, there was only a reduction in size. Addition of TTX (2×10^{-6}g/ml) further reduced the contraction. In another series of experiments, higher concentrations of TTX were tried. Fig. 49 shows that the atropine resistant component could be completely eliminated by TTX at 8×10^{-6}g/ml . This tolerance to high TTX was in contrast to the excitatory response in LM and the inhibitory nerves in CM which were sensitive to TTX at 10^{-7}g/ml. In the CSs that exhibited this TTX component with single pulses, atropine readily abolished the "on" contractions upon repetitive stimulation leaving this component intact (Fig. 50).

3) "Off" Excitation - Repetitive stimulation at 5 to 10 HZ (0.5 to 5msec pulse durations) led to an "off" contraction after termination of the stimulation (Fig. 46, 48, 51, 54). This response was present in all the strips tested, but its amplitude was very variable as was the length of the stimulation period required to produce this response. Fig. 51 demonstrates the effect of stimulation period on the "off" responses. In a, no "off" response was observed. With prolongation of the stimulation period (b,c,d), the "off" response became progressively larger. The "off" contraction did not appear to be related to the "on" contraction. In preparation that did not exhibit "on" contractions, "off" responses were still present (Fig. 52). Furthermore, the "off" excitation was also resistant to atropine, unlike the "on" contraction (Fig. 46,48). The "off" response was very

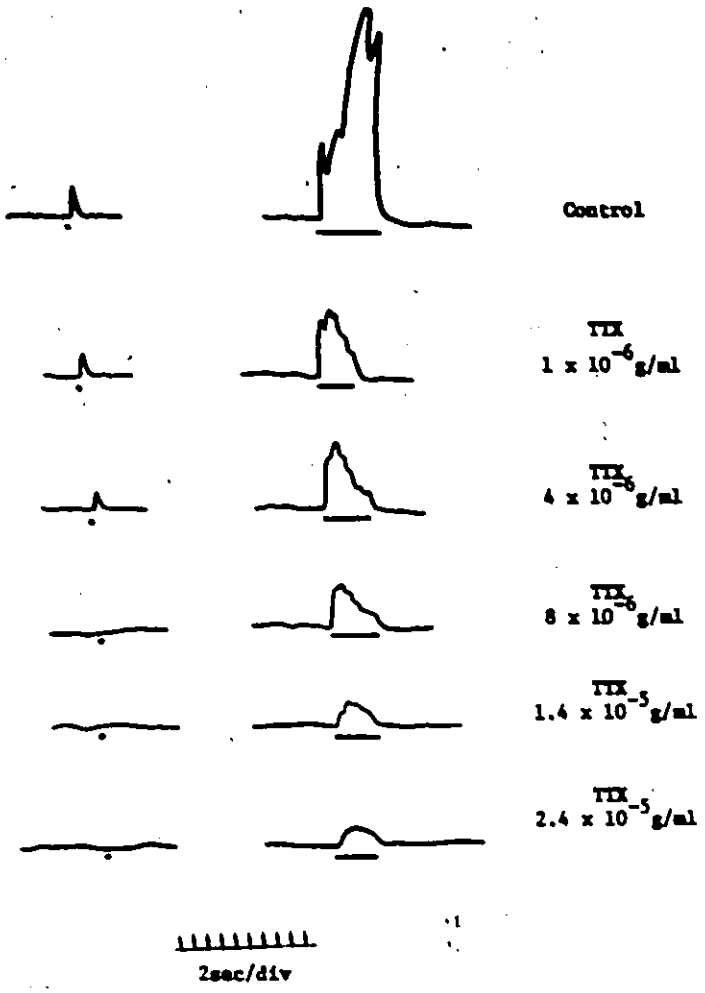


Fig. 49. The effect of TTX on the contractile responses to single and repetitive stimulation. High concentration of TTX abolished the response to single stimulation. With repetitive stimulation, there was a residual contractile component. This component was probably due to direct muscle stimulation (see text).

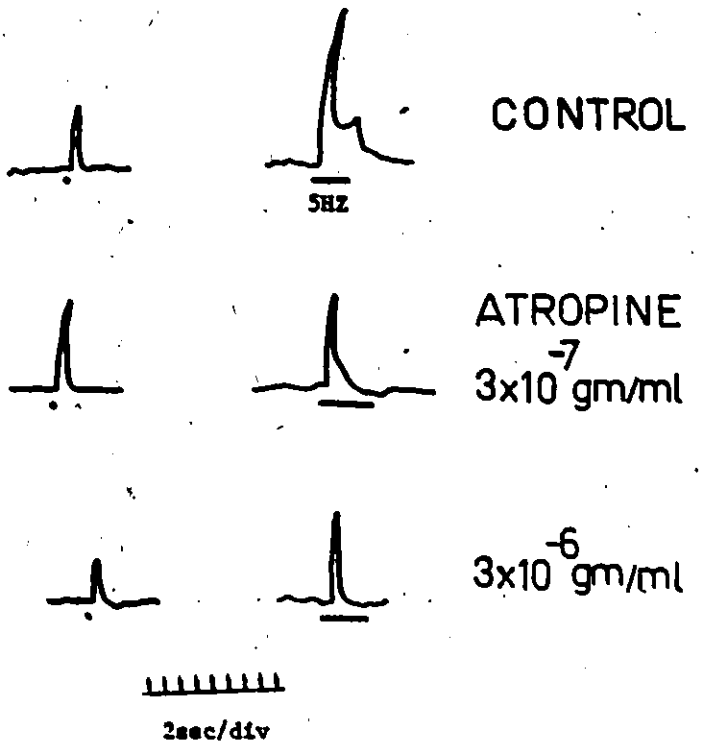


Fig. 50. The cholinergic "on" contraction can be dissociated from the TTX-tolerant contraction with atropine.

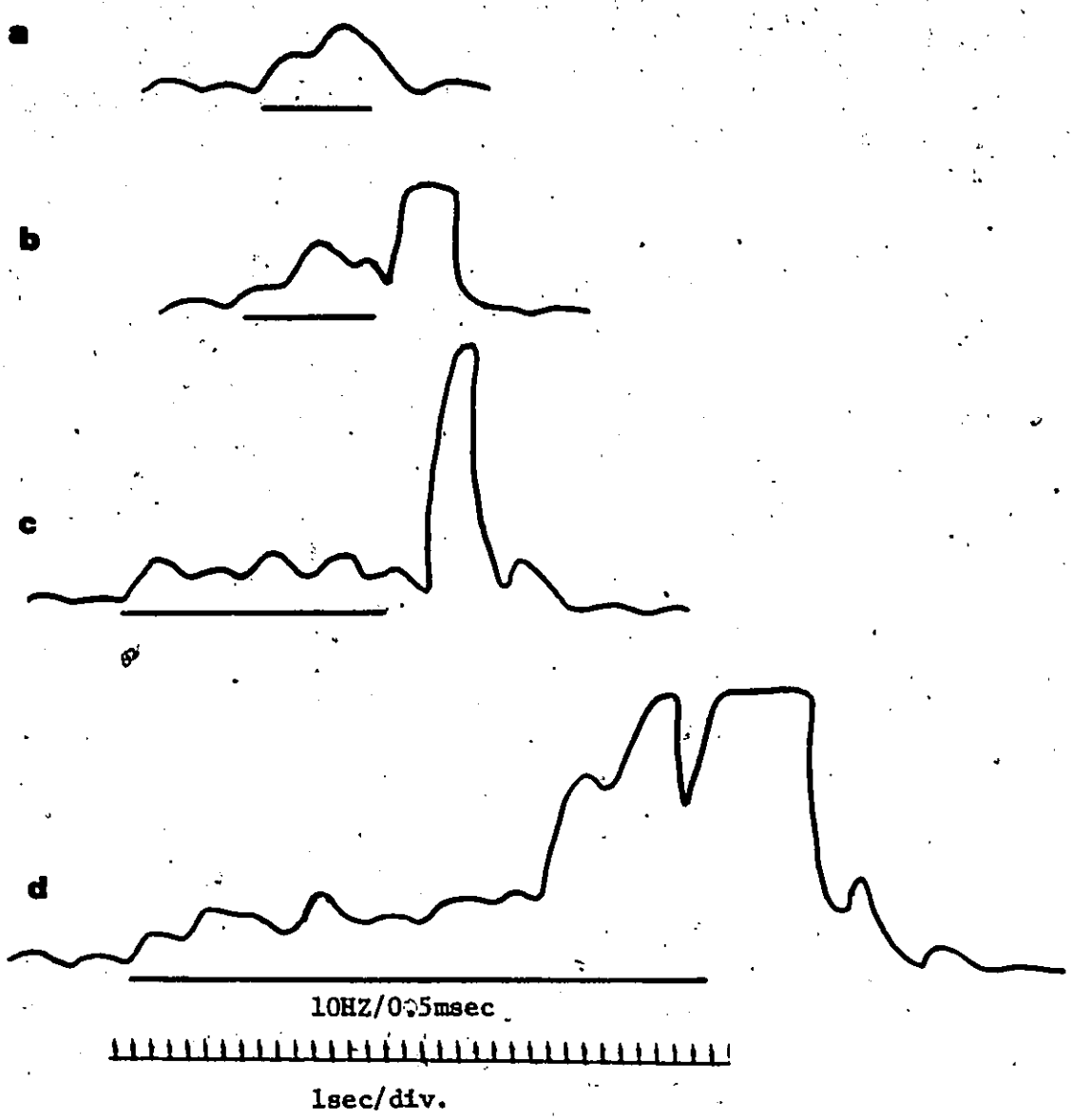


Fig. 51. The emergence and the intensity of the "off" contraction was dependent upon the duration of the stimulation period. The "off" contraction in b. and d. went off scale.

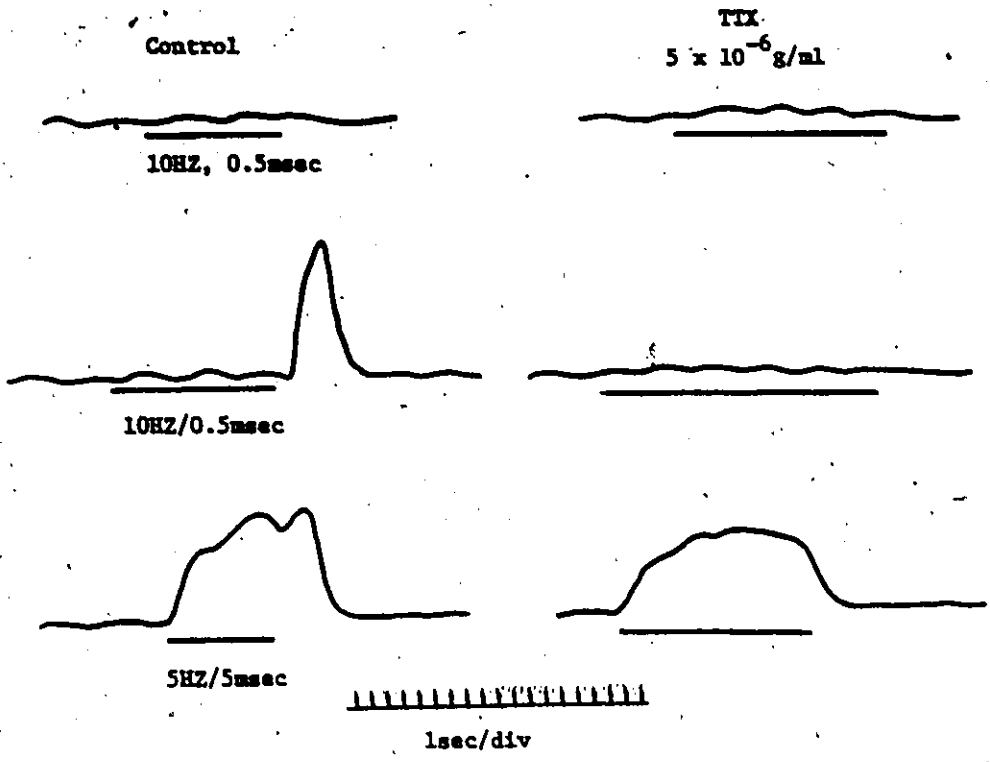


Fig. 52. "Off" contraction is not associated with the "on" contraction because it can be elicited in the absence of the "on" contraction.

sensitive to TTX and could be abolished at concentrations as low as 10^{-7}g/ml (Fig. 46, 48, 52). In electrical recordings off depolarization and firing of action potentials were usually associated with IJPs during the stimulation period (Fig. 53).

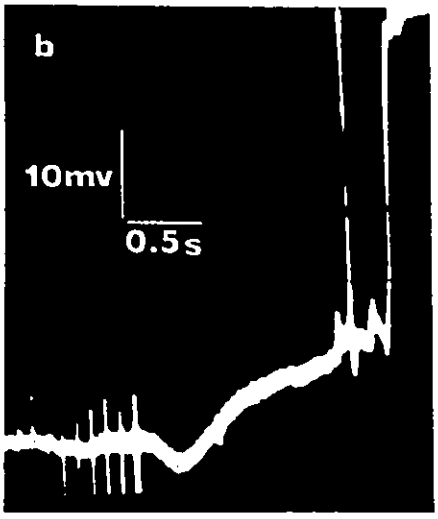
With repetitive stimulation, there remained a residual contraction component not sensitive to TTX and atropine (Fig. 46, 48, 52). With stimulation pulses of 5msec duration, this component became more prominent (Fig. 48, 52). TTX and atropine had little effect on the size of this type of contraction. Therefore, the residual component was probably due to direct stimulation of the muscle cells and not related to any neural mechanisms.

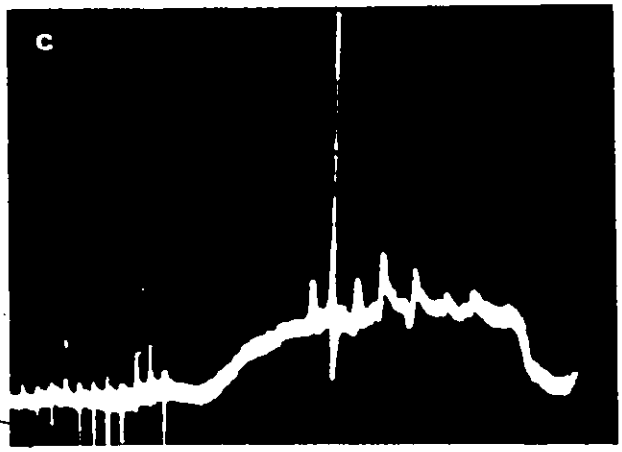
Since CM of rabbit duodenum did not maintain tone, the mechanical response to the stimulation of the inhibitory nerves could not be readily demonstrated. However, if the intramural nerves were stimulated during a contraction period of the CS, inhibition of the mechanical contraction could be observed (Fig. 54).

Fig. 53.

"Off" responses from CM cells after long periods of repetitive stimulation.

a. a single pulse produced a single IJP. Repetitive stimulation led to summation of the IJPs. Action potentials were observed after the stimulation period and the resulting contraction dislodged the electrode. The picture represents two sweeps of the oscilloscope. vert: 20mv; horiz: 2sec.
b. This is an enlargement of the excitatory response of a.
c. Another example of "off" excitation from another cell.





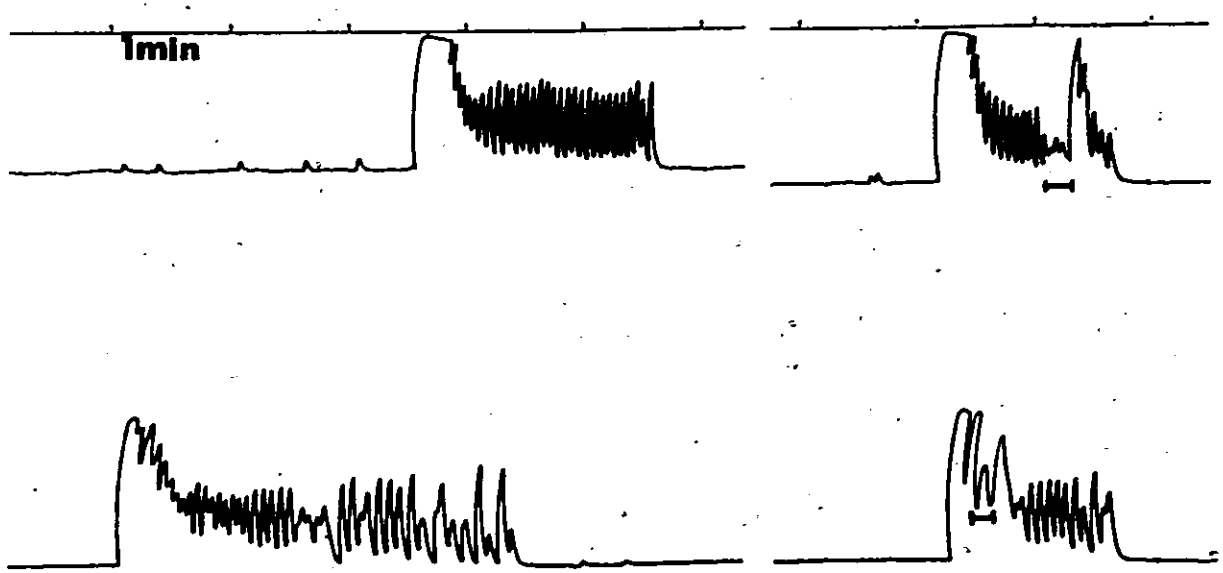


Fig. 54. Circular strips did not maintaine tone, therefore relaxation due to the stimulation of the inhibitory nerves (10HZ, 0.5msec) could only be demonstrated during a contraction period. In the examples shown here, the contractions were induced by carbachol (10^{-6}g/ml).

Discussion

The only reported electrophysiological investigation of the innervation of the muscle layers of the small intestine was carried out on the guinea-pig, a species that does not show ECA (Hirst et al., 1975). In this study the innervation of the muscle layers of the small intestine of the rabbit, a species that possess ECA, has been examined. By characterizing the electrical activities of LM and CM first, it was possible to define unequivocally the response of each muscle layer to field stimulation of its intramural nerves. A cell can be classified as either LM or CM according to the following criteria: 1) depth of penetration, 2) membrane potential, 3) spontaneous spiking activities, 4) electrotonic coupling in relation to the alignment of the muscle strip. The MP of LM was also more sensitive to temperature change than CM. The results obtained in this study showed that the innervation of the two muscle layers were different.

A. Innervation of LM

The functional innervation of LM was excitatory and cholinergic. Anatomically, the innervation of the rabbit small intestine has not been studied in detail. From EM studies, Richardson (1958) found that nerve fibres in small bundles did not usually come in close contact with the muscle cells. Only in two instances did he find the bundles close to muscle fibres. In such cases, the vesiculated nerve fibres were found "lying within an indentation on the surface of a muscle fibre". There is apparently no Schwann membrane interposed

between nerve and muscle, the interval between their surface membranes being of the order of 200A". Thus it appears that, in general, LM of rabbit small intestine is not densely innervated by close neuromuscular junctions. However, on rare occasions, close neuromuscular contacts do exist.

The electrophysiological investigation of the innervation from this study is consistent with the findings of Richardson. In over 107 cells sampled randomly, only 8 cells showed prominent EJP activity i.e. a clear depolarization response to single stimulation. Presumably these cells were closest to the neuromuscular junctions. The rest of the cells did not show clear depolarization with single stimuli. However, repetitive stimulation did increase the frequency of spiking in these cells. The atropine-sensitive excitatory response in such conditions may be the result of overflow of transmitters from the close neuromuscular junctions.

Burnstock (1970) had proposed a model for the neuromuscular interaction of the intestinal LM in which the neural influence is mediated through a few "key cells" (the muscle cells to which the nerve makes close contact) distributed in limited areas (Fig. 55). Through electrotonic coupling, the cells of the immediate vicinity (the "coupled cells"), being distant from the key cells, may be activiated by propagated action potentials generated by cells close to the key cells. They may also be affected by the diffusion of transmitters released at the key cells.

The data from this study can be fitted into the above model. According to the model SEJPs would be expected to occur only at close

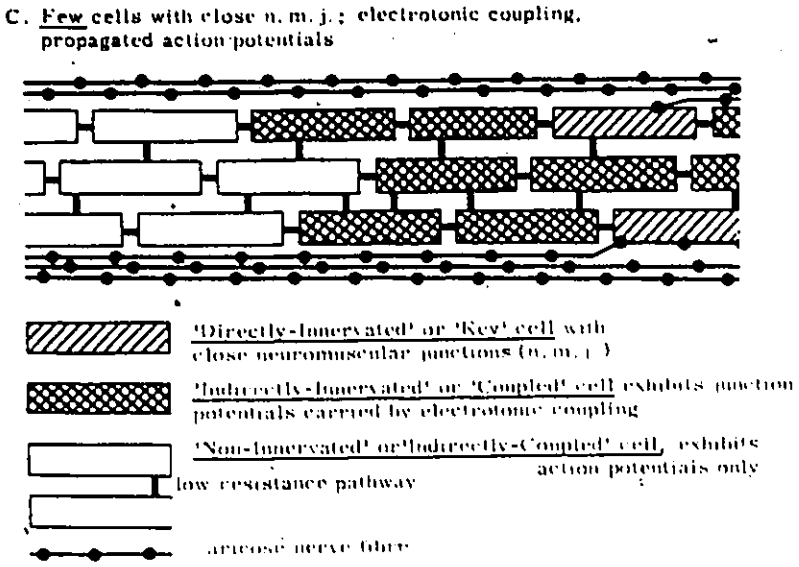


Fig. 55. Burnstock's model for the innervation of intestinal smooth muscle in which only very few cells were directly innervated. (Burnstock, 1970).

neuromuscular junctions. In only 16 out of 107 cells the SEJP was prominent. Such cells then may fit into the classification of "key cells". In response to field stimulation, the cells that exhibited good EJPs may be classified accordingly as either "key cells" or "coupled cells". Indeed, in those cells that demonstrated the largest EJP responses, SEJPs were also present (Table 5). SEJPs were also closely associated with the other evoked cholinergic excitatory responses such as crowding, change of spike amplitude and the increase of spike frequencies (Table 5). The rest of the LM cells (close to 90%) were not associated with SEJPs. They may fit into the category of "indirectly coupled cells". Repetitive stimulation produced increased activities in some of these cells, probably because of the increased availability of transmitters due to the overflow of transmitters at the "key" sites of due to the propagation of action potentials originated at the "key cells".

The SEJPs and EJPs recorded in LM were similar to those in guinea-pig vas deferens in having a fast and slow component (Bennett, M.R., 1972). The guinea-pig vas deferens is densely innervated with small axon bundles with occasional close contacts with the muscle cells. The neuromuscular junctions are less than 50nm apart in 20% of the cells (Merrilles, 1968). Based on experimental and theoretical considerations, Bennett (1972) proposed that the fast components were due to transmitter release from close contact sites as they occurred in 20% of the cells. The slow component of the EJP was suggested to be due to transmitter release from the remaining varicosities in the axon bundle and to produce the potential change by electrotonic spread.

	Responses Induced by Stimulation of the Intrinsic Nerves				
	SEJP	Depolarization	Crowding	Decrease in spike amplitude	Increase in spike frequency
SEJP	16	8	16	14	14
Depolarization	8	8	8	8	8
Crowding	16	8	16	15	14
Decrease in spike amplitude	14	8	15	14	15
Increase in spike frequency	14	8	14	15	15

Table 5. The relationship of spontaneous EJPs and the excitatory responses evoked by neural stimulation. If cells that exhibit SEJPs were the sites of close neuromuscular contacts, then these cells should respond best to electrical stimulation of the intramural nerves. This was found to be the case. There were 16 cells with SEJPs from a sampling of 107 LM cells. All of them showed conspicous excitatory responses - depolarization, crowding of spikes, increase in the number of spikes, and decrease in spike amplitude (section A2) - to stimulation. All the cells that showed the best evoked responses (those with large EJPs and therefore likely to be very close to the nerve terminals) were within this group of cells with SEJPs.

EJPs in guinea-pig vas deferens also appeared as a mixture of the two components, having a fast and slow time courses of decay (Fig. 56). In the rabbit small intestine, most of the SEJPs and some of the EJPs resembled the mixed type with an initial fast component followed by the slower component (cf. Fig. 27 and Fig. 56). Thus it is very likely that the cells with SEJPs and good EJPs are the sites with close neuromuscular contacts, similar to those of the guinea-pig vas deferens.

EJPs were most visible on the CP and at the inter-CP period. Repetitive stimulation did not produce an overall depolarization as in rabbit colon (Gillespie, 1962b). Thus in cells with the best responses, there appeared an EJP complex in between CP in addition to the increase in the number of action potentials on the CP. The EJP complex probably resulted from the activation of some neuronal excitatory circuitry similar to the ones described by Hirst, Holman & McKirdy (1975). In any case the individual CPs could still be distinguished with repetitive stimulation. However, the discrepancy between this study and that on the rabbit colon may be due to the different methods of stimulating the extrinsic pelvic nerves; a technique which is probably more effective in eliciting cholinergic responses than is field stimulation. Thus it remains to be proven whether the excitatory nerves can induce a sustained depolarization in the rabbit small intestine if the extrinsic cholinergic nerves are activated.

No IJP was observed in LM, even in the presence of atropine. This is an agreement with the findings of Hirst, Holman & McKirdy (1975) for the guinea-pig small intestine. However, mechanical studies of LSs

Fig. 56a. Examples of theoretical (fig. 74 from Bennett) and experimental findings (figs. 62 and 63) of the type of EJPs encountered with the type of innervation as in guinea-pig vas deferens.

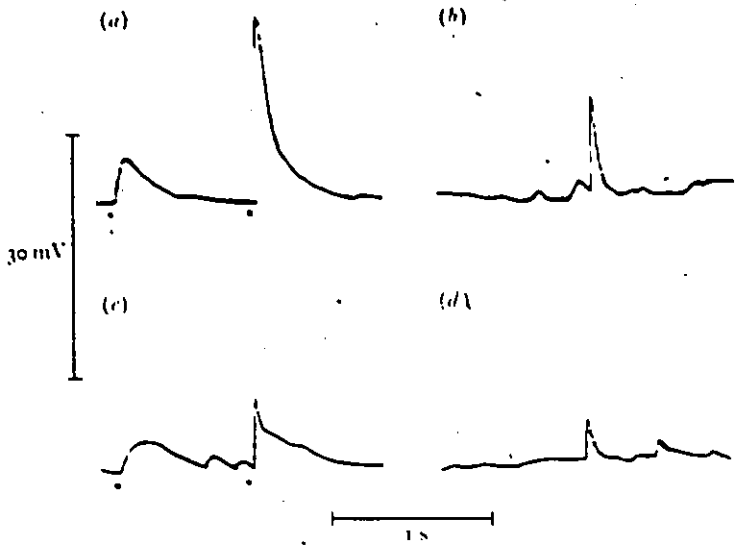


Fig. 63. Evoked junction potentials and spontaneous junction potentials recorded in a single smooth muscle cell in the guinea-pig vas deferens. (*a*) and (*c*) Junction potentials evoked by stimulation of the hypogastric nerve (indicated by the dots) at 1 Hz; the second junction potential in both (*a*) and (*c*) shows a second fast component superimposed on the normal slow junction potential. (*b*) and (*d*) Spontaneous junction potentials; the time course of these potentials is similar to that of the fast component in (*a*) and (*c*), which suggests that they have a common origin.

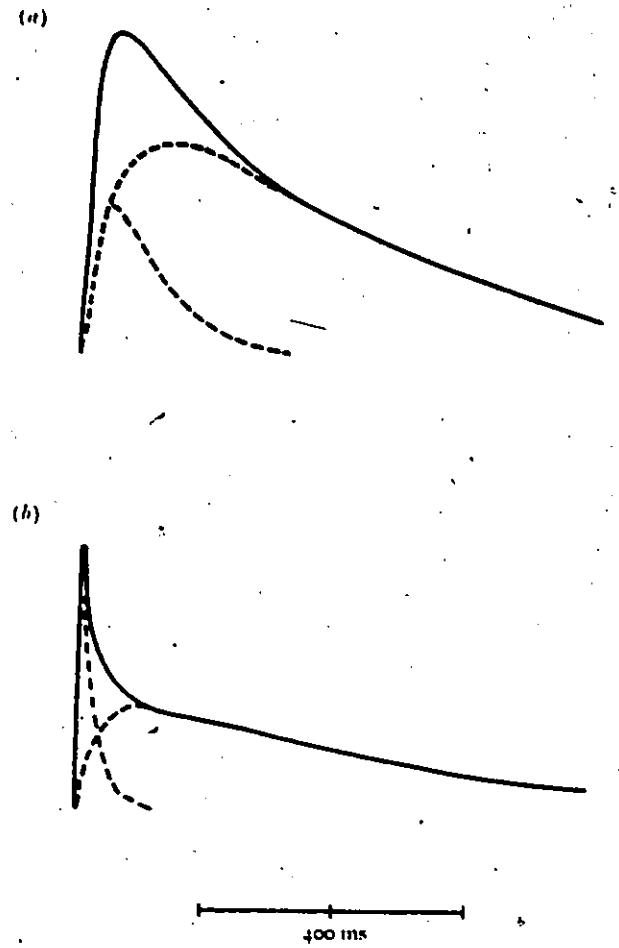


Fig. 74. Theoretical predictions of the time course of junction potentials in a smooth muscle which has neuromuscular junctions formed by both close-contact varicosities and small axon bundles. (*a*) A junction potential in a smooth muscle in which 20 % of the cells have a close-contact varicosity and small axon bundles ramify throughout the muscle; if all these nerves are excited, the junction potential consists of the addition of slow and fast components shown by the broken lines, and decays at first with a time constant of 100 ms and then with a time constant of 400 ms. (*b*) A junction potential in a smooth muscle cell in which only the close-contact varicosity on that cell is excited, together with small axon bundles within the muscle; the junction potential consists of the addition of fast and slow components shown by the broken lines, and decays at first with a time constant of 30 ms and then with a time constant of 400 ms. Junction potentials with the time course of (*b*), and intermediate between (*b*) and (*a*), are observed in the guinea-pig vas deferens.

Fig. 56b.

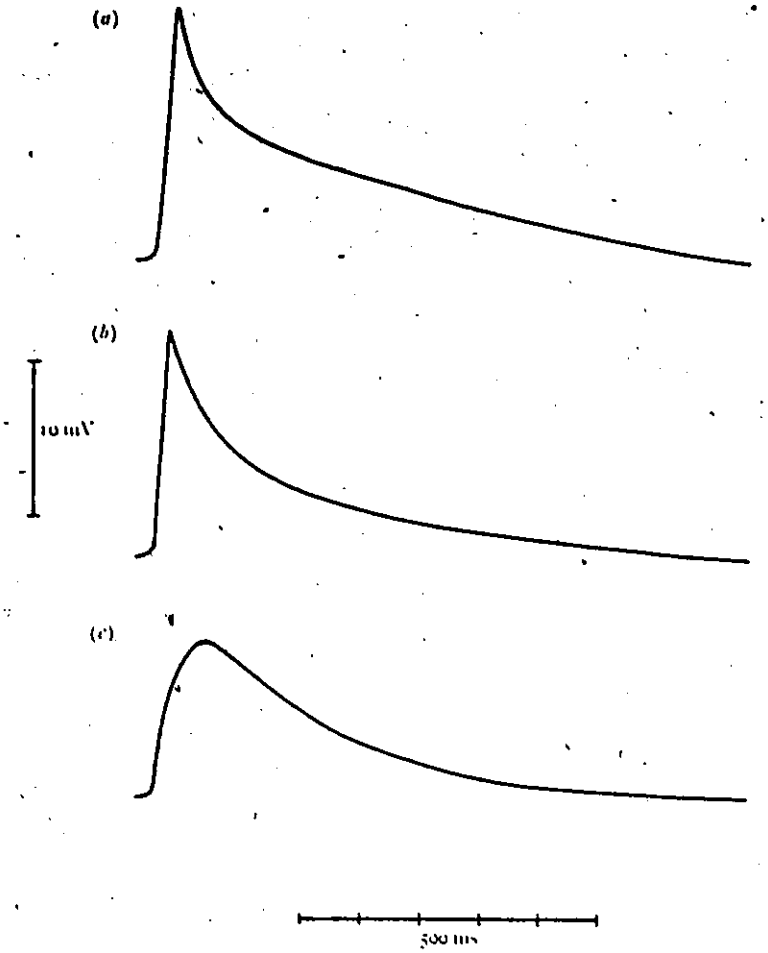


Fig. 62. Junction potentials with different time courses which can be recorded in different smooth muscle cells of the guinea-pig vas deferens. This muscle has neuromuscular junctions consisting of small axon bundles and occasional close-contact varicosities. The most commonly recorded junction potential type in the muscle cells to stimulation of the sympathetic nerve terminals with single pulses, is shown in (*c*), in which the potential decays with a time constant of 300 ms; (*a*) and (*b*) show junction potentials which can be recorded in about 20% of the cells in the vas deferens. They have initially fast components of decay (with time constants of 50 ms) followed by slow components of decay which have the same time constant as the junction potential in (*c*) (300 ms).

Fig. 56c.

showed marked relaxations with repetitive stimulation in the presence of atropine. Since neither inhibitory potentials nor any other change in electrical activity (inhibition of spiking) was observed electrophysiologically, it is difficult to reconcile this discrepancy. One possible explanation would be that the relaxation of LM is a mechanical artefact, (Wood & Perkins, 1970; Wood & Harris, 1972). As demonstrated in tubular intestinal segments (Wood & Perkins, 1970) as well as strips (Wood & Harris, 1972), there are passive mechanical interaction between the two muscle layers. A decrease in the length of one dimension should be accompanied by a corresponding increase in the second dimension, according to the constant surface area law (Wood & Perkins, 1970). For example, Wood & Perkins (1970) demonstrated with a tubular intestinal segment, that a small decrease in the radius, due to the contraction of the CM, passively elicited an increase in length of as much as 180%. This hypothesis was tested with LS preparations in which the longitudinal muscle layers were physically separated from the underlying circular muscle layers. In such preparations, the amount of circular muscle that could produce the contraction leading to the "relaxation artefact" of the LSs was reduced to a minimum. In twelve such preparations tested, all responded with excellent relaxation in the presence of atropine (Fig. 57). These results indicated that the relaxation of LS was probably not a mechanical artefact due to the contraction of CM.

An alternate explanation would be that the LM was directly innervated by inhibitory nerves. However, there are few or no descending pathways. In electrophysiological recordings made downstream

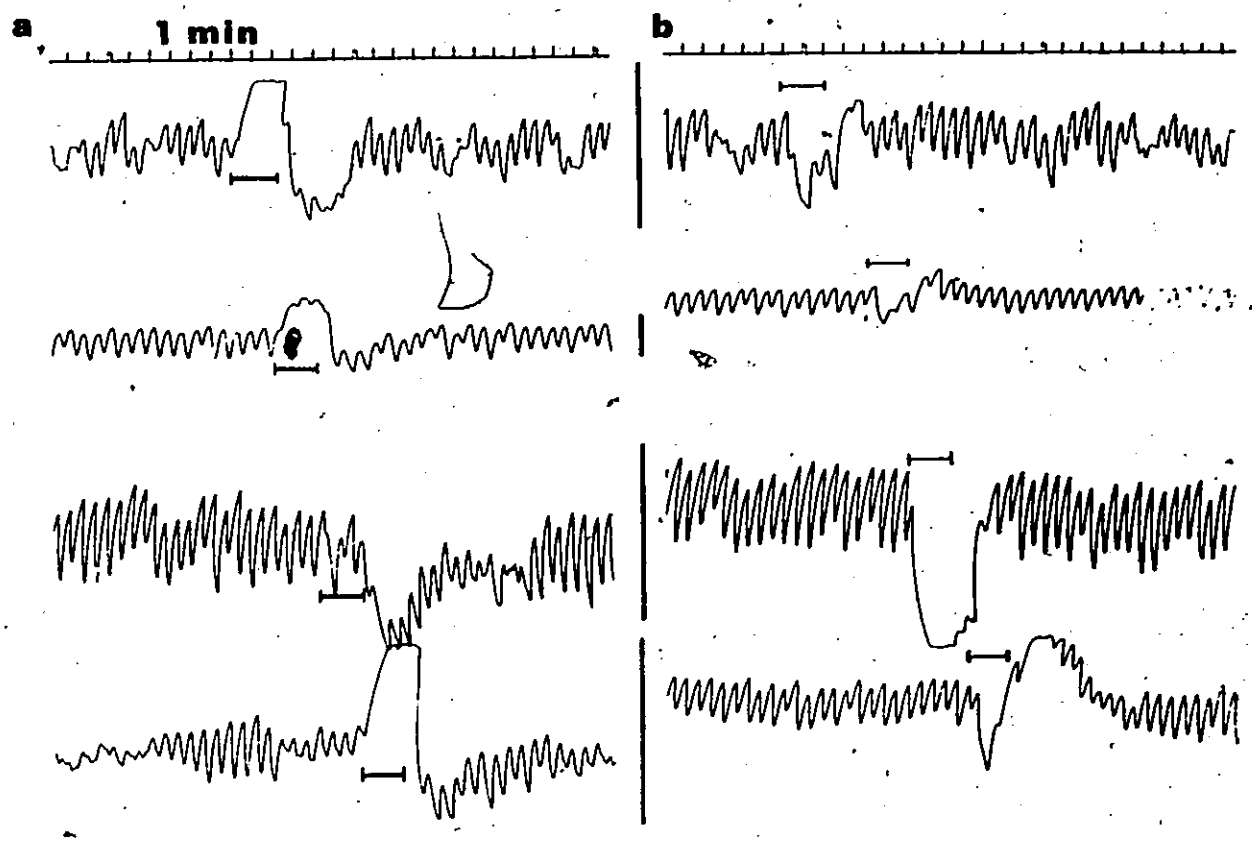


Fig. 57. a. Effect of repetitive stimulation on regular LSs (2 & 4) and LSs in thich the longitudinal layers were physically separated from CM (1 & 3). In 3, an "on" relaxation was observed. In 9 out of 12 striped LSs, relaxation was the observed response.

b. Effect of atropine (3×10^{-7} g/ml) on the same preparations as a. Relaxation was observaed in all strips.

of the stimulating electrodes, no inhibitory responses were observed. Hirst, Holman & McKirdy (1975) also did not observe IJP in the LM of guinea-pig small intestine (stimulation in this case was by inflating a balloon orad to the recording site). In another study of the innervation of the LM of guinea-pig small intestine, Kuriyama et al.,(1967) reported the presence of both EJPs and IJPs. Stimulation in this case was applied by placing one electrode directly on the muscle and the other 3-4 cm away. In this way, the recording electrode could be placed within the field of stimulation and presumably the inhibitory nerves could be directly activated without relying on any neural pathways. Similarly, the relaxation of LSs could be due to the direct activation of inhibitory nerves between the two stimulating electrodes. It should also be noted that in the presence of verapamil, small IJPs were sometimes observed in LM of rabbit jejunum using the same experimental procedure reported here (Daniel & Taylor, 1975). Hirst & McKirdy (1974) also found that with repetitive stimulation, inhibitory potentials were observed in guinea-pig small intestine in the presence of atropine although there was no response to single stimuli. They postulated that the hyperpolarization in LM with repetitive stimulation was due to the overflow of transmitters originating from the CM. Whether the LM was directly innervated by inhibitory nerves therefore required further testing.

B. Innervation of CM

The predominant response of CM to field stimulation is the IJP. The IJP in rabbit small intestine is similar to that in other intestinal preparations such as guinea-pig taeni coli (Bennett et al.,

1966a), guinea-pig small intestine (Kuriyama, Osa & Toida, 1967) and the circular muscle of guinea-pig caecum (Osa & Kuriyama, 1975). The IJP in rabbit small intestine was blocked by TTX but not by atropine or guanethidine (Daniel & Taylor, 1975). IJPs in other visceral muscles were very likely due to selective conductance change of potassium permeability and had a reversal potential of about 80-90mv (Tomita, 1972; Hidaka & Kuriyama, 1971; Creed & Gillespie, 1977). The size of IJP would then be expected to change according to the MP. During ECA, the MP oscillates in cycles. It was predicted that the IJP would be largest on top of CP and smallest during the inter-CP period when the MP was closest to the reversal potential of the IJP. However, this was not always true especially at the inter-CP period where large variations were constantly observed. One possible explanation is that the muscle membrane itself underwent permeability changes periodically during the ECA. In such a case, the size of IJP would also depend on the membrane resistance, as well as the MP. For the rabbit small intestine, Mills & Taylor (1971) reported conductance changes during the ECA cycle. Therefore, both the MP and the conductance change of the membrane at different phases of the ECA may contribute to the variation of the size of IJP.

The contractile responses of CM to field stimulation are complex. At least three types of neurally mediated contraction could be differentiated: a cholinergic response (the "on" response) readily abolished by 3 to 6 X 10^{-7}g/ml atropine; a contraction response that was resistant to atropine (6 X 10^{-6}g/ml) and abolished by high concentration of TTX (8 X 10^{-6}g/ml); and an "off" response not sensitive

to atropine but blocked by TTX (3×10^{-7}g/ml). A highly atropine-resistant excitatory component (10^{-4}M of atropine required to block the effect) was found in rabbit colon (Garry & Gillespie, 1955; Gillespie, 1968). Furness (1970) also reported spontaneous and stimulation-related excitatory activities in the rabbit colon that were not sensitive to TTX (10^{-7}g/ml) and hyoscine (2×10^{-7}g/ml).

These excitatory responses were difficult to study electrophysiologically because of the resulting powerful contraction of CM. The electrophysiologically data reported here is therefore far from complete. However, electrical events that probably correspond to the mechanical activities were also observed. In the presence of atropine, the size of IJPs increased significantly. This implies that field stimulation activated both the cholinergic and inhibitory nerves although the IJPs were dominant. Therefore, the presence of a cholinergic excitatory component is implicated. In response to a single stimulus an EJP with action potentials was sometimes observed. The action potentials induced by field stimulation of nerves had a prominent hump, which was very different from the action potentials produced by a long depolarizing pulse. Thus the action potentials in response to field stimulation were unlikely to be due to direct muscle stimulation. At the end of long periods of repetitive stimulation, excitatory responses leading to action potentials were also observed electrophysiologically. This would correspond to the "off" response in mechanical studies.

Similar to the study of the longitudinal muscle, there are discrepancies between the electrical and mechanical responses from the circular muscle. Electrically, the dominant response to field stimulation was the IJP whereas mechanically, the dominant response was contraction. However the arrangement of the set-ups for electrical and mechanical recordings were different. In electrical recording, all the measurements were made downstream from the stimulation site. The events happenning between the stimulation plates were not monitored. With ring electrodes used for mechanical recording, about 80% of the muscle strip lay between the stimulating rings. Therefore the mechanical response reflected more closely of the activity within the stimulation region rather than downstream from the stimulation region. Further experiments in which the electrical events happenning within the stimulation region is studied would be required to resolve the discrepancy.

Another possible reason for the predominance of excitatory mechanical response in the circular muscle is that the circular muscle can only exhibit excitatory contractions and not inhibitory relaxations because of the lack of tone. From electrical recordings, it was found that 70-80% of the cells were dominated by IJPS. However, in the remaining 10-20% of the cells, excitatory responses were observed. It is therefore likely that the excitatory mechanical response reflects the action of this small group of cells dominated by the excitatory input.

In the GI tract, only the cholinergic, adrenergic, and the non-adrenergic inhibitory neural influences have been studied in detail. However, many other known or putative transmitters (e.g. serotonin, substance P, VIP, etc.) are also present in the GI system and the functional roles of these agents are not known. In the CM, two other types of excitation (the TTX-tolerant and the "off" excitation) not related to the cholinergic system were observed. The "off" response had been referred to as "rebound excitation" in the guinea-pig taeni coli (Bennett, 1966a). The "rebound excitation" was ascribed to a membrane effect following the hyperpolarization due to the IJP (Bennett, 1966a). Recently this interpretation has been challenged and a separate neural origin proposed (Bywater, 1978). In the guinea-pig small intestine, Bywater reported that after hyperpolarization beyond the reversal potential of IJP, or after blocking of IJP with apamine (a drug supposedly to block IJP), the excitation process persisted. Also the excitation could not be reproduced by electrical hyperpolarization mimicking the potential change during IJPs. The neural transmitters responsible for these excitatory actions remain to be identified.

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