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# THE GENERATION AND CONDUCTION OF ACTIVITY IN SMOOTH MUSCLE

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## INTRODUCTION

Unlike normal skeletal muscle, smooth muscle systems normally generate electrical activity without neuronal inputs. Furthermore, the organization and integration of this myogenic activity can provide an effective system that controls the organization of contractile events in time and space (1, 2). This type of control is most evident in the gastrointestinal tract.

For effective analysis of such control systems it is essential to obtain information about the intrinsic electrical activity of uncoupled systems, i.e. those not driven or influenced by activity from other active components. Such information should include analysis of the tissue or cellular origins of electrical events as well as of their timing mechanisms. The effect of known electrical or chemical inputs on this intrinsic activity should also be studied and should provide information about the limitations imposed by refractoriness of active or driven electrical events.

In addition, the coupling mechanisms between intrinsically active systems should be analyzed in terms of the structures that participate in this process and their passive electrical properties. This can become a difficult undertaking in a complex organ such as the small intestine, which has three participating muscle layers (outer longitudinal, main circular, and inner dense circular). The analysis of coupling cannot overlook the occurrence of possible (transmission of strain) or necessary (physical constraints) mechanical coupling imposed by laws of conservation of volume or mass. For example, distension of an intestinal segment may compel what appears to be passive longitudinal shortening, and active contraction of circular muscle of the intestine necessarily stretches and appears to relax longitudinal muscle (3–5).



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To avoid or limit complexities of inputs and physical coupling problems, many investigators have chosen to study simplified systems, e.g. a thin, elongate muscle strip. The results of such studies provide essential information about the properties of the systems as simplified; also this information can legitimately be assumed to apply to the simple system functioning as part of a complex whole. However, new properties are bound to arise from more complex levels of organization in the intact system that cannot be predicted from those of the simple system; that is, the properties of a segment of intestine cannot be deduced in toto from the properties of isolated longitudinal or circular muscle bundles.

Ultimately, it is necessary to analyze also the properties of the intact coupled systems. Such analysis in the gastrointestinal tract has shown that many properties can be analyzed in terms of a system of coupled relaxation oscillators. In much of the gut, control of excitability can be shown to have the properties expected of such systems. Furthermore, these systems interact with neurogenic and hormonal controls to provide control systems of considerable diversity and sophistication.

This review considers critically the nature of the origin of electrical activity in gastrointestinal smooth muscle systems, its coupling between cells and tissues, and the nature and applicability of the relaxation oscillator model to them.



## THE COUPLED RELAXATION OSCILLATOR MODEL

Relaxation oscillators are a type of nonlinear oscillators that have been used to explain the nature of myogenic electrical oscillations in the smooth muscle cells of the gastrointestinal tract, and the coupling between them (1, 2, 6). These oscillators are distinguished from linear oscillators (sinusoidal oscillators described by a linear differential equation) and are amenable to modeling the gastrointestinal electrical control activity (ECA)[1] because when two of these oscillators are coupled (unidirectionally or bidirectionally), the frequency of oscillation is changed without a major change in their waveform. This permits the frequency of waves to be altered by coupling without interfering with the use of a particular waveform to control a local phenomenon, in this case, excitability. Thus, the waveform can control the local membrane excitability to occurrence of spikes and contractions in the time domain irrespective of frequency. Furthermore, if sufficient coupling exists to allow oscillators (see below) to become phase-locked, a local oscillator will maintain a definite phase relationship with all other oscillators coupled to it and driving it or being driven by it. Oscillators of higher frequency when uncoupled, have phase lead when phase-locked, so the local phenomenon can become related in time over a large

[1]The terminology for electrical activity of muscle in the gastrointestinal tract has recently been discussed (7). *Electrical control activity* corresponds to what some call *slow wave activity* or *pacesetter activity*. The outdated term *basic electrical rhythm* (BER) was applied to the same phenomenon. *Electrical response activity* (ERA) refers to the spike potentials normally associated with contraction.

region or an entire organ. The result is a global-spatial as well as temporal control system.

The relaxation oscillators used to model the gastrointestinal ECA are the Van der Pol oscillators and modifications of these (see 7, 8–16). If such an oscillator is uncoupled, its potential slowly drifts towards a threshold voltage and then undergoes a rapid depolarization called a *control potential,* collectively *electrical control waves* (*EC waves*). This behavior occurs because these oscillators have a nonlinearity that is voltage dependent; that is, at a threshold voltage it changes its characteristics and initiates an oscillation. The rate of drift and the difference between the threshold potential and the potential after an oscillation ends determines the intrinsic frequency of the oscillator. In the coupled state, a neighboring oscillator or oscillators may provide an electrical input to this oscillator before it reaches its threshold potential and may force its potential to the threshold prematurely or delay it. In the gut, the inputs from other oscillators usually seem to produce a premature oscillation. This input probably involves a local depolarizing current flowing across the extracellular space between cells or through low resistance junctions connecting cells. Thereafter, the depolarization occurs as usual and the cycle restarts. As a result of coupling in the gut, the period of oscillation is shortened and hence the frequency of the driven oscillator is increased towards the frequency of the driving oscillator.

Whether the driving oscillator, which must have a higher intrinsic (uncoupled) frequency than the driven one, can actually drive the other oscillator or not is determined by whether the input from it is sufficient to force the potential of the oscillator of lower intrinsic frequency to threshold. If the intrinsic frequencies of the two oscillators will oscillate at the same frequency; that is, they will become enally be applied when the potential of the other oscillator is sufficiently close to its threshold and will force this potential to threshold. Thereafter, the input from the higher frequency oscillator will arrive just before the other is ready to oscillate. The two oscillators will oscillate at the same frequency, that is, they will become entrained or phase-locked. On the other hand, if the difference in intrinsic frequencies is larger, the input from the higher frequency oscillator (even if both started oscillating at nearly the same time) will be applied earlier in the cycle of oscillation of the low frequency oscillator. There will be a larger difference between the potential present and the threshold potential, and a greater input will be required to the threshold and/or a longer time (greater phase lag) will ensue before the driven oscillator reaches threshold. A longer output current or a stronger coupling will be required in this case to achieve entrainment. In the event the input is insufficient, the second oscillator will not become phase-locked to the high-frequency oscillator. However, the input current will shorten the period to the next cycle in some cycles when the potential of the lower frequency oscillator is near threshold; that is, its frequency will be pulled up.[2]

[2]In the large bowel, in contrast to the stomach and small intestine, proximal lower frequency oscillators may pull down the frequency of distal higher frequency oscillators (see below).

Thus, there can be two outcomes of coupling between the relaxation oscillators at different intrinsic frequencies depending on the magnitude of this difference, the extent of coupling, and the strength of coupling current. In one, favored by good coupling between the two oscillators and a small difference of intrinsic frequencies, each oscillation of the higher intrinsic frequency drives the lower intrinsic frequency oscillator, resulting in an entrainment and phase-locking of the two oscillators. In the other, favored by poor coupling and too large a difference in intrinsic frequency between the coupled oscillators, the depolarization of the higher intrinsic frequency oscillator can drive the lower intrinsic frequency oscillator only when its potential is near threshold. This results in frequency pulling. Both of these patterns are observed in the gastrointestinal tract (Table 1).

Record work of Sarna et al (17) in vivo has pointed out the possible presence of another type of oscillator in the gastrointestinal smooth muscle. These oscillators have been referred to as the *one shot oscillators.* These oscillators are not inherently unstable and self-excitable, but when stimulated with sufficient strength, they undergo one oscillation and then come to rest. If appropriately coupled, there will be a sequence of oscillations in time and the perturbation will appear to be propagated. The nonpacemaker cells of the rest of the gut and all the smooth muscle layers in the esophagus may belong to this class. The basic operation of these oscillators is similar to those described above, that is, the potential of these oscillators must be brought to the threshold to undergo a depolarization.

Models using either the differential equation representation or electronic circuits can represent these oscillators (6). In both cases the resemblance of models to the

**Table 1** Organization of coupled relaxation oscillator systems in gut

| Region | Locus of high frequency oscillator | Nature of response of low frequency oscillators |
|---|---|---|
| Gastric corpus[a] and antrum | Proximal, corpus on greater curve | Phase-locked; phase lag decreases distally |
| Upper small[a] intestine | Upper duodenum | Phase-locked; phase lag increases distally (human?)[c] |
| Lower small?[a,b] intestine | Proximal oscillators | Pulled up; short regions of transient entrainment |
| Proximal large?[a,b] intestine | May be distal (human?)[c] | Pulled up (high frequency oscillations also pulled down) |
| Distal large?[a,b] intestine | May be distal (human?)[c] | Pulled up (high frequency oscillations also pulled down) |

[a] In most regions, oscillations are phase-locked around the gut circumference with little phase lag. The remainder of this table refers to phase relations in the distal direction.
[b] A question mark indicates lack of conclusive data.
[c] "Human?" means human data are not complete.

physical system is primarily qualitative but the similarity of these models to those of Hodgkin-Huxley, which quantitatively describe the phenomena in nerves, has been shown (18).

In the differential equation on the electronic models, coupling means the feeding of the input of one oscillator into another oscillator. In the physical system a similar phenomenon must occur; that is, there must be a mechanism whereby the voltage or the current change produced by one cell can affect another cell. This is considered below.



## ORIGIN OF ELECTRICAL ACTIVITY

### *Spontaneous Oscillations*

Many smooth muscles are rhythmically or intermittently active when stretched. Stretch activates smooth muscles in some cases by depolarizing them (19, 20) and in other cases when nerves and other tissues are also present by releasing mediators such as acetylcholine (see 21, 22) or serotonin (5-hydroxytryptamine) (see 23), or prostaglandins (see 24–26). In the arteries and possibly other tissues such as sphincter, spontaneous active tone may be related to prostaglandin release under unstretched conditions, but critical experimental data are not available.

In the gastrointestinal tract, many smooth muscles have spontaneous electrical activity without stretch or known nervous input. Wood and others (27–29) postulate that intrinsic neurones are tonically active in intestine, that there is a resultant predominant tonic inhibition, and that the natural state of the intestinal muscle after withdrawal of this inhibition is electrically active with contractions. The data cited to support this hypothesis, which are activation of muscle after administration of tetrodotoxin (TTX), atropine, local anesthetics, etc, are interpreted on two unproven assumptions: (*a*) that all the effects of these agents result from withdrawal of nervous inputs and (*b*) that these procedures inhibit all release of nerve mediators or all effects of such release. Not all gastrointestinal muscle is activated by TTX or other inhibitors of neuronal activity, for example, opossum esophagus in vivo or in vitro (personal observations). So far no one has found such strips to have continuous release of mediator or junctional potentials affecting most circular muscle cells as this hypothesis requires. In any case, none of the procedures used to withdraw tonic nervous activity abolish spontaneous electrical activities of the intestine (27, 28). Thus, there is no reason to assume that the intestinal electrical activity to be discussed requires nervous inputs. Most of these studied so far (Table 1) have possessed the characteristics described above of coupled relaxation oscillators (CRO). The guinea pig intestine is an interesting exception in this table since its electrical oscillatory activity which depends on release or addition of acetylcholine is blocked by atropine and/or TTX (30–32). Acetylcholine seemed to act by initiating a cyclical increase in $Na^+$ conductance.

Whether the spontaneous electrical activities arise from an inherent metabolic instability of membrane potentials of a single or a few smooth muscle cells (pacemakers) or from the cooperative activities of groups of cells has not been determined and awaits appropriate tissue culture studies, or detailed microelectrode analysis

and modeling. There also remains the remote possibility that some unknown specialized structures release activating chemicals in these systems to account for the spontaneous electrical activity.

### *Origin*

In the small intestine, early dissection studies led to the conclusion that spontaneous electrical activity originated in the longitudinal muscle. Bortoff (33) found that flat strips of intestinal wall initiated ECA or slow waves that were in phase in adjacent longitudinal (LM) and circular muscle (CM), that partial removal of the outer layer resulted in the disappearance of oscillators in the exposed circular muscle except near the residual longitudinal muscle, and that such activity diminished exponentially with distance from longitudinal muscle (34). Kobayashi et al (35) made similar findings with cylindical preparations, and numerous investigators have found that isolated longitudinal strips generate spontaneous ECA.

However, all the above studies suffer from a methodological defect. The assumption was made that the various dissection procedures actually separated circular from longitudinal muscle. When examined histologically, the "longitudinal strip" usually contains myenteric plexus as well as the outermost layer of circular muscle (36). Any one of these tissues may be the site of origin of ECA. Recently, Connor et al (37) found spontaneous
from the cat intestine. Taylor et al (36) found that a few of many longitudinal strips that were pulled off rabbit intestine were devoid or nearly devoid of circular muscle. They had no ECA. The underlying circular muscle possessed ECA. They also found a few cells that developed very large EC or slow waves (30–40 versus 10-20 mv elsewhere) and seemed to be located just beneath the myenteric plexus. They suggested that these might be pacemakers. CM cells could be identified because they had higher membrane potentials and much larger inhibitory junction potentials and because they often generated larger EC waves [Taylor et al (36)] than LM cells. Kreulen (38) also found smaller, slower-rising, sometimes nonexistent EC waves in isolated cat longitudinal intestinal muscle compared to larger ones when CM and LM were intact. They also found very large EC waves from both layers, but did not make clear their criteria for determining the locus of recording electrodes. They interpreted their data as electrotonic spread from LM into CM where EC waves were larger because of regenerative $Ca^{2+}$ conductance changes and augmented EC waves in both LM and nearby CM. This model requires that CM alone be inactive and that adequate coupling exist between layers. The same data are also consistent with the origin of EC waves in the outer layer of CM. In localizing the origin of EC waves in LM, Bortoff (2, 34, 39) places great weight in experiments which he interprets as indicating that in the LM, EC waves are accompanied by an outward passive and then inward active membrane current while in the CM, the current is entirely outward and passive. The findings would also be expected if the EC wave was driven by pacemaker cells located in the external portion of CM near LM or if the LM responded to such pacemakers by generating an active current while CM did not.

Whether intestinal ECA originates in LM or in certain CM cells requires further study. Preparations of relatively undamaged (judged by membrane potentials, spiking, junction potentials, etc) LM or CM can be found devoid of ECA, or with two uncoupled pacemakers (36, 37, 40). Also, most cells of rabbit intestinal strips with ECA show no phase of gradual depolarization between control waves (40, 41). These results strongly suggest that generation of ECA is not a property of all intestinal cells and that some cells are "one-shot" oscillators (see above).

Any model that postulates one or a few cells driving other cells must explain how this can occur if these cells are coupled like those in taenia coli (42–44). There, current input into a single cell by a microelectrode rarely could initiate an action potential even in the impaled cells. Tomita (43, 44) suggested that this was due to the presence of the cell in a syncytium with good coupling in all directions, providing a large area of cell membrane over which input current was dissipated. Pacemaker cells might be located between muscle layers with coupling only to a limited number of other cells. Alternatively, the problem may arise from the use of an inappropriate model of coupling or of the coupled system and/or from the differences in coupling of active and passive currents.

Bortoff (2) has emphasized that both in flat (4, 34, 45) and cylindrical (35) preparations, EC waves and contractions are in phase in LM and in underlying CM. Further, as mentioned above, when LM is stripped away (along with plexus and some CM in most cases) there seems to be exponential decay of EC amplitude with distance from LM (or from surviving pacemakers). This implies some electrical coupling between layers either from LM to CM if ECA originates in LM or from CM to LM if it originates in CM pacemakers. Gabella (46) has reported that nexuses sometimes occur between LM and CM in guinea pig intestine, but one of us has never found any between LM and CM in dog intestine after many years of searching, nor in rabbit intestine after a briefer search. Indeed, in most regions these layers are widely separated by ganglia, nerves, blood vessels, and/or layers of interstitial cells or fibroblasts. In longitudinal strips of rabbit intestine, most circular muscle cells do not show coupling to the muscle between electrode plates in a partitioned bath, presumably because there is poor transverse coupling between CM bundles and because the sites of coupling to LM are rare (36). Also in circular strips, most LM cells are similarly not coupled to the muscle between the plates (G. S. Taylor, D. Cheung, and E. E. Daniel, unpublished observations). Connections between intestinal muscle layers must be rare and localized. The location of pacemakers in or near such connections may allow effective coupling of these to both layers.

In dog stomach, both longitudinal and circular strips can generate ECA (47) and this may be true of guinea pig stomach as well (48–50). Cat stomach was reported to generate EC waves in LM, based on procedures like those used in cat intestine (51), but histological controls were not done. In guinea pig stomach circular strips, ECA consists of a spike component superimposed upon what appears to be a control potential. Ohba et al (49, 50) suggested that there was another underlying oscillating component. They used a double-sucrose gap to hyperpolarize the membrane. This abolished about 30 mv of the 40 mv control potential. In addition to this voltage-

dependent component, they found a residual 10–15 mv component, especially in antral preparations, which could not be so abolished. When membrane potentials were voltage clamped at or near the resting potential, rhythmic waves of inward current at the same frequency as ECA were recorded. Variations in clamp potential had little effect on these periodic inward currents. They concluded that there was a component of myogenic activity not involving a conductance change since its magnitude was not markedly affected by altering the electrochemical gradient. It was presumed to initiate a conductance change which generated the major voltage-dependent component abolished by hyperpolarization. However, the frequency of this "voltage-independent" component was modestly affected by voltage changes; hyperpolarization slowed and depolarization accelerated its frequency.

The simplest explanation for these results is that there were some cells that were arranged geometrically or so coupled to other cells as to be imperfectly voltage-clamped. Ohba et al (49, 50) normally used mixed strips of circular and longitudinal antral muscle, but stated that similar results were obtained with pure circular strips. Histological controls of this dissection were not mentioned. If pure circular strips were obtained, it does not follow that all cells were of appropriate geometry and coupling to be adequately clamped.

Ohba et al (49, 50) imply that all muscle cells have voltage-dependent and -independent components of myogenic activity. If the frequency of control potentials in guinea pig stomach is set by a voltage-independent activity of all cells, it is difficult to see how these oscillations become synchronized over many cells. If this voltage-independent activity originates from a few pacemaker cells and spreads electrotonically into others, it is also difficult to envisage how the various voltage-independent pacemakers cells become phase-locked, even if they are coupled to one another. However, these problems disappear if voltage clamping of driven cells was inadequate to control the membrane potentials of pacemaker cells and if there was some coupling between pacemakers via the same voltage-dependent mechanisms, which influence

Clearly, further study of the mechanism underlying these results is required. Studies of the coupled and uncoupled frequencies of the guinea pig stomach in vivo are essential. If, like other species, the guinea pig stomach has a system of coupled relaxation oscillators, the explanations of Ohba et al (49, 50) will need reconsideration. Also the extent to which various preparations of guinea pig stomach can be driven electrically would be of great interest. The stomachs of other mammals can easily be paced above their normal frequencies by short electrical pulses (11, 52–54); if the guinea pig stomach or muscle strips from it can be paced, it is difficult to imagine that its frequency is normally controlled by a voltage-independent mechanism.

In large intestine, studies carried out mostly in the cat, which has a continuous longitudinal muscle coat, suggested that the origin of spontaneous electrical activity was the circular muscle (55–61; for review see 62). However, these experiments bear repeating with careful histological controls; also, species with taenia coli require further study.

### *Ionic Mechanisms*

The ionic mechanisms underlying ECA in the intestine are still controversial. Initial studies of dogs in vivo (63) demonstrated that in the small intestine, EC waves were susceptible to removal of $Na^+$ (Li substitution) and $Ca^{2+}$ with EDTA, and to inhibitors of the Na pump, e.g. ouabain. Perfusion with $Cl^-$- or $K^+$-free solution had little perceptible effects on EC waves recorded in vivo by extracellular electrodes. In vitro studies of intestine from cats with pressure electrodes or double sucrose gap (37, 64–66) and from rabbits with microelectrodes (67) or double sucrose gap (40) have led to generally similar but sometimes inconsistent results: (*a*) Removal of $Na^+$ (e.g. Li substitution) or marked reduction of $Na^+$ to $<20$ mM (34, 40, 64–67) abolishes or seriously diminishes EC waves; (*b*) Inhibitors of the Na pump such as ouabain or zero $K^+$ also abolish EC waves (34, 40, 63, 65, 67); (*c*) $Ca^{2+}$ removal abolishes or diminishes EC waves (or slows their rise rate) as well as spikes (37, 65, 67, 68), but in rabbit intestine, verapamil, a $Ca^{2+}$ conductance blocking agent (69), does not inhibit them except in doses larger than required to inhibit spikes (40, 67); (*d*) The frequency of EC waves is highly temperature-sensitive (40, 64, 67, 70), and in rabbit intestine (67) the components with the high temperature sensitivity ($Q_{10} > 2$) are the EC wave duration and interwave period, but not the wave amplitude or depolarization time ($Q_{10} < 2$); (*e*) In rabbit intestine, replacement of $Cl^-$ with nonpenetrant anions ultimately reduces the duration of EC waves and accelerates depolarization, though initially it may enhance the amplitude of the plateau portion of EC waves (67); (*f*) In rabbit intestine, the EC wave may consist of two components, an initial rapid depolarization, followed by a plateau. These two components appear to be separated at low temperature and by the selective suppression of the plateau potential by substitution of impermeant anions for $Cl^-$ (67). El-Sharkawy & Daniel (67) suggested that this EC wave results from a $Ca^{2+}$-dependent transient increase in $Na^+$ conductance followed by a transient, but slower, increase in chloride conductance which was triggered by the initial increase in sodium conductance. Repolarization may involve turning off of chloride conductance or Na pump activity or both. Bortoff (2) criticized the suggestion that increased chloride permeability might be involved because alteration of external chloride may affect permeability to other ions (74).

Connor et al (37) studied longitudinal strips of cut intestine in a double sucrose gap. No mention was made of compensation for the series resistance (internal longitudinal resistance plus solutions and reference electrode resistances). In the 20% of strips that had spontaneous EC waves, the applications of a voltage clamp at the resting membrane potential did not abolish repetitive spontaneous inward currents that occurred at the frequency of the EC waves. Hyperpolarization of 30 mv or depolarization of 10 mv did abolish them. Their occurrence over a limited voltage range was similar to results of Mills & Taylor (71) in rabbit intestine. Ouabain or removal of external $K^+$ depolarized cat intestine 5–8 mv and abolished EC waves, even after restoration of membrane potential (37). After $K^+$ depletion, restoration of $K^+$ produced 20 mv (ouabain-sensitive) hyperpolarization in inactive

preparations. The outward current associated with this hyperpolarization under voltage clamp decreased with increasing hyperpolarization of the membrane. These authors could detect no conductance change associated with EC waves. They proposed that EC waves originate by an oscillating voltage-sensitive Na pump which turns off to initiate and on to terminate EC waves. However, failure to compensate for the series resistance could have resulted in failure of the clamp to control membrane potential so that the recorded current will have a configuration like an inverted EC wave as it did (2, 40, 72).

A more recent study (40), when the series resistance in the gap was not compensated for, yielded results from longitudinal strips of rabbit intestine under voltage clamp which resembled those of Connor et al. However, when the series resistance was compensated for, clamping at the membrane potential led to random current oscillations. Clamping at a potential equivalent to the peak of the EC wave caused disappearance of current transients. Clamping at a membrane potential 5–10 mv more negative than the resting potential restored rhythmic current transients. That adequate voltage clamping of pacemaking cells occurred in these experiments seems unlikely, but if it was achieved, these results are difficult to reconcile with either a voltage-dependent conductance change or an oscillating voltage-independent pump current.

In longitudinal strips of rabbit intestine, El-Sharkawy & Daniel (67) found that ouabain, K-free solutions, Na-free solutions, DNP, and cooling to 15°C all turned off EC waves averaging 18 mv but at membrane potentials 8–15 mv more negative than the peaks of the EC potential (which was about 37 mv in a cell with 55 mv resting potential and 18 mv EC wave). If these procedures abolished EC waves solely by inhibiting the Na pump, such inhibition does not lower the membrane potential sufficiently to account for the depolarization during the EC wave. Subsequent studies (36, 40) showed that after ouabain or $Li^+$ substitution for $Na^+$ had depolarized cells 5–8 mv and abolished EC waves, they could be restored, at least for many minutes, by hyperpolarization of the cells. Furthermore, El-Sharkawy & Daniel (67) found that after $K^+$ depletion and readmission the membrane hyperpolarized by 50–60 mv to 75–80 mv and EC waves began immediately at a frequency of 18/min but they were of only 2–8 mv amplitude in such preparations. If 50–60 mv of the membrane potential originated from pump current, these EC waves were too small to be explained by its turning off. Small EC waves would be expected if the hyperpolarization reduced their transmission from pacemakers or if their size depended on conductance changes. The ionic gradients that drive current flows following conductance changes were diminished in Na-rich tissues. The ability of hyperpolarization initially to restore EC waves (see above) after such gradients were diminished by Na pump inhibitors would be explicable in similar terms; that is, hyperpolarization would restore the gradients. Eventually because of excessive depletion of ionic gradients or because of permeability changes secondary to altered ionic contents, cell hyperpolarization would be unable to restore these gradients. Replacement of external $Cl^-$ by an impermeant anion should increase transmembrane resistance and enhance electrogenic currents. In fact, the procedure decreased EC amplitude slightly and duration markedly, but did accelerate repolarization (67). Increased

repolarization could reflect
from abolition of a $Cl^-$ conductance increase during the EC wave.

In addition, very large EC waves up to 40 mv have been recorded both in rabbit cells (36) and cat (38) intestinal muscle. There is no experimental (see 36, 37, 67, 75) or theoretical (73) basis for believing that the electrogenic pump current accounts for a membrane potential contribution of this size in the normal $K^+$-rich cell.

The inhibition of intestinal EC waves by reducing $Na^+$ (to $< 20$ mM) could be explained by abolition of Na pump current or by abolition of an inward Na current resulting from a permeability change. Restoration of $Na^+$, unlike restoration of $K^+$, did not lead to hyperpolarization, but did restore EC waves (37, 67). In tissues studied to date, reduction of $Na^+$ by half had little or no effect on EC waves (37, 40, 65, 67) or inward currents (37), either because the inward $Na^+$ current-carrying mechanism is still saturated at this extracellular $Na^+$ concentration or because the gradient is rapidly readjusted by loss of intracellular $Na^+$.

If $Na^+$ permeability changes during the depolarization phase of the EC wave, decreases in transmembrane resistance (Rm) are expected. Mills & Taylor (71) could not measure conductance during depolarization but found decreased electrotonic responses in a double sucrose gap to hyperpolarizing currents during the depolarized phase of EC waves in rabbit intestine. They assumed that this represented decreased $R_m$ since the space constant or electrotonic potential is proportional to $\sqrt{R_m/R_i}$ when $R_m$ is effective transmembrane resistance and $R_i$ is internal longitudinal resistance. If this resulted from a decrease in $R_m$, there would be a shorter membrane time constant ($= C_m R_m$) at the EC wave peak compared to the trough, and pulse height would be relatively larger because of closer proximity of the pulse height to the steady state value. This would result in underestimation of the conductance change. However, Connor et al (37) have pointed out that the EC depolarization might bring the membrane potential into a region of delayed rectification, causing an increase in conductance secondarily. These authors found no evidence of changes in electrotonic potentials and thus in conductance during EC waves in cat intestine. The time course of the initial depolarizing current and any underlying conductance change is unknown. Whether conductance changes at the slow-wave peak are related to those during depolarization is uncertain. In considering this problem it must be kept in mind that the size of the intracellular electrotonic potential produced by a given current pulse applied elsewhere is affected by the longitudinal internal resistance ($R_i$) (inversely) as well as by the transmembrane resistance (Rm). $R_i$ in turn reflects
other resistances unrelated to the tissue in the node in physiological saline may also affect it. Thus, the amplitude of the electrotonic potential is not uniquely related to transmembrane resistance, and its interpretation is dependent on the equivalent circuit assumed to apply. Further, it appears probable that not all cells in an intestinal strip are actively participating in EC wave initiation. Many may be responding passively to currents generated elsewhere. Certainly in the preparations of Connor et al (37) not all were generating EC waves. Caution in interpreting changes or lack of changes of electrotonic potentials in strips during EC waves as reflecting

The strongest evidence of a permeability change underlying generation of EC waves, is the fact that, in intestine and stomach, EC waves can be initiated in electrically quiescent preparations or driven faster (11, 12, 37, 40, 52, 71, 75, 76) in active preparation of intestine or stomach by short current pulses. No experimental or theoretical evidence supports the proposition that the pump current could be turned off or on in this way.

EC waves have been clearly demonstrated to show refractoriness. Thus, in the dog stomach, which normally has a period of 12 sec between EC waves, another cannot be initiated by current pulses during the first 4–6 sec after the preceding EC wave (11). The maximum rate at which they can be driven is limited by such refractoriness and by relative refractoriness of the oscillators. Similariy in the small intestine, the rate at which EC waves can be driven is limited by their refractoriness (2). Such refractoriness might be explicable in terms of increased ionic conductance during the EC wave, but it is difficult to see how decreased electrogenic Na pumping unaccompanied by a conductance change might prevent driving.

The role of $Ca^{2+}$ in EC wave generation requires further analyses. In rabbit intestine (67), $Ca^{2+}$ withdrawal depolarized cells and abolished EC waves, but verapamil had no effect on EC waves in doses that inhibited spikes (ERA). Thus, if Ca entry is involved in EC wave generation, the $Ca^{2+}$ enters by a different channel than is utilized by spikes. Further, the amount is too small to trigger contraction. Subsequent studies (40) showed that $Sr^{2+}$ could substitute for $Ca^{2+}$ in maintaining ECA.

In the stomach of dog and cat the EC wave consists of a definite initial spike-like component followed by a plateau. The ionic mechanisms underlying these components have not been adequately studied. In the cat stomach, in vitro the first component was eliminated by reduction of $Na^+$ to 20 mM, but not much affected when 38 mM $Na^+$ was present (51). Ouabain also ultimately inhibited this component but no special sensitivity was observed. Elimination of $Ca^{2+}$ inhibited both components, but the first was stated to be less susceptible to reduced concentrations. $Mn^{2+}$ also selectively inhibited the plateau component. In the dog stomach (47), both components were reduced in amplitude, and the plateau was reduced in duration by $Ca^{2+}$ reduction. Ouabain was not very rapidly effective in inhibiting either component. Partial reduction in $Na^+$ by intraarterial perfusion had little effect (77). Conceivably the first component may involve an increase in $Na^+$ conductance while the second may involve an increase in $Ca^{2+}$ conductance.

In the guinea pig stomach, Ohba et al (50) have recently presented evidence that complete Na removal depolarized the cells and inhibited the EC wave. Restoration of 5–10 mM $Na^+$ restored the EC wave even in the presence of ouabain. Removal of $K^+$ or ouabain (up to $10^{-5}$ M) depolarized the membrane and decreased the size of but did not eliminate EC waves; frequency changes, usually a slight increase, also occurred. Even ouabain ($5 \times 10^{-7}$ M) plus $K^+$-free solutions did not abolish EC waves, and ouabain did not abolish effects of $K^+$ concentration changes in EC waves. Ouabain did abolish or diminish the depolarization on $K^+$ or $Na^+$ removal and the hyperpolarization on their restoration. $Ca^{2+}$ removal diminished EC waves. Elevated $Ca^{2+}$ slowed the frequency of EC waves. There were some quantitative differ-

ences in the effects of experimental procedures on voltage-dependent and voltage-independent components of these EC waves (see above). Low $Na^+$ prolonged both parts, but prolonged the "voltage-independent" part more; however, this was not evident in all records. Removal of $K^+$ shortened this component, and restoration of $K^+$ initially prolonged it even when ouabain was present or when $Na^+$ was reduced and before any membrane potential change occurred in response to $K^+$. High $Ca^{2+}$ prolonged this voltage-independent component but did not interfere with the effect of restoring $K^+$. Removal of $Ca^{2+}$ and its restoration with $K^+$ diminished the prolongation of the voltage-independent component. They interpreted these results to mean that the $Na^+$-$K^+$ pump was not directly involved in EC wave generation or in its prolongations on restoration of $K^+$ but suggested that some other pump or metabolic process involving $K^+$ and $Ca^{2+}$ was involved. In different experiments, different concentrations of ouabain were used, and it was not clear when the $Na^+$-$K^+$ pump was fully blocked. Moreover, none of the variables introduced were able to abolish selectively the voltage-dependent component. Since this, in contrast to the voltage-independent component of EC, is supposed to result from a conductance change, its persistence except when both components were abolished is hard to understand.

To sum up the situation, EC waves of the intestine probably are generated by some but not all cells. The locus of these cells may be in the longitudinal muscle, or in circular muscle near it. The initiation of EC waves may involve a conductance increase to $Na^+$ (or less likely to $Ca^{2+}$) ions, but not all cells may be involved in active electrogenesis. The plateau portion of the EC wave may involve other conductance changes, for example, to $Cl^-$. Also, the Na pump is involved, either because of its role in maintaining membrane potential and ionic gradients or restoring them after depolarization or, less likely, because of its ability to oscillate when membrane potentials are fixed. The EC waves can be driven electrically, show refractoriness, and behave as expected of a system of coupled relaxation oscillators. The origin and ionic mechanisms of EC waves in stomach and large intestine require further study.

## COUPLING OF OSCILLATORS

### *Morphological Basis*

In a variety of tissues, electrical coupling has been shown to be correlated with the existence of gap junctions (see 78–82). In gastrointestinal muscle, electrical coupling within and between layers is a prerequisite for any model of coupled relaxation oscillators. Numerous gap junctions have been found in circular muscle of intestine (83–87), stomach (88, 84), and esophagus (84, 86) of various species. However, few or no gap junctions have been observed by transmission electron microscopy (TEM) in longitudinal muscle (83–88), but there are exceptions (86). Phase lags around the circumference of intestine and stomach in vivo are negligible (1, 2, 35, 89, 90). Less coupling would be required to explain the short phase lag of oscillation around the circumference if intrinsic frequencies were nearly identical (see above). In most regions the intestine and stomach are being driven above their intrinsic frequency by proximal oscillators. It is probable that coupling in the longitudinal axis requires

longitudinal muscle (36) and cannot be provided for by transverse coupling between bundles of circular muscle. Longitudinal muscle cells are poorly coupled circumferentially. Thus, to avoid any falling out of phase circumferentially would require good circumferential coupling in circular muscle since simultaneous circumferential input from longitudinal muscle would not be guaranteed. The substantial number of gap junctions within circular muscle bundles could provide for this coupling. However, measurements of space constants in the circular muscle compared to longitudinal muscle (35, 43, 91) do not reveal drastic differences (e.g. 2–3 mm in CM and 1–2 mm in LM). This may reflect inadequacy of the model used for interpretation of space constants or the dependence of coupling on structures other than TEM-visible gap junctions. Burnstock and co-workers (92) have recently reported that very small junctions invisible by TEM may provide for coupling in guinea pig taenia coli. These small aggregations of membrane particles observed by freeze-fracture raise the question of what constitutes a gap junction: how many particles, their size and spacing, and so on. There are many similarly sized particles on the PF face of fractured smooth muscle membranes; no one has suggested that all are capable of participating in coupling by forming gap junctions. Further, no evidence has been given that the small aggregates of particles on the surface of one cell are in contact with similar aggregates on adjacent smooth muscle cells. Thus, any critical evidence that these small aggregates of particles provide a possible structural basis for coupling remains to be obtained.

The coupled cells that have no TEM-visible gap junctions are often observed to be connected by thin, elongate arms of protoplasm even when relaxed. Gap junctions at the termini of such arms would be difficult to see either by TEM or by freeze-fracture.

Further, the conventional wisdom that gap junctions form the only basis for coupling between cells has been challenged in relation to smooth muscle (85). Other structures such as close appositions or even desmosomes may allow for a lower resistance pathway between cells relative to a higher resistance pathway to extracellular space. Such, after all, is the minimal requirement for cable-like behavior of cellular strips (6). Recently, Sperelakis & Mann (93) have presented a model of the potential in the cleft between two cells during excitation of one of them. The resistivity of the membrane adjacent to the cleft was the same as the remainder of the surface membranes. Sperelakis & Mann have presented evidence that, provided the cleft is narrow enough and there was an appropriate delay in firing of part of the surface membrane adjacent to the cleft, the cleft potential became negative when the cell was activated. The membrane of the second cell adjoining the cleft was thus depolarized relative to the extracellular space and could be activated. Furthermore, Stibitz & McCahn (94) have presented calculations showing that signal transmission can occur between cells that have capacitative coupling without low resistance junctions. No one has attempted such a model for coupling of EC waves in smooth muscle.

### *Cable Properties and Local Circuit Current*

There is clear evidence that both LM and CM in gut show properties that can be considered consistent with a cable-like arrangement. With appropriate current input

(e.g. simultaneously across a strip in a partitioned bath), there is evidence that currents decay exponentially with distance, have space constants in the order of millimeters, and show time courses consistent with such properties (35, 43, 91, 95). Also, currents can be recorded extracellularly near longitudinal muscle during EC waves in a way consistent with flow of local circuit current (2, 34, 39), i.e. outward (passive due to local circuit currents) followed by inward (active due to an influx of $Na^+$ or $Ca^{2+}$ ions) membrane current; slow waves can be triggered electrically by cathodol currents or anodal break currents (11, 12, 37, 40, 52, 71, 75, 76). It has been claimed (2, 96) that slow-wave propagation velocities are inversely related to longitudinal resistance, which is the resistance to local circuit flow.

The significance of such properties can be overinterpreted. The only requirements for cable-like behavior are a source of parallel capacity and a resistance in the axial direction along the strip that is lower than that in the radial direction. Very simple models (97) show such behavior, and coupling between cells by any mechanism that provides lower resistance to current flow between cells than to the extracellular space will suffice. The real system may show discontinuous rather than exponential decay of current. There will be a voltage drop across the resistance drop at every cell-to-cell junction, but this will not be detected. Bartoff (2, 96) has analyzed impedance data on gastrointestinal muscle assuming that there are low-resistance junctions between cells and the resistance at each junction is shunted by a capacitance. His method and analysis of data was based on earlier studies by Tomita (97, 97a). Impedances of a strip placed in a narrow tube were studied in normal Krebs solution and in one containing half sucrose and half $Na^+$ Krebs solution. The difference was used to calculate shunting by extracellular fluid, on the assumption that muscle impedance and cell volume are the same in both solutions. Tomita (44) has recently rejected these assumptions (44, 98). Also, no justification is given for locating the only capacitance parallel to the junctional resistance. This seems especially dubious in tissues without gap junctions. Furthermore, sequential activation of phase-locked oscillators can provide outward passive followed by inward active currents as an effect rather than a cause (6).

The coupling currents between relaxation oscillators in the gut must behave like currents anywhere. What is uncertain is the extent to which the active response of the driven but spontaneously active oscillator to the coupling current is comparable to that of a segment of squid axon to local circuit current. For example, it is difficult to explain the nature of the frequency gradient in the intestine if it is determined solely by core conduction. The refractory period of the oscillators clearly limits their ability to be driven. It has been shown in the proximal intestine of the dog or cat (12, 75, 76, 99) that the greater the frequency of ECA the shorter the length of the chain of phase-locked oscillators; distal oscillators with greater refractory periods drop out. Below the jejunum (12), entrainment to an electrical pacemaker can occur but the maximum frequency cannot be increased and there are normally numerous small and time-variable frequency plateaus. Bortoff (2, 96) has argued that the increase in phase lag or reduction in propagation velocity along the intestine is due to increasing resistance to local circuit current flow rather than to increasing refractoriness. Bartoff's results from isolated segments of cat intestine suggested that the maximum driven frequency is determined by the longitudinal resistance rather than

by refractoriness since the maximum frequencies (limited by refractoriness) of segments of duodenum and mid-jejunum seemed to converge while phase lags at decreasing frequency (when refractoriness should become negligible) did not. The maximum driven frequency of ileum did decrease. However, in vivo, the maximum driven frequencies of jejunum near the end of the first long frequency plateau clearly are less than in proximal regions (12, 75, 76, 99). Also, the range of experimental frequencies Bartoff studied in vitro was limited and maximum driven frequency and maximum velocity were obtained by a linear extrapolations, which may not be justified.

Another difficulty, if coupling of electrical events in the intestine is determined by core conduction, is the failure of spikes or response activity in stomach or intestine to spread or to be coupled except over much shorter distances than ECA (14, 15, 70, 89, 90, 100). Since the same circuitry is available for spread of both electrical events, this is unexpected if spread is similar to that in a cable. On the other hand, the frequency of spiking is sufficiently high (Ca 10 Hz) that the entrinsic frequencies of the gut oscillators (3 to 19/min) cannot follow it. Within a region that is in the excitable phase of an oscillation, spread of spikes seems to occur (1, 2, 90), but usually with the same phase lag as the EC waves.

The existence of frequency pulling is another factor that does not corroborate with the proposal that coupling of gut muscle occurs by core conduction. If core conduction were involved, an EC wave should be conducted or not. In fact, in a region of pulling, an EC wave may follow for a few cycles, each cycle having an increasing phase lag, and then fall out.

One of the strongest points against the argument for core conduction is the finding that if intestine is surgically uncoupled and then reanastomosed end-to-end with approximation of layers, coupling is immediately restored. This ultimately fails again, owing to inflammatory changes and wound healing (G. Scott, personal communication). A similar series of events occurs in stomach in which it may be sufficient to approximate the cut edges to restore coupling (E. E. Daniel, and S. Sarna, unpublished observations). In such cases, current flow is clearly not via low resistance junctions between cells.

## INTERACTIONS WITH NERVES AND HORMONES

Electrical control potentials appear to control motility of intestine by making the electrical potentials of cells closer to threshold for spiking (ERA) in the intestine (1, 2, 101–103). Acetylcholine release brings about an excitatory junction potential or depolarization; this can add to the EC wave-induced depolarization to reach threshold. In the intact stomach, the major change produced by acetylcholine when contractile activity is initiated seems to be an increase in the amplitude and duration of a second plateau potential, and spikes are often superimposed on this plateau at least in vivo (11, 102, 103). However, this description is based on only a few intracellular records (102).

In the intact dog stomach, studied with monopolar extracellular recording, acetylcholine, like an electrical stimulus, can also induce a premature EC wave if the muscle is not refractory (1, 14, 77, 89, 103), but blockade of the effects of acetylcho-

line does not reduce the normal frequency of ECA (1, 102, 103). Thus, acetylcholine action is not involved in the origin of the normal ECA. Vagal stimulation at frequencies less than 6–8 Hz causes contractile activity associated with increased duration and amplitude of the plateau phase of the EC wave accompanied on many occasions by spiking superimposed in that phase (100). At higher frequencies, which produces greater acetylcholine release, vagal stimulation causes premature control potentials wherever the muscle is not refractory and thus uncouples distal regions from the proximal pacemaker. No clear difference in threshold has been found between small intraarterial doses of acetylcholine which initiate premature EC waves or contractions (77, 89, 103), but when vagal stimulation is used, the contractions occur at lower frequencies than the premature control potentials (100). Caution is indicated in interpreting this last result to mean that effects on plateaus have a lower threshold to acetylcholine than effects in spikes; vagal stimulation activates fibers innervating noncholinergic nerves as well as cholinergic ones. Release of inhibitory mediator from these nerves may affect thresholds to acetylcholine.

In vitro (47), strips of longitudinal muscle of dog stomach devoid of nerve plexus oscillate at slow frequencies, presumably their intrinsic frequencies. Acetylcholine in low doses can increase frequency as well as initiate contractions associated with elevated, prolonged plateau potentials. Usually there was also initial depolarization between EC waves associated with increased tone in the strip, but both rapidly disappeared. Frequency of EC waves also declined with repolarization, and application of continuous depolarizing currents was stated to produce increases in ECA frequency which were similarly related to membrane depolarization. This study (47) does not clarify fully whether acetylcholine always produced an increase in the initial spike-like potentials as well as in the plateau phase of the EC wave. Some records suggest it did not. Certainly, the increase in the plateau phase amplitude and duration was more marked and the onset of contraction was temporally related to this phase. An outward, depolarizing current, like addition of acetylcholine, could apparently bring the plateau phase to the EC wave to threshold for contraction. In vivo (102, 103), catecholamines in moderate doses or atropine inhibits contractions in association with reduction of the plateau phase and little or no change in the spike phase of the EC wave. Large doses of catecholamines produce rapid, spike-like waves unaccompanied by contraction (103). Thus, the initial spike phase, though often of greater amplitude than the plateau phase during contractions, does not seem to be able to initiate contractions. Either different conductance changes occur in the two phases, with $Ca^{2+}$ entering only during the plateau, or the duration of the initial spike is too short to allow a threshold amount of $Ca^{2+}$ to enter.

In the stomach EC waves therefore seem to control motility by providing a myogenic, periodic spike-like depolarization and a smaller, following plateau phase. The initial spike phase does not initiate contraction. When no acetylcholine is being released, the plateau phase provides too little and too short a depolarization to initiate contraction, but it can reach contraction threshold by additional depolarization or spiking on release of acetylcholine. Thus, as in the intestine, a combination of myogenic and neuronal depolarization is normally required to trigger contraction. Naturally, any procedure that produces sufficient depolarization could

trigger contraction by adding to the EC wave. Unlike the intestine, acetylcholine can trigger an EC wave as well as add to the plateau phase.

Hormones such as gastrin or motilin (104) can also release acetylcholine in the dog stomach, apparently by an action partially or wholly at a preganglionic site. Thus both hormones can accelerate the frequency of ECA and initiate contractions by an atropine-susceptible mechanism. Gastrin and its analogues have an additional action to increase EC wave frequency which is independent of acetylcholine; that is, it is atropine-insensitive (104) and present in nerve plexus-free strips of LM (47). In contrast to acetylcholine, which apparently accelerates ECA by depolarizing the membrane potential, gastrin usually increases frequency without a change in membrane potential (47). Since the EC wave in stomach is initiated by the spike component, the generation of these spikes can occur either by a membrane potential-independent or -dependent mechanism and the membrane independent mechanism accelerates occurrence of spikes without increasing plateau amplitude or duration. Thus, spike and plateau components can be controlled independently. There is no evidence that gastrins are involved in driving gastric ECA in vivo. Gastrins and motilins also stimulate ERA in the intestine of dog by releasing acetylcholine (M. Cook, unpublished observations).

Intrinsic, nonadrenergic inhibitory nerves innervate the whole gastrointestinal tract; so far there is little information (101) about their interactions with ECA. In all cases studied (e. g. 101) they seem to provide the predominant innervation in circular muscle; that is, if transmural stimulation is applied, the major or only junction potential is the inhibitory junction potential (IJP). The difficulty in recording EJPs in circular muscle may derive from a predominance of the membrane effects of IJP mediator over those of acetylcholine or there may be a mechanism that turns off acetylcholine release while nonadrenergic nerves are activated. Further study is required.

IJPs are larger during the depolarized phase of EC waves in the rabbit intestine than between such waves (101), presumably because they increase potassium permeability and, the membrane potential being further from $E_K$ on depolarization, they are displaced more by the permeability. IJPs will inhibit ERA and contractions by hyperpolarizing the membrane by increasing its $K^+$ permeability. We do not consider the wiring arrangements whereby excitatory or inhibitory neuronal events are organized in time and space.

## SUMMARY

Smooth muscle, especially gastrointestinal smooth muscle, spontaneously generates oscillatory electrical activity that can control contractions in time and space by altering excitability. The origin and ionic mechanisms underlying these electrical control activities are still controversial, but they behave as coupled relaxation oscillators and they control muscle excitability. Normally, contractions are produced by the addition, during the depolarized phase of the oscillations, of further depolarization by acetylcholine or other means. Pharmacologists who wish to study drug actions on such muscles must be aware of the possibility that drug effects may be

determined by these oscillations and may influence contractions by affecting these oscillations as well as by releasing, mimicking, or inhibiting the effects of nerve mediators or by affecting excitation-contraction coupling. Also the use of simplified organ bath preparations may eliminate or alter these control potentials so that results in vitro may not apply in vivo.

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