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EXCITATION AND CONDUCTION IN THE SMOOTH MUSCLE OF THE ISOLATED TAENIA COLI OF THE GUINEA-PIG

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The theories concerning conduction of excitation in smooth muscle may be divided in two groups—those based on the assumption that the cells behave as separate units (multi-unit theories), and those which assume that the cells behave as a syncytium, at least from the electrical point of view (unitary theories). In the multi-unit theories the spread of excitation has been suggested to take place within an interstitial network (Keith, 1915; Tiegs, 1925; Fischer, 1944; Ambache, 1947) or to be due to diffusion of a transmitter substance (Rosenblueth, 1936). On the other hand, Bozler (1938*a*, *b*, 1941, 1948) has been the chief advocate of the unitary theory and he, as well as Bülbring (1955, 1956), Greven (1955), Prosser, Smith & Melton, (1955), Prosser & Sperelakis (1956), have emphasized that conduction by an intercellular nerve network was unlikely.

Further questions remained, however; first whether the responses to electrical stimulation were 'all-or-none', or whether they were graded, and secondly, what was the mechanism of conduction.

There is no doubt that different types of smooth muscles behave differently, and the present work has been carried out entirely on a spontaneously active type in which the problem of excitability and conduction has been investigated in relation to the nature and the initiation of spontaneous activity. A short account of some of the results has been communicated to the Physiological Society (Bülbring, Burnstock & Holman, 1958).

METHODS

Isolated intestinal smooth muscle, the taenia coli of the guinea-pig, was used in all experiments. A modified Krebs's solution (Bülbring, 1953) was used throughout.

Recording

Intracellular electrodes. The methods have been described previously (Bülbring, 1954, 1957; Holman, 1958). When two micro-electrodes were used they were impaled at right angles to each other and at 45° to the horizontal preparation, entering the fibres radially. The resistance of the electrodes varied from 20 to 70 MΩ.

Monophasic extracellular recording. The sucrose-gap method (Stämpfli, 1954) was used to record from small strips of the taenia (2·0 cm length and 0·5 mm diameter), as described by Burnstock & Straub (1958).

Diphasic extracellular recording. Strips from the superficial (serosal) surface of the taenia (of 1·5–2·0 cm long, and less than 0·5 mm in diameter) were teased off (not cut) and suspended in oil. Ag:AgCl electrodes were connected to the preparation through glass tubes containing Ringer's solution holding bristles from a natural-bristle toothbrush (Trautwein, Kuffler & Edwards, 1956) which made contact with the muscle over a length of less than 0·2 mm. The pair of recording electrodes were connected by cathode followers to the input of a balanced DC amplifier which fed one beam of the oscilloscope. One electrode was fixed near the caudal end of the strip and the other end was held by the micromanipulator and was therefore movable. A layer of oil floated on the surface of the bathing solution, and when measurements were made the level of the bathing solution was lowered so that the muscle and electrode assembly were immersed in a layer of warmed oil.

Stimulating

When the recording was in oil, the stimulating electrodes, which were of the same type as the recording electrodes, were fixed about 5 mm apart, at the oral end of the strip.

The external stimulating electrodes used in conjunction with intracellular and sucrose gap recording were two rings of Ag wire, 1·5–3·0 mm apart, embedded in Perspex. The taenia was threaded through these rings.

When the stimulus was applied through a micro-electrode, a low-resistance electrode (10–20 MΩ) was used and a switch was incorporated in the circuit to monitor the membrane potential before and after stimulating.

The stimulator was of a conventional design (square wave output) and was coupled to the preparation by an anode modulated RF oscillator, as described by Perkins (1955). When it was required to monitor the current using external electrodes a series resistance was included in the stimulating circuit. The voltage drop across the resistor was displayed by one beam of the oscilloscope which was not isolated from earth.

RESULTS

Spontaneous activity

Fig. 1 shows typical patterns of spontaneous activity. Fluctuations in spike shape, as described previously (Bülbring, 1957), were recorded with external as well as internal electrodes. In (*a*), obtained with wick electrodes in oil, the potentials varied from simple diphasic spikes, showing a constant interval between the peaks of negativity at each electrode, to polyphasic waves. In (*b*), obtained with the sucrose gap method, and (*c*), recorded intracellularly, large simple spikes were intermingled with complex spikes of variable size. Extracellular records rarely showed a completely regular discharge, but in individual fibres this was seen in about one third of the records (see Fig. 2*b–d*).

Slow rhythmic fluctuations of the membrane potential were observed in both intra- and extracellular records. They occurred at the same range of frequency as the spike potentials but the relation between slow waves and spikes was not necessarily fixed (Fig. 2*a*). In Fig. 2*b–d* the slow waves were synchronous with the spikes, but while in (*b*) the spikes occurred during the rising phase of the slow waves, they occurred much later in (*c*). In (*d*) the spikes appeared to be initiated by the slow waves and 'wiped' them out. In

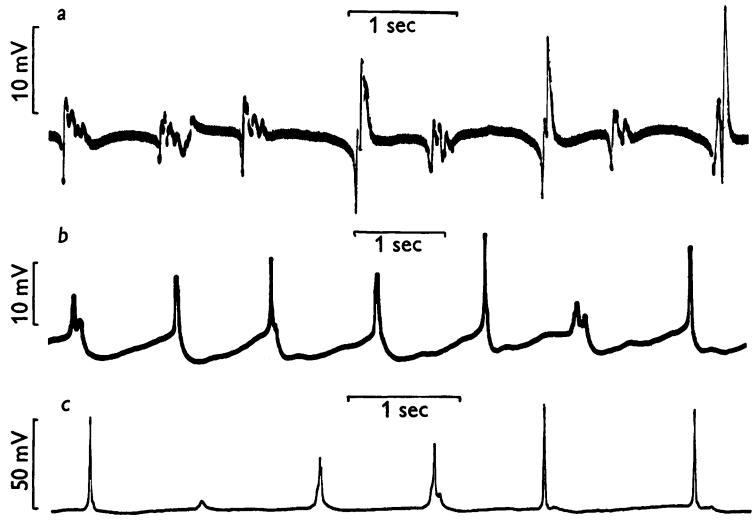


Fig. 1. Spontaneous activity of guinea-pig taenia coli. (*a*) Extracellular diphasic recording with wicks in oil (electrode separation 2 mm; temp. 37° C); (*b*) extracellular monophasic recording with the sucrose-gap method (electrode separation 7 mm; temp. 34° C); (*c*) intracellular recording, temp. 35° C. (Records in this and subsequent figures retouched).

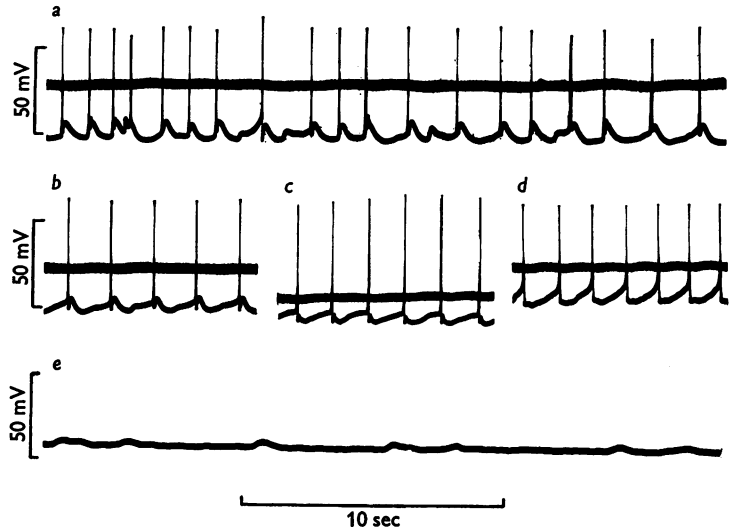


Fig. 2. Patterns of spontaneous activity recorded intracellularly. For description see text.

such records, in which the spikes were probably generated by the slow waves quite close to the point of impalement, the firing threshold was consistent; but in such records as Fig. 2*a* when the spikes were partly locally initiated, partly conducted, the firing threshold was variable. Fig. 2*e* shows slow waves in the absence of spikes. The five records serve to illustrate that spike potentials and slow waves could not be considered as separate events though they could be recorded independently (see also Figs. 15 and 16) and had a completely different time course. While the duration of the spikes was about 15 msec (mean half duration 6·75 msec according to Holman (1958)), the duration of the slow waves was 0·5–1 sec.

Extracellular stimulation

Excitability. When a spontaneously active muscle was stimulated with a single shock of 100–200 msec duration, several different effects were observed when recording extracellularly. On some occasions there was a brief disturbance of the spontaneous discharge shown by irregularity and a more complex shape of the spikes (Fig. 3*a*). On many occasions there was no detectable change (Fig. 3*b*). On rare occasions the muscle responded with a single large diphasic spike (Fig. 3*c*). Most of our preparations showed spontaneous activity even if allowed to cool to 27° C. In one preparation activity was absent and in others there were long pauses between bursts of spikes. During inactivity these preparations responded to a single stimulus with a single diphasic spike with a latency proportional to the distance from the stimulating electrode (Fig. 3*d*). If a burst of brief pulses was given (1–5 sec, at a rate of 5–15/sec, 50 msec pulse duration) an inhibition or irregularity occurred (Fig. 4). After the stimuli were stopped there was usually a short pause followed by a prolonged phase (lasting several minutes) when the frequency of the potentials was greater than normal.

If the muscle was stimulated with brief pulses at a rate approximating that of the spontaneous activity it was possible to set up a consistent response to each stimulus and to 'drive' the muscle at a constant frequency for long periods. Driving converted the most irregular discharge to a regular one. In general the first stimulus would be given out of phase with the spontaneous activity. It usually produced no effect. The second or third stimulus, however, was followed by an irregular response, and after the seventh or eighth stimulus a large regular diphasic spike appeared. The latency of the spikes and their configuration were unchanged as long as the driving was continued (over 2 hr in one experiment).

Fig. 5 shows records taken from three different preparations which were driven by extracellular electrodes. In Fig. 5*a* the spikes were recorded in oil with wick electrodes, in (*b*) the sucrose-gap method was used, and in (*c*) recording was intracellular. Under optimal stimulating conditions the greatest

negativity when recording in oil was 15 mV, with the sucrose-gap method 25 mV, and with intracellular electrodes 60 mV.

The responses to rhythmic stimulation were initiated at the cathode. The records in Fig. 6 were taken with wick electrodes, recording in oil, and show the effect of altering the polarity of the stimulus. The spikes which occurred

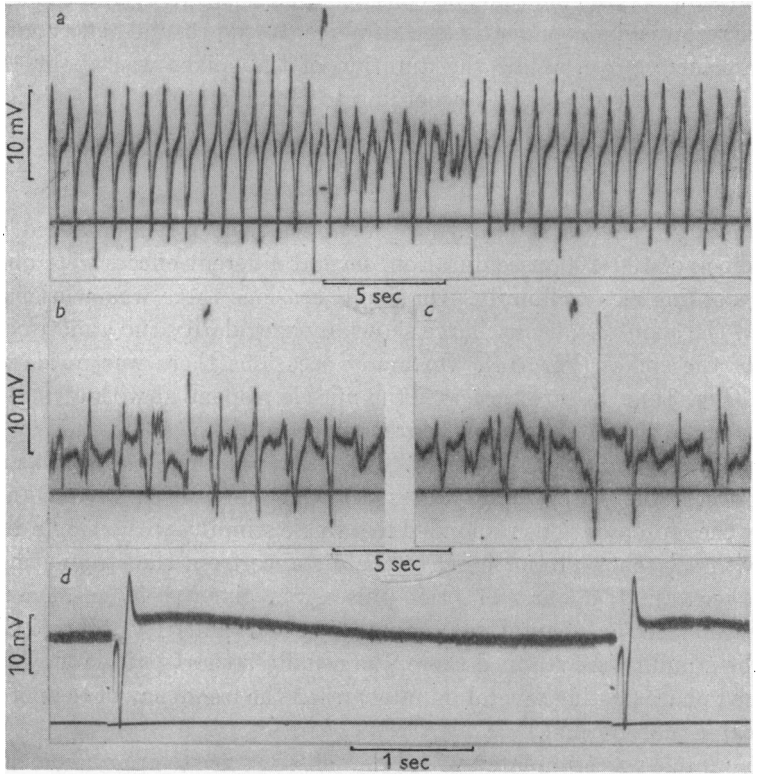


Fig. 3. The effect of applying a single stimulus: external stimulation, extracellular recording with wicks in oil. (*a*) During synchronous spontaneous activity stimulus caused brief disturbance (stimulus 150 msec, temp. 38° C); (*b*) during asynchronous spontaneous activity stimulus was ineffective; or (*c*) elicited diphasic spike (stimulus 150 msec, temp. 35° C); (*d*) during inactivity single stimulus elicited response of constant size and latency (stimulus 55 msec, temp. 33° C).

after switching from cathodal to anodal pulses were small, complex and out of phase with the stimuli. On switching back to cathodal pulses spikes reappeared in phase with stimulation.

It was found that the taenia could be driven satisfactorily over only a relatively small range of frequencies. At 34° C stimulation at frequencies of about 25% above or below the spontaneous rate was ineffective. For example, in Fig. 7 the response to different frequencies of stimulation is illustrated. A rate of 36/min was too slow to drive the spikes which were unrelated to the

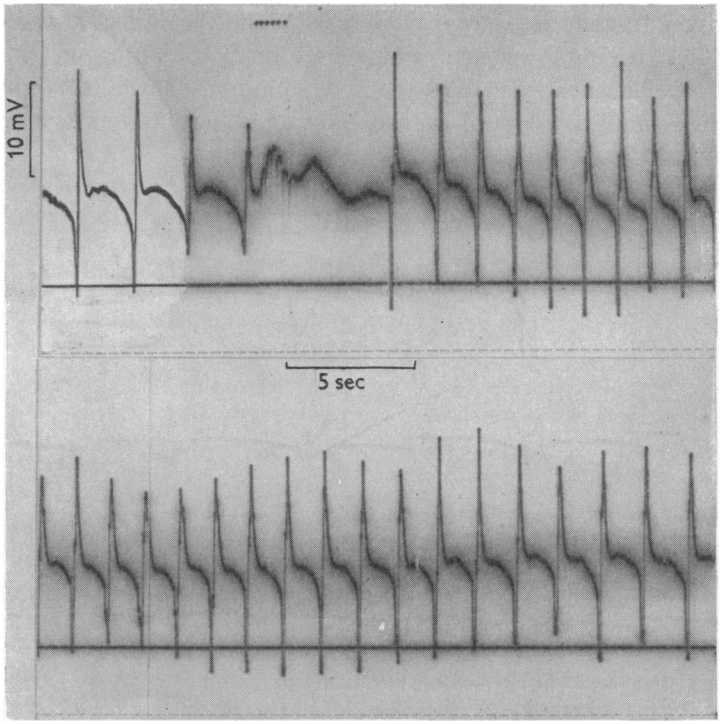


Fig. 4. The effect of applying a burst of six impulses (55 msec) at 5/sec; temp. 35° C; continuous record (external stimulation, extracellular recording with wicks in oil). Note brief inhibition and subsequent acceleration of activity.

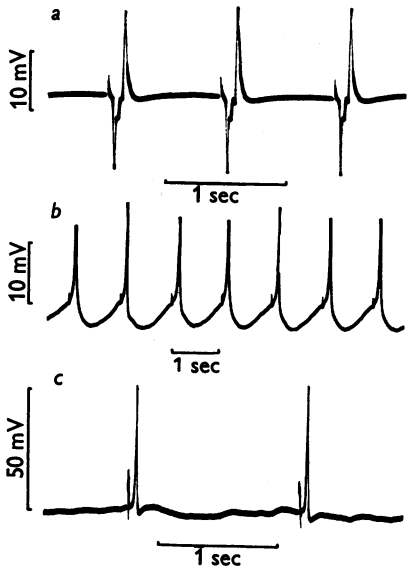


Fig. 5. Spikes in response to 'driving'. External stimulation (pulse duration 55 msec). Recording (*a*) extracellular in oil, 27° C; (*b*) by sucrose-gap method, 34° C; (*c*) intracellular, 35° C.

stimulation (*a*). Over a range of 48–72/min the muscle could be driven. At the lower driving frequencies the spikes were large and the latency short (*b*), while at the higher driving frequencies spikes were small and appeared after longer latencies (*c*). The effect of driving too fast (96/min) is shown in (*d*) where only every other stimulus produced a spike. If stimulation was very fast (132/min)

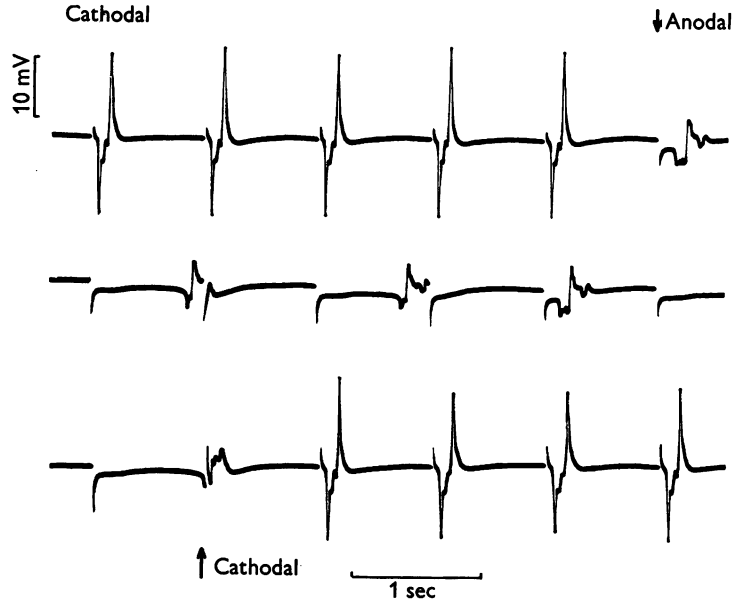


Fig. 6. The effect of reversing the polarity of the stimulus (55 msec). External stimulation; extracellular recording in oil; temp. 34° C. For description see text.

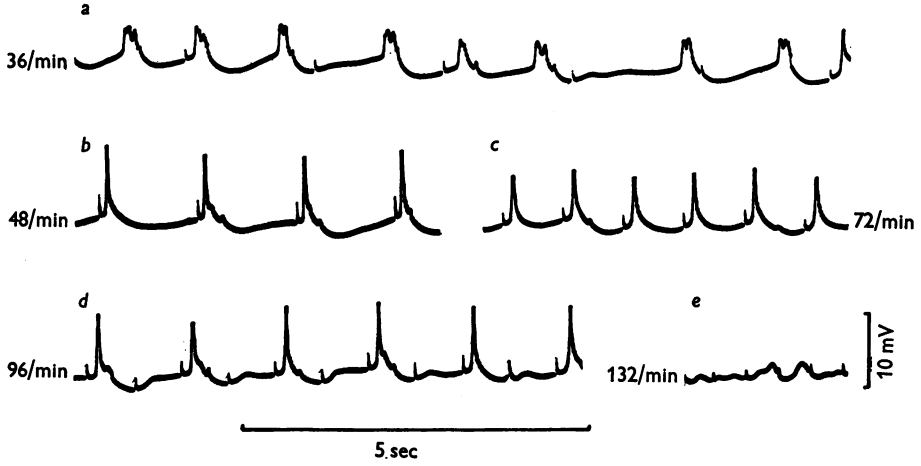


Fig. 7. The effect of different rates of a stimulation (external stimulation, sucrose-gap method). Note that preparation could be driven at a rate between 48 and 72/min. Temp. 34° C. For further description see text.

all synchronous activity was abolished (*e*). At higher temperatures the optimal driving frequency, i.e. the frequency at which the spike was maximal and the latency constant, fluctuated from time to time and was very critical. At lower temperatures the optimal frequency varied less with time and the muscles could be driven over a wider range of frequencies. The optimal driving rate fell as the temperature decreased. At 38° C frequencies of 90–100/min were required compared with 20–50/min at 30° C.

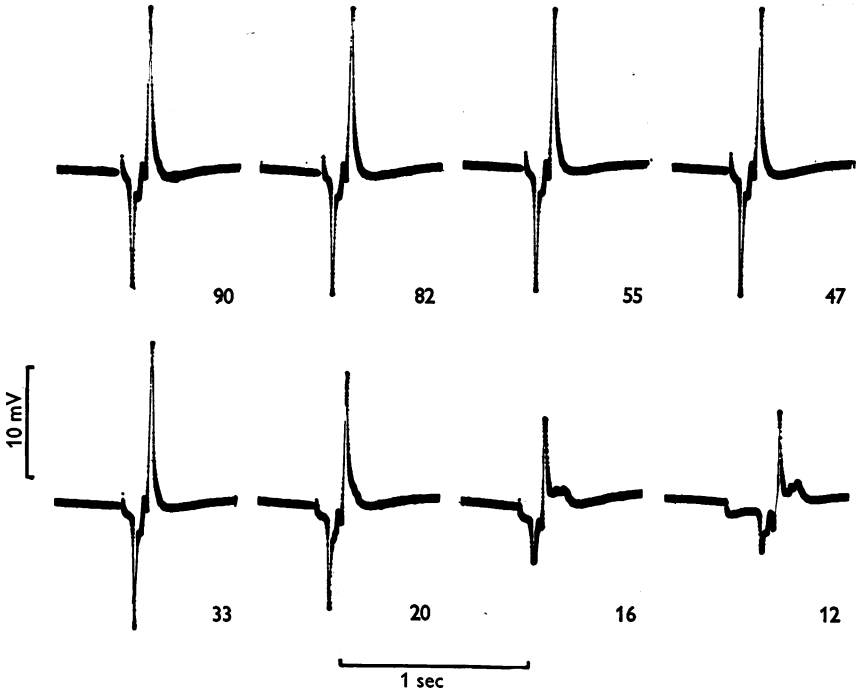


Fig. 8. The effect of changing the stimulus duration (msec, shown in each record); external stimulation, extracellular recording in oil; stimulus strength and electrode position unaltered throughout; temp. 36° C.

The value of the threshold strength and duration was variable in different preparations and also within the same experiment. Relatively large stimuli were needed to establish driving but later the duration or strength of the stimulus could be reduced without changing the response. In Fig. 8 the strength of the stimulus was kept constant while the duration was varied. The threshold duration in this experiment was between 20 and 33 msec. It can be seen that there was no decrease in latency or in spike amplitude as the duration was increased. Thus the spikes were all-or-none. Similar experiments were done in which the strength of the current was varied while the duration was kept constant.

Conduction. The latency was measured from the stimulus artifact to the foot of the spike or, in some cases, to the peak of negativity at the proximal electrode. Plots of latency against the distance from the stimulating electrode were linear for distances of over 1 cm (Fig. 9). Conduction velocity at 38° C varied from 6·7 to 8·8 cm/sec. At lower temperatures the velocity fell. Within the range of 28–38° C the Q_{10} was approximately $=2$. There was no decrement in response over distances of up to 2·0 cm, the maximum length studied.

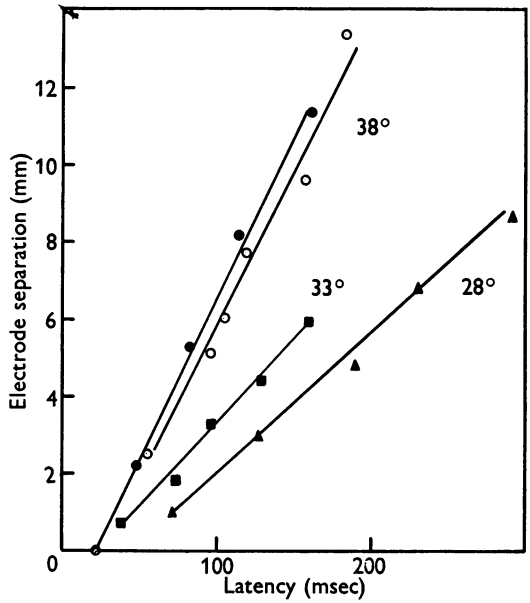


Fig. 9. Graph showing the relation between the latency, measured from stimulus artifact to the peak of negativity at the proximal electrode (abscissa), and the distance of the proximal electrode from the stimulating electrode (ordinate). Four different preparations.

For conduction over long distances the time of initiation of the spike, approximately 15 msec, is short relative to the conduction time, as can be seen from the graph. Conduction velocity was therefore calculated directly from the measurements of latency when the distance between the stimulating and recording electrodes was more than 5 mm.

Excitability and conduction were normal when nervous participation was excluded by applying atropine 10^{-5}, or by using ganglion-free strips of taenia. In one experiment a small strip of taenia was kept in modified Krebs's solution at room temperature for 27 hr. It was allowed to 'warm up' for an hour at 37° C and then set up in oil. It still showed vigorous spontaneous activity and could be driven in the usual way. Conduction velocity was normal.

Relation between the activity of neighbouring cells

Spontaneous activity. When the spontaneous activity of two cells within the same bundle and no more than 0·5 mm apart was recorded simultaneously, it was found that the spikes were generally discharged at the same frequency, but they did not necessarily coincide. This is shown in Fig. 10. At times cell *A* led cell *B* and vice versa. In addition, cell *B* appeared to be influenced by other sources of excitation than those affecting *A*. The activity in both cells was irregular, but every spike in cell *A* was associated with a small graded

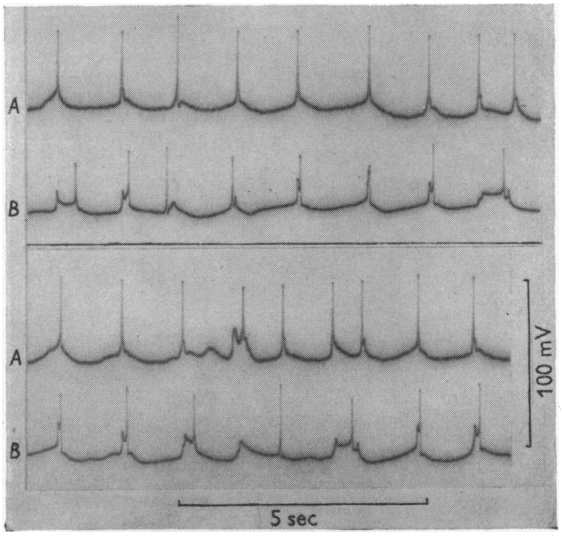
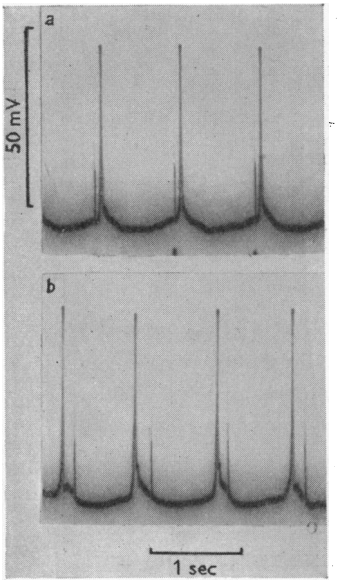


Fig. 10. Intracellular records of spontaneous activity of two cells (*A* and *B*) within the same bundle, 0·5 mm apart; temp. 35° C. For description see text.

potential in cell *B*. This either died away without initiating a spike or it led to double spikes in *B* of which either the first or the second coincided with the spike in *A*. Some of the spikes in *A* showed very pronounced pre-potentials. This and other records taken with two micro-electrodes suggested that the activity of one cell was generally reflected to some extent in the activity of another cell a few cell lengths away. The fluctuating phase difference of the two cells and the varying degree of pre-potentials suggested that the influence of local pace-makers was not fixed but fluctuated from time to time and from place to place with respect to the two cells.

Intracellular stimulation. When the stimulus was applied through a micro-electrode of low resistance (10–20 MΩ) responses could be recorded in neighbouring cells. The distance between the two micro-electrodes was determined in longitudinal direction, and also their position in relation to the fibre bundles which had an average diameter of 20μ. The responses were of three different

types. When the two electrodes were in the same bundle and not more than 0·7 mm apart, a conducted response of a short latency, comparable to that observed during extracellular stimulation and proportional to distance, was obtained (Fig. 11*a*). If, however, electrodes were placed in adjacent bundles, a conducted response of much longer latency was recorded which was also constant (Fig. 11*b*). Fig. 12 shows how, under these conditions, when the rate of stimulation was changed from 50 to 60/min the latency was not altered. When the electrodes were impaled at still greater distances, either longitudinally or laterally, a third situation was observed. Responses were recorded which did not show a constant latency. Nevertheless, an indirect influence was apparent; i.e. the rate, but not the latency of the responses, was correlated



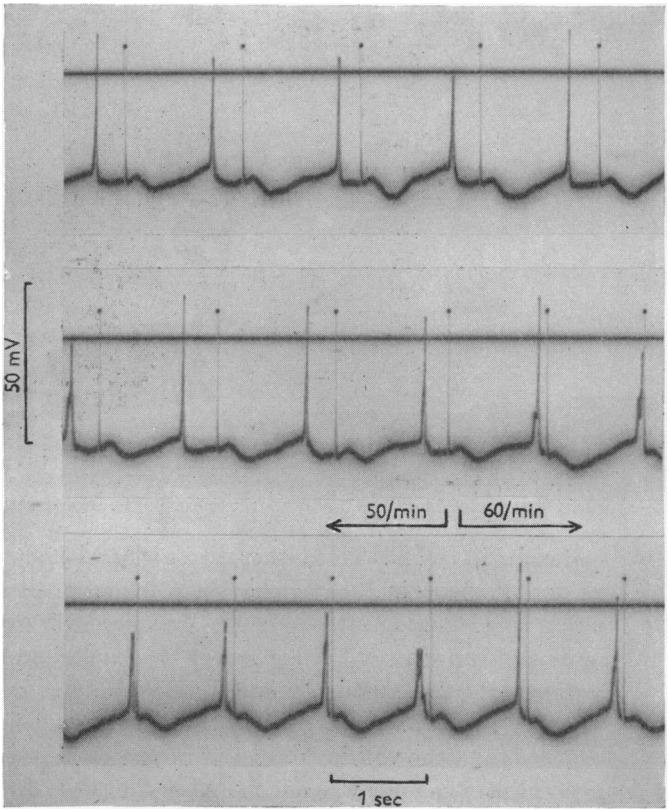


Fig. 11

Fig. 12

Fig. 11. Intracellular stimulation (55 msec pulse duration) and recording: (*a*) in the same bundle 250μ apart; (*b*) in adjacent bundle 450μ apart. Note difference in latency. Temp. 35° C.

Fig. 12. The effect of changing the frequency of intracellular stimulation (55 msec pulse duration) on the response of another cell situated in an adjacent bundle, continuous record. Note constant latency. Temp. 36° C.

with the stimuli (Figs. 13 and 14). In these conditions it was still possible to show that the activity was dependent on the stimulus, because, if the polarity of the stimulus was reversed spikes were immediately abolished and they reappeared promptly on returning to normal polarity.

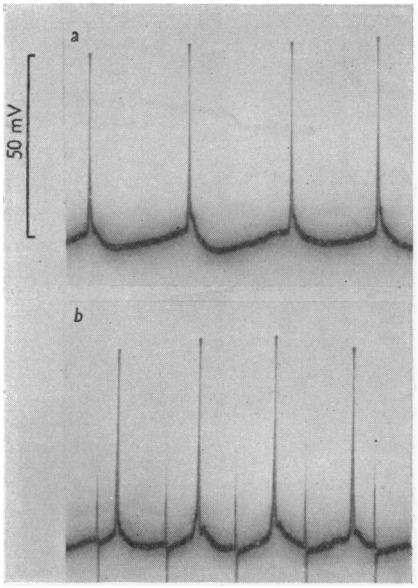


Fig. 13

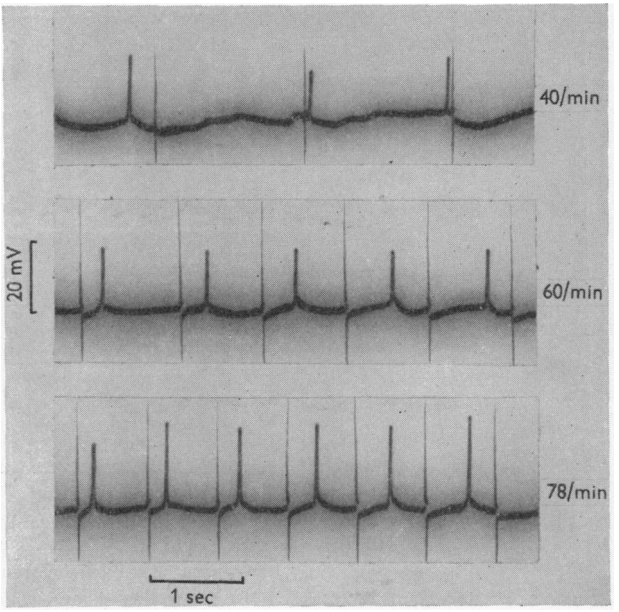


Fig. 14

Fig. 13. Intracellular recording: (*a*) spontaneous activity; (*b*) when stimuli at 70/min were applied through a micro-electrode separated by several fibre bundles, at a distance of 450 μ. Temp. 36° C.

Fig. 14. Intracellular records during stimulation (pulse duration 55 msec) through a micro-electrode separated by several fibre bundles; temp. 36° C. Note change in frequency but no constant latency.

Relation between slow waves and spikes during spontaneous activity and during electrical stimulation

Records illustrating a close relation between slow waves and spikes are shown in Fig. 15. The spontaneous activity in (*a*), consisting of a mixture of slow waves and spikes, may be compared with the activity in (*b*), when the preparation was driven. Electrical stimulation elicited slow waves and, occasionally, these gave rise to a full spike. In Fig. 15(*c*), taken from another experiment, the gradual development from slow wave to full spike in response to driving is shown.

In contrast, Fig. 16 illustrates an experiment in which the slow waves could be separated from the spikes elicited by stimulation. In (*a*) spontaneous spikes arose from the top of the slow waves. When stimulation was applied

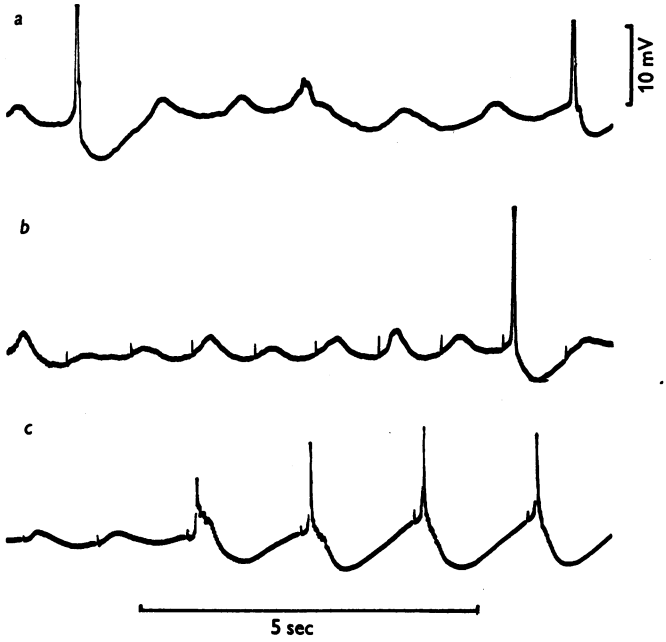


Fig. 15. Extracellular records obtained with sucrose-gap technique; temp. 34° C. (*a*) Spontaneous activity showing slow waves and spikes; (*b*) during electrical stimulation (pulse duration 55 msec) producing slow waves or spikes; (*c*) from another preparation, showing gradual development from slow wave to spike, at the beginning of driving.

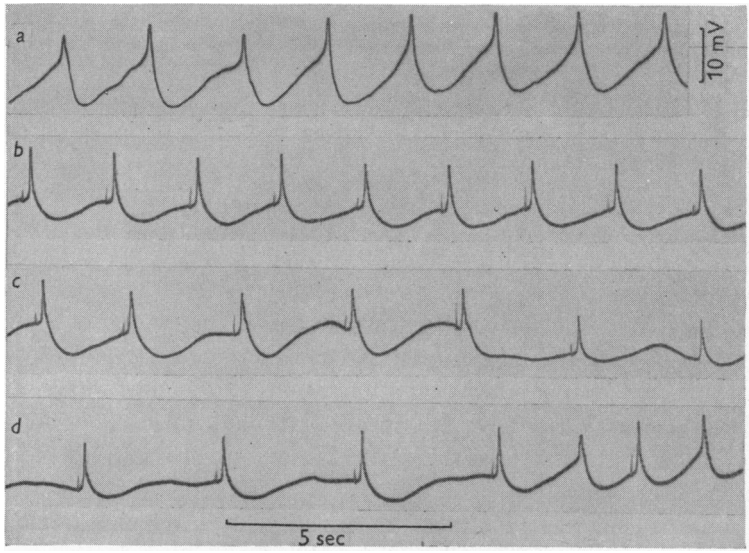


Fig. 16. Extracellular records obtained with sucrose-gap technique; temp. 34° C. (*a*) Spontaneous discharge of spikes from top of slow waves; (*b*) spikes in response to driving (pulse duration 55 msec), synchronous with slow waves; (*c*) slower rate of driving, out of phase with slow waves; (*d*) two spontaneous spikes initiated by slow waves occurring between conducted spikes at end of record. Records *b–d* are continuous.

at a frequency similar to the spontaneous rate of discharge (*b*), the spikes were still synchronous with the slow waves but did not appear to be generated by them. This became clear in (*c*), when the preparation was driven at a slower rate and the conducted spikes were out of phase with the slow waves. In (*d*) the spontaneous spikes generated by the slow waves are seen side by side with the conducted spikes set up by stimulation.

DISCUSSION

The experiments have shown, in agreement with previous workers (Bozler, 1938*a*, *b*, 1941; Greven, 1956; Prosser, Sperelakis & Bergman, 1955; Prosser & Sperelakis, 1956) that it is possible to set up a conducted response in smooth muscle as shown by the linear relationship between electrode distance and latency. The conduction velocity was found to be of a similar order as that reported for other smooth muscles.

We found that a response could be set up which was conducted without decrement over many cell lengths, provided that (1) a large number of cells were stimulated, as with external electrodes and (2) the frequency of stimulation was not more than about 20% above or below the spontaneous rate of discharge. When a preparation was inactive, which was rare, a single stimulus produced a conducted response identical with the driven responses.

A conducted response over a distance of only a few cell lengths was still obtainable if only one or a small number of cells was stimulated through a low resistance micro-electrode. In these conditions, however, two further possibilities of influencing the activity of neighbouring cells were detected. First, if the recording electrode was placed in an adjacent bundle, a response of constant, but much longer latency was recorded. This was similar to that described by Brune & Kotowsky (1956), who found a conduction velocity of 4 mm/sec for the spread of excitation during spontaneous activity in the taenia coli. Secondly, if the recording electrode was inserted at greater distances from the stimulating electrode, the rate of discharge might still be dictated by the stimulation but the latency was no longer constant. Thus, in an area of about 1·5 mm diameter, the activation of one cell influenced the behaviour of another cell either directly, or by a more devious pathway.

An influence between neighbouring cells was also noticeable during spontaneous activity. First, the spikes recorded intracellularly showed varying degrees of pre-potential and arose from different potential levels, which suggested that the spike discharge recorded in each fibre was a mixture of locally initiated and conducted activity. Secondly, the discharge in two neighbouring cells was found to be of the same frequency but not necessarily synchronous. Frequently small graded potentials were seen to occur synchronously with the spikes in the neighbouring cell. The time course of these abortive potentials was comparable with that of the spikes, but was quite different from that of

the slow waves. They appeared to be genuine membrane potentials because they could not be recorded when the electrode slipped out of the cell.

Slow potential fluctuations—the slow waves—were always seen to occur spontaneously, usually at the same rate as the spike discharge. They have previously been recorded by Bozler (1942*a*, *b*) in the intestinal muscle from several species, and recently in uterine muscle by Jung (1956) and by Melton (1957). Bozler found that spikes could be discharged simultaneously from many regions in the guinea-pig intestine, and that the origin of the discharge was continuously variable. The spikes arose from the slow potential waves which were non-conducted, regular and could be recorded 'almost continuously and from all regions of the muscle'. Greven (1953) recorded similar waves and agreed with Bozler's interpretation that they were local potentials. Intracellular records (Bülbring, 1957) supported this point of view. In the present work slow waves were recorded not only during spontaneous activity but also in 'driven' preparations. They were frequently elicited by driving. On many occasions, during spontaneous activity and also during electrical stimulation, the spikes were generated on the top of the slow waves. On other occasions the spikes appeared to be conducted and were out of phase with the slow waves.

It may be that the optimal driving frequency of the taenia was related to the frequency of the slow waves. No attempt has been made to determine the absolute refractory period, but the fact that pairs of spikes could be recorded, separated by a few msec, suggested that it was very short and comparable with the duration of the spike. The maximal rates for driving suggested that the muscle had a long relative refractory period of about 0·7 sec at 37° C.

From our experimental results it appeared that excitation, whether initiated spontaneously or imposed by driving, could spread in two ways. First, by slow waves: if these local potentials reached threshold, spikes were generated. Local depolarization might be produced by stretch deformation resulting from the activity of adjoining cells. Thus each cell might be stimulated by the contraction of the cell behind it. Such a mechanism of interfibre spread of excitation has recently also been discussed for uterine muscle by West & Landa (1956). The second mechanism is provided by the conducted response. Conduction might be expected to depend partly on the properties of the region of contact between adjacent cells—of which nothing is known—partly on excitability. An important limiting factor for conduction was the spontaneous activity of each cell which seemed to determine not only whether a stimulus could set up a response, but also whether this was 'all-or-none' or a graded potential.

The possibility of conduction through an intercellular nerve net was unlikely, since the velocity was slow and not affected by atropine 10^{-5}. Conduction was normal in very small strips from the superficial layers of the taenia

which were ganglion-free, and also in a strip which had been kept for 27 hr at room temperature.

Since there is no histological evidence for a true muscular syncytium, an alternative method must exist by which excitation can pass from cell to cell. Fatt (1954), writing about electrical transmission at invertebrate synapses, described the conditions for a high degree of interaction between cells. 'If the cells were actually touching, and if their membranes in the region of contact had a low resistance compared with that of the neighbouring parts of the cell, the synapse would serve to direct current between the interiors of the two cells while the active membrane changes would occur in neighbouring regions.' This situation is implied in Bozler's 'unitary' theory, and Prosser's suggestion of 'ephaptic' conduction is consistent with this idea. Though there is no direct proof for such a mechanism in smooth muscle, it is the simplest explanation that has been put forward and appears to provide an adequate explanation for the experimental results.

SUMMARY

1. Excitability and conduction has been studied in isolated smooth muscle preparations from the taenia coli of the guinea-pig.

2. In one series of experiments stimulation was extracellular, and recording either extracellular diphasic, extracellular monophasic (sucrose-gap method) or intracellular. In another series of experiments stimulation was applied through a low resistance micro-electrode and recording was intracellular.

3. 'All-or-none' responses were set up and conducted along many cell lengths if extracellular stimulation was applied at a frequency approximating the rate of spontaneous discharge. The conduction velocity was 6·7–8·8 cm/sec at 38° C.

4. Responses conducted at a similar velocity were also set up by intracellular stimulation but were limited to cells within the same fibre bundle and to a distance of not more than 0·7 mm. Responses conducted with much longer latency were recorded in cells situated in adjacent bundles at a distance of up to 0·5 mm. At greater distances an indirect influence was apparent, i.e. the rate but not the latency of the responses was correlated with the stimuli.

5. When two neighbouring cells were impaled simultaneously it was found that their spontaneous spike discharge was of the same frequency though not necessarily synchronous. Locally initiated spikes alternated with conducted spikes and the spikes in one cell were reflected as small graded potentials in the other cell.

6. Slow waves were frequently set up by electrical stimulation and gave rise to spikes, as during spontaneous activity. On other occasions the conducted spikes were out of phase with the slow waves. The role of slow waves for the initiation of spikes and for the spread of excitation is discussed.

7. Both excitability and conduction were normal when nervous participation was excluded (*a*) by applying atropine 10^{-5}, (*b*) by using ganglion-free strips of taenia, (*c*) by using a preparation which had been kept at room temperature for 27 hr.

8. The experiments support the view that conduction takes place by electrical transmission from cell to cell.

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