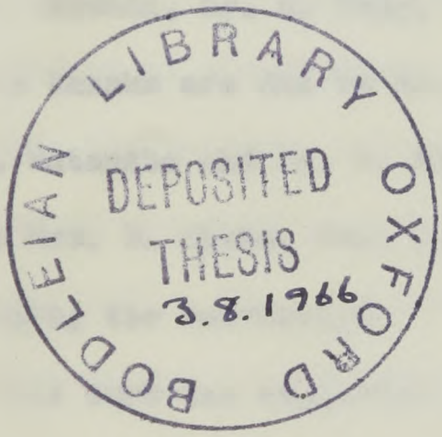
A STUDY OF THE SPECIFIC MEMBRANE PROPERTIES IN DIFFERENT TYPES OF SMOOTH MUSCLE

Thesis submitted for the degree of Doctor of Philosophy



By

TODAO TOMITA

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ABSTRACT

The primary question which I have attempted to answer by the experiments described in this thesis was: What are the electrical properties of the smooth muscle membrane?

I. Intracellular stimulation

The first approach consisted of a study of the responses to intracellularly applied currents recorded intracellularly, with the same microelectrode using the Wheatstone bridge method.

a) Effect of intracellular current application on single cells.

The experiments were carried out on the spontaneously active taenia coli of the guinea-pig. The spontaneous discharge consisted of a slow and a fast (spike) component. They were clearly related. The slow component usually preceded the spike, often triggered it but not always, and persisted as a depolarization delaying the polarization of the membrane following the spike.

When the membrane was depolarized by the internal current application the spike component became smaller and its rate of rise decreased while, when the membrane was hyperpolarized, the spike component became larger and its rate of rise increased. With very strong hyperpolarization the spike component was blocked. This change of the spike amplitude and its rate of rise was just as expected from our knowledge of the effect of the membrane potential on the sodium carrying system in other excitable tissues. However, the slow component was scarcely affected by the membrane polarization and no frequency modulation of the spontaneous discharge was observed. Even when the spike component was blocked by strong hyperpolarization,

the size and frequency of the slow component remained normal.

The effective membrane resistance, calculated from the current-voltage relationship, was about 40 MΩ. The time constant of the electrotonic potential was 2-4 msec.

In the majority of the cells, no active response was observed in response to intracellular stimulation, even though a normal resting potential and spontaneous spikes with overshoot were recorded with the same micro-electrode which was used for stimulation. Only occasionally a graded action potential was produced. The amplitude of the evoked spike was increased by conditioning hyperpolarization exactly like that of the spontaneously generated spike.

b) Electrical interaction between cells.

For these experiments, two independent Wheatstone bridge circuits were used, one for each micro-electrode. Since the spontaneous electrical activity of two cells within 50 μ separation was well synchronized, it was expected that there would be electrotonic spread between cells. However, intracellular current application to one cell had almost no effect on the membrane potential of adjacent cells. Even if the stimulating current evoked a spike, no effect was observed in a neighbouring cell.

From the results of these experiments it is difficult to explain how the spike is evoked, what the slow component is, or how the spike propagates from cell to cell. The possibility suggested itself that the cell membrane is not homogeneous but composed of different areas with different properties and, furthermore, that a single cell is not

the unit of excitation. If so, external field stimulation might produce more information than intracellular stimulation.

Therefore, the second approach in the investigation of the membrane properties of the smooth muscle was made by external current application.

II. External Field stimulation in normal Krebs solution

Large stimulating electrodes were used, the arrangement of which was varied according to the experimental requirements.

a) Polarization of the membrane by long current pulses.

When current was applied externally, the spontaneous spike frequency was modulated. In contrast to intracellular stimulation, depolarization increased the frequency of the spike discharge while hyperpolarization decreased it. Moreover, depolarizing current easily triggered a spike in every cell.

These phenomena might involve the effect of nerve stimulation because the tissue has intrinsic nerve fibres. In fact, it has been shown that a short current pulse (< 1 msec) applied externally causes a transient hyperpolarization ("inhibitory potential") of about 1 sec duration and a latency of 100-200 msec. Therefore it was necessary to discriminate the direct response of the muscle membrane to external stimulation from the indirect (nerve mediated) response.

b) The inhibitory potential

When two stimulating electrodes were placed, one at each end of the tissue, which was therefore exposed to a uniform longitudinal potential field, the inhibitory potential behaved quite differently

from the response of the muscle membrane to polarization.

Though the muscle membrane was depolarized near the cathode, hyperpolarized near the anode and not polarized in the centre of the tissue (about 3 cm length), the inhibitory potential was recorded everywhere with the same amplitude in response to both polarities of the stimulating current if its parameters were kept constant. If the length of the tissue was reduced to about 1 mm, the spike became graded and threshold current intensity was increased, while the inhibitory potential was produced with little change in size and threshold current intensity.

The strength duration curves for the inhibitory potential and for the spike were obtained. The chronaxie for the inhibitory potential was about 1 msec and that for the spike was about 30 msec. A short current pulse of less than 1 msec produced only the inhibitory potential but not the spike. Tetrodotoxin (10^{-7} g/ml) abolished the inhibitory potential without affecting the resting potential nor the spontaneous and evoked spike potential of the muscle fibre.

From the results, the inhibitory potential can be explained as a response to stimulation of nerve fibres and can easily be discriminated from the direct response of the muscle fibres.

III. External field stimulation

Hypertonic solution (Krebs solution containing 10% sucrose) abolishes the spontaneous spike activity. A normal spike can still be evoked but there is no mechanical response. The inhibitory potential is abolished within 20 min. Therefore, the membrane properties can be

studied using external current application without interference from spontaneous activity, or movements, or the inhibitory potential.

a) Electrotonic potential

Interpolar polarization was studied with preparations of more than 10 mm length placed between two stimulating electrodes, one at each end. It was found that cells near the cathode were depolarized, those near the anode were hyperpolarized and those in the middle part of the tissue were not polarized.

When an insulating partition was placed between the stimulating site and the recording site, the electrotonic potential not only decreased in size roughly exponentially with the distance from the partition, but also in its rate of rise and fall. It was also found that the electrotonic potentials had a time course similar to that predicted from the cable theory applied to a nerve fibre. The space constant of the electrotonic potential was 1.6 mm (range 1.4-1.9 mm); the time constant was 60-100 msec.

b) Spike generation and propagation

When the membrane was depolarized by about 25 mV, a spike was evoked in every cell. The minimum longitudinal potential gradient to produce the spike was 2-3 mV/100 µ. Since the spike was propagated over the whole tissue (conduction velocity: 7 cm/sec) and the electrotonic potential decayed with a space constant of 1.6 mm, the apparent firing level was very low far away from the stimulating electrode.

There were some qualitative differences between the responses to

long and short stimuli. When the stimulating current was short (less than 10 msec), the spike was produced in a graded manner with a short latency. Spikes thus produced did not show a clear slow component. When the stimulating current was long (more than 20 msec) the spike appeared in an all-or-none manner and its latency was very long (up to 1 sec) at threshold intensity.

During external conditioning hyperpolarization the spike amplitude was decreased stepwise and notches or humps appeared on the rising or falling phases of the spike.

These phenomena may be related to the different mechanisms and sites involved in the generation of the slow component and the spike.

On the basis of the results obtained by intracellular and external current applications, the following two hypotheses are presented. One is that the smooth muscle cell membrane is not homogeneous but composed of areas with different electrical properties. The second is that many cells are electrically interconnected forming a functional bundle.

IV. Evidence for different areas in a single cell membrane

Since the properties of the electrotonic potential produced by field stimulation (See V) are direct evidence that cells are electrically interconnected and act as a functional syncytium, there must be some membrane areas which act as a current pathway between cells.

Spontaneous electrical activity consists of two clearly different components, the slow and spike components. Intracellularly applied current affects only the spike component. Frequency modulation is

observed only by external but not by intracellular current application. These results indicate that the slow component is generated in a different area from that in which the spike is produced, and that this area which generates the slow component must be near the area which acts as current pathway between cells.

In smooth muscle the spike amplitude is small and its rate of rise is slow compared with most other excitable membranes. The spike is insensitive to the external Na concentration, and it is very difficult to trigger a spike by intracellular current application. These facts suggest that the smooth muscle membrane is less excitable probably due to a poorly developed sodium carrying system.

The hypothesis is as follows (Terms A, B and C are introduced for convenience):

Areas A may be the nexuses i.e. patches of membrane very close to neighbouring cells. When a external current is applied longitudinally to the tissue, most of the current flows through these areas from cell to cell.

Areas B may surround A. They would produce the slow component and have a long time constant. Current passing through area A might affect area B. If this is depolarized, a local potential, the slow component, is initiated. If it is hyperpolarized, the slow component is suppressed. Hence the frequency modulation.

Area C may be the remaining part of the membrane. It would produce the spike when the slow component has reached threshold level. It would have a shorter time constant than area B and might be

electrically less excitable. During intracellular stimulation, most of the current would flow through area C.

On the basis of the hypothesis, when a long current pulse is applied externally, area B which has a long time constant is excited and a slow component is produced which then generates an all-or-none spike. However, when a short current pulse is applied through an external electrode, this only stimulates area C which has a short time constant and produces a spike in graded manner.

V. Evidence for electrical interconnections between cells

Although the electrotonic potential produced by intracellular current application is not detected in the neighbouring cell, the facts that the space constant (1.6 mm) of the electrotonic potential, produced by external current application, is much longer than the cell length (100 μ), and that interpolar polarization is the same as that expected from a long core conductor, are direct evidence for the electrical interconnections between muscle fibres.

Furthermore, from the minimum longitudinal potential gradient necessary to produce the spike (2-3 mV/100 μ) and the critical firing level of the cell membrane (about 25 mV), it must be assumed that the fibres are interconnected, because the potential gradient per cell length (100 μ) is too small to produce a depolarization of 25 mV.

The failure to obtain evidence for cell-to-cell interaction by intracellular current application is not necessarily a contradiction to the idea of the presence of interconnections between cells, because

the spatial decay of the intracellularly applied current would be very sharp due to the three-dimensional current spread in a muscle bundle composed of many aggregated cells.

This situation was simulated using analogous electrical model circuits and the results obtained with these models agreed with the above prediction, i.e. the spatial decay of the current distribution becomes very sharp if there are interconnections between cells.

VI. Deduction of the membrane parameters from direct and indirect evidence.

The membrane resistance could not be measured directly because, when current was applied intracellularly, there was no accurate method of calculation due to the three-dimensional current spread, and because, when current was applied externally, the intensity of the current which passed through the membrane could not be measured with the present method. However, it may be possible to estimate the membrane resistance if the membrane capacity of the taenia is assumed to be the same as that of the slow skeletal muscle fibre (about 2 $\mu F/cm^2$) because both muscle types have no sarcoplasmic tubular system which causes the high membrane capacity in the twitch fibre. From the capacity of 2 $\mu F/cm^2$ and the time constant of 60 - 100 msec, the membrane resistance of the muscle fibre is calculated to be 30 - 50 $K\Omega\ cm^2$ which is of the same order as that of the slow skeletal muscle fibre (28 $K\Omega\ cm^2$). From the data of K^{42} efflux, a high membrane resistance (7 - 27 $K\Omega\ cm^2$) is also predicted. The low K permeability may be responsible for the high membrane resistance and also for the

high ratio of Na permeability to K permeability compared with other excitable membrane. The smooth muscle fibres are aggregated in functional bundles of about 30 μ diameter. The capacity of a bundle would then be 12 $\mu F/cm^2$ (because of the large surface area) and the membrane resistance of the bundle would be 5 - 8 $K\Omega\ cm^2$.

VII. Final conclusion

In spontaneously active muscle (guinea-pig taenia coli), intracellular stimulation causes no frequency modulation; the time constant of the electrotonic potential is 3 msec; a spike is triggered only occasionally; it has no slow component; and no electrotonic spread is detected in adjacent cells. On the other hand, external current application modulates spike frequency; a spike with a slow component is evoked in all cells; and the electrotonic potential fits the cable theory. The space constant is 1.6 mm and the time constant is 60 - 100 msec.

The difference between intracellular and external stimulation could be explained by differences in current distribution, if many fibres with three-dimensional inter-connections were aggregated to form functional bundles, and the possibility that a single muscle fibre membrane is composed of areas with different properties. Participation of many muscle fibres appears to be necessary for spike production and propagation, the fundamental mechanism being electrotonic spread, as in the ephaptic junction between neurones of low animals. A single muscle fibre of the taenia has several such junctions,

and the electrotonic spread from many surrounding cells must summate to generate first an active slow component, which then triggers the spike.

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Chapter I

Introduction

Knowledge of the electrophysiology of smooth muscle is far behind that of striated muscle. This is probably due to the small size of the individual cells and the complexity of their arrangement in the structure of the smooth muscle organs. Spike conduction in vertebrate skeletal muscle is explained by the local circuit current along the membrane of the fibre which has core conductor (or cable) properties (Fatt & Katz, 1951) like a nerve fibre (Hodgkin & Rushton, 1946); and each fibre can be activated independently from other fibres. Since smooth muscle usually consists of many short fibres (100-200 μ in length) in series and in parallel, the excitation necessary for physiological function must be transmitted from cell to cell.

Bozler (1948) tried to classify vertebrate smooth muscle into two categories: (1) multiunit smooth muscle in which excitation is conducted along nerves and (2) unitary muscle in which it is conducted from muscle fibre to muscle fibre. The first category includes such muscles as the nictitating membrane, iris, cilliary muscle, and pilomotor muscle, and the second category includes most visceral smooth muscles, those of the digestive tract, ureter, and uterus. However, it may be that a fundamental mechanism for the spread of excitation is common to all smooth muscle and that any differences between different types of smooth muscle can be related to additional factors, such as the structural arrangement of the fibres and the density of their innervation.

The relation of the individual smooth muscle cells to each other is of great functional importance. Is there a true syncytium, i.e. a protoplasmic continuity, between fibres? If not, what is the nature of the interrelation between cells which allows the whole muscle to act as a 'functional syncytium'?

The term 'intercellular bridge' was first used to describe an apparent connection between smooth muscle cells as seen with the light microscope (McGill, 1909). Early electron microscope studies, however, disagreed as to whether there was complete protoplasmic continuity across the process towards a neighbouring cell (Thaemert, 1959) or whether the process or 'bridge' was spanned by intact membranes (Prosser, Burnstock & Kahn, 1960). Yamamoto (1960) working with human processus vermiformis, Caesar, Edwards & Ruska (1957) working with mouse urinary bladder and uterus, and Gansler (1961) working with rat uterus and guinea-pig colon, failed to find intercellular bridges of any kind but they did see areas of close contact between membranes of adjacent cells.

Regions of fusion of plasma membranes of adjacent smooth muscle cells were described in smooth muscle incubated in a physiological solution and fixed with permanganate (but not with OsO_4). (Dewey & Barr, 1962, 1964). The structure at the region of fusion was termed a 'nexus'. The nexus appears as three dark lines of uniform thickness separated by two light lines. The overall thickness of the nexus is remarkably constant (about 140 A).

In investigations of the intestinal longitudinal smooth muscle of the mouse, Rhodin (1962) estimated that each cell makes at least 150

lateral side contacts with neighbouring cells. The surface area of each contact was approximately 0.13 square microns. The summed surface area of contact was then about 20 square microns. Since an intestinal smooth muscle cell has a total surface area of about 400 square microns, it may be assumed that at least 5 per cent of this area has contact with the membranes of neighbouring cells.

At the present stage of our knowledge it is difficult to correlate the electrophysiological results with the histological observations. Prosser (1962) proposed the following possibilities for spike propagation through smooth muscle. (1) Conduction mediated through nerve fibres which run parallel to the muscle fibres, (2) Conduction by mechanical pull from muscle fibre to muscle fibre, (3) Conduction by a chemical transmitter between muscle cells, and (4) Electrical conduction through the smooth muscle as in a functional syncytium. The last possibility is generally throught to operate in visceral smooth muscle, i.e. spike propagation by local circuit current through low resistance connections between cells (Prosser, 1962; Nagai & Prosser, 1963a, b; Barr & Berger, 1964). However, so far, there is no direct evidence for this idea. Moreover, the lack of a morphological syncytium (Caesar, Edwards & Ruska, 1957; Dewey & Barr, 1962; Prosser, Burnstock & Kahn, 1960) and the very weak electrotonic interaction between neighbouring cells as measured by intracellular micro-electrodes (Sperelakis & Tarr, 1965) seem to be evidence against the above hypothesis.

The main aim of this thesis was to throw light on the conduction

mechanism of the spike in visceral smooth muscle. In order to do this the electrical properties of a single muscle cell had to be analysed first (Chapter III). It was surprising to find that most of the cells examined produced no spike in response to intracellular stimulation (Chapter III, B) and that almost no electrotonic spread was observed between cells if current was applied intracellularly (Chapter III, C). These results were the opposite to what had been expected from previous studies of the spontaneous electrical activity.

Experiments were then carried out in which current was applied externally using large stimulating electrodes and an insulating partition between stimulating and recording sites, to eliminate the stimulus artifact (Chapter II). It was then found than an electrotonic potential was easily observed, with a spatial decay which had a space constant of 1.6 mm (Chapter V, B), and that a spike was produced in every cell in response to external stimulation (Chapter V, C).

The results obtained with external stimulation strongly suggested that the tissue had cable properties and that the muscle fibres were electrically interconnected as a functional bundle (Chapter VI, B). The conduction mechanism in such a bundle, therefore, appeared to be similar to that in a single nerve or skeletal muscle fibre. However, a complication, possibly arising from the complex structure, was that the electrical activity, not only the spontaneous discharge but also that evoked by external stimulation, consisted of a slow component and a spike component. The experimental results suggested the possibility that different parts of the cell membrane might have different electrical

properties (Chapter VI, A).

The question remained why the response to intracellular stimulation was so different from that to external stimulation. This can also be explained by the arrangement of many cells with three-dimensional interconnections as a functional bundle. If such bundles are stimulated externally, the current spreads only in one direction, whereas if the current is applied to one single cell it spreads in all directions (Chapter VI, B).

When external field stimulation is applied, it is natural to assume that muscle fibres as well as nerve fibres are stimulated, because the muscle receives innervation. Current conceptions of the structure of the peripheral components of the autonomic nervous system are not clear and the electrophysiology of the terminals is not known. Another section of this thesis, therefore, was concerned with distinguishing the direct responses of the muscle fibre to external field stimulation from the responses mediated by the nerve fibres (Chapter IV).

The experiments were confined almost entirely to one type of smooth muscle, the taenia coli of the guinea-pig, because the taenia is a typical example of visceral smooth muscle with parallel fibres and few other tissue components. It has spontaneous activity, its mechanical contraction is slow and nervous contribution to excitation is negligible.

As an example of another type of the smooth muscle, a few experiments were carried out on the vas deferens of the guinea-pig.

It has no spontaneous activity, its contraction is quick and it is excited by nervous stimulation. Nevertheless it was found that the main feature of the passive membrane properties were similar in both types of smooth muscle.

Some of the results included in this thesis have been published (Kuriyama & Tomita, 1964a, b, 1965; Bulbring, Kuriyama & Tomita, 1965).

Chapter III

Method

The smooth muscle of isolated taenia coli of the guinea-pig was used as described by Bülbring (1954). Pieces of 4-6 mm length were usually mounted isometrically, slightly stretched, the central portion being immobilized by tying the underlying circular coat on a perspex ring. The preparation was placed in an organ bath, through which oxygenated Krebs solution flowed continuously. The organ bath was 7 mm in depth and surrounded by a warming bath filled with oil, the temperature of which was kept constant at 42°C, using an electric heater with negative feed-back control. Krebs solution entered the organ bath through a polythene tube (2 mm in diameter) of about 20 cm length which was immersed in the warming bath. With a flow rate of 2 ml per min, the temperature of the solution near the preparation was about 36°C. Slight differences in temperature between the site where the solution entered the organ bath and the site where it was sucked out in order to maintain a constant level of solution did not seem to affect the results.

A modified Krebs solution of the following composition was used (mM): Na 137.4; K 5.9; Ca 2.5; Mg 1.2; Cl 134; HCO_3 15.5; H_2PO_4 1.2 and glucose 11.5; equilibrated with 97% O_2 + 3% CO_2.

Hypertonic solution was made by adding 6 - 10 g sucrose to 100 ml Krebs solution. Since the muscle movements were abolished in hypertonic solution, the perspex ring for fixing the preparation was usually not

used. This did not affect the results.

All records were taken intracellularly using capillary micro-electrodes of 40 - 80 MΩ filled with 3 M KCl. The micro-electrode was suspended from a silver wire of 100 μ diameter according to the flexible method described by Woodbury & Brady (1956). The micromanipulator was of the type designed by Matthews (1952).

(Fig. 1) A) Intracellular stimulation. A single intracellular micro-electrode was used for electrical recording as well as for stimulating by means of the Wheatstone bridge method (Araki & Otani, 1955). Fig. 1 is a block diagram of the experimental arrangement. In order to supply a constant current into the cell the resistance of one bridge arm, in series with the micro-electrode, was 1000 MΩ which was very high compared with that of the micro-electrode (40 - 80 MΩ). The range of applied current was between 10^{-10} and 10^{-8} A. The current intensity was calculated from the division of the voltage applied to the bridge by 1000 MΩ plus the electrode resistance. The error in this calculation introduced by a possible resistance change of the micro-electrode during the experiment was probably less than 10% since the high resistance of one bridge arm was in series with that of the micro-electrode. Before inserting the micro-electrode into a cell the bridge was balanced so that the potential changes produced in the micro-electrode were zero when the current pulse passed through the electrode. However, since the resistance of the electrode was usually increased by its insertion into the cell and by passing a strong current through it, the potential shift observed by applying a current was too large and

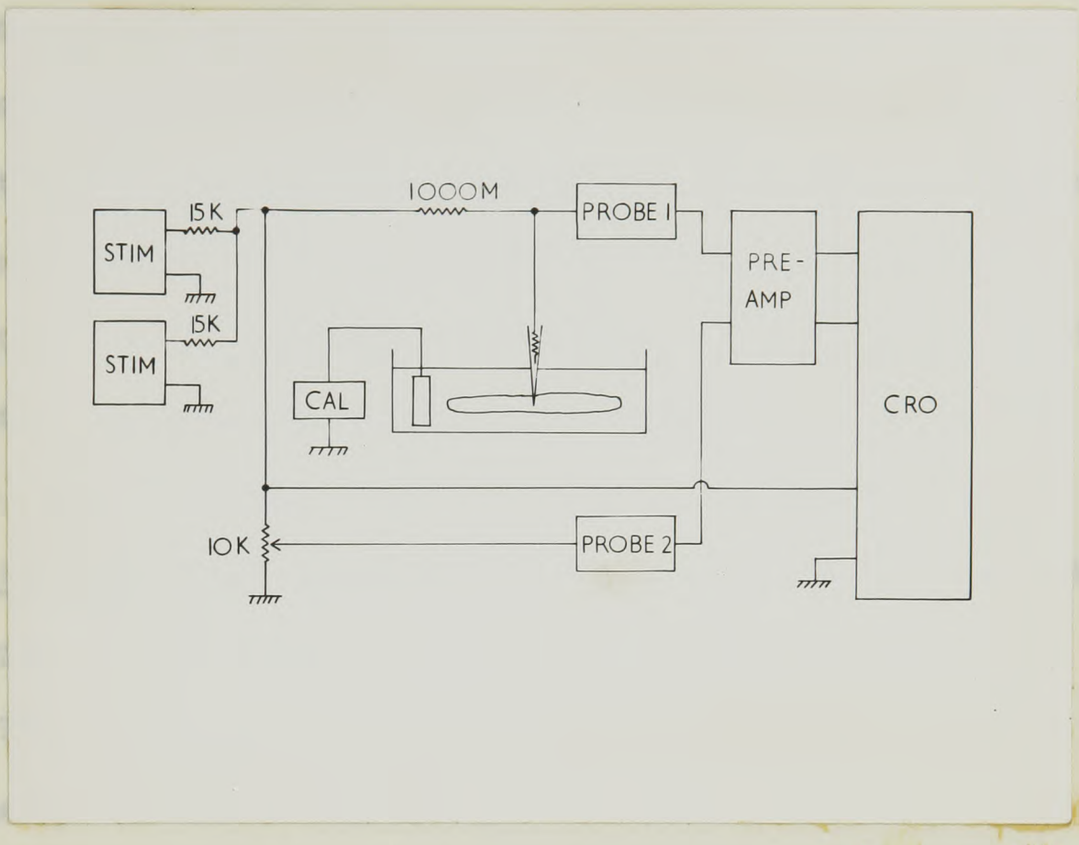


Fig. 1. Diagram of the experimental arrangement used for recording and stimulating with a single micro-electrode. The circuit consists of a Wheatstone bridge, one arm of which is composed of 1000 MΩ and the micro-electrode, and the other arm is a 10 KΩ potentiometer. By adjusting the potentiometer, the output potential of the pre-amplifier displayed on one beam of the cathode-ray oscilloscope (CRO) was reduced to zero when the stimulating current was applied to the bridge, with the micro-electrode in the bathing solution. The output of each of the two isolators of the two stimulators (Stim) was supplied to the bridge through a 15 KΩ resistor in order to reduce the interaction between the two isolators. The current intensity was calculated from the supplied potential which was displayed on the second beam of the CRO.
A calibrating potential (Cal) was applied between the bath and ground.

could therefore not always be taken as the true measurement of the potential change across the membrane.

When electrical interaction between cells was studied two independent bridge circuits were used, one for each micro-electrode. In these experiments, when the stimulus was strong, it was very difficult to eliminate completely the artifact which was produced by an interaction between the recording system of one bridge and the stimulator (especially the isolator) of the other bridge.

(B) External field stimulation. Three different arrangements for electrical stimulation were used.

(1) The taenia of various length (10 - 28 mm) was placed longitudinally between two Ag-AgCl plates (3 x 4 mm), one at each end. The distance between the electrodes and the end of the tissue was usually 1 - 2 mm at both ends. The distance became greater when the effect of reducing tissue length was studied, the inter-electrode distance being kept at 30 mm.

(2) Two rings of platinum wire (of 0.4 mm thickness), 3 - 5 mm apart, were placed round one end of the tissue.

In (1) and (2), the potential shift due to the stimulating current was reduced to a minimum by appropriate spacing of the indifferent electrode in relation to the micro-electrode before it was inserted into the cell. If the artifact was still recorded extracellularly its amplitude was subtracted from the intracellularly recorded potential.

(3) Two rings of platinum wire were placed round one end of the

tissue. This was then put through a small hole in a celluloid film which served as an insulating partition between the stimulating site and the remaining length of the tissue from which records were taken. The partition abolished the artifact caused by the external electrical field. Sometimes only one stimulating electrode was placed on the tissue and the other was placed a few mm away in the bathing solution.

Since the stimulating electrodes were immersed in the bathing solution most of the current flowed through the solution and absolute values for the intensity of the stimulating current which flowed through tissue cannot be given. Relative values of stimulating current intensity were obtained by recording the potential gradient in the solution with two silver electrodes, 2 mm apart, placed in the stimulating bath. The maximum voltage applied to the stimulating electrodes was 30 V.

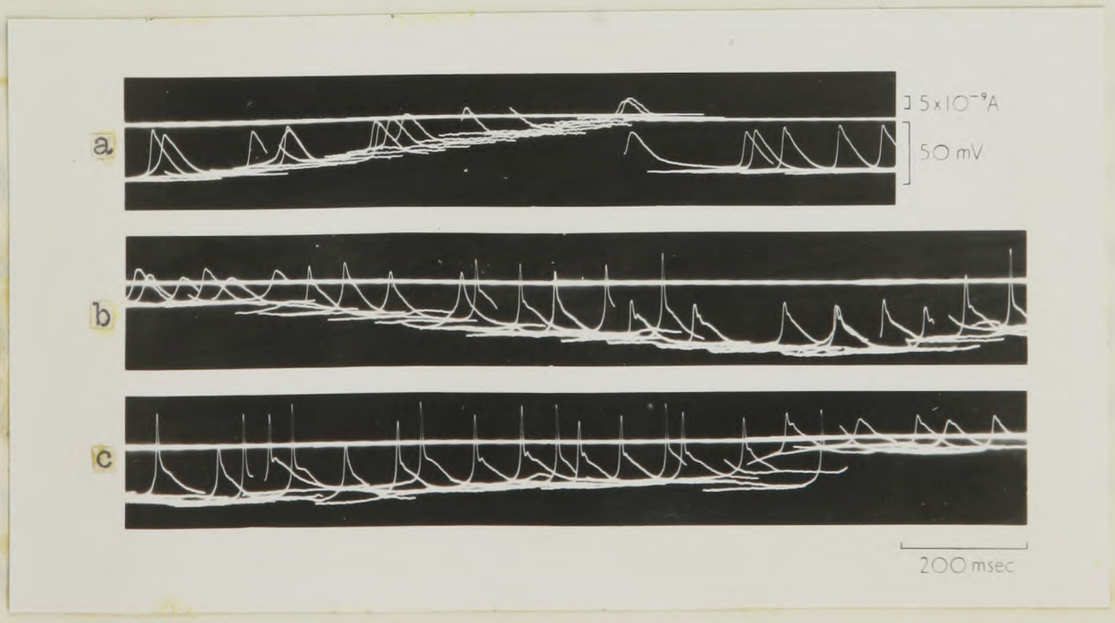
Chapter III

Intracellular stimulation

(A) The effect of polarizing currents on the spontaneous electrical activity. In the taenia coli, the spontaneous electrical activity consists of slow potentials and spikes. The slow potential has been called the local potential, slow wave or generator potential (Bülbring, 1956, 1959; Holman, 1958; Bülbring & Kuriyama, 1963). In the present thesis it will be called the 'slow component' hereafter, in contrast to the spike component. Its amplitude and its shape varies not only from preparation to preparation, but also from cell to cell and it is sometimes difficult to distinguish clearly between the slow component and the spike component, especially when the spike is abortive.

Fig. 2 shows the effect of polarising currents on the spontaneous electrical activity of two different fibres. The upper trace monitors the current, the shift of the trace being small due to the low gain. In both fibres a large slow component with a small spike was seen in the normal condition, and the amplitude of both components fluctuated spontaneously. When a depolarizing current of gradually increasing intensity was applied through the micro-electrode (a), the spike components became smaller and disappeared. The amplitude of the slow component was relatively less reduced but, if the current intensity was sufficiently strong, the slow component became very small. The rate of rise of the slow component was reduced by depolarization, but

Fig. 2. Effect of intracellular current application on spontaneous electrical activity. Upper trace = current intensity. Lower trace = intracellular records. (a): Effect of depolarizing current. The current intensity was gradually increased and then suddenly returned to zero. (b) and (c): Effect of hyperpolarizing current. In (b) current intensity was gradually increased and slightly decreased towards the end of the record, and in (c) hyperpolarizing current was at first gradually decreased, then suddenly returned to zero. (b) and (c) show parts of a continuous record taken from the same fibre, (a) from a different fibre in the same preparation. Records were taken by fast sweeps (50 msec/cm on the screen of the CRO) on a moving film (5 mm/sec). The time scale is the sum of both and refers to individual spikes, but not to spike frequency. Note the increase of spike amplitude during hyperpolarization, and then block of the spike components with very strong hyperpolarization, but little change of the slow component.



the rate of fall was less affected. On the other hand, a hyperpolarizing current of gradually increasing intensity increased the amplitude and the rates of rise and fall of the spike, while the slow component remained almost unchanged (b and c). These effects were completely reversible when the current was discontinued. When a strong current was applied causing hyperpolarization beyond a critical level, which was about 40 mV more negative than the normal membrane potential, most of the spike component was blocked (b), while the slow component remained unaffected. The properties of the slow component were thus quite different from those of the spike component.

In some cells, the spike component was clearly distinguishable from the slow potential as shown in Figs. 3 and 4. Sometimes the amplitude of the slow potential decreased spontaneously and then no spike was generated. Polarizing current had two different types of effect on such fluctuating electrical activity. One type is shown in Fig. 3. The polarizing currents changed only the amplitude of the spike but not that of the slow component. Neither was the fluctuation of the spike generation influenced by depolarization or by hyperpolarization. The other type of polarizing effect is shown in Fig. 4. In this preparation, the spike generation was affected by polarization. When the membrane was depolarized the slow component triggered a spike almost every time, and when the membrane was hyperpolarized the spike component was dropped out more frequently. The slow component also seemed to be larger with hyperpolarization, although the peak might be the remaining part of the spike component.

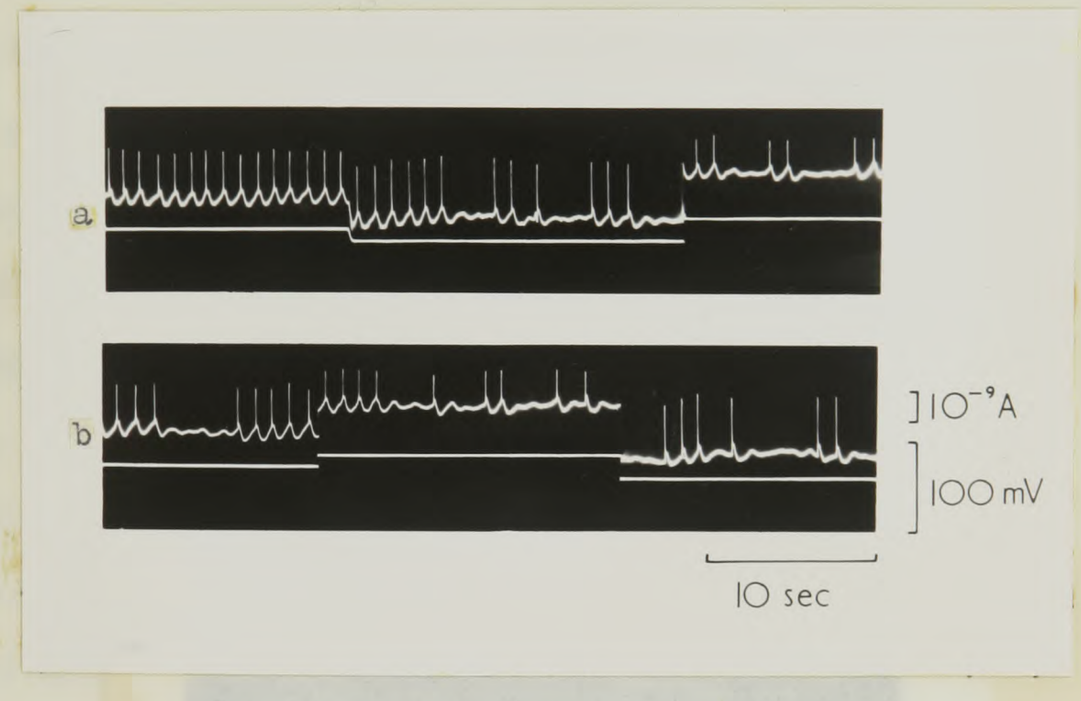


Fig. 3. Intracellular recording and stimulation, using a single micro-electrode. (a) Effect of hyperpolarization followed by depolarization. (b) Reversed order. Note that polarizing current changed spike amplitude but had no influence on the amplitude of the slow components nor on their occasional failure to generate a spike. Both records were taken from the same fibre. The potential shift during current application cannot be taken as the true membrane polarization because of a slight unbalance of the bridge.

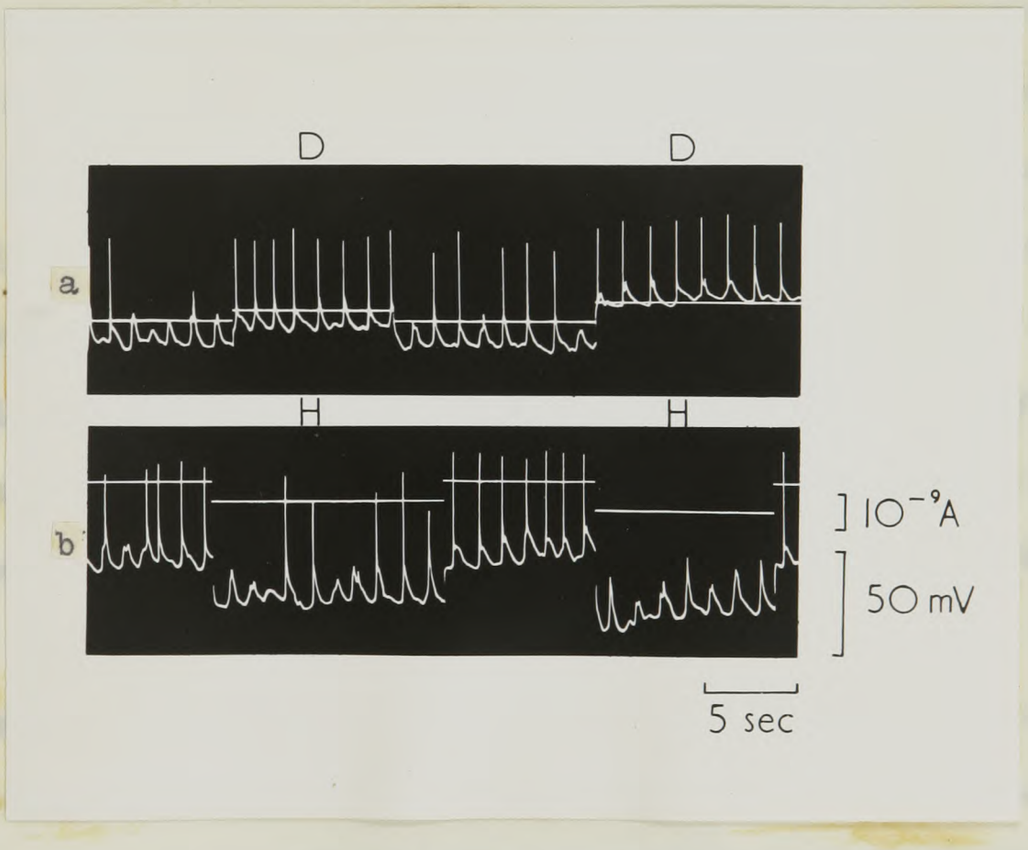


Fig. 4. Effect of polarizing current applied intracellularly. (a) during depolarization (D) spikes were generated on every slow component. (b) during hyperpolarization (H) the spike generation failed more frequently. No change of the frequency of the slow component was seen.

As shown in Fig. 2 and 4, the spike generation could be affected by the polarizing current. However, a change in frequency of the slow component, was never observed, even when the spike component was dropped. In contrast, if polarizing current was applied by large external electrodes the spike frequency was easily modified, and was associated with a similar modulation of the frequency of the slow component, as will be described later.

In a fibre in which the spontaneous electrical activity consisted of large slow components and small spike components, as for example in Fig. 2, the change of the amplitude and the maximum rates of rise and fall were roughly proportional to the intensity of hyperpolarizing current. However, the depolarizing current was much less effective. This is probably due to the fact that in this type of cell the hyperpolarization affects mainly the spike component, while the depolarization affects both the spike and the slow component (probably due to a decrease in membrane resistance). If the electrical activity consisted mainly of spike components, as for example, in Fig. 3, the relation between spike height and current intensity was linear in both directions, as seen in Fig. 7.

The effect of polarization varied greatly in different cells. However, the decrease of the spike amplitude by hyperpolarization, observed by Nagai & Prosser (1963b) in the circular intestinal muscle of the cat, was never seen, though excessive hyperpolarization blocked the spike component (Fig. 2b and Fig. 4b). The maximum increase of the spike amplitude was about 50 mV per nA. The biggest effects were

observed in cells in which the spike amplitude and its rate of rise were low, and the slow component was relatively large as shown in Fig. 5. This effect might be due to the polarizing current exerting its main effect on the inactivation process of the sodium carrier system (Hodgkin & Huxley, 1952b). Hodgkin & Huxley (1952b) studied, in the squid giant axon, the effect of conditioning polarization of the membrane on the sodium current using the voltage-clamp method. At the normal resting potential about 40% of the sodium-carrying system is inactivated and not available for carrying sodium ions when the membrane is excitated. Conditioning hyperpolarization decreases the inactivated fraction, thus the sodium current is increased by hyperpolarizing the membrane up to 30 mV, when it reaches a steady level. The sodium current is decreased by conditioning depolarization because a greater fraction of the sodium carrier system is inactivated.

In some preparations, the change of spike amplitude and of the rates of rise and fall produced by a constant polarizing current were gradual, reaching a steady state only after 5 sec or more, as shown in Fig. 6. This very gradual change in amplitude and rate of rise of the spike may be explained mainly by a very slow change of the inactivation process of the sodium carrier system according to the ionic theory (Hodgkin & Huxley, 1952b), and seems to correspond to the slow time course (1 - 10 sec) of spike restoration by applying hyperpolarizing current in nerves and skeletal muscles in which the spike had been reduced in size or blocked by sustained depolarization or exposure to low Ca or high K (Koketsu & Noda, 1960; Narahashi, 1964).

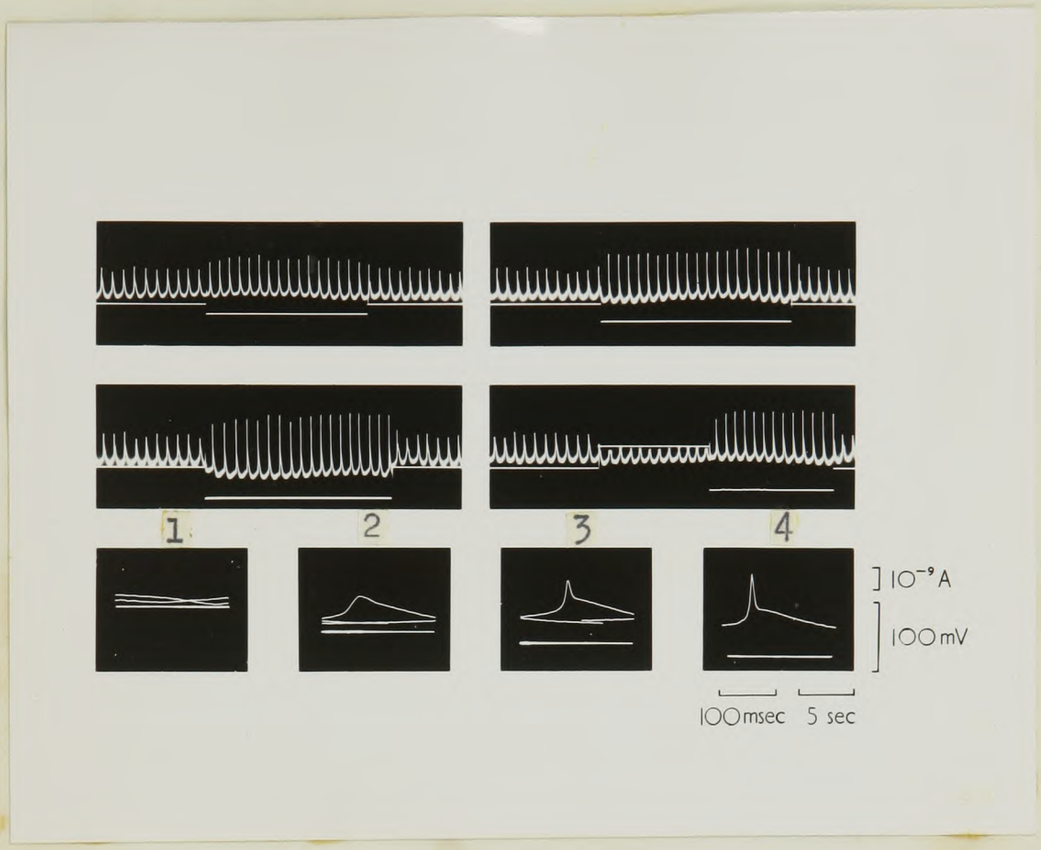


Fig. 5. Marked effects of polarizing current on the spike amplitude. A single intracellular electrode was used for recording (upper trace) and stimulating. Downward shift of current record (lower trace) indicates hyperpolarizing current, and upward shift, depolarizing current. Bottom records were taken on fast time base (100 msec calibration) to show effect of polarization on the spike shape. (1) depolarization, (2) control, (3) weak hyperpolarization (corresponds to left top record) and (4) strong hyperpolarization (corresponds to left middle record).

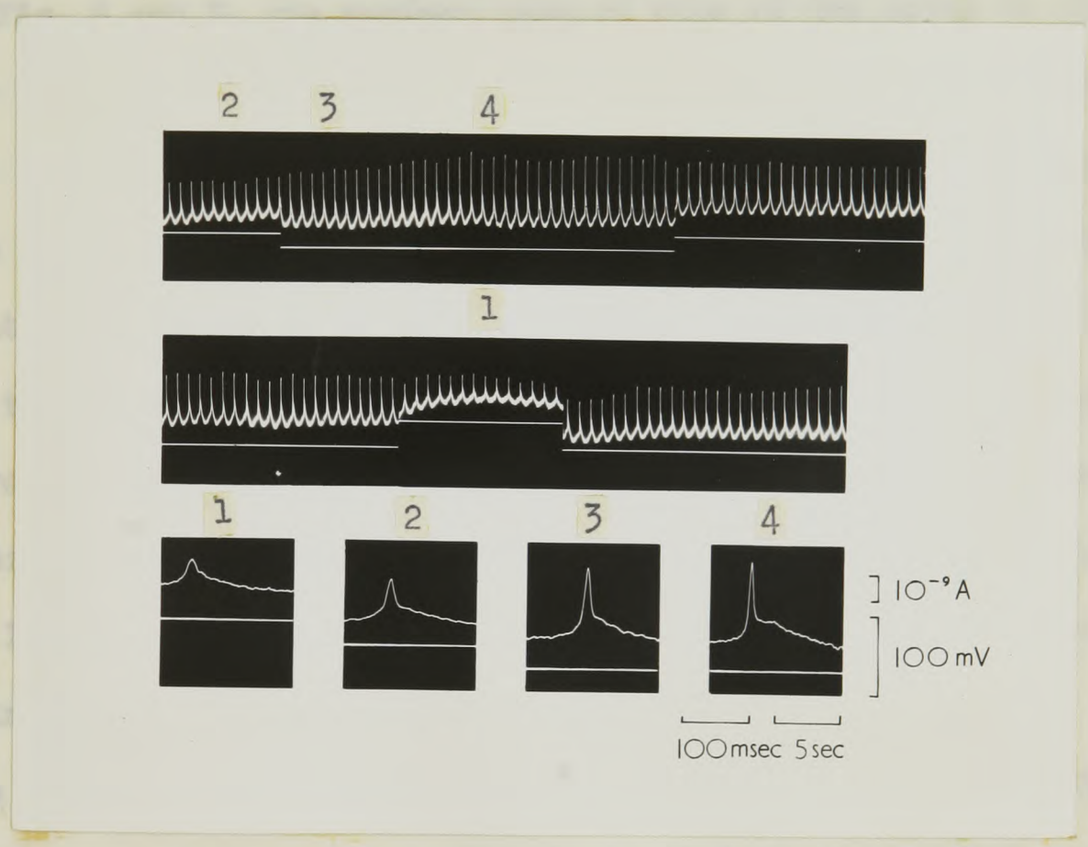


Fig. 6. Effects of intracellularly applied hyperpolarization (top) and depolarization (middle) on spike amplitude. Note gradual change of the spike amplitude during polarization but constant frequency. Bottom records on faster time base: effects of polarization on spike shape. Each record corresponds to the spikes which have the same number in the upper records.

When an action potential is propagated along a fibre of uniform structure bathed in a large volume of conducting fluid, the membrane current density is proportional to the maximum rate of rise of the action potential. In the squid giant axon, the inward current density is known to be related to the membrane potential (Hodgkin & Huxley, 1952b). The rate of rise of the action potential, which is indicative of the inward sodium current, should also be related to the membrane potential. As seen in Fig. 6 and 7, the maximum rate of rise of the spike in the taenia coli was increased by hyperpolarization and decreased by depolarization, which is analogous to the relation between the rate of rise and the membrane potential in Purkinje fibre (Weidmann, 1955) and also to the inactivation curve in squid nerve fibre (Hodgkin & Huxley, 1952b).

In the taenia coli, the rate of fall was changed in the same manner as the rate of rise when a polarizing current was applied. However, the values of the rate of fall are very variable (Holman, 1958; Bülbring, 1959). In some fibres, the rate of fall was faster than the rate of rise; in others, the reverse was found.

The membrane resistance in the resting state may be calculated from the change of the spike amplitude divided by the applied current intensity if it is assumed that the membrane resistance becomes very low during the spike phase (Coombs, Curtis & Eccles, 1959; Frank & Fuortes, 1956; Johnson & Tille, 1961). This assumption is based on an analogy with the spike in squid giant axons (Hodgkin & Huxley, 1952a), frog muscle fibres (Fatt & Katz, 1951), and the supramedullary neurones of the puffer fish (Hagiwara & Saito, 1957), where the membrane resistance

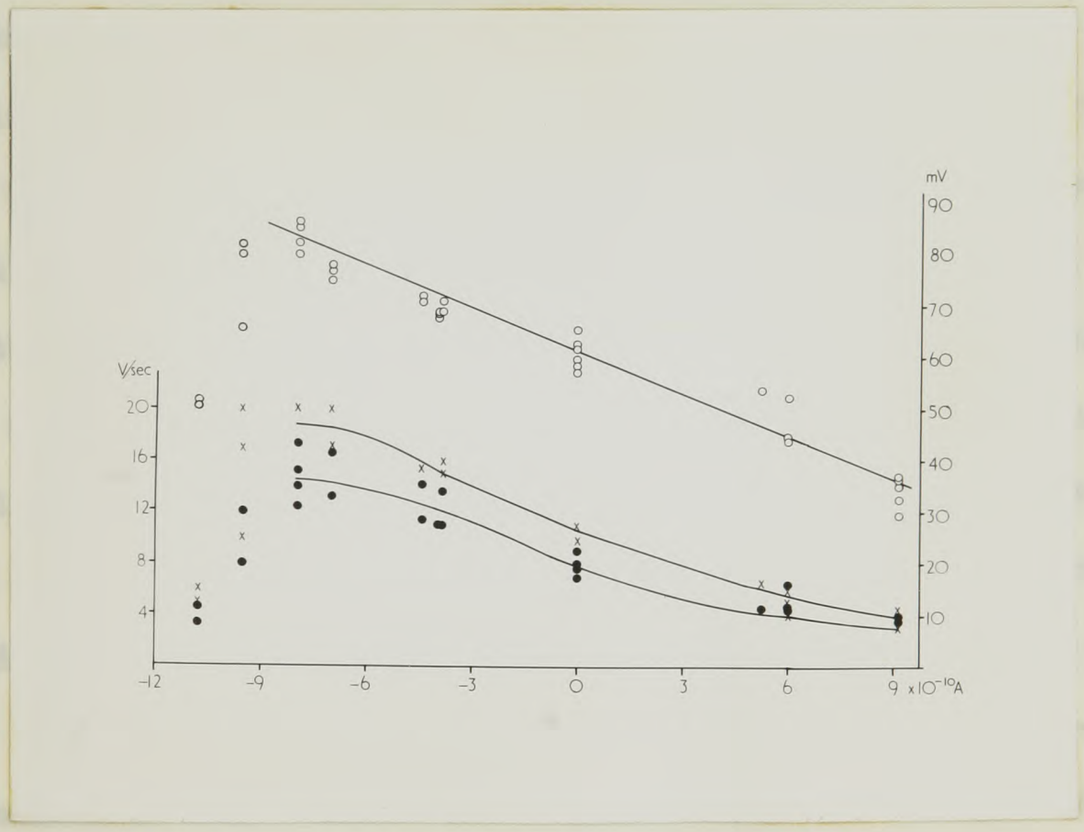


Fig. 7. Relation of amplitude (°) and maximum rates of rise (•) and fall (x) of the spike (ordinate) to depolarizing (abscissa, right side) and hyperpolarizing current (left side).

at the peak of the spike is less than 5% of the resting value, and where the ionic mechanism generating the spike is little affected by the current flow across the membrane.

For the purpose of calculation, the fibres which had a large spike amplitude of about 60 mV were selected, not only because the resistance during the spike might be lower in a large spike, but also because the effect of the polarizing current on the inactivation process of the sodium carrier system of large spikes might be smaller. An example of the relation between the current and the spike amplitude used for the calculation of the membrane resistance is shown in Fig. 7. The highest value of the effective membrane resistance obtained by this method was about 30 MΩ (range 10 - 30 MΩ in 10 cells).

In the smooth muscle the membrane resistance during the spike is probably not as much reduced as in other excitable tissues, because the small size and slow rate of rise of the spike, indicates less increase of Na conductance during the spike. The resistance measurement with an internal electrode might also show less reduction of the membrane resistance during the spike if the muscle fibre has two- or three-dimensional connections with neighbouring cells (George, 1961; Noble, 1962, ~~1966~~). Furthermore, the polarizing current has a marked effect on the rate of rise of the spike (Figs. 2 and 6) indicating a strong influence on the sodium carrier mechanism which controls the spike amplitude. The relatively small reduction of the membrane resistance during a spike, giving a lower value, and the effect on the sodium carrier, giving a higher value than true membrane resistance,

seem to cancel each other out. Thus the measurement of the effective membrane resistance from the relation between applied current and the change of spike amplitude gave a similar value (30 MΩ) to that obtained by the direct method (40 MΩ, see the next page) in which the resistance was calculated by dividing the electrotonic potential by the applied current.

(B) The response to intracellular electrical stimulation. When a weak current was applied to the membrane through the intracellular micro-electrode an electrotonic potential with a time constant of between 2 to 4 msec in different fibres was produced. In most cells the current-voltage relation showed no rectification up to 30 mV polarization. In some cells the membrane resistance seemed to be slightly decreased by depolarization, but, when a strong depolarizing current was applied, the resistance appeared often to be slightly increased. However, using the Wheatstone bridge method, a change of resistance of the micro-electrode could not be distinguished from a change of the membrane resistance, since the electrode resistance was usually increased by passing a strong depolarizing current through it.

The effective membrane resistance, calculated from the current-voltage relation, was for weak currents 20 - 70 MΩ (40 MΩ ± 12 s.e. in 10 cells).

In most cells no spike could be triggered by intracellular electrical stimulation even if the membrane was depolarized by more than 50 mV or the stimulating current was increased to more than 5×10^{-9}A. In such cells the effect of polarizing currents on the

amplitude of the spontaneously generated spike was, however, clearly seen (Fig. 8). Moreover, it is unlikely that this inexcitability was due to cell damage, because the membrane potential was high and the spontaneously generated spikes had a high amplitude and overshoot.

According to Bülbring, Burnstock & Holman (1958), intracellular stimulation with low resistance electrodes (10 - 20 MΩ), produced responses in neighbouring cells. However, their records suggest that the stimulation was extracellular rather than intracellular, because the stimulation occasionally elicited an inhibitory potential, which is produced by nerve stimulation, as will be described later. Moreover, the latency of the evoked spike was long which is a property of the response to external stimulation.

Neither depolarization nor hyperpolarization up to 30 mV produced instability or oscillation of the membrane potential as seen in the circular muscle of the cat by Nagai & Prosser (1963b). Occasionally irregular deflexions in the potential tracing were observed with strong polarizing currents but they were proved to be due to a resistance change of the micro-electrode since they were also observed when the electrode was taken out of the cell.

In some cells a graded response was recorded when the stimulating current intensity was increased. In a few cells the spike was triggered in an all-or-none fashion. This was more frequent if the preparation was bathed in a solution containing excess calcium (7.5 or 12.5 mM). Fig. 9 shows the effects of conditioning depolarization (upper row) and of conditioning hyperpolarization (middle row) on the spike

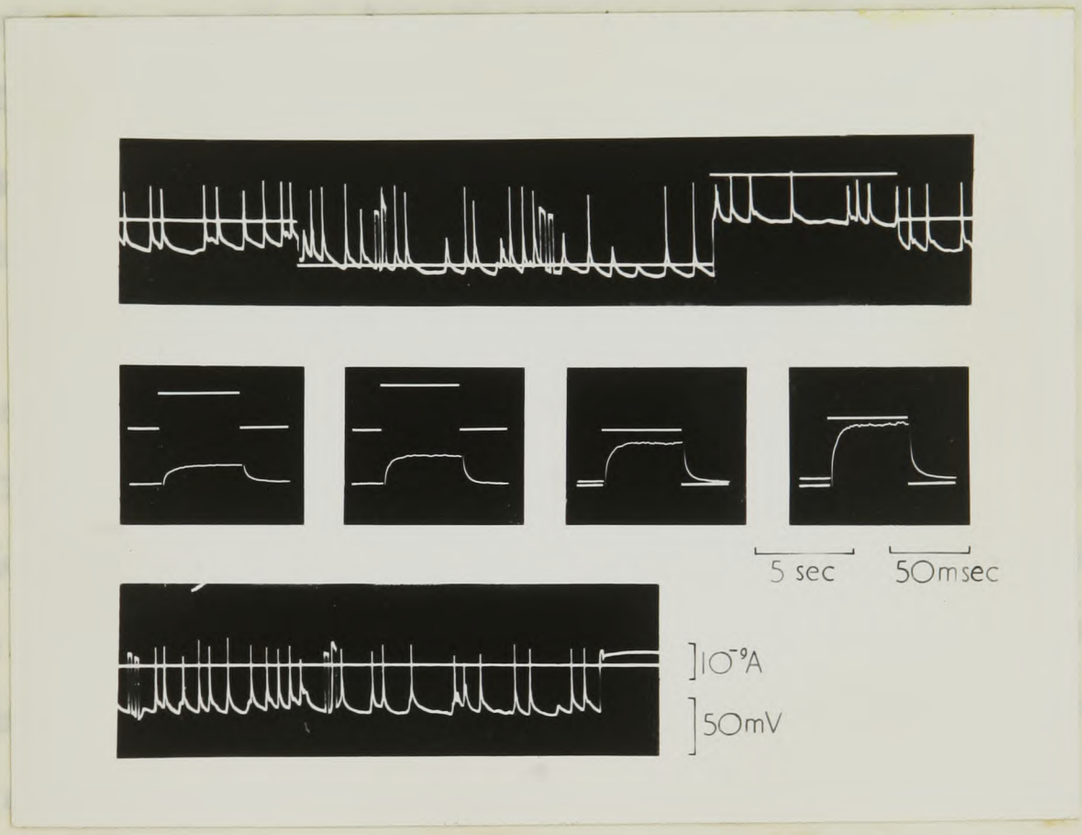


Fig. 8. The effect of polarization on the spontaneous spike amplitude (top) and responses to intracellular stimulation (middle). In the course of the top and bottom records calibrating pulses of 50 mV are applied. At the end of the bottom record the electrode is taken out to show zero potential level. Note no active response to the stimulation, even though effect of polarization on spike amplitude is clear, and spontaneous spike shows overshoot.

triggered by intracellular stimulation. Conditioning hyperpolarization increased the evoked spike amplitude and its rate of rise and fall, while conditioning depolarization decreased them. The effect developed gradually and was completed in a few seconds after the application of the polarization (Fig. 9, bottom row). These effects were similar to those observed on the spontaneously generated spikes (cf. Fig. 6).

The maximum amplitude of the evoked spike during conditioning hyperpolarization was 50 mV from the firing level which was usually -35 to -30 mV. The maximum rates of rise and fall were 18 and 20 V/sec respectively and the spike duration at half spike amplitude was 7 msec. These figures are similar to those of the spontaneously generated spike (Holman, 1958; Bülbring & Kuriyama, 1963) and also to those of crustacean muscle fibres recorded by Fatt & Katz (1953a).

The latency of the spike was very short (a few msec). Repetitive firing was never observed. In a few cells damped oscillations following the spike were recorded during conditioning hyperpolarization. During conditioning depolarization a spike was occasionally evoked in some cells as a break response upon cessation of a sufficiently strong anodal pulse, as observed in the Ranvier node (Coyama & Wright, 1961). Intracellular stimulation never produced the slow component which was seen in the spontaneous electrical activity.

(C) Electrical interaction between cells. When the spontaneous electrical activity of two cells within 50 μ separation was recorded simultaneously with two independent micro-electrodes, it was found that the spikes were generally discharged at the same frequency, but they

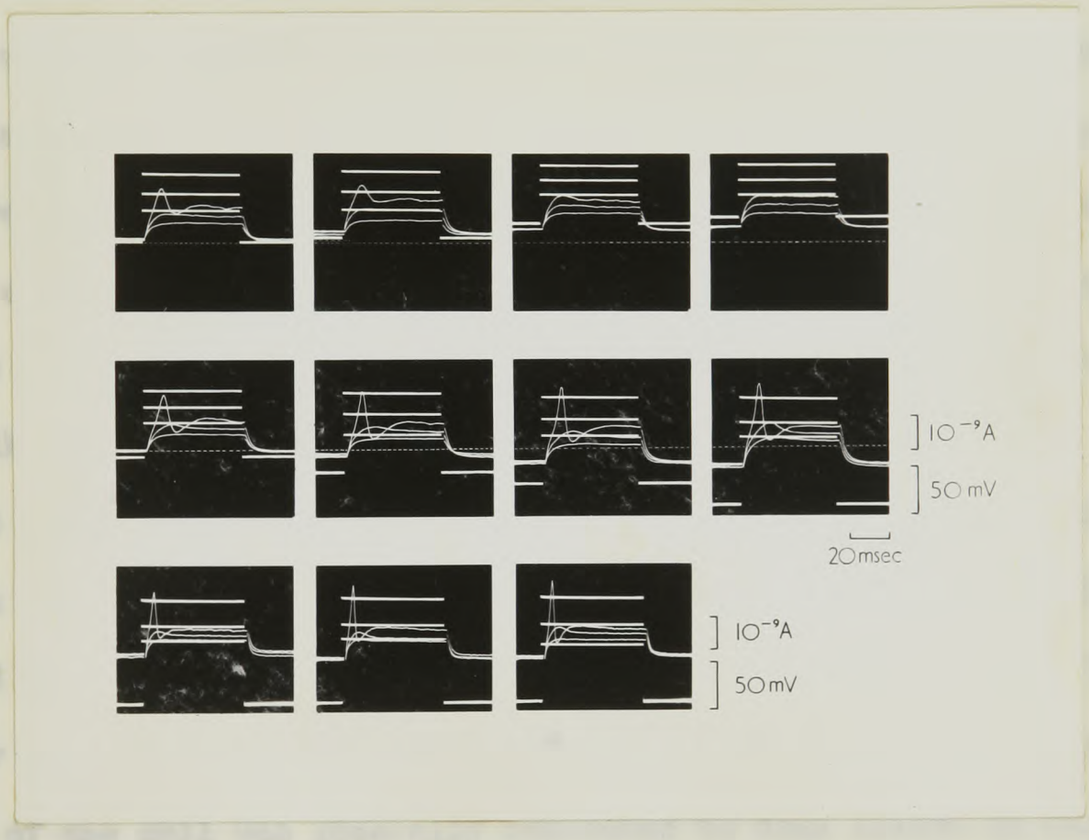


Fig. 9. Spikes triggered by intracellular stimulation. In each record three responses to depolarizing current pulses of increasing intensity are superimposed. Interrupted line is the original membrane potential. Top: left to right: effect of increasing conditioning depolarization. Middle: effect of increasing conditioning hyperpolarization. Bottom: records taken after 1, 3 and 5 sec constant conditioning hyperpolarization.

did not necessarily coincide. These results agreed with the observations by Bülbring, Burnstock & Holman (1958). When the separation of the electrodes was in the longitudinal direction (possibly within the same bundle) synchronization of the spike activity was nearly perfect as shown in the lower row of Fig. 10. If one cell failed to discharge the spike component and only produced the slow component, the other cell followed in a similar manner, although the amplitude of the electrical activity fluctuated without a strict relation between two cells. However, when the electrode separation was in the transverse direction, synchronization was less perfect, as shown in the upper row in Fig. 10. In this record, the cell (A) shown in the upper trace discharged usually a little earlier than the cell (B) shown in the lower trace, and the discharge of the cell (B) was more and more delayed. When this delay reached about 300 msec the cell (B) could not discharge the spike component and produced only the slow component. Once the spike discharge failed in the cell (B) the next spike came earlier so that the spike coincided with, or occurred earlier than, that of the cell (A). 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. The interaction was stronger in the longitudinal direction than in the transverse direction and also when the distance between the cells was shorter.

If the cellular interaction were due to electrotonic spread of the spike from one cell to another, like the spread by local circuit current in the nerve fibre, one would expect that any change of membrane

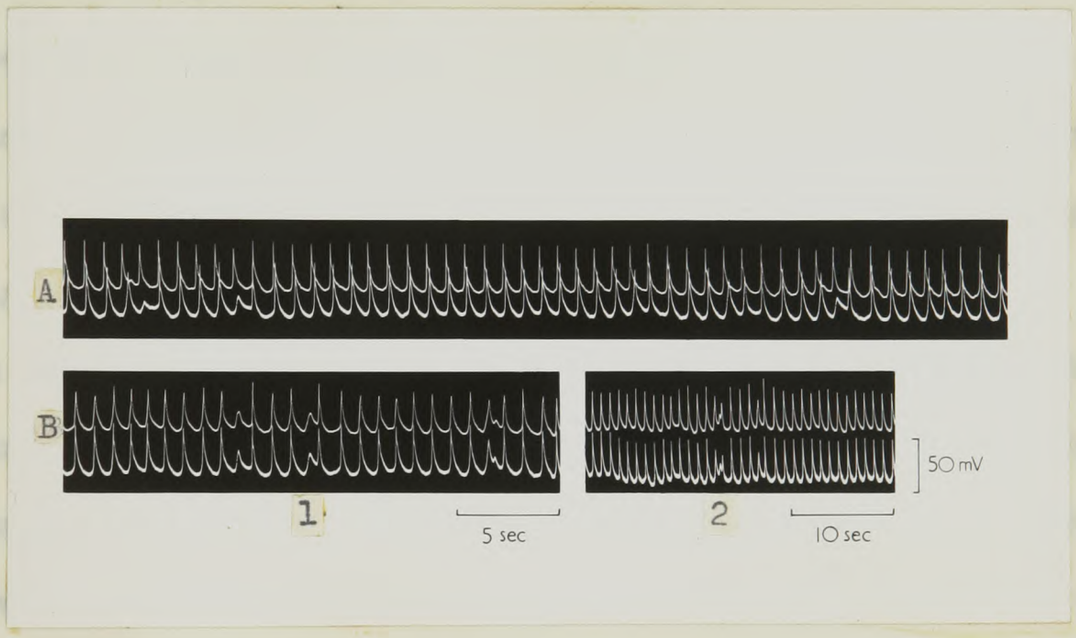


Fig. 10. Simultaneous records taken with two intracellular electrodes separated by about 50 μ, (A) transversely, (B) longitudinally. Note better synchronization of the spontaneous electrical activity in (B) than in (A). In B2, taken from the same cells as in B1 but on a slower time base, no strict relation between the spike amplitude of two cells is seen.

potential produced in a single cell by intracellular current application would also be reflected on the membrane potential of another cell nearby. Such an electrotonic interaction has actually been demonstrated among the nerve cells which have an ephaptic junction, such as the lobster cardiac ganglion (Hagiwara, Watanabe & Saito, 1959; Watanabe, 1958; Watanabe & Bullock, 1960) in ganglion cells of Aplysia (Tauc, 1959), in leech ganglion (Hagiwara & Morita, 1962, Eckert, 1963) and in the giant synapse of the crayfish (Furshpan & Potter, 1959).

To examine this possibility in the taenia coli, two micro-electrodes were inserted within 50 μ distance. The effect of applied current on the cell in which the stimulating electrode was inserted was easily seen from the change of the spike amplitude as described before. However, as shown in Fig. 11, no effect of the current applied to one cell was detected in other cells nearby, neither on the membrane potential nor on the spike. However, the precise distance between the tips of the two micro-electrodes could not be determined since the exact depth of the tip of the micro-electrode inside the tissue could not be measured, so that the two electrodes might be in different bundles even when they were inserted in longitudinal direction through the surface of the tissue.

In the circular intestinal muscle of the cat, Nagai and Prosser (1963b) applied current intracellularly and recorded an electrotonic potential in another neighbouring cell. They concluded that there were relatively low-resistance interfibre connections. However, Sperelakis & Tarr (1965) using the same preparation, reported that, as in the taenia coli, current flow through one cell did not have any substantial effect

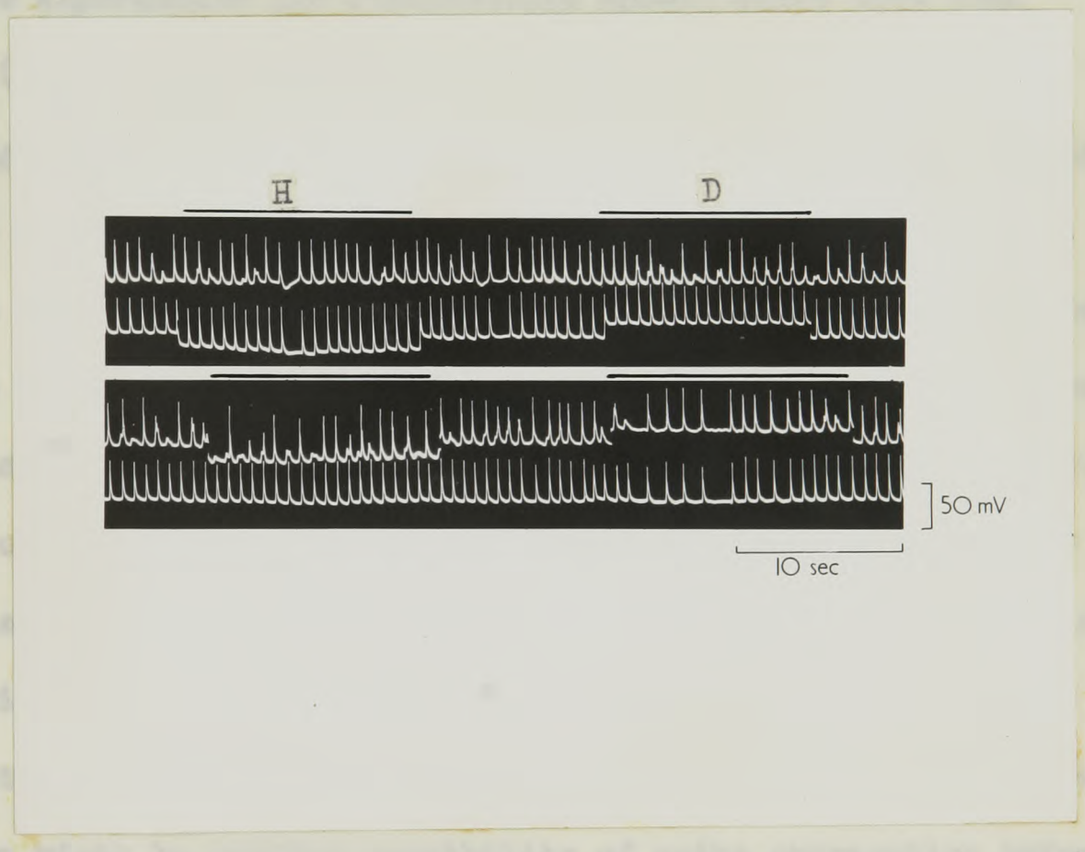


Fig. 11. Records taken simultaneously from two single cells, cell A (upper trace) being separated transversely by less than 30 μ from cell B (lower trace). In the top record, hyperpolarizing current (H) and depolarizing current (D) were applied to cell (B) as shown by horizontal bars. In the bottom record the current was applied to cell (A). Note no effect of polarization on the other cell in either direction.

on the transmembrane potentials of adjacent cells.

There are several possible explanations for the close relation between the spontaneous electrical activities of two cells in spite of the failure to produce a spread of the electrotonic potential by intracellular stimulation. (1) The electrical interaction may be limited only to the next cell (transversely and longitudinally) and the two electrodes may have been inserted several cells apart. In other words, if the two electrodes had been inserted much closer than in the above experiments the electrotonic spread might have been observed. (2) Single cell activity may not be able to affect the neighbouring cells because of the sharp spatial decay of the potential spread. It would then be necessary for many grouped cells to be involved in the electrical activity, as under physiological condition, in order to influence the activity of other cells. This is the idea of a functional bundle and will be discussed later. (3) The passive properties of the membrane may be different from the active ones so that the electrotonic potential cannot spread to other cells but the spike activity can. The electrical resistance of the close contact membrane between cells might be reduced during the spike. (4) Chemical transmission might be another possibility of spike propagation between cells.

In order to test the third possibility a spike was triggered by intracellular stimulation in one cell (the lower trace in Fig. 12) and the membrane potential was observed in a neighbouring cell (the upper trace in Fig. 12). No spike, slow component nor electrotonic

potential could be detected in the neighbouring cells during the strong current application which produced a spike in a cell directly stimulated, as shown in B4 and 5 in Fig. 12. This result made the third possibility rather unlikely. The fact that, when current was applied externally, an electrotonic potential was easily observed from every cell near the stimulating electrode, suggested that the second possibility was the most probable one.

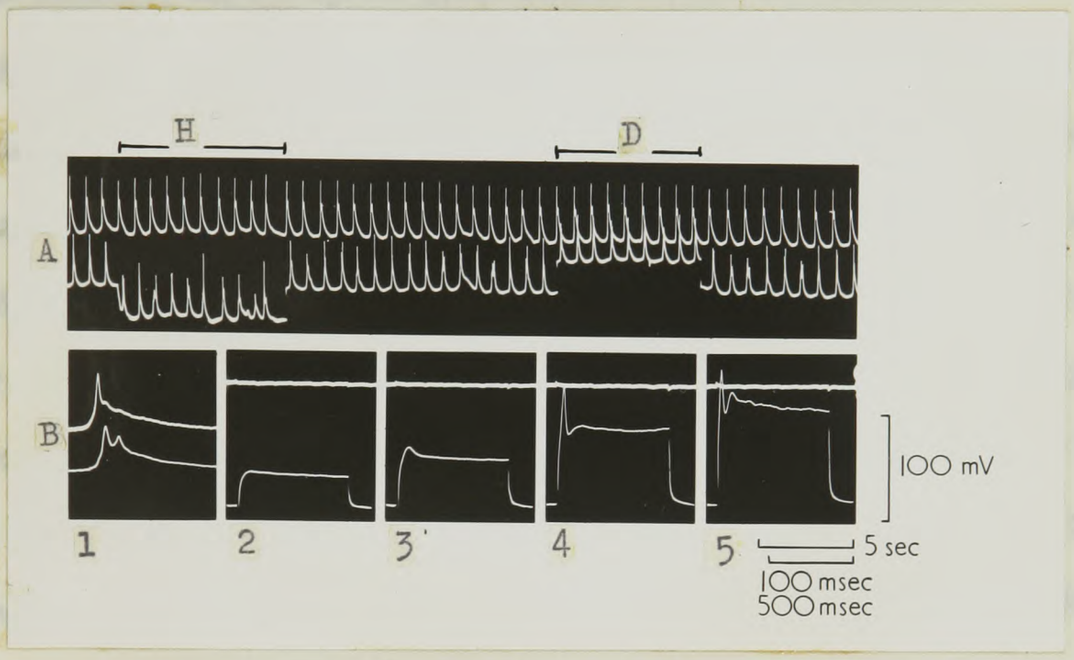


Fig. 12. A: Simultaneous recording of the spontaneous spikes with two intracellular electrodes (transverse separation of about 30 μ) and effect of hyper- (H) and depolarization (D) applied to one cell (lower trace) producing no effect on a neighbouring cell (upper trace). B1: Spontaneous spikes from both cells taken by fast sweep. B2 and 3: a depolarizing current pulse was applied to one cell (lower trace), but no change in membrane potential was seen in the other cell (upper trace). B3 and 4: a spike was triggered by intracellular stimulation without any change in the cell nearby.

Chapter IV

External stimulation. Part I

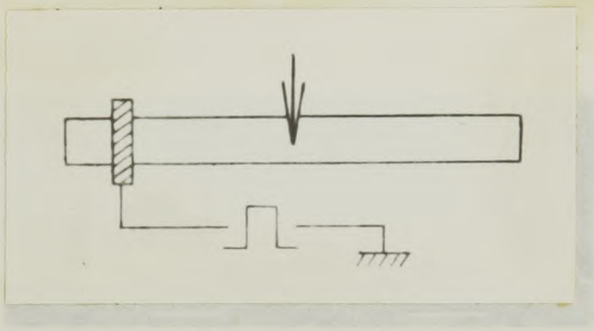
As described in Chapter II (Methods), external stimulation was always field stimulation using large electrodes. Focal stimulation, with an extracellular micro-electrode, was never applied.

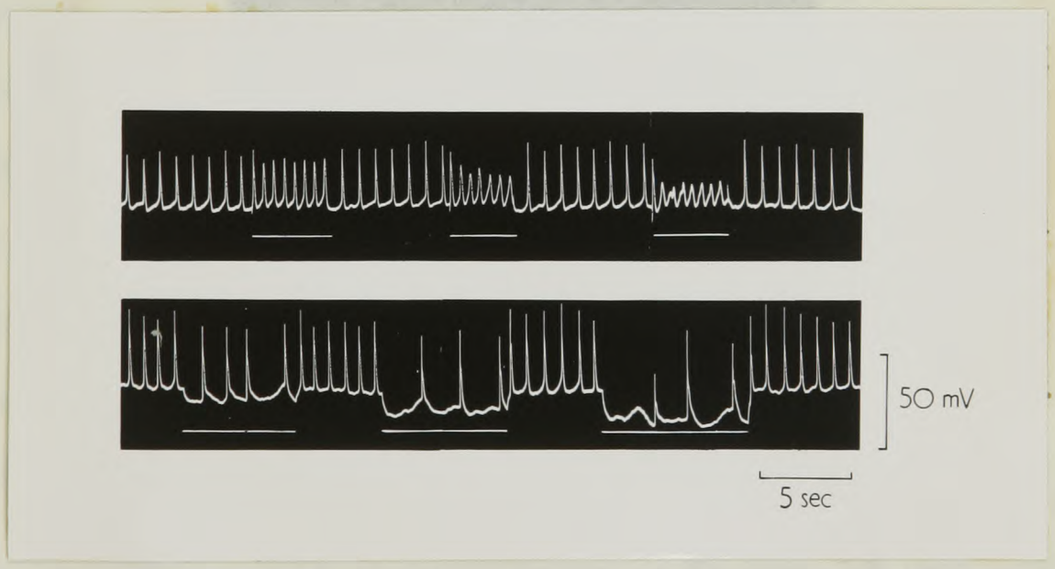
(A) Polarization of the membrane. It has been shown in the preceding section that in the taenia coli of the guinea-pig intracellular stimulation evokes a spike only in very few cells and causes no frequency modulation of the spontaneous discharge. External stimulation, however, easily triggers a spike and changes the spontaneous spike frequency (Bülbring, 1955, 1957; Bülbring, Burnstock & Holman, 1958). Bülbring (1955) used Ag-AgCl wire inserted into glass tubes as polarizing electrodes, one of which was placed at one end of the preparation and the other was dipped in the bathing solution. Anodal polarization slowed or stopped the spontaneous spike discharge while cathodal polarization increased the frequency. The polarization of the muscle (for example, hyperpolarization) was determined by the electrode dipping in the bath (anode), and not by that which touched the preparation (cathode). This was confirmed in the present experiments. The relation between arrangement of the stimulating electrodes and the polarization of the muscle fibre was found to be complicated. When both stimulating electrodes were placed on one end of the tissue, the polarization became reversed along the tissue. (This phenomenon was investigated in experiments in which spontaneous activity was abolished by exposure to hypertonic solution and these will be described in detail later

(p 36 and Fig. 28).) Thus, with this stimulating arrangement, some part of the tissue was depolarized while the other part was hyperpolarized. Also, the spike might be propagated through the whole tissue or it might be blocked by hyperpolarization while the electrotonic potential spread passively with a space constant of only a few mm.

Due to the above phenomena, the response pattern produced by external polarization differed with different arrangements of the stimulating electrodes or with different distances of the recording site from the stimulating electrodes. Two examples of the effect of polarization are shown in Figs. 13 and 14, which were obtained with one stimulating electrode placed round one end of the tissue, and the other immersed in the bathing solution. In the preparation shown in Fig. 13 the spike amplitude was reduced very much by depolarization while the spike frequency was increased less than twice. In another preparation (Fig. 14) which had a high membrane potential, a large spike amplitude and a slow spontaneous frequency, the reduction of the spike amplitude was less marked and the increase of the spike frequency was more pronounced when depolarizing current was applied. The maximum frequency produced by depolarization was about 3 c/sec. and the increase seemed to depend on the spontaneous frequency before stimulation. Hyperpolarization sometimes reduced the spike size. This could be due to a partial block of the spikes which were propagated from the depolarized part of the tissue. Usually the membrane was more hyperpolarized by anodal current than it was depolarized by cathodal current of the same current intensity. This may be explained partly by rectification of the

Fig. 13. Intracellular records of the effect of polarization applied with a large external electrode (arrangement shown in the inset). Duration of external current application is shown by white bars. Upper record: effects of depolarizing current. Lower record: effects of hyperpolarizing current. Note the modulation of spontaneous spike frequency.





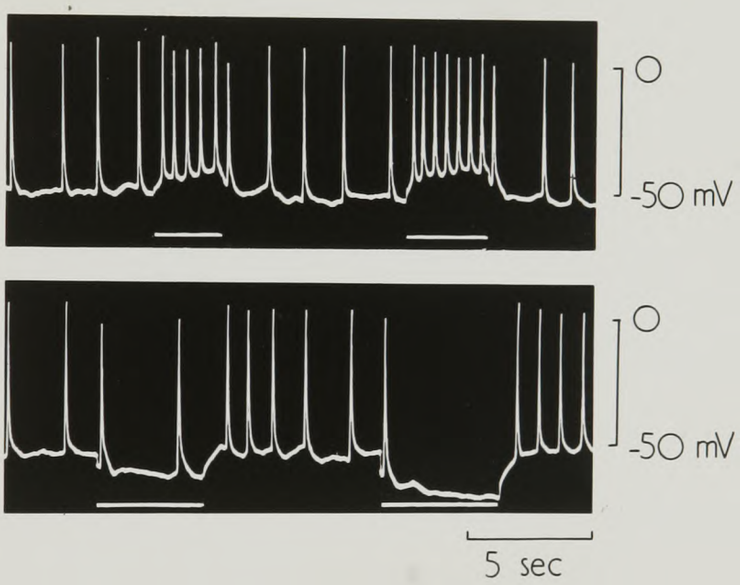


Fig. 14. Effect of polarization applied with a large external electrode. Experimental arrangement as in Fig. 13, but with a different preparation which had a lower spontaneous spike frequency. Upper record: depolarization; lower record: hyperpolarization, which stops spontaneous discharge.

membrane resistance and partly, as will be described in the next section (p 27), by the inhibitory potential which might persist due to repetitive firing of the nerve fibres.

Just after the insertion of the micro-electrode a low resting potential and small spike amplitude were sometimes observed in normal solution (see also Gillespie, 1962a). Then, during 10 - 30 sec, the resting potential and the spike amplitude increased progressively. If during that time external current of either polarity was applied, the electrotonic potential also increased in a similar manner to the spike. Therefore, if the resting potential and the spike potential represent phenomena which are related to the properties of the cell membrane, then the electrotonic potential must also be produced at the membrane.

The effects caused by polarization of the membrane were studied in greater detail in hypertonic solution. Hypertonicity abolished the spontaneous electrical activity and therefore made the analysis of the electrotonic potential easier. However, before describing these results, it is necessary to describe experiments designed to differentiate responses due to nerve stimulation from responses of the muscle membrane to the flow of current through the whole tissue.

(B) <u>The inhibitory potential</u>. It has been shown that a short pulse of external field stimulation (< 0.1 msec) produces a transient hyperpolarization and blocks spontaneous spikes. This transient hyperpolarization has been called 'inhibitory potential'. It has been interpreted as a post-synaptic potential produced by intrinsic

inhibitory nerve fibres, releasing an unknown transmitter since the effect is resistant to bretylium and guanethidine which are adrenergic blocking agents (Bennett, Burnstock & Holman, 1963; Burnstock, Campbell, Bennett & Holman, 1963, 1964; Suzuki, 1964). However, on this evidence alone, it could not be concluded that the inhibitory potential was the result of stimulation of additional intrinsic, non-adrenergic, inhibitory nerves without knowing more about the electrophysiological properties of the inhibitory potential.

(a) Experiments in which part of the tissue with the stimulating electrodes was separated by an insulating partition from the remaining part in which records were taken.

In preliminary experiments, no convincing evidence as to the nature of the inhibitory potential could be obtained and, indeed, the following evidence seemed to be against the idea of nerve stimulation (Bülbring, Kuriyama & Tomita, 1965). In the first place, external field stimulation easily polarized the muscle fibre and modified its spontaneous spike activity (Figs. 13 and 14). Secondly, when the extreme end of the tissue was stimulated by placing one electrode on the cut end of the tissue, the other electrode being 1 - 2 mm away in the bathing solution, anodal current which hyperpolarized the muscle membrane was more effective than cathodal current in producing the inhibitory potential, as seen in Fig. 15. If the stimulus duration was more than 10 msec it caused an electrotonic potential which was usually followed by the inhibitory potential. If a long stimulus (more than 500 msec) was applied there was no clear distinction between

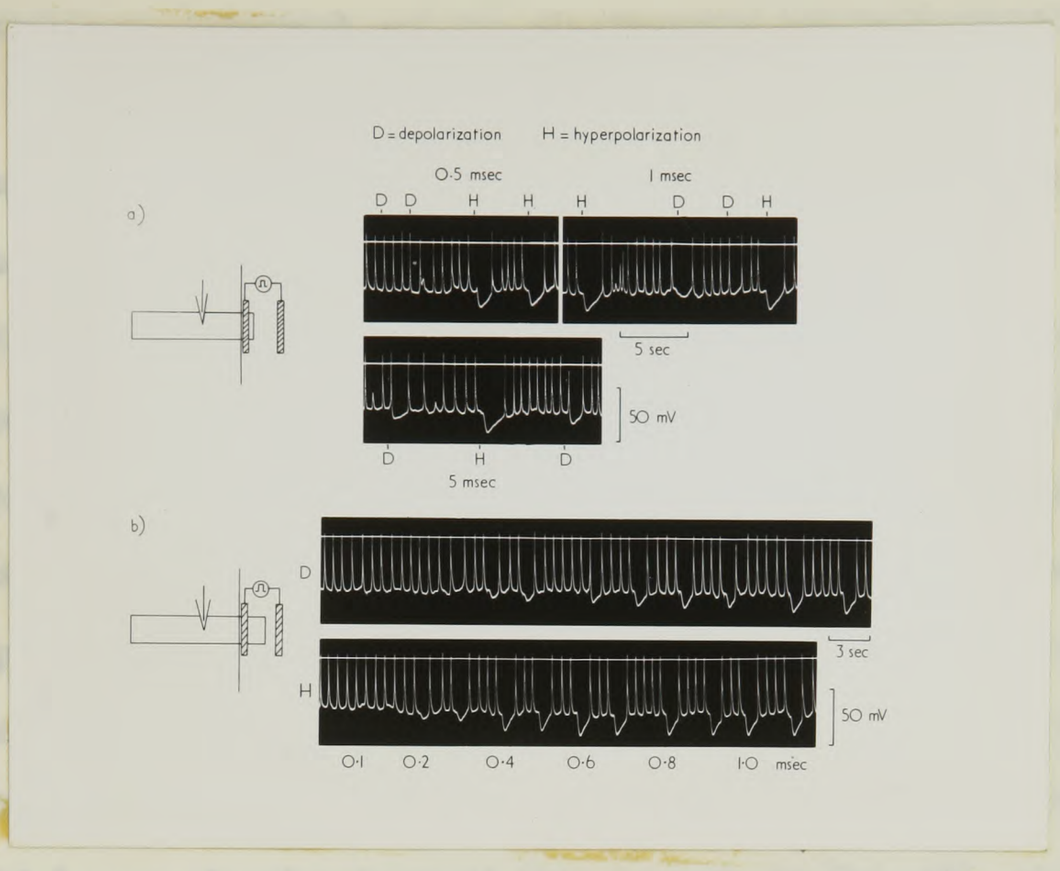


Fig. 15. (a): The inhibitory potential in response to external stimulation applied to one end of the tissue, which was separated by an insulating partition from the recording site, as shown in the inset. Note depolarizing current (D) is less effective than hyperpolarizing current (H). Stimulus duration was changed from 0.5 to 5 msec but current intensity was kept constant.

(b): The same preparation as shown in (a) but the end of the tissue was moved nearer to the second electrode so that a longer part of the tissue was stimulated than in (a), as shown in the inset. Stimulus duration was increased from 0.1 to 1.0 msec, each stimulus of the same duration being applied twice, but the intensity was kept constant. Upper record (D): depolarizing current, and lower record (H): hyperpolarizing current. Note that in (b) depolarizing current became more effective than in (a).

the inhibitory potential and the electrotonic hyperpolarization (Fig. 16). From these results, the inhibitory potential seemed to be a secondary active response to the hyperpolarizing current which flowed across the muscle membrane.

However, if the inhibitory potential were a direct response of the muscle membrane produced by the stimulating current, the difficulty remained that a special mechanism had to be postulated for the conduction of the inhibitory potential along the muscle fibres, because the spatial decay of the inhibitory potential was much slower than that of the passive electrotonic potential (Fig. 17). At 1 mm from the stimulating electrode, both the electrotonic potential and the inhibitory potential were clearly observed (top records in Fig. 17), while at 4 mm from the stimulating electrode only the inhibitory potential was observed (bottom records in Fig. 17), although the spikes produced by depolarizing current were seen. At 10 mm distance from the partition, also the inhibitory disappeared.

In these preliminary experiments an insulating partition was placed between the stimulating site and the recording site in order to eliminate the complications caused by the external stimulating field. With this arrangement the reversed polarity phenomenon mentioned before (P.24) was no longer observed (see Fig. 28). However, the current flow in the tissue close to the stimulating electrode might still be complicated, since the electrode was a ring surrounding the tissue. Furthermore, the distribution of the stimulating current inside the muscle preparation might be different from that in a nerve fibre, because of the different

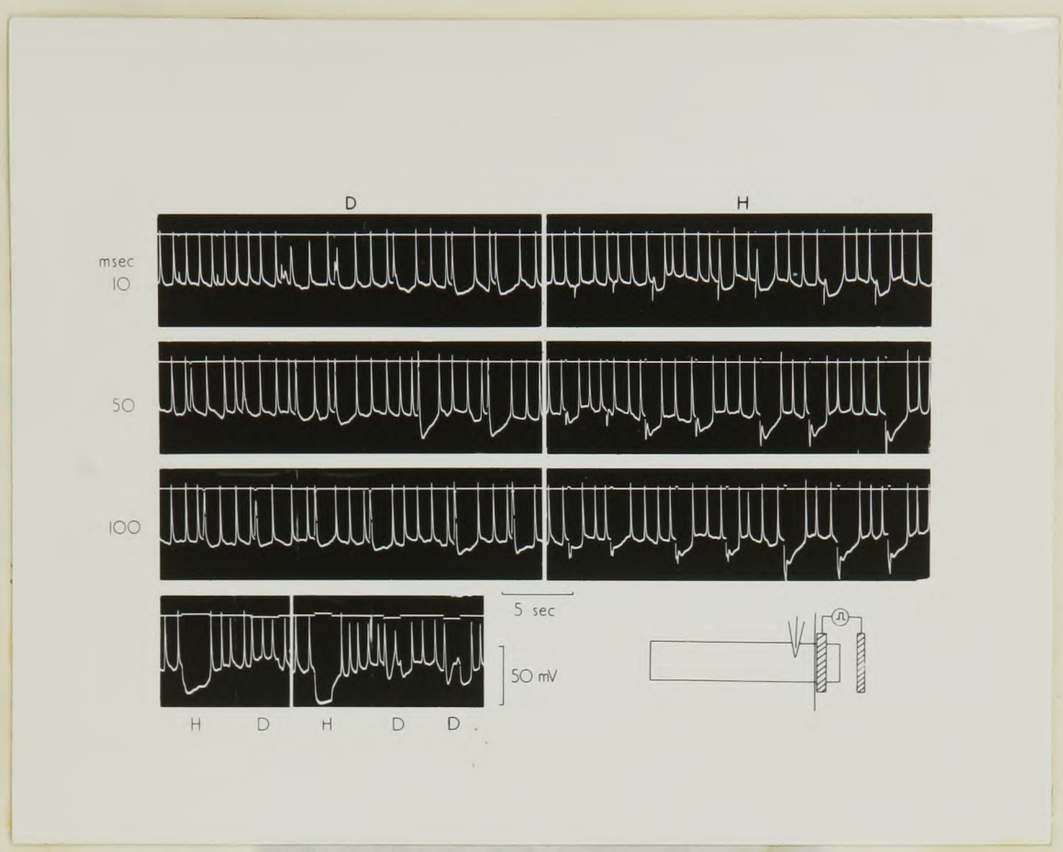


Fig. 16. The inhibitory potential and the electrotonic potential. The stimulating and recording arrangement is shown in the inset. All records were taken from the same single cell. The upper trace shows the relative intensity of externally applied currents which, in each record, was increased from left to right. The following effects are illustrated: The electrotonic potential preceded the inhibitory potential; depolarizing current (D) produced a spike and was less effective than hyperpolarizing current (H) in producing the inhibitory potential; a long hyperpolarizing stimulus obscured the inhibitory potential; a long depolarizing current produced the inhibitory potential as on- and off-responses (bottom record).

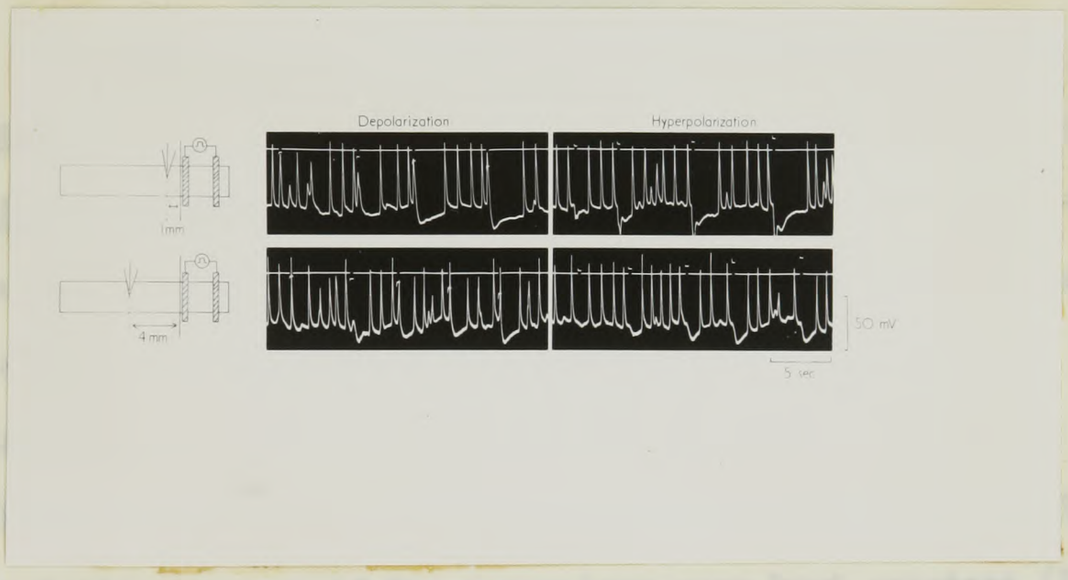


Fig. 17. The inhibitory potential recorded at two different distances from the insulating partition. Upper record: 1 mm and lower record: 4 mm, as shown in the inset. The upper trace in each record shows increasing relative intensity of the externally applied current (duration: 200 msec). Note clear electrotonic potential at 1 mm (especially in response to hyperpolarizing current) but no electrotonic potential at 4 mm from the partition; a spike was produced by depolarizing current.

electrical properties (e.g. space constant) and the difference in structure (e.g. the cell diameter, its length and the direction of the arrangement in the tissue) so that the current which hyperpolarizes the muscle membrane might depolarize nerve fibres running in a different direction. Further experiments were therefore carried out, designed to control current flow through the tissue more strictly than before in order to investigate whether the inhibitory potential was the result of nerve or of muscle stimulation or both.

(b) Experiments in which the stimulating electrodes (Ag-AgCl plates) were placed at each end of the tissue which was therefore exposed to a uniform longitudinal potential field. Though the muscle membrane was depolarized near the cathode, hyperpolarized near the anode and not polarized near the centre of the tissue (see Fig. 27), the inhibitory potential was recorded everywhere in the tissue of about 3 cm length with almost the same amplitude in response to both polarities of the stimulating current if its parameters were kept constant (Figs. 18 and 19). The threshold current intensity for producing the inhibitory potential was also almost the same at both polarities (1 and 2 in Fig. 19). This result excludes the possibility that the inhibitory potential is produced in the muscle membrane as a direct response to hyperpolarizing current and is conducted to the recording site. If the inhibitory potential were produced in response to direct hyperpolarizing current, the latency of the inhibitory potential would be long and its size would be small when the record is taken near the cathode since it would be conducted decrementally from the anode about 3 cm away (cf. p. 28).

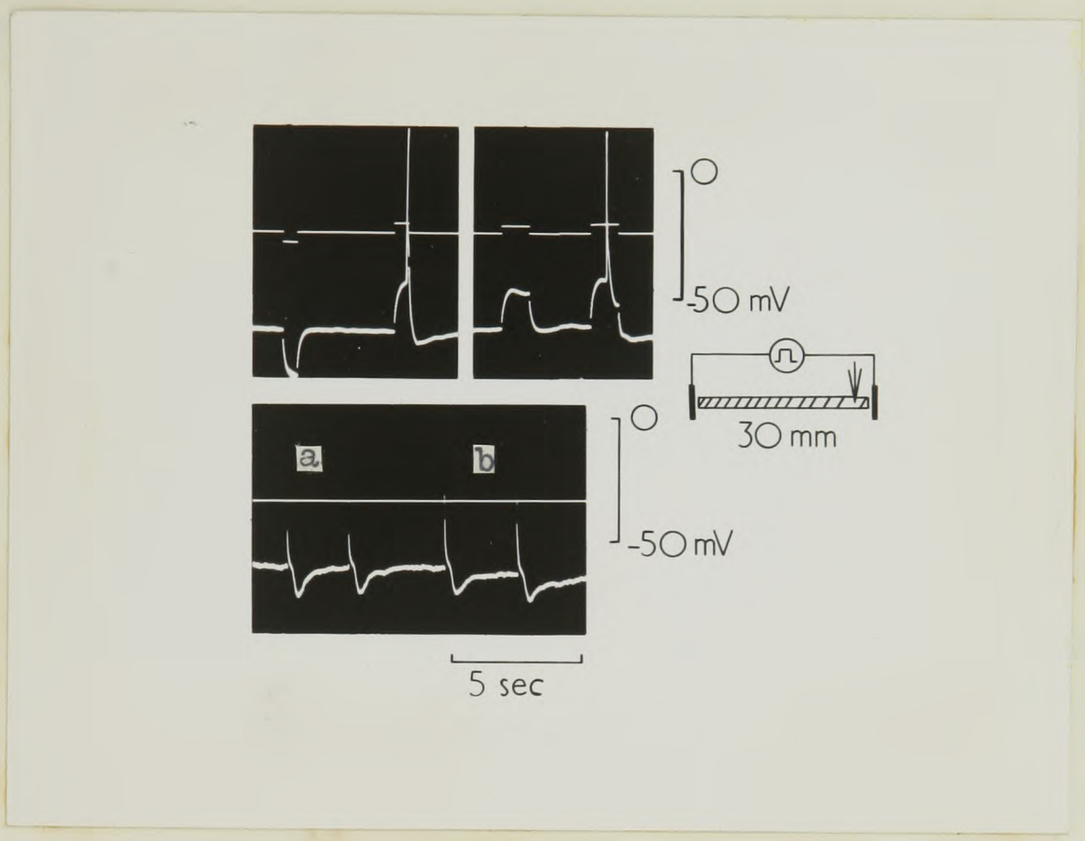


Fig. 18. The electrotonic potential (upper records) and the inhibitory potential (lower record) from the same cell near the end of the tissue of about 3 cm length. (Electrode arrangement shown in the inset). Upper trace shows stimulating current. With long current pulses, when the stimulating electrode near the recording site was the anode, the cell was hyperpolarized, when it was the cathode, the cell was depolarized and, at a given threshold, produced a spike (upper records). With short current pulses, almost the same inhibitory potentials were produced by hyperpolarizing (a) and by depolarizing current (b). Two stimuli of 0.5 msec were repeated at each polarity.

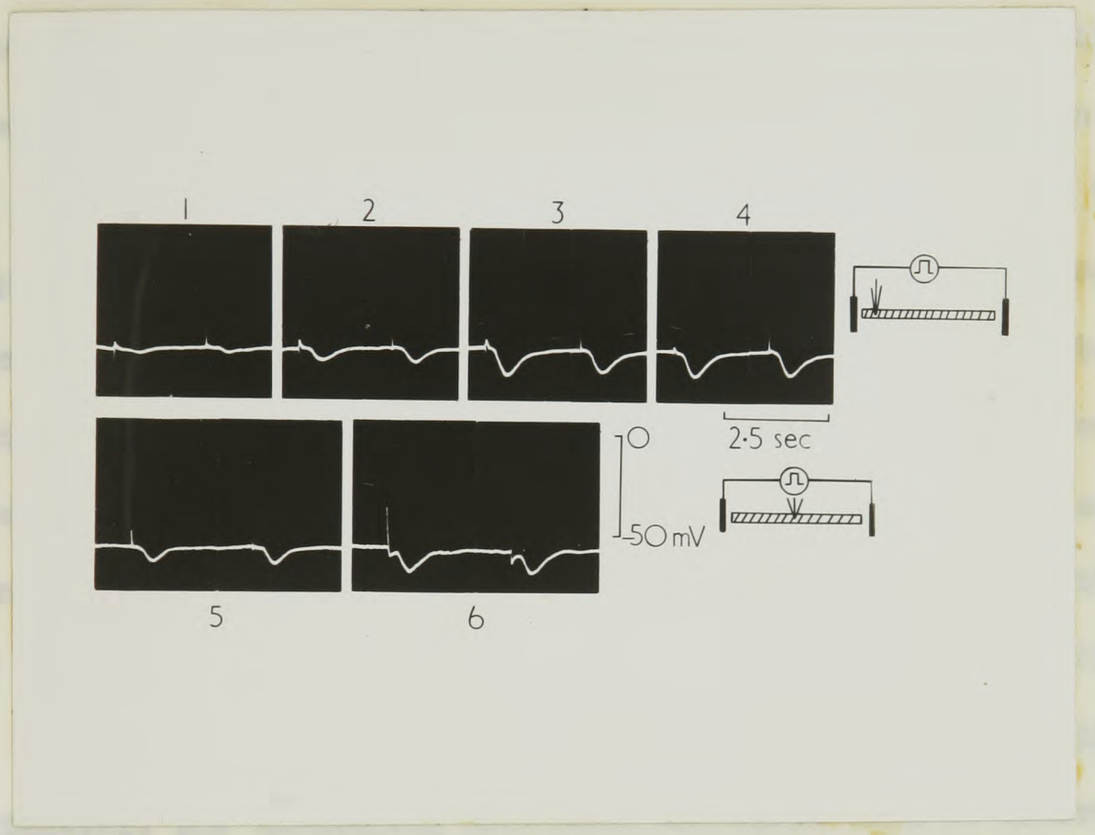


Fig. 19. The inhibitory potential recorded from a cell near the end (1-4), and from a cell near the centre (5 and 6) of a tissue 3 cm long, as shown in the insets. In each record, the first stimulus was by depolarizing current, the second by hyperpolarizing current, of the same duration and intensity. 1 = 0.01; 2 = 0.03; 3 = 0.05; 4 = 0.07; 5 = 0.03; and 6 = 0.05 msec. Note that stimuli of either polarity produced the same inhibitory potential, if the duration was the same.

When a long piece of tissue was used it was difficult to keep the micro-electrode inside a cell due to large movements. This difficulty was overcome by using a solution which was made hypertonic by adding sucrose to the Krebs solution (see also Chapter V, p. 34 - 35). In Krebs solution containing 10% sucrose the inhibitory potential became gradually smaller and finally disappeared after 15 - 20 min as shown in Fig. 20. The reduction of the size was probably caused by hyperpolarization of the muscle membrane since the inhibitory potential recovered when the membrane was depolarized by adding KCl to the external solution. When Krebs solution containing 6 - 8% sucrose was used, the reduction of the inhibitory potential was much less, but some slight tissue movement still remained. Records from this series of experiments, done in hypertonic solution, are shown in Figs. 18 - 24.

In order to study the latency and the time course of the inhibitory potential, the insulating partition was again used as described under (a) because this method abolished the stimulus artifact which disturbed the early phase of the response. In these experiments, a long piece of tissue was exposed to the external stimulating field (12 mm) and the record was taken at various distances from the partition in the recording bath. The stimulation of such a long piece of tissue produced the inhibitory potential at both polarities with almost the same threshold intensity. Occasionally with this arrangement the threshold current intensity for producing the inhibitory potential was also much lower to anodal current than to cathodal current, but the size of the inhibitory potential became the same at both polarities when the current intensity

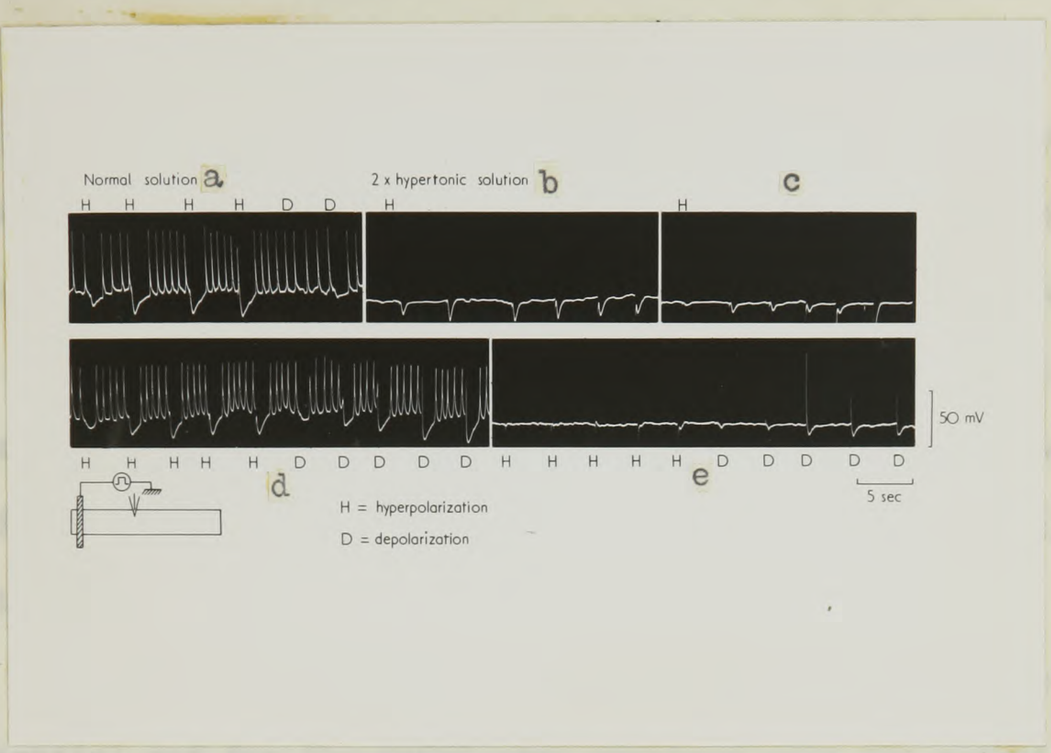


Fig. 20. The effect of hypertonic solution (Krebs solution containing 10% sucrose) on the inhibitory potential. H: hyperpolarizing, D: depolarizing current application. (a) Control (H for 1, 2, 3 and 4 msec; D for 3 and 5 msec). Exposure to hypertonic solution hyperpolarized the membrane and stopped the spontaneous activity. The inhibitory potential could be recorded without spike activity, but the size became small. (b) after 10 min (1, 3, 5, 10, 15, 20 msec); (c) after 20 min in hypertonic solution (1, 5, 10, 20, 50, 100 msec). (d) Another preparation, control: H and D for 1, 3, 5, 10 and 20 msec; repeated in (e) after 25 min in hypertonic solution.

was increased as shown in Fig. 21. Since the latency and the time course of inhibitory potentials with the same amplitude were nearly the same in response to anodal and cathodal stimulation, as seen in Fig. 22, the origin of the inhibitory potential seemed to be at the same site with both polarities. The time to reach the peak of the inhibitory potential seemed to be the same, independent of the size, as long as the recording was taken from the same cell (Fig. 21). An accurate measure of the absolute value of the latency, however, was difficult due to the initial low rate of rise of the inhibitory potential.

The strength-duration curves (Fig. 23) for the inhibitory potential and for the spike were obtained with the stimulating electrodes placed at each end of the tissue applying a uniform longitudinal field. A short current pulse (less than 1 msec) produced only the inhibitory potential but no spike. The chronaxie for the inhibitory potential was about 1 msec and that for the spike was about 30 msec. In the preliminary experiments in which the partition method was used (see (a)) they were 1 - 5 msec and 20 msec respectively (Bülbring, Kuriyama & Tomita, 1965).

If the length of the tissue was reduced to about 1 mm keeping the distance between the stimulating electrodes (3 cm) constant, the spike became graded and current threshold was increased while the inhibitory potential could be produced with little change in size and threshold current intensity as shown in Fig. 24.

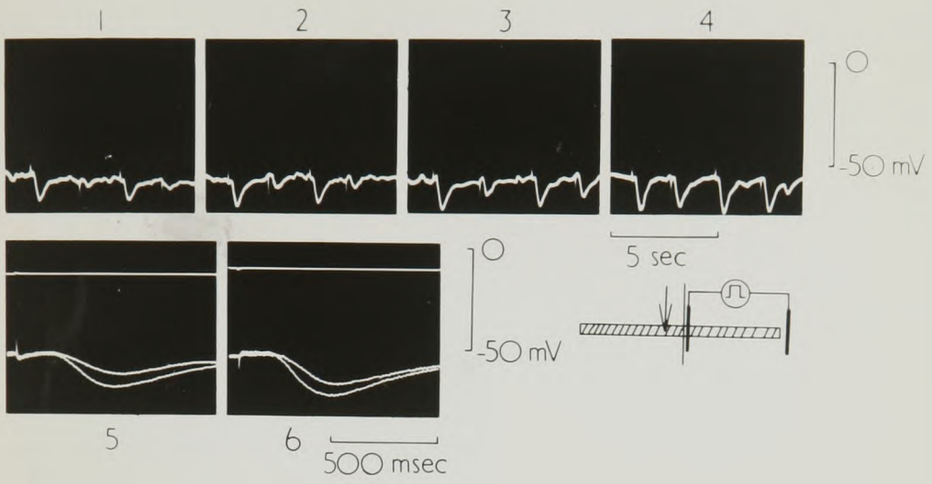


Fig. 21. The inhibitory potential produced by stimuli of constant intensity, different polarities and increasing duration. A 12 mm length of taenia was stimulated and separated from the recording site by an insulating partition, as shown in the inset. In the records 1 to 4, four stimuli of the same duration, but of alternating polarity (+, -, +, -) were applied, the stimulus duration being in 1 = 0.03; 2 = 0.05; 3 = 0.07; 4 = 0.1 msec. In 5 the responses to hyperpolarizing currents of 0.01 and 0.03 msec are superimposed and in 6 the responses to depolarizing currents of 0.07 and 0.1 msec are superimposed.

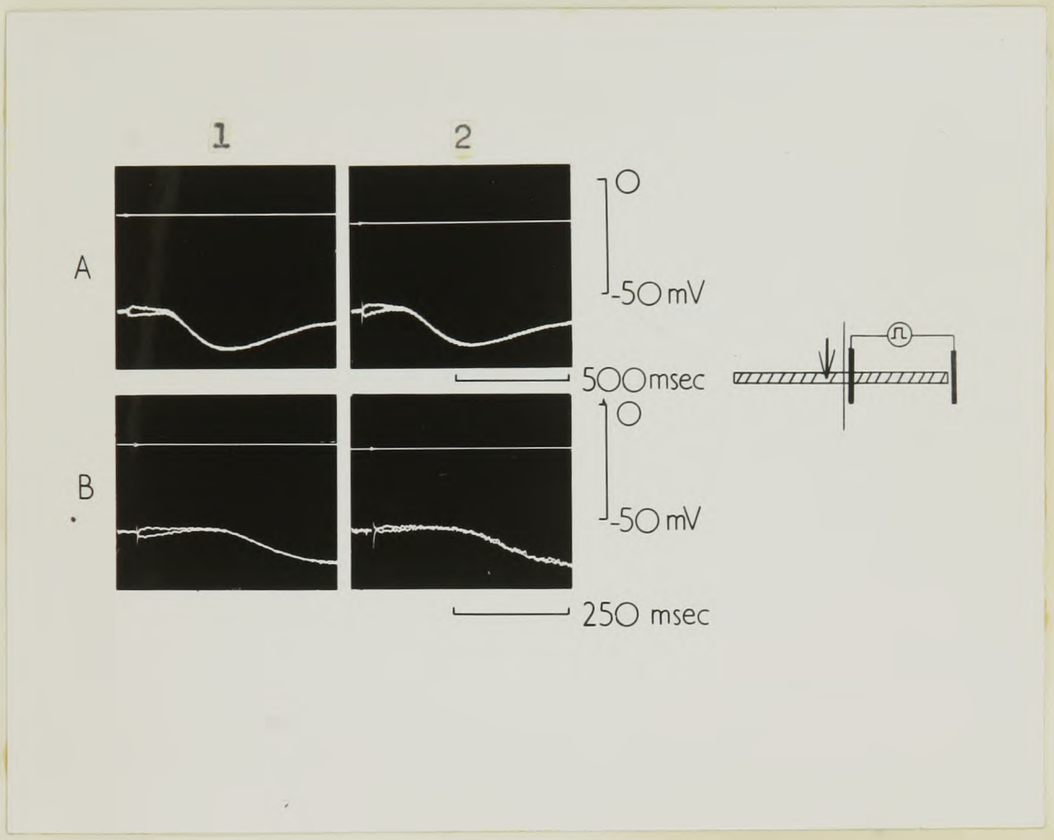


Fig. 22. The time course of the inhibitory potential from four different cells, produced by stimuli of different polarities. Stimulating and recording arrangement is shown in the inset. In each record, the responses to depolarizing and hyperpolarizing currents of the same intensity and duration were superimposed. A: recorded at 0.5 mm from the partition, stimulus duration: (1) = 0.05 msec, (2) = 0.07 msec. B1: at 0.5 mm distance, 0.07 msec; and B2: at 1.3 mm distance, 0.15 msec.

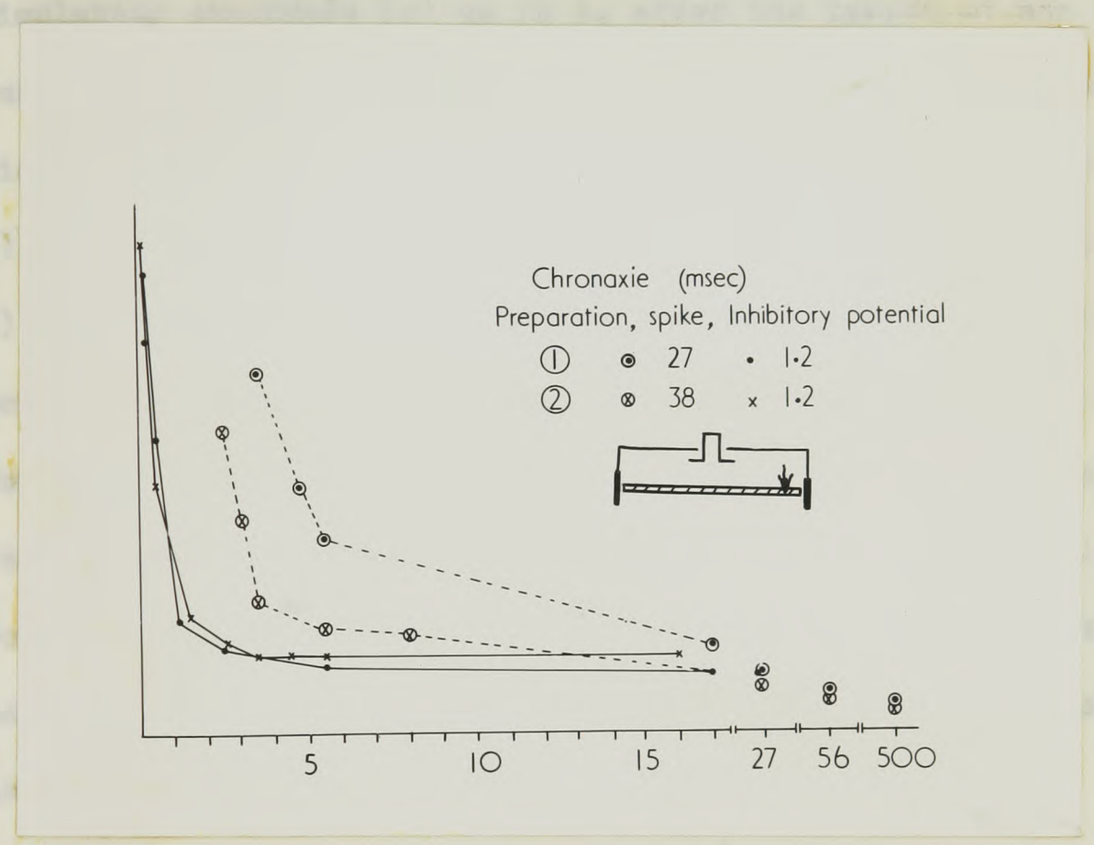
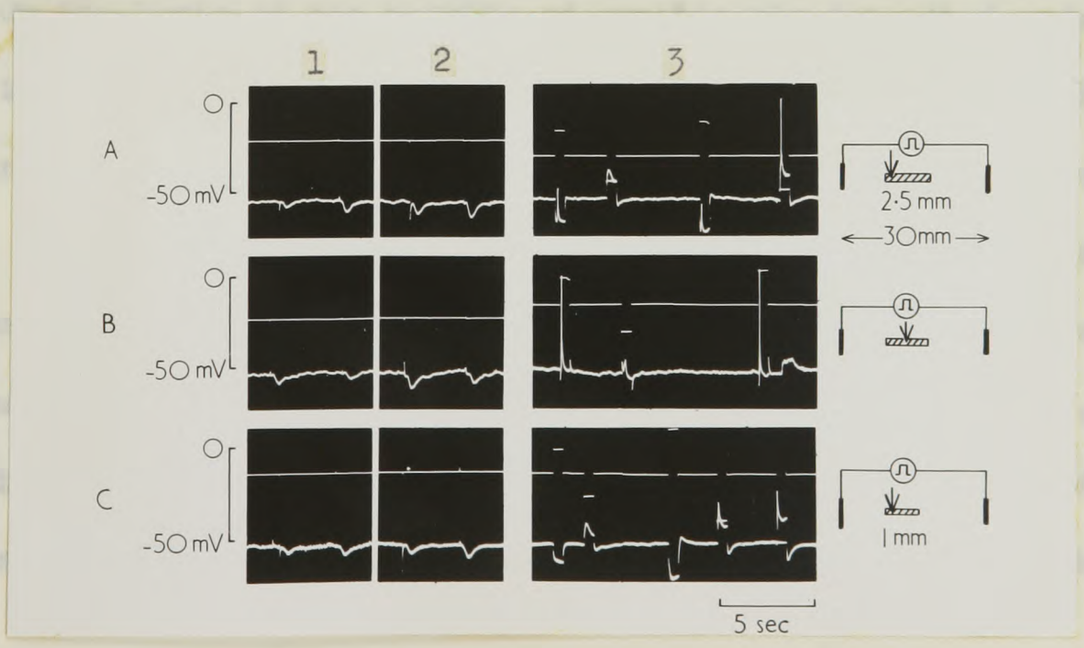


Fig. 23. The strength-duration curves for the inhibitory potential (•, x) and for the spike (⊙, ⊗) obtained from two different preparations (•, ⊙, and x, ⊗). Ordinate: current intensity in arbitrary units; abscissa: current duration in msec. The stimulus arrangement is shown in the inset.

Fig. 24. Inhibitory potentials (1 and 2), electrotonic potentials and spikes (3) recorded from a short length of taenia (2.5 mm) placed at equal distance between two stimulating electrodes (a and b) 30 mm apart. Upper traces = current monitor; lower traces = intracellular recording. A: Records from the end of the tissue near stimulating electrode (a). B: From the centre of the tissue. C: From the end near stimulating electrode (a) as in A, after the length of the tissue had been reduced to 1 mm. Records 1 and 2 show responses to two short stimuli (in 1: 0.03 msec; in 2: 0.05 msec), the first with electrode (a) being the cathode, the second with (a) being the anode. Records (3) show responses to long stimuli. Note, when electrode (a) was the anode, the membrane was hyperpolarized in A and C, but not in B; and when electrode (a) was the cathode, the membrane was depolarized in A and C, but not in B. Also note that the tissue of 1 mm length (C) produced only a graded response in contrast to the all-or-none response in A and B, but that the size of the inhibitory potential was similar to that in A and B.



(c) Experiments in which a small centre portion (1 - 2 mm) of the tissue was exposed to Krebs solution, while the two remaining parts were immersed in oil, and current was applied between the centre part and one end of the tissue. Records were taken between the centre part and the other end of the tissue, an arrangement being similar to that used for a single nerve fibre (Hodgkin & Rushton, 1946). The inhibitory potential was produced as an on-response to cathodal stimulation and generally as an off-response to anodal stimulation (Fig. 25). However, sometimes, it was noted that a depolarization with the same time course as that of the inhibitory potential was produced as an on-response to anodal stimulation and that its amplitude increased with increasing hyperpolarization, as shown in Fig. 26. This depolarization observed during the application of hyperpolarizing current may be explained by assuming that the inhibitory potential is a post-synaptic potential whose polarity can be reversed when the membrane potential becomes more negative than the synaptic equilibrium potential. (Fatt & Katz, 1953b; Coombs, Eccles and Fatt, 1955; Hoyle & Wiersma, 1958; Hagiwara, Kusano & Saito, 1960).

On the basis of the results of experiments described under (b) and (c), the inhibitory potential can be explained as a response to stimulation of nerve fibres rather than of muscle fibres. However, the following facts have to be considered: When the inhibitory potential was recorded with method (b) from a long piece of tissue (3 cm), it was the same everywhere, in the centre part and at both ends of the tissue, at both polarities of the stimulating current. Reducing the length of the tissue to 1 mm had little effect on the inhibitory potential. The observations

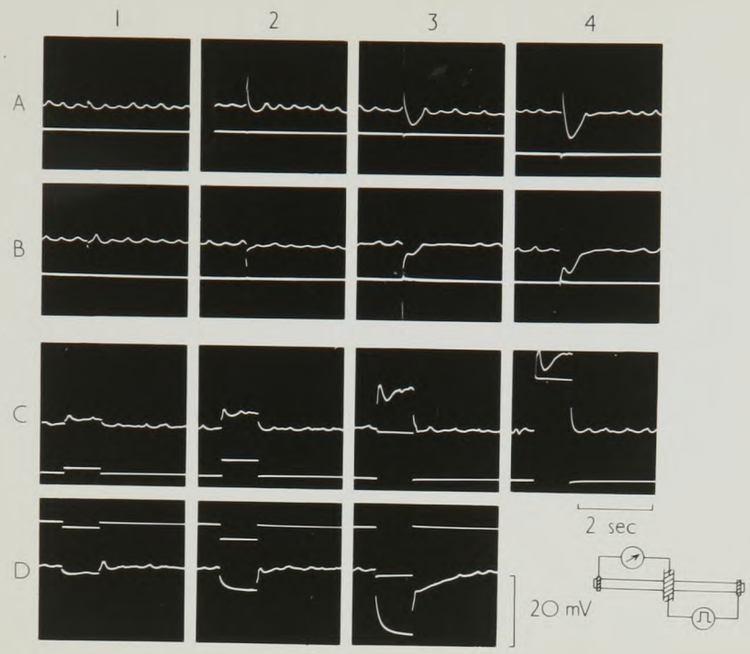


Fig. 25. Externally recorded inhibitory potentials. Stimulating and recording arrangement is shown in the inset (description see text). A: responses to depolarizing current (the stimulating electrode at the centre part of the tissue was the cathode) of increasing intensity from 1 to 4, and constant duration (10 msec). Records in B were obtained by reversing the polarity of the stimuli (hyperpolarizing current). C: Responses (upper trace) to depolarizing current pulses (lower trace) of 1 sec duration and increasing intensity. D: The same as C, with reversed polarity (and reversed traces). Note similar responses to short current pulses of both polarities and the on-response to depolarizing and off-response to hyperpolarizing current of long duration.

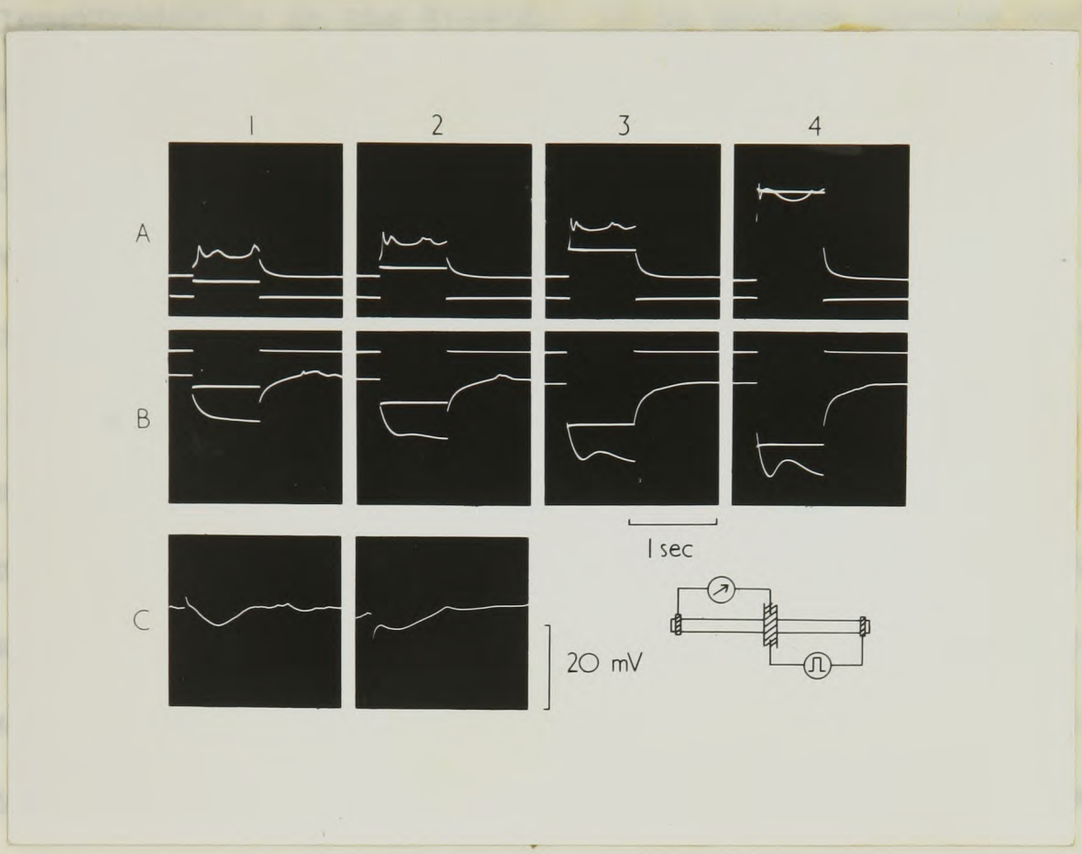


Fig. 26. Externally recorded responses to external stimulation. A: Responses (upper trace) to depolarizing current (lower trace) and B: Responses (lower trace) to hyperpolarizing current (upper trace), of 1 sec duration and increasing intensity from 1 to 4. C1: Response to depolarizing current, C2: to hyperpolarizing current of the same duration (10 msec) and the same intensity. Note the slow depolarization during the hyperpolarizing current pulse (B2, 3, 4), the time course of which is similar to that of the slow hyperpolarization during the depolarizing current pulse (A2, 3, 4). The inhibitory potential produced by short current pulses (C) is similar at both polarities.

suggest that the nerve fibres must be rather short and that they must run longitudinally in the tissue. It is unknown whether such inhibitory nerve fibres are connected with the ground plexus which consists of a dense axon plexus embedded in a network of sheath cells surrounding the muscle fibres (Hillarp, 1946, 1959) and how far excitation of this plexus is responsible for the inhibitory potential.

In a few experiments guinea-pigs were anaesthetized and a length of taenia up to 10 mm was undercut to separate it from Auerbach's plexus. After allowing a week for nerve degeneration the taenia was exised and used for electrical recording. At the end of the experiment it was examined histologically. Other pieces of taenia from normal animals were kept at 5°C for up to 96 hours and then examined. It was found that there was a good correlation between the density of surviving nerve fibres, observed in preparations vitally stained with methylene blue, and the size of the inhibitory potential after denervation or cold storage.

It has been known that tetrodotoxin blocks the spike activity in nerve (Narahashi, Moore & Scott, 1964; Nakamura, Nakajima & Grundfest, 1965). The reffect of tetrodotoxin (5×10^{-8} - 2×10^{-7} g/ml) was examined on the taenia coli in the hypertonic solution. Tetrodotoxin abolished the inhibitory potential within a few minutes, while it had no effect on the resting potential and the evoked spike in the muscle, confirming the observation by Hotta and also by Kuriyama (personal communications). The effects of denervation and tetrodotoxin are both in favour of nerve stimulation, as postulated by Bennett, Burnstock and Holman (1963).

Chapter V

External stimulation

Part II

(A) The effect of hypertonic solution. To study the electrical properties of the taenia coli more precisely the spontaneous electrical activity and the mechanical movement of the tissue were eliminated by using a hypertonic solution. The electrical responses could be obtained without any mechanical disturbance, like in the frog skeletal muscle (Hodgkin & Horowicz, 1957; Howarth, 1958). Exposure to Krebs solution containing 10% sucrose reduced the spike frequency, and then stopped spontaneous electrical and mechanical activity within 5 - 10 minutes, confirming the observation by Holman (1957). The membrane was hyperpolarized by 10 - 15 mV. The tissue remained quiescent for several hours. The effect was completely reversible.

When the external K-concentration in the hypertonic solution was raised to 18 or 24 mM, the membrane was depolarized, and then the spontaneous electrical activity reappeared although the mechanical response did not recover. The response to intracellular stimulation using the Wheatstone bridge method was the same as that in normal Krebs solution, i.e. the membrane time constant was 2 - 4 msec and a spike response was as rare as in physiological solution. In contrast, a spike was easily triggered by external stimulation, and the fast spike component could be distinguished from the slow component as during spontaneous discharges in normal Krebs solution. The amplitude and the maximum rates of rise and fall of the spike were generally greater

than those in normal solution. However, in preparations in which a very sharp spike had been observed in Krebs solution hypertonicity increased its duration mainly by decreasing the rate of fall.

It has been reported that hypertonic solution decreased the nexal area and electrotonic coupling between the cells of the taenia coli and abolished the propagation of the action potential (Barr, Dewey & Evans, 1965). However, in the present experiments, although the spontaneous electrical activity stopped in 2 times hypertonic Krebs solution (10% sucrose), the spike was still propagated at the same speed (7 cm/sec) as in physiological solution (Bülbring, Burnstock & Holman, 1958). In electron micrographs (K. Shoenberg, unpublished observations) the number of nexuses was not noticeably reduced though the intervening extracellular spaces seemed to be enlarged and the cells shrunk.

The cessation of the spontaneous activity in the hypertonic solution may be due to hyperpolarization produced by a loss of cell water and an increase of the internal K-concentration (R. Casteels & J. Setekleiv, personal communication). It may also be due to structural changes of the membrane near the nexus where the slow component (pacemaker activity) is probably produced (Chapter VI, A). The fact that increasing the external K-concentration restores the spontaneous electrical discharge in the hypertonic solution suggests that the main factor is hyperpolarization of the membrane.

(B) Electrotonic potential. Fig. 27 shows the responses to stimuli of 2 sec duration at three different intensities recorded at three different sites when the tissue was placed between two stimulating

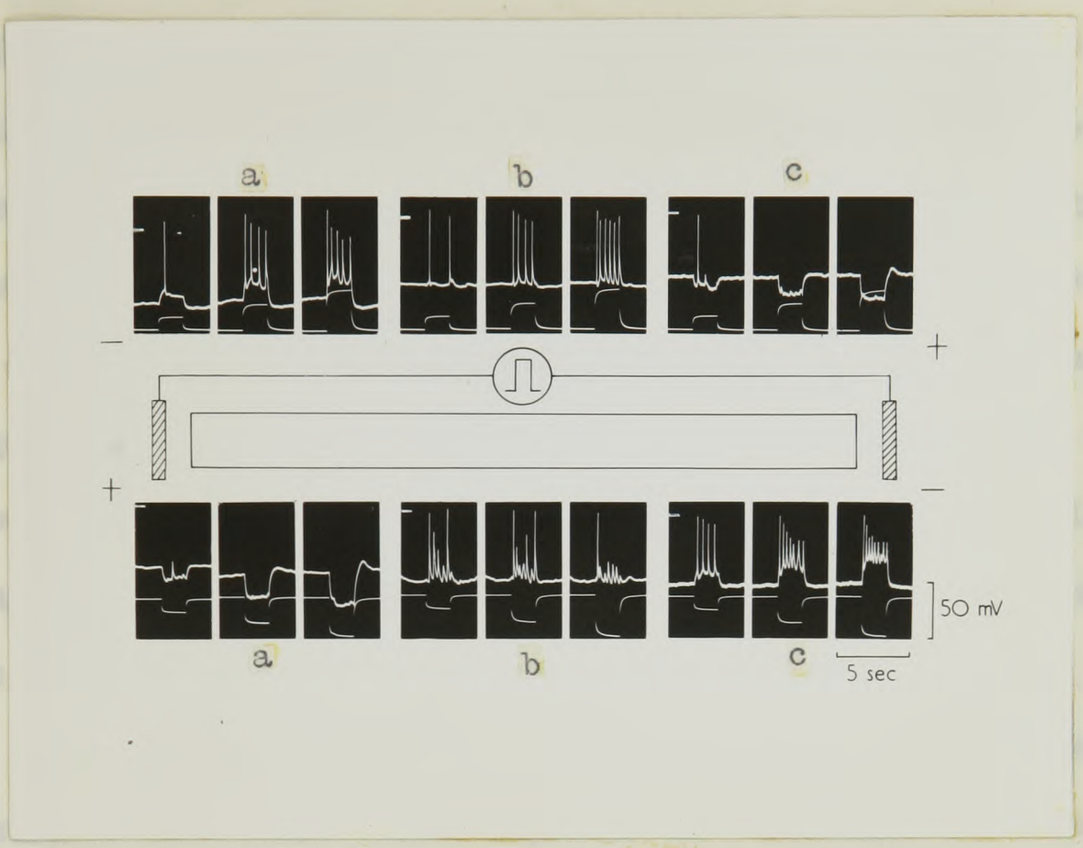


Fig. 27. Electrical responses to external stimulation recorded intracellularly (upper trace) from three different cells (a, b, c). The arrangement of the stimulating electrodes is shown in the inset; the relative current intensities are shown in the lower trace. The top row was recorded when the left stimulating electrode was the cathode and the right electrode the anode; the bottom row at reversed polarity. In each row, the three records on the left (a) were taken from the same cell near the left stimulating electrode, the three middle records (b) from a cell near the centre of the tissue, and the three right records (c) from a cell near the electrode on the right. Zero potential is indicated on the left of each group of records.

electrodes one at either end. The cells near the cathode were depolarized, those near the anode were hyperpolarized, while those in the middle part of the tissue showed no polarization. With a weak stimulating current spikes were recorded over the whole length of the tissue. The minimum potential gradient for triggering the spike was 200 - 300 mV/cm. As the stimulus intensity was increased the spike frequency increased near the cathode and in the middle part of the tissue, but spikes were abolished near the anode. Here small potential changes could still be discerned at a frequency which was the same as that of the spikes in the cells near the cathode.

The electrotonic potential was also studied when both stimulating electrodes were placed close together (3 - 5 mm apart) at one end of the tissue. In Fig. 28a, the extrapolar polarization was plotted against the distance between the recording site and the nearest stimulating electrode. It was found to change its polarity at about 1 - 2 mm from the nearest electrode. When the recording electrode was inserted far from the stimulating electrode, for example at a distance of 3 mm or more, a weak current produced a spike only when the nearest electrode was the cathode (cathodal stimulation). When the current intensity was increased, however, cathodal stimulation produced hyperpolarization which blocked the spike propagation. On the other hand, with strong currents, only anodal stimulation, which depolarized the cells far away, produced a spike. This phenomenon of reversed polarity seemed to be the same as that observed by Bülbring (1955) when one electrode was touching one end of the tissue and the other electrode was immersed in the bathing solution.

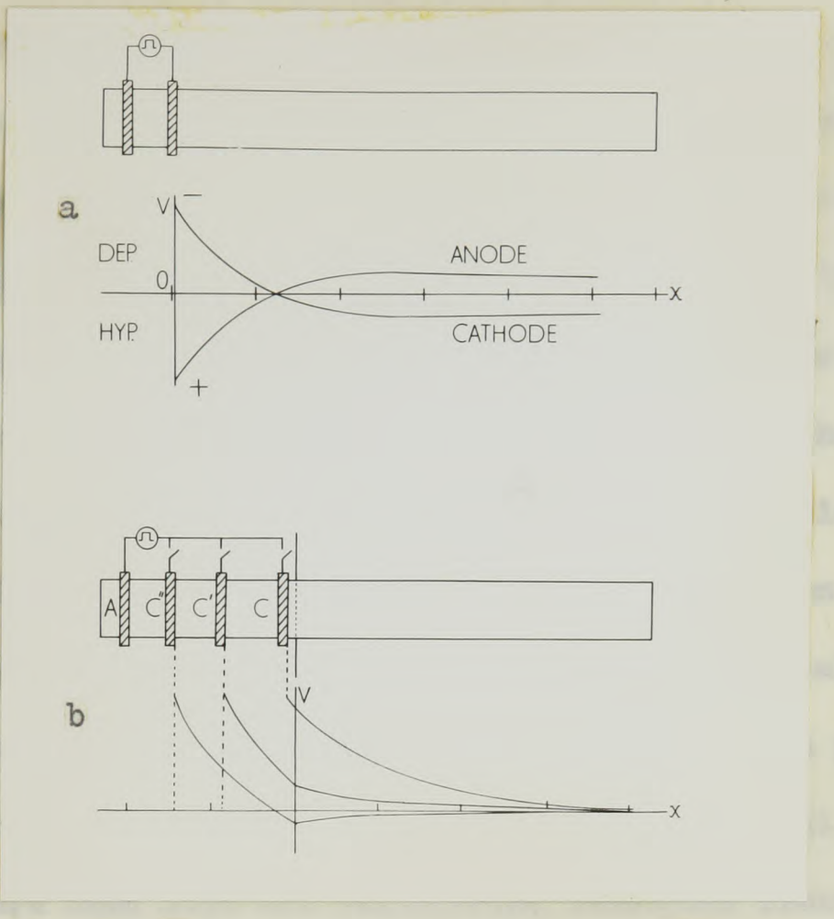
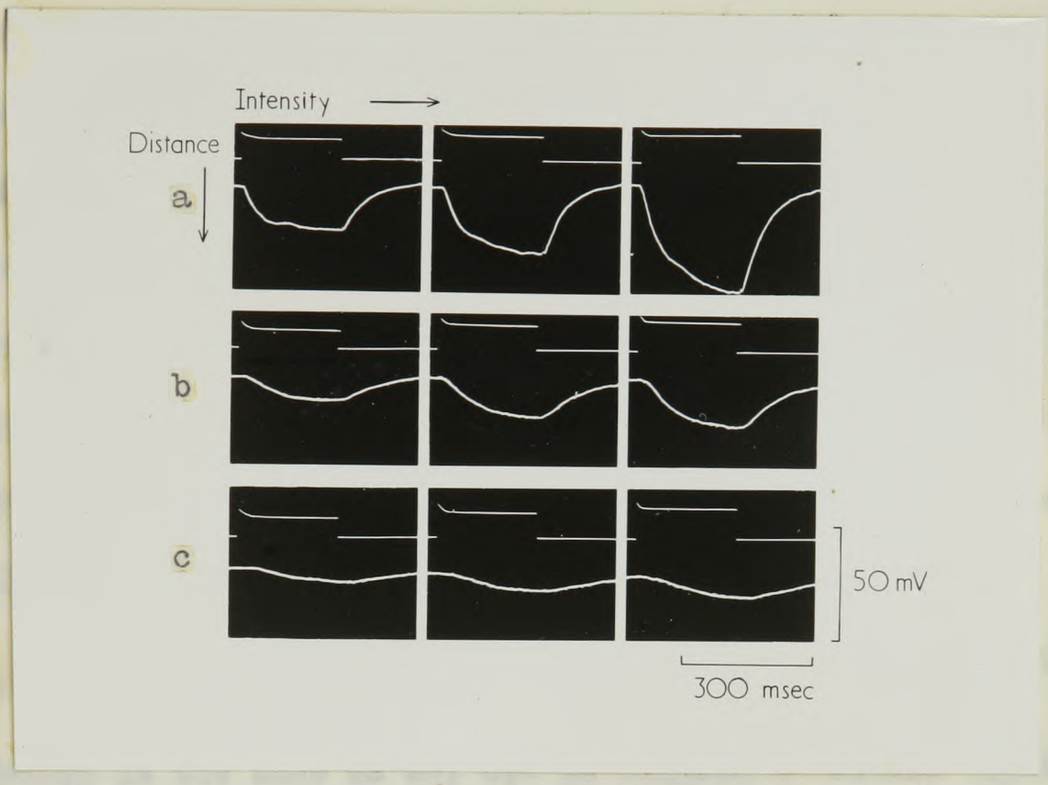


Fig.28. Schematic diagram of electrode arrangement (top) and extrapolar membrane polarization (bottom). (a) without, (b) with, insulating partition. V: voltage, x: distance between nearest stimulating electrode and recording electrode in mm. C: cathode, A: anode. Depolarization upwards (DEP); hyperpolarization downwards (HYP). (a) When the stimulating electrode nearest to the recording site was the cathode (-), the membrane polarization labelled 'Cathode' was obtained; when the polarity was reversed, the curve labelled 'Anode' was obtained. (b) Stimulating arrangement with cathode nearest partition. The curve showing membrane polarization changed with the distance of the cathode from the partition (C, C', C'').

When a partition was placed between the stimulating and the recording site, the reversed polarity was no longer observed and the electrotonic potential decreased roughly exponentially with the distance of the recording electrode from the partition, as shown in the lower diagram of Fig. 28b. When this arrangement was used, the magnitude and the sign of the electrotonic potential depended on the distance between the nearest stimulating electrode and the partition. As the nearest stimulating electrode was moved further away from the partition ($C \rightarrow C' \rightarrow C''$), the amplitude of the electrotonic potential decreased and finally reversed its polarity. This could have been predicted from the relation shown in the lower diagram of Fig. 28a. All the following experiments were done with the stimulating electrode close to the partition.

Fig. 29 shows examples of the electrotonic potential produced by anodal stimuli of 250 msec at different intensities and recorded intracellularly at different distances from the partition. The electrotonic potential not only decreased in size roughly exponentially with distance, but also in its rates of rise and fall. It was found that the electrotonic potentials had a time course similar to those predicted from the cable theory applied to a nerve fibre by Hodgkin & Rushton (1946).

The space constant (λ), the distance at which the electrotonic potential was reduced to 1/e, was measured from the exponential spatial decay of the electrotonic potential along the tissue. It ranged from 1.4 to 1.9 mm in 6 experiments (mean 1.6 mm). The electrotonic



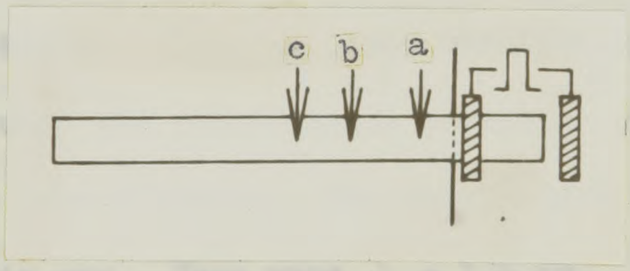


Fig. 29. Intracellular records of electrotonic potentials (lower trace) produced by externally applied anodal current of three different intensities (monitored in upper trace). (a) at 0.7 mm, (b) at 2.2 mm, (c) at 3.3 mm from the partition. Recording and stimulating arrangement are shown in the inset.

potentials obtained near the insulating partition had the time constant (τ_m) of 55 msec (range: 38 - 85 msec) which was measured from the time reached to 84% of the maximum. However, this value could not be taken as the true value of the time constant because current flow near the partition might be complicated due to imperfect insulation around the tissue and the distance between the stimulating electrode and the partition. The time constant (τ_m) was calculated from the following cable equation inserting an appropriate value for T (= t/τ_m) to get the theoretical curve (V_m) which fitted the actual records of electrotonic potentials at three different distances from the partition.

$$V_m = V_{m\,t=\infty,\,x=0}\,\frac{1}{2}\left\{e^{X}\left[1+\mathrm{erf}\left(\frac{X}{2\sqrt{T}}+\sqrt{T}\right)\right]-e^{-X}\left[1+\mathrm{erf}\left(\frac{X}{2\sqrt{T}}-\sqrt{T}\right)\right]\right\}$$

This equation is the same as eq. 6.0 in Hodgkin & Rushton (1946) and the derivation is presented in detail in Chapter VI C, of the present thesis. The steady level of the electrotonic potential at zero distance from the stimulating electrode, $V_{m\,t=\infty,\,x=0}$, was obtained by extrapolating the relationship between the electrotonic potential and the distance from the partition to zero distance. The values from two preparations were calculated in this way. They were λ = 1.6 mm, τ_m = 60 msec; λ = 1.4 mm, τ_m = 80 msec. When the time to reach half-maximum of the electrotonic potential was plotted against distance the relation was a straight line as observed in nerve (Hodgkin & Rushton, 1946) and in skeletal muscle fibre (Katz, 1948). From this somewhat longer time constants were obtained, i.e. 70 and 100 msec. These values are consistent with Nagai & Prosser's (1963b) observations in the circular

intestinal muscle of the cat in which they used pressure electrodes; they found the space constant to be 1.03 mm and the time constant, 133 msec.

Some slight deviations from the time course of the electrotonic potential predicted by the cable theory were observed. For example, with strong anodal stimulation, electrical activity often appeared as a result of the conducted activity generated near the cathode, as will be described on p.45 and Fig. 39. Furthermore, if the anodal current pulse was shorter than 200 - 300 msec the rate of decay of the electrotonic potential was usually slower than the rate of onset. An increase of the membrane resistance due to hyperpolarization may be one of the reasons for the slow decay or it may be due to the remaining part of the inhibitory potential. However, when a strong anodal current of long duration was applied, the rate of fall became faster than the rate of rise due to anodal break response. The size of the slow depolarization produced on the cessation of anodal current increased in a graded manner with the strength of the applied current (Fig. 30). The response was clearer when the record was taken near the stimulating electrode, and about 1 sec in duration and up to 20 mV in size. Sometimes, the large slow depolarization triggered a spike as seen in Fig. 30. This might correspond to the anode break excitation in nerve fibre (Ooyama & Wright, 1961).

When the external K concentration was increased from 5.9 mM to 66 mM (replacing Na which was correspondingly reduced) the current-voltage relationship of the electrotonic potential shifted towards the

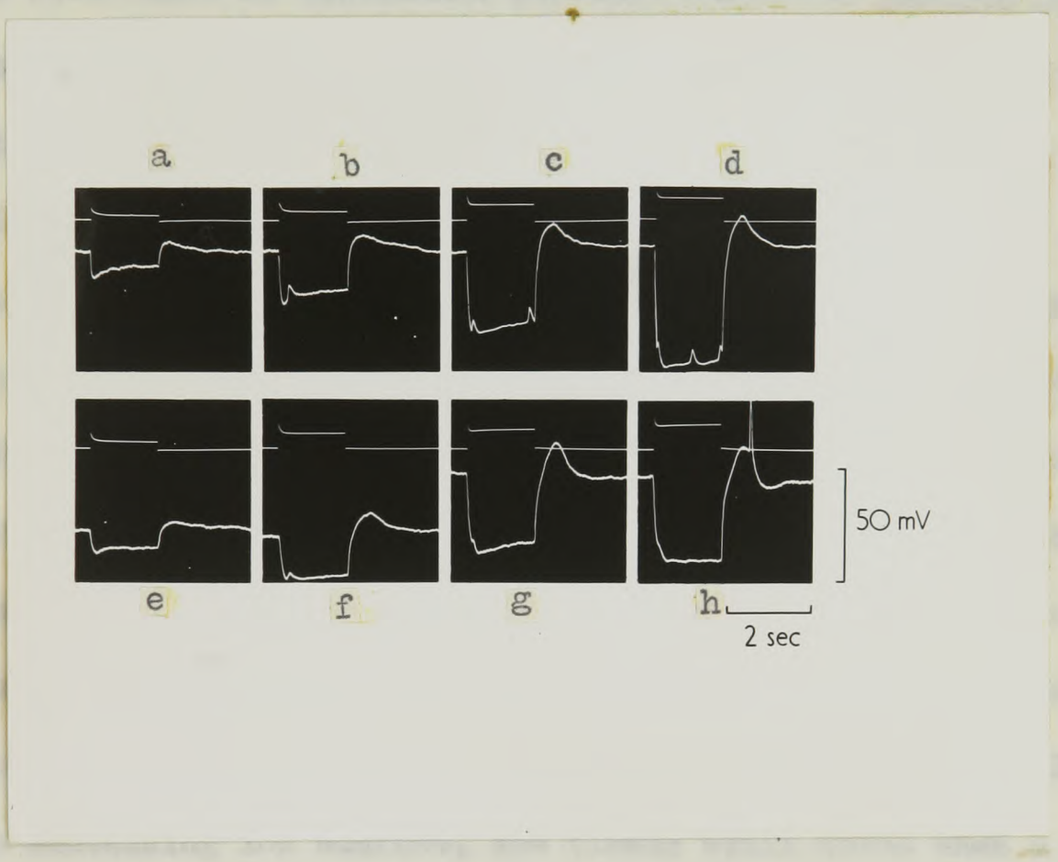


Fig. 30. Intracellular records of responses (lower trace) to long anodal current pulses of increasing intensity (monitored in upper trace) applied externally. (Arrangement as in Fig. 29) a-d at 0.2 mm; e-h at 0.7 mm distance from the partition. Note the appearance of small potential changes during hyperpolarization and, after cessation of current pulse, the slow depolarization which, in the last record (h), triggered a spike.

current axis and the space constant was reduced from 1.7 mm to 0.95 mm. This change of the space constant in high external K corresponds a reduction to 1/3.2 of the membrane resistance, since it is proportional to the square of the space constant (eq. (10) in chapter VI, p. 76). Frankenhaeuser & Waltman (1959) obtained a reduction to 1/4 of the membrane resistance of desheathed peroneal nerves of Xenopus laevis in isotonic KCl solution, calculated from the decrease of the space constant from 2 mm to 1 mm.

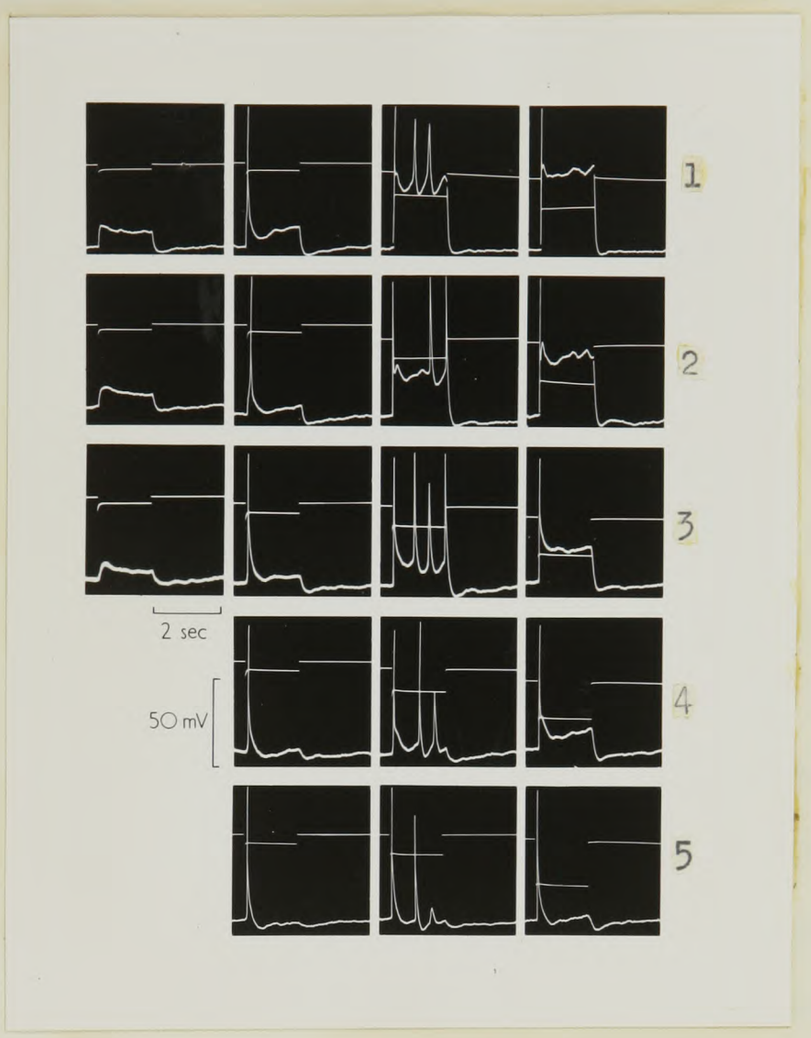
Reducing Na (replaced with tris) or Cl (replaced with ethanesulphonate) had little effect on the shape of the electrotonic potential, the current-voltage relationship or the space constant.

A few experiments were done on the vas deferens of the guinea-pig in hypertonic solution. As in taenia coli, the electrotonic potential was observed at a distance of many cell lengths from the insulating partition. Depolarization produced a spike. When the stimulus duration was more than 1 sec, repetitive spikes were easily produced. In Krebs solution containing 10% sucrose, the tissue still moved when a spike was evoked, although it was much weaker than in normal Krebs solution. This was probably due to incomplete penetration of the sucrose solution into deep parts of the tissue which is thicker than the taenia and for this reason less suitable. Since the properties of the electrotonic potential were found to be similar to those of the taenia, though the vas deferens is normally quiescent and is physiologically excited through nerves, it is assumed that all visceral smooth muscles have cell-to-cell connections.

(C) Spike generation and propagation. Repetitive spikes usually appeared when a depolarizing stimulus was sufficiently long (more than 1 sec). Fig. 31 shows the responses to cathodal stimulation of 2 sec duration and different intensities recorded at different distances from the partition. Up to a certain limit, the number of spikes produced by a stimulus increased with its intensity, but beyond this, increasing the intensity reduced the number of spikes to one (also cf. Fig. 39). The observed maximum frequency was usually less than 2/sec. Far away from the partition the electrotonic potential was small as expected from the electrotonic spread, but the frequency pattern of the spikes was similar to that in the cell near the partition. After cessation of a long cathodal stimulus, there was a transient hyperpolarization the time course of which was very similar to the positive after-potential following a spike.

In Fig. 32, the amplitudes of the intracellularly recorded electrotonic potential as well as of the action potential are plotted against the intensity of externally applied current. Since the peak potential of the spike remained almost constant, it may be assumed that a true electrotonic potential across the membrane was actually recorded. The observed rectification shows that membrane resistance decreased with strong depolarization. In some preparations the resistance increased slightly with strong hyperpolarization.

Since the strength-duration curve was a simple hyperbola as shown in Fig. 33, and the chronaxie was 16 - 23 (mean 19) msec in four experiments, a nervous contribution to excitation was unlikely. This



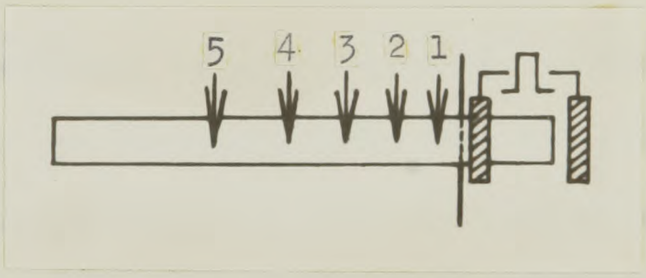


Fig. 31. Intracellular records (lower trace) of responses to external cathodal stimulation of four different intensities (monitored in upper trace). (Arrangement see inset) Row (1) at 0.2 mm; row (2) at 0.7 mm; row (3) at 1.5 mm; row (4) at 2.2 mm; row (5) at 3.3 mm distance from the partition. Note that the size of the electrotonic potential diminished but that the spike frequency pattern remained the same at different recording distances.

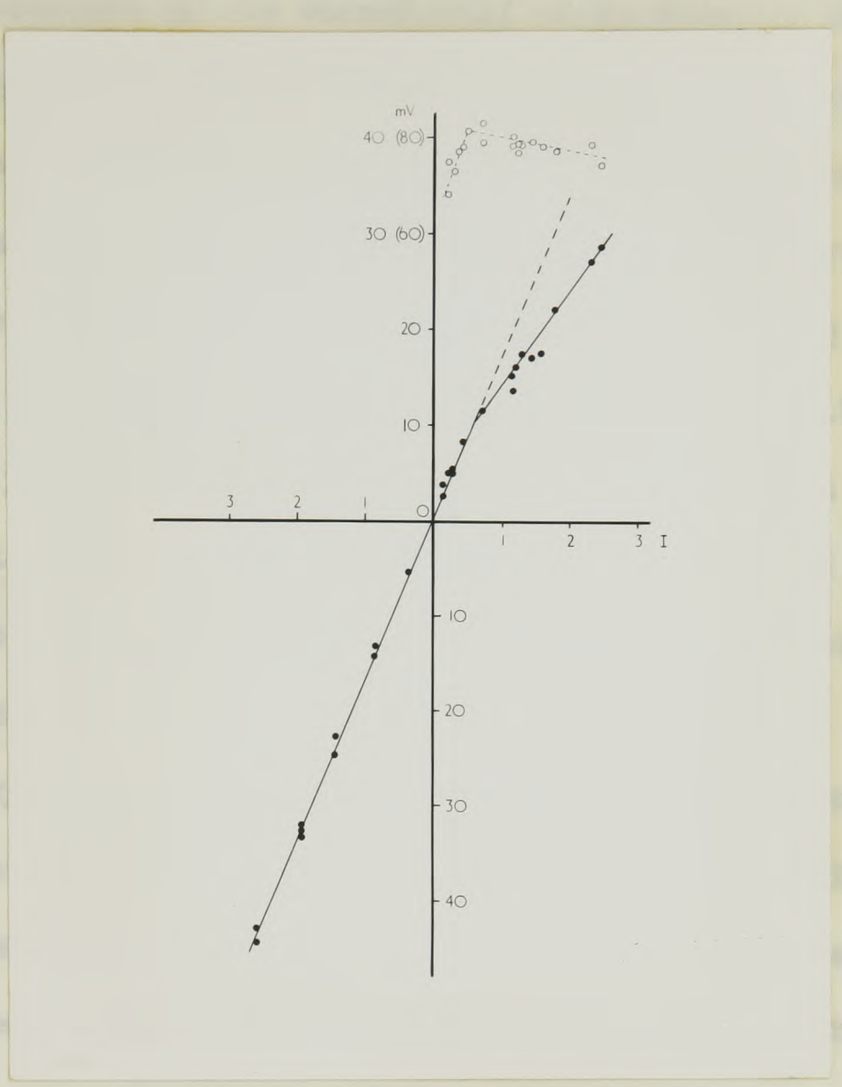


Fig. 32. Relationship between externally applied current (I, arbitrary units) and intracellularly recorded potential (mV). The values were obtained at 0.3 mm from the partition (stimulating arrangement of Fig. 31) and are shown by the closed circles. Depolarization upward, hyperpolarization downward. Open circles: peak of the spike potential (voltage scale in brackets).

chronaxie was obtained by the stimulating arrangement in which one ring electrode was placed at the end of the tissue and the other electrode was placed in the bathing solution as shown in the inset of Fig. 33. A similar strength-duration curve and chronaxie were observed when both electrodes were touching the tissue and the recording site was separated from the stimulating site by an insulating partition. When the tissue was placed between two stimulating electrodes a little longer chronaxie was obtained (see Chapter IV, p. 31 and Fig. 23). There were some qualitative differences between the responses to long and short stimuli. When the stimulating current was short (less than 10 msec), the spike was sometimes produced in a graded manner with a short latency as shown in Fig. 34. Spikes thus produced did not show a clear slow component (see also Fig. 35); their properties being similar to those of the spike triggered by intracellular stimulation (Fig. 9). When the stimulating current was long (more than 20 msec) the spike appeared in an all-or-none manner and its latency was sometimes very long (up to 1 sec) at threshold intensity. The threshold potential at which the spike arose was higher for the spike with short latency than for that with long latency (see the bottom right hand record in Fig. 34).

When records were taken near the stimulating electrode (within 1 mm), spikes of small amplitude, irregular shape and variable latency appeared sometimes in response to stimuli of near threshold intensity, as shown in Figs. 34, 35 and 36A. These irregular spikes arose on a slow potential change which is probably the slow component (see Fig. 35).

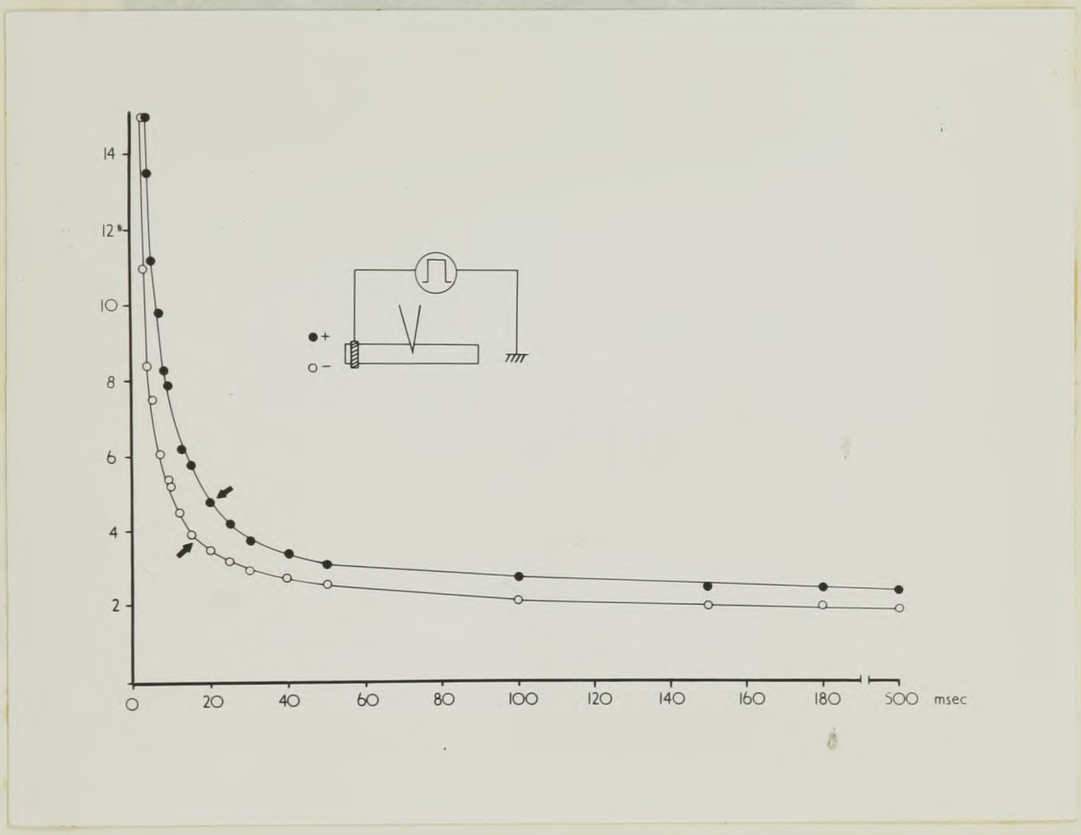


Fig. 33. Strength-duration curve for the spike obtained by the stimulating arrangement shown in the inset. Ordinate: current intensity in arbitrary units; abscissa: current duration in msec. Values shown by closed circles were obtained when the electrode at the end of the tissue was the anode; open circles when it was the cathode. Chronaxie was 20 and 17 msec respectively, indicated by arrows.

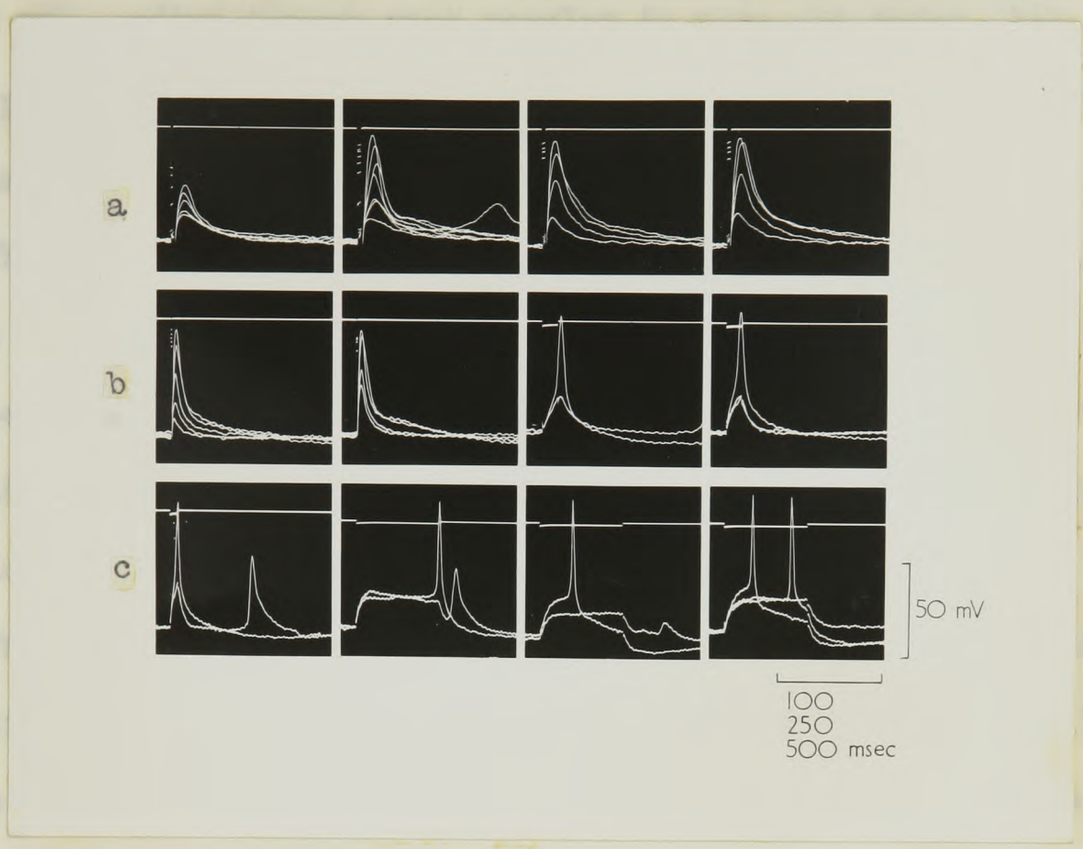


Fig. 34. Threshold responses to external current pulses of different duration and intensity (monitored in upper trace), recorded intracellularly from the same cell (lower trace). Stimulating arrangement is shown in the inset. From left to right: row (a) 2, 3, 3, 3 msec; row (b) 3, 5, 30, 30 msec; row (c) 30, 400, 400, 400 msec. Note different time bases, 100 msec for (a), 250 msec for (b), and 500 msec for (c).

When records were taken far from the stimulating electrode (more than 3 mm), spikes became more regular in size and shape (Fig. 36B). It is possible that spikes of small size near the stimulating electrode increased to normal size or died out during conduction.

The spike was propagated over the whole tissue in both directions at the same speed. The conduction velocity was measured with two micro-electrodes which were inserted 3 - 5 mm apart. The conduction velocity was 7.3 ± 0.7 cm/sec, the same as that obtained in Krebs solution by Bülbring, Burnstock & Holman (1958).

From the difference in latency at different distances from the partition and also from the difference in the critical firing level it was evident that the spike was generated near the partition and conducted along the tissue.

The refractory period of the conducted spike was much longer than that of the spike which was recorded near the partition. The absolute refractory period of the spike which was directly produced by the stimulating current was nearly as short as the duration of the spike (22 - 36 msec, mean 29 msec in 5 experiments), while that of the conducted spike was 180 - 1300 msec (mean 620 msec) in 5 experiments. When repeated stimuli were applied in short succession, a second small spike was observed near the partition, i.e. near the stimulating electrode, while no spike was observed far from the stimulating electrode.

Since the properties of the electrotonic potential strongly suggested that muscle cells were electrically interconnected it was of interest to study effect of cutting the tissue. Reducing the width

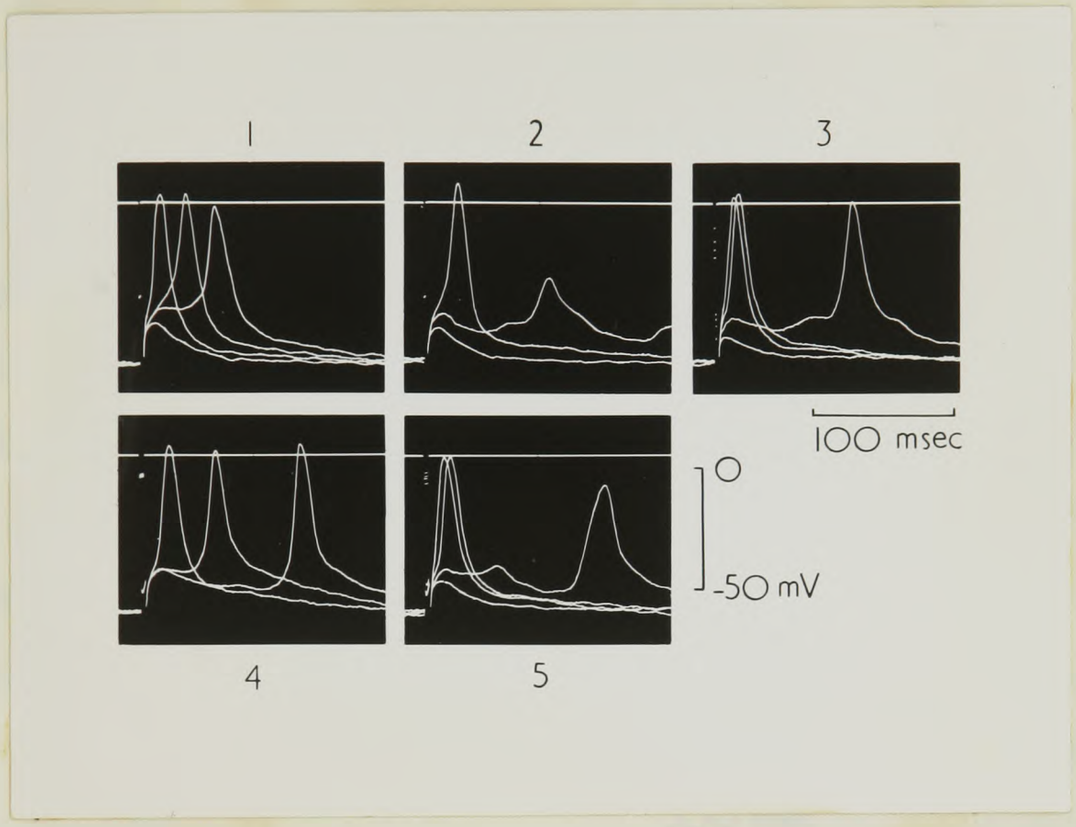


Fig. 35. Threshold response to short external current pulses. (Upper trace: current monitor, lower trace: intracellular record). Stimulating arrangement is shown in the inset of Fig. 34. Records were taken from the same cell at 0.5 mm distance from the partition. Stimulus duration: (1) and (2) = 1 msec; (3) = 1.8 msec; (4) and (5) = 3.5 msec. Note irregular spike with long latency on the slow potential change.

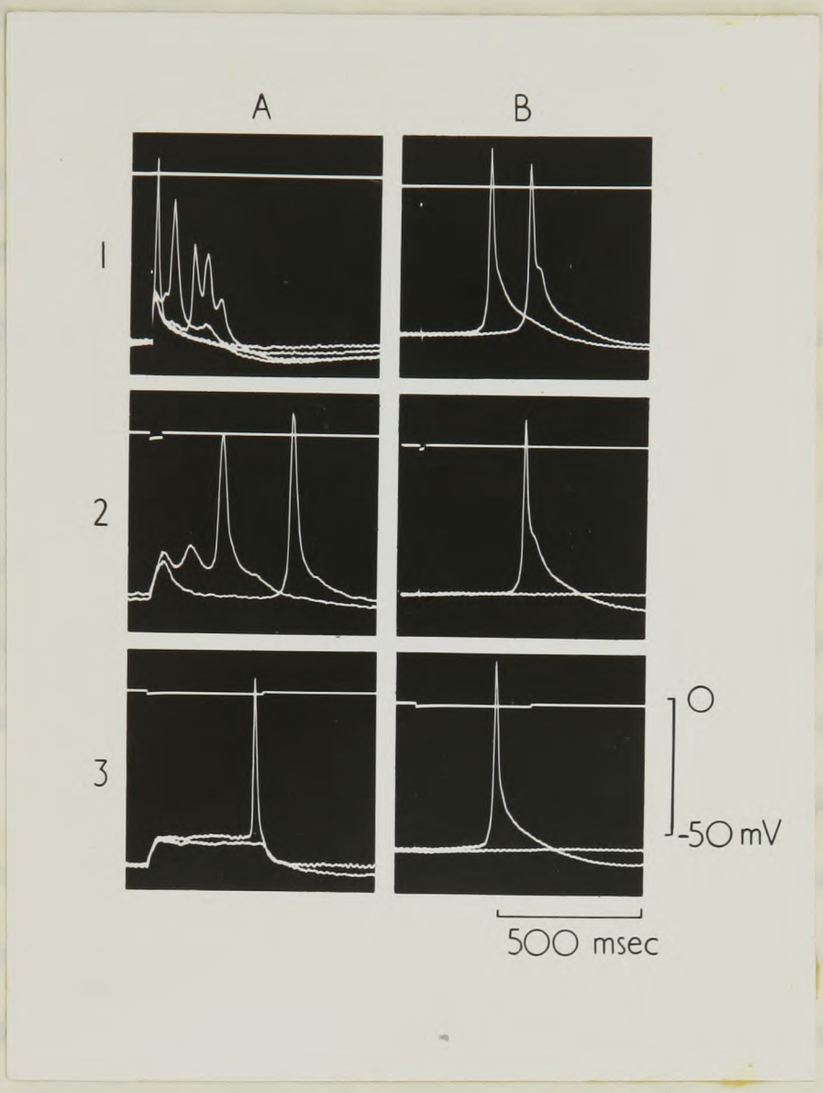


Fig. 36. Threshold responses to external stimulation obtained at different distances from the stimulating electrode. Stimulating arrangement is shown in the inset of Fig. 34. Upper trace: current monitor, lower trace: intracellular record. Stimulus duration: (1) = 2 msec; (2) = 15 msec; (3) = 400 msec (Time scale for 2A is 250 msec). Recording distance in (A) was 0.3 mm, in (B) 5 mm from the partition. Note irregular response to short current pulse near the partition (A), but regular spike far from the partition (B), and also note the difference in the electrotonic potential.

of the tissue by cutting the muscle longitudinally, parallel to the fibre axis, did not affect spike generation, provided the strips were wider than 150 μ. However, in a strip of less than 100 μ in width, the spike was produced in a graded manner (Fig. 37). This result might be either due to damage or to the small size of the tissue being a limiting factor for the excitability or for the spike amplitude. The importance of tissue size was also suggested by the finding that a graded spike was produced when a monopolar stimulating electrode of 50 μ diameter was used, while an electrode of 100 μ diameter produced a full spike in an all-or-none manner, confirming the observations by Nagai & Prosser (1963a) in the cat's intestinal circular muscle.

When part of the tissue was cut transversely, at right angles to the axis, and the micro-electrode was inserted into the bundle which was cut, no electrotonic potential could be recorded (Fig. 38, 5 - 9), but it was seen when recording was taken from an intact bundle (Fig. 38, 1 - 4). The bundle, 20 - 40 μ in width, was clearly observed through the microscope (cf. Bülbring, 1954). As far as passive electrical connection was concerned therefore, each bundle appeared to be independent, suggesting a very poor electrotonic spread across side connections between the bundles. In contrast, the spike evoked by the electrotonic potential (Fig. 38, 3 and 4) was recorded even in the transversely cut bundle (Fig. 38, A 8, 9; B 9), suggesting that spike conduction occurred across side connection between the bundles.

During a long anodal current pulse small repetitive potential changes were often seen, especially near the stimulating electrode, as

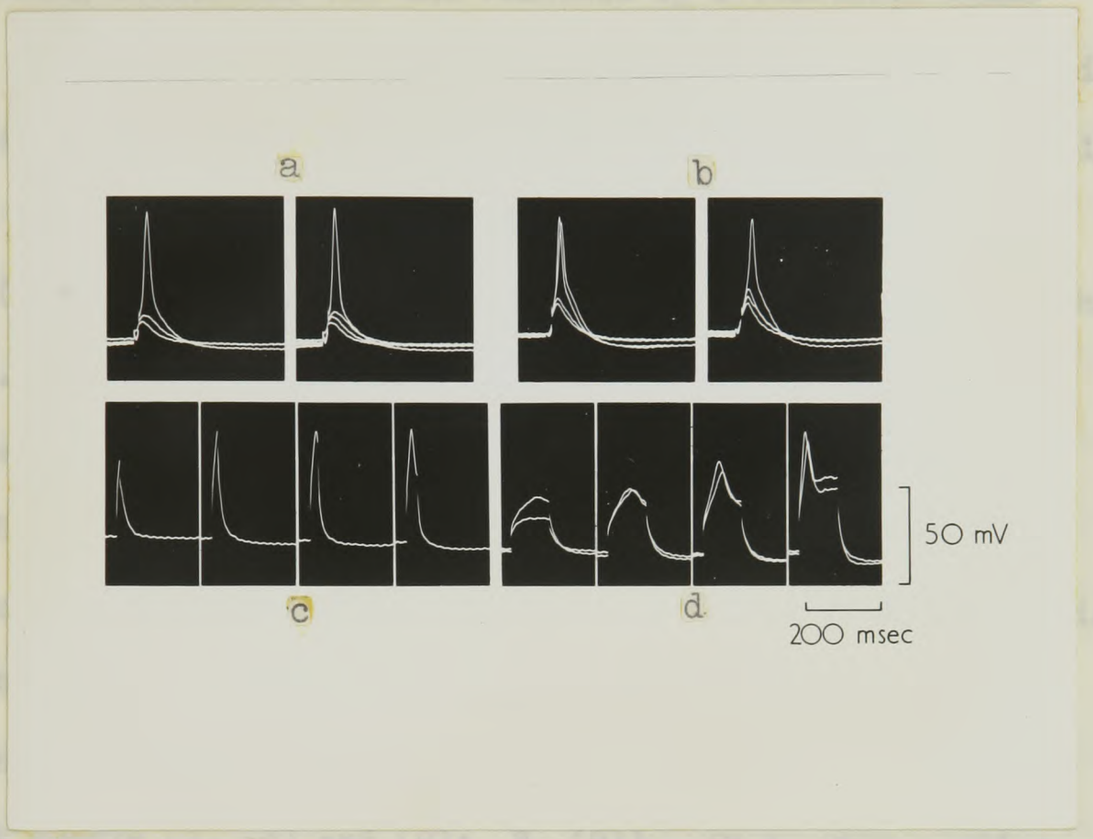
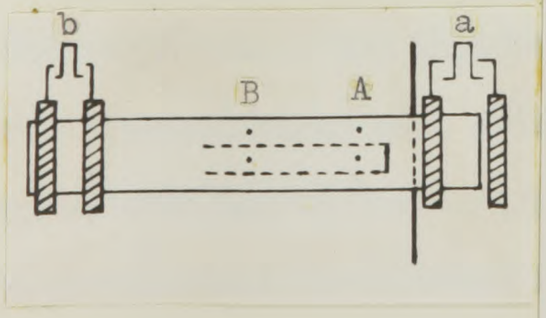
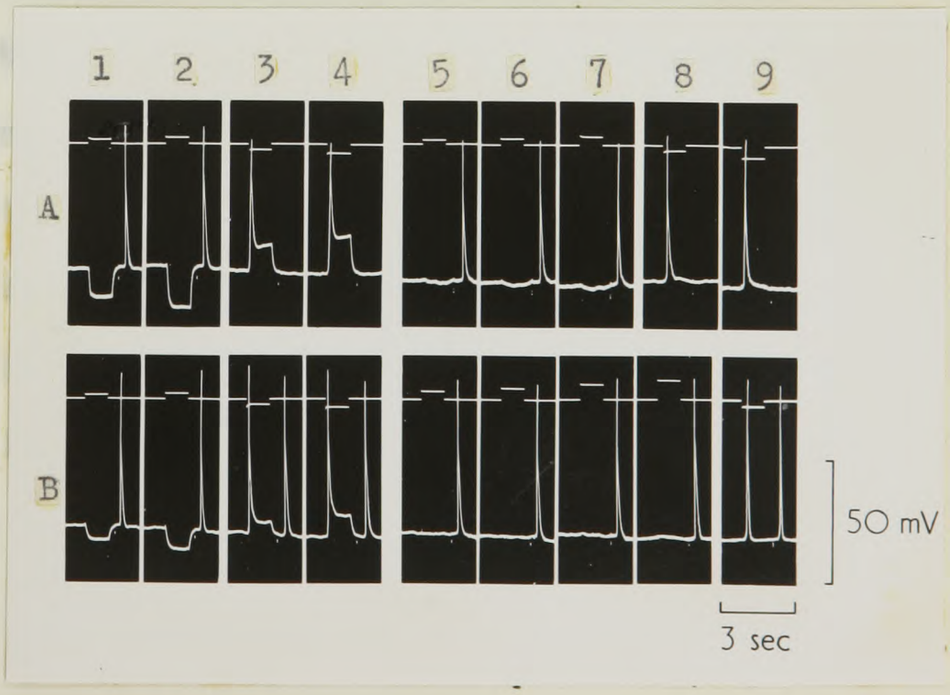


Fig. 37. Effect of reducing the width of the tissue, by cutting longitudinally, on spike generation. Records were taken intracellularly from three different fibres. (a): Two records from the same cell showing superimposed responses to stimuli of increasing intensity in a strip of 220 μ width; (b): When the width was reduced to 130 μ. The eight records in (c) and (d) were obtained from the same cell in a strip of 70 μ width, (c) with increasing stimulus duration, (d) with increasing stimulus intensity. The stimulus intensity was adjusted to near threshold. Stimulus duration: (a) and (b) 15 msec; (c) 10-30 msec; (d) 60 msec.

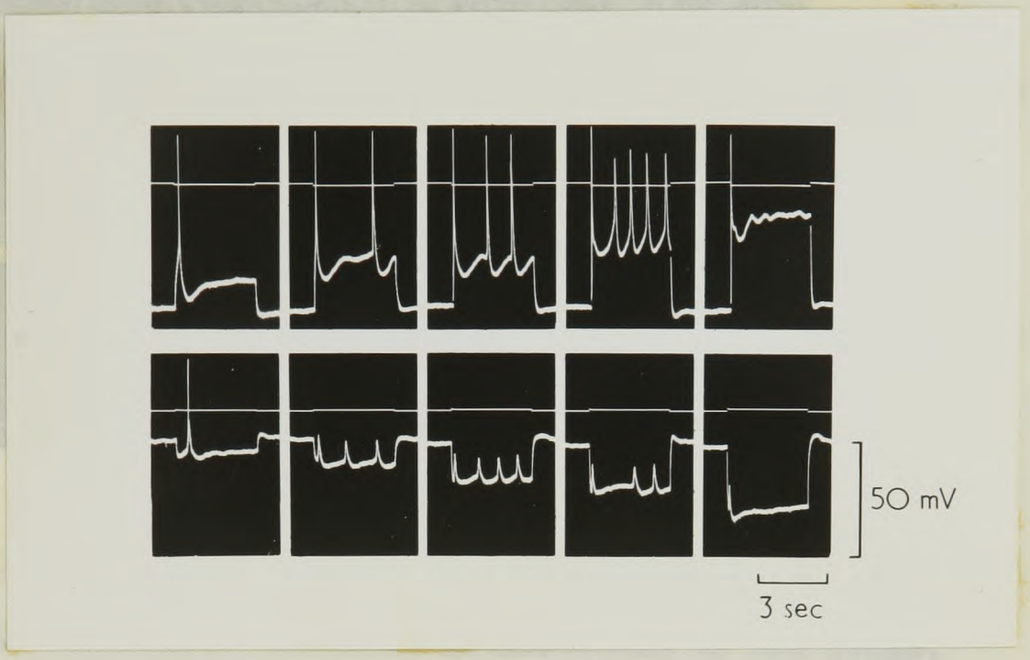
Fig. 38. Effect of cutting the tissue transversely. Electrode arrangement shown in inset. The electrotonic potential was produced by stimulating electrodes (a) and followed by a conducted spike evoked by a test shock of 5 msec from electrodes (b) at the opposite end of the tissue. A small part of the tissue was cut transversely at 0.3 mm distance from the partition. The micro-electrode was inserted (A) at 0.8 mm distance and (B) at 1.5 mm distance from the partition. Records 1-4 were taken from an intact bundle and, by moving the micro-electrode less than 50 μ across, 5-9 from a cut bundle. Upper trace = relative polarizing stimulus intensity, lower trace = intracellular record. Note, no electrotonic potential in the cut bundle (5-9), but the spike which was triggered by the electrotonic potential in the cut bundle in (3) and (4), appeared in the cut bundle (A, (8) and (9); B, (9)). In A, when a spike had been triggered by the electrotonic potential produced by electrode (a), electrode (b) failed to evoke a conducted spike, probably because it fell into the refractory period.





shown in Fig. 39. In the upper row of Fig. 39 are responses to cathodal stimulation from the same cell. With increasing stimulus intensity repetitive discharge of increasing frequency was observed, but with too strong intensity this was absent. The lower row shows responses to anodal stimulation. When the anodal stimulus intensity was weak, a large spike was observed though its amplitude was smaller than that of the spike produced by the cathodal stimulation. As the intensity was stepped up small repetitive responses appeared. These responses seemed to be due to the spike activity produced near the cathode, because the frequency patterns of the top and bottom records shown in Fig. 39 were similar. Such low amplitude spike activity is also seen in Figs. 27 and 30.

In order to study these small responses the following experiment was carried out. Two sets of the stimulating electrodes were placed, one at each end of the tissue and an insulating partition separated one end with one pair of electrodes from the remaining part of the tissue from which records were taken, as shown in the inset of Fig. 40. The electrodes (A) in the recording part (left) were used for triggering the spike and those (B) in the stimulating part (right) were used for application of the electrotonic potential. As shown in Fig. 40 (a), the amplitude of the conducted spike was decreased when the membrane was hyperpolarized with external electrodes. This reduction of the spike size may be due to a process similar to that operating during hyperpolarization as shown in Fig. 39. The middle row of Fig. 40 (b) shows a similar response to that in the top row, but taken by fast



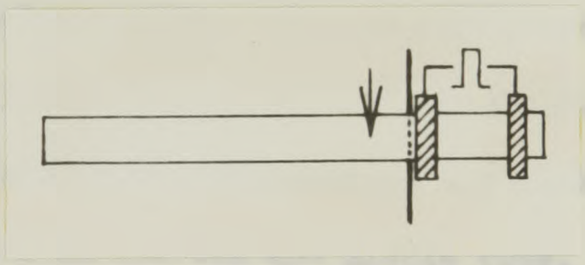


Fig. 39. The response to depolarizing (top row) and hyperpolarizing current pulses (bottom row) from the same cell close to the partition (about 200 μ distance). Stimulating arrangement is shown in the inset. Upper trace, monitor of stimulus; lower trace, intracellular record. Note the appearance of small potential changes during hyperpolarization at a frequency pattern similar to that of the spikes produced by depolarizing current.

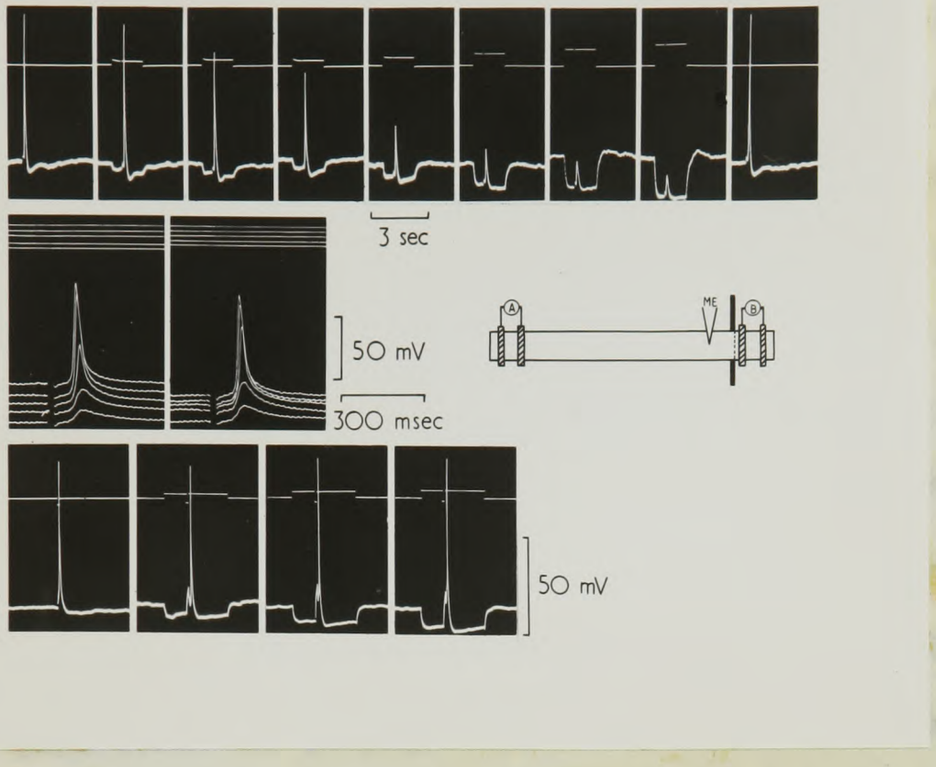


Fig. 40. Effects of external polarization on the spike. Electrode arrangement shown in inset. Upper trace, stimulus; lower trace, intracellular record. The recording micro-electrode (ME) was inserted at about 0.5 mm from the partition. During hyperpolarization applied with electrodes B, the spike was triggered by electrode A in the top and middle rows, and by electrode B in the bottom row. In the top and middle rows, the polarizing current intensity was successively increased, while the current intensity for triggering the spike was kept constant. In the bottom row, the stimulus current intensity was adjusted to threshold intensity whenever the polarizing current was increased. Note change of the amplitude and shape of the conducted spike by conditioning hyperpolarization. Also note that the time to reach the peak remains constant.

sweep to show that the time course was almost the same though the spike amplitude was reduced during hyperpolarization. If the spike was generated by the same electrode as that used for the membrane polarization (electrode B), the spike amplitude remained constant during anodal polarization as shown in the bottom row of Fig. 40 (c). Therefore, externally applied hyperpolarization affected only the conducted spike (Fig. 40a), probably due to reducing the effectiveness of the cell-to-cell interaction, because when the test shock caused enough depolarization (Fig. 40, c), the cell produced a full spike.

Spike generation and propagation in the taenia was found to be very complicated: The response produced near the partition had different properties from that produced far from the partition. Reducing the width of the tissue strip had effects on the spike generation. External polarization changed the amplitude of the conducted spike. All these phenomena suggest that muscle fibres are electrically interconnected and that the electrical activity of each single cell is affected by that of several neighbouring cells. This would be expected from the passive properties described in Chapter V, B. Further examples of the complicated electrical interaction between cells can be demonstrated by the fact that during external application of conditioning hyperpolarization, the conducted spike was sometimes produced with notches during the rising or falling phase and was blocked stepwise as shown in Fig. 41. Similar changes of the spike shape were also occasionally observed upon changing the stimulus intensity (Fig. 42) when no partition was used and this can probably be

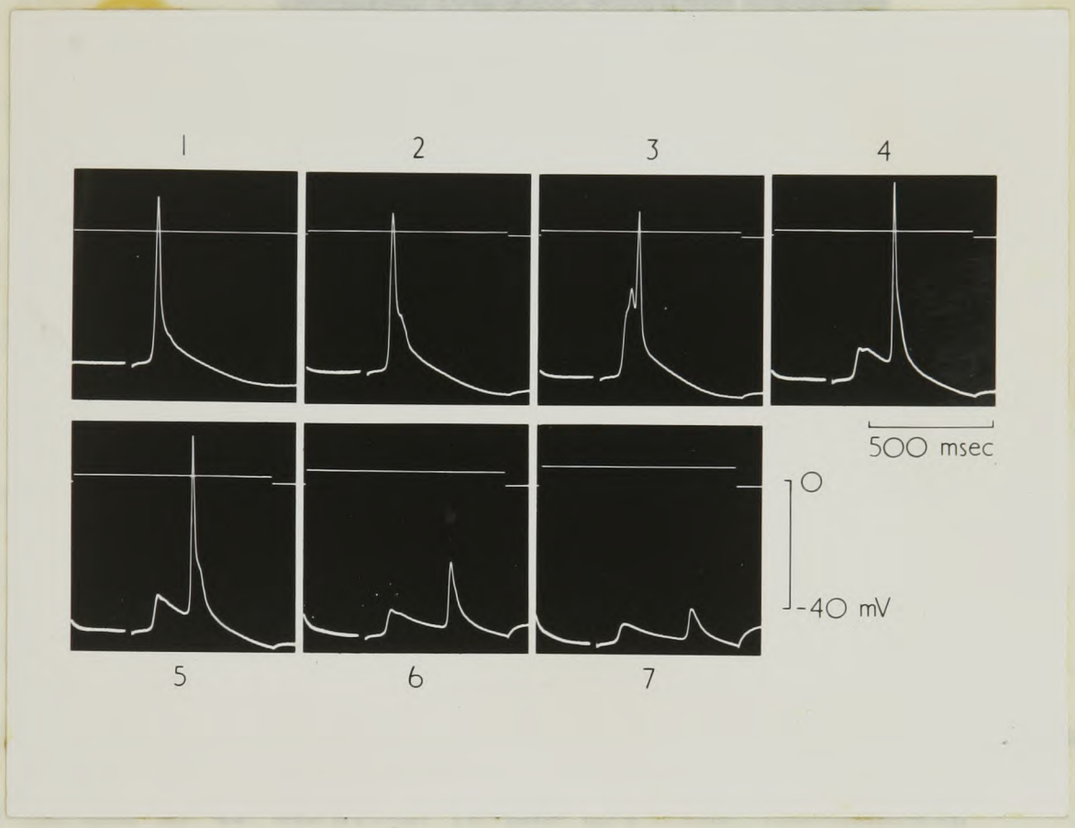


Fig. 41. Effect of conditioning hyperpolarization on the shape of the conducted spike. Stimulating arrangement the same as in Fig. 40. Upper trace = conditioning hyperpolarizing current which was applied just at the beginning of each picture; lower trace = intracellular record. Note discontinuities on rising and falling phases and stepwise block of the spikes during strong hyperpolarization.

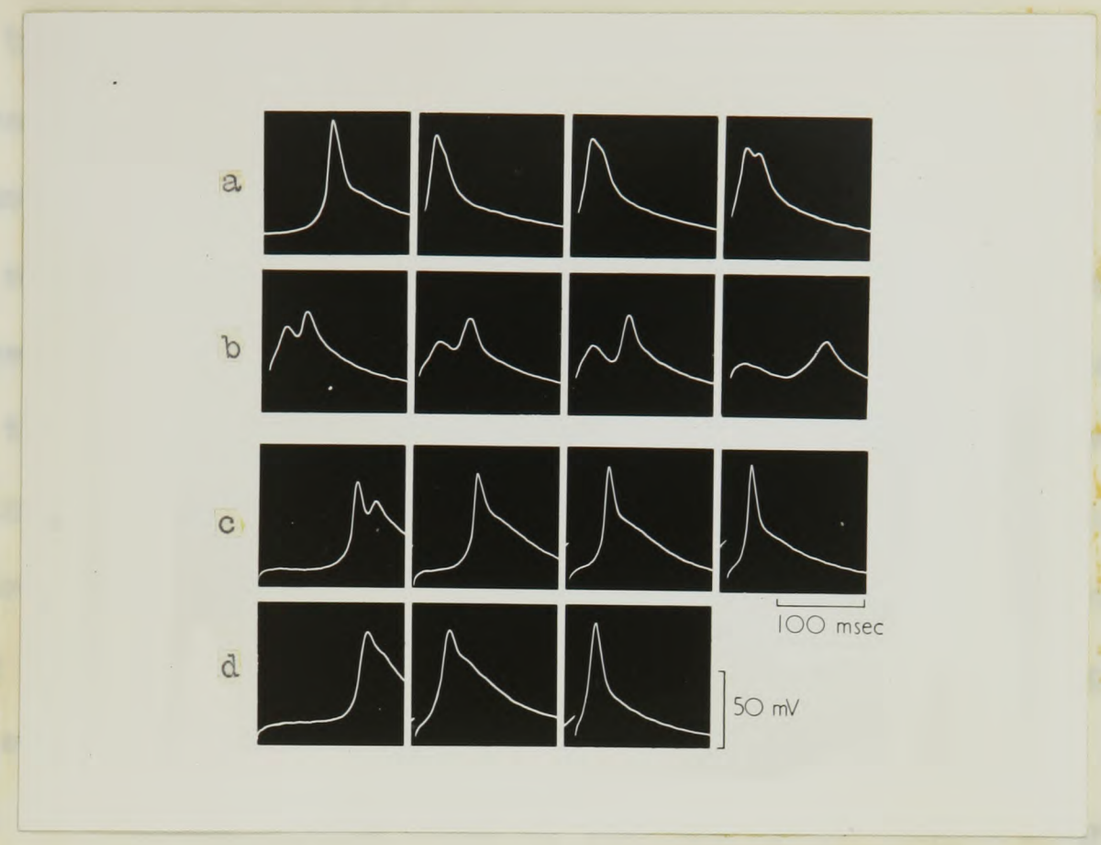


Fig. 42. Change of spike shape produced by changing stimulus intensity (a and b) or duration (c and d). The stimulating arrangement is shown in the inset. (a) and (b) = Spikes recorded from the same cell in response to stimuli of 10 msec duration and successively increased intensity. The stimulating electrode nearest to the recording site was the cathode. (c) and (d) Spikes produced in two different cells by stimuli of fixed intensity, but successively increased duration from 2 to 10 msec. In (c) and (d) the stimulating electrode nearest to the recording site was the anode.

explained by the phenomenon of reversed polarity (see Fig. 28). Such notch formation on the spike potential appeared also frequently during spontaneous electrical activity (see Fig. 45). This suggests that there are several sites on a single muscle fibre where the interaction takes place. When two spikes travelling from each end of the tissue were made to coincide at the recording site by adjusting the interval of the stimuli which triggered the spikes at both ends of the tissue, the recorded spike became a little larger and sharper than the spike travelling along the same recording site in one direction only (Fig. 43). The effect of spike collision may also be explained by the electrical interaction between cells so that the spike becomes larger when more cells are involved. It is also possible, that the summation of the local circuit currents by the spikes in neighbouring cells produced a larger spike if the cell had a graded electrical activity.

Finally, it must be emphasized that the electrical interaction may not be simple electrotonic spread of the electrical activity as in a core conductor, but that it may be mediated by another process, an active slow depolarization in a rather complicated network of muscle bundles composed of many fibres.

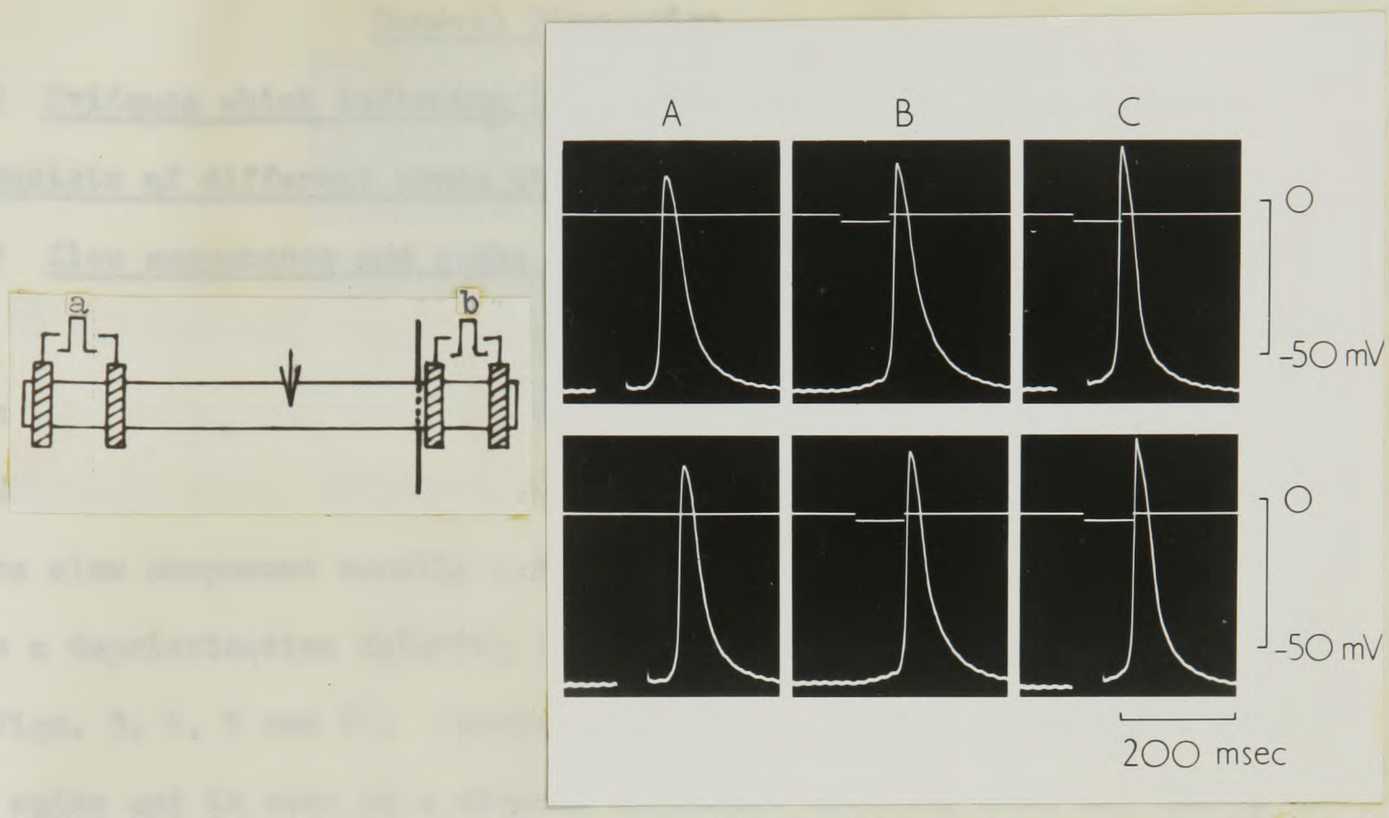


Fig. 43. Effect of spike collision on spike amplitude and shape. The stimulating arrangement is shown in the inset. Upper and lower records from two different cells. A = the spike triggered by electrode (a), B = the spike triggered by electrode (b). In C, the time interval between stimuli from (a) and (b) was adjusted to produce the largest spike. The micro-electrode was inserted at 3 mm distance from the partition and at 5 mm distance from the nearest electrode of (a).

Chapter VI

General Discussion

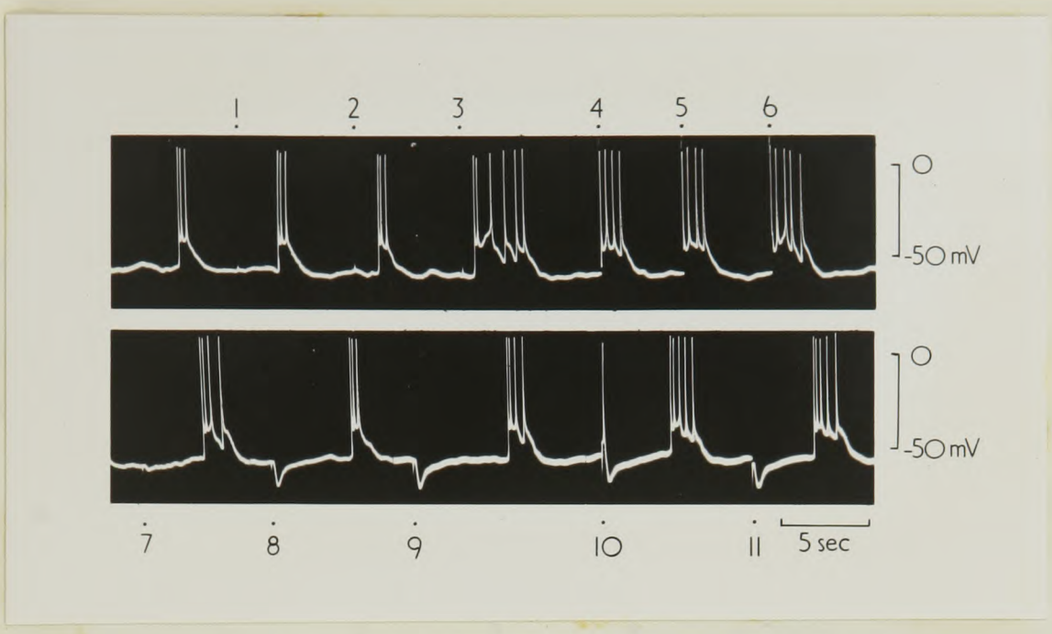
A) Evidence which indicates that the smooth muscle cell membrane consists of different areas with different properties.

1) Slow components and spike components of the spontaneous activity.

The slow components and spike components generated spontaneously in the taenia coli usually occur in close relation to each other (Bülbring, 1959; Bülbring, Burnstock & Holman, 1958; Holman, 1958). The slow component usually precedes a spike, triggers it and persists as a depolarization delaying the full recovery of the membrane potential (Figs. 3, 4, 5 and 6). However, sometimes the slow component follows a spike and is seen as a clearly different activity from the spike, or it appears on the falling phase as a notch (4, 5 and 6 in Fig. 45).

When the tissue has been kept at low temperature (5°C) spontaneous activity is absent. During the recovery process at normal temperature (36°C) at first only small, irregular slow components appear either spontaneously or in response to electrical stimulation. They become gradually larger and regular, and the spike component is produced if the slow component reaches a critical level. The slow components and spikes are also differently affected by changes in the ionic composition of the external solution (Bülbring & Kuriyama, 1963). In hypertonic solution, large, long-lasting slow components can be produced triggering repetitive spikes as seen in Fig. 44 (cf. Bülbring & Kuriyama, 1963).

At least one type of the slow component, A, may be a pacemaker or generator potential (1, in Fig. 45), since the taenia has spontaneous



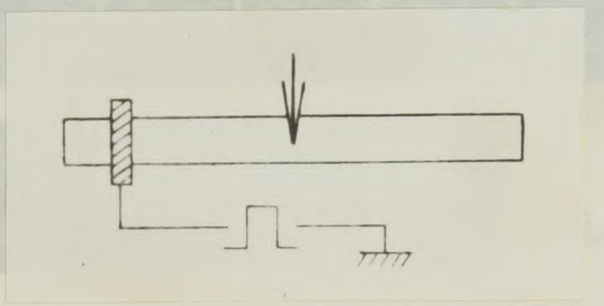


Fig. 44. Example of spontaneous and evoked long slow components in hypertonic solution (Krebs solution containing 6% sucrose). The stimulating arrangement is shown in the inset. At each dot electrical stimulation; in upper record depolarizing current; and in lower record hyperpolarizing current. Stimulus duration: (1) = 0.5; (2) = 1; (3) = 2; (4) = 3; (5) = 5; (6) = 10; (7) = 0.5; (8) = 1; (9) = 3; (10) = 5; and (11) = 10 msec. Note that depolarizing current of more than 3 msec produced the slow component and spikes, while hyperpolarizing current produced the inhibitory potential.

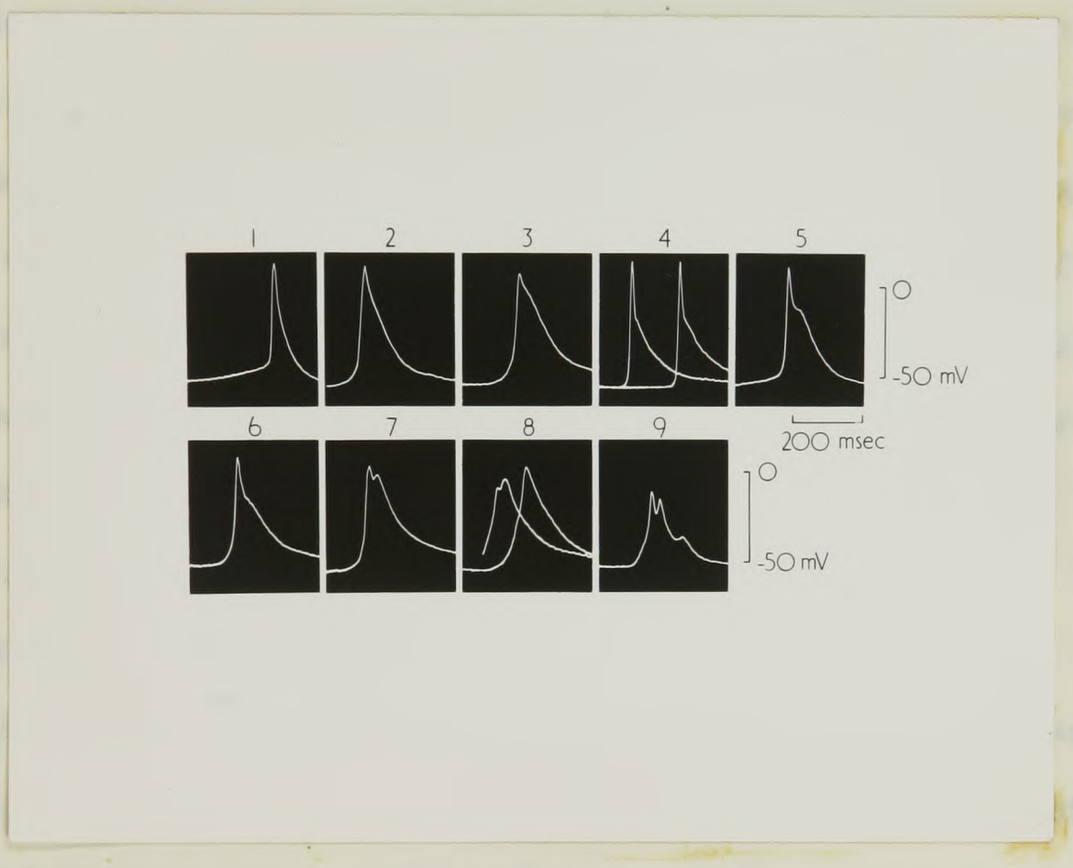


Fig. 45. Various shapes of spontaneous spikes from four different cells. Cell A = 1; cell B = 2 and 3; cell C = 4 and 5; cell D = 6 - 9. Note: slowly rising pre-potential in 1; long spike duration and discontinuity in falling phase in 2 and 3; notch in falling phase in 4 and 5; fluctuation of spike shape in a single cell in 6-9.

electrical activity. This component A may be produced in a special area of the cells at the pacemaker region of the tissue and trigger the spike there. The spike is then conducted to all the muscle fibres in the whole tissue, while the slow component A spreads electrotonically in a limited portion of the tissue, so that in the non-pacemaker regions the relationship between the slow component and spike changes. At the same time, the slow component can be produced independently in another part of the tissue (at another pacemaker region). However, it may not trigger a spike if it occurs during the long refractory period of the preceding spike or if its amplitude is too small. At other times it may succeed in setting the pace and thus the pacemaker region may shift from one part of the tissue to another. The slow component A is important for controlling the spontaneous spike frequency.

It has been shown that, in the taenia coli, there is a good correlation between the spike frequency and the tension development (Bülbring, 1954, 1955). The spike appears to be the initial event in the excitation-contraction coupling (Holman, 1958). Since depolarization itself does not cause any rise in tension and the tension rises only when spikes occur (Bülbring, 1959), slow components observed without a mechanical response (Burnstock & Prosser, 1960) may be the type A which is in no way connected with spike activity in neighbouring cells.

On the other hand, it is likely that there is also another type of slow component, B, the underlying mechanism of which is quite different. Since the electrical activity of the cells lying closely together is well synchronized, and the electrotonic potential produced

by external current application is detected intracellularly over a distance of many cell lengths, there must be some electrical interaction between cells. The electrotonic spread of the electrical activity (slow component and spike component) in neighbouring cells may initiate an active response (like a local response) and this then triggers spikes. The most frequently observed slow component is probably this type of the slow component, B, and important for the spike propagation. The size and shape is very variable and may depend on the condition of the cell (e.g. degree of stretch, previous history). Sometimes it is very difficult to distinguish between the spike and slow component as shown in 2, 3, 7, 8 and 9 in Fig. 45. Some of these could be abortive spikes on large slow components. Notches and humps during the spike suggest that there are several sites on each single cell for electrical interaction between cells (7, 8 and 9 in Fig. 45; and also Figs. 41 and 42).

2) <u>The effect of polarizing current on the spontaneous electrical activity</u>.

(i) <u>Intracellular polarization</u>. It was found that intracellularly applied polarizing currents strongly influenced the spike amplitude but had little effect on the slow component (Fig. 2, 3, 5 and 6). The spike could be evoked in only a few cells while the spontaneous electrical activity was observed in every cell. The intracellularly evoked spike does not show the slow component.

Prepotentials in cultured chick embryonic cardiac cells can be arbitrarily classified into small and large types (Sperelakis & Lehmkuhl,

1964). Neither depolarization nor hyperpolarization affected the spike of the small prepotentials, while hyperpolarization increased and depolarization decreased the large prepotentials. The authors suggested that both types may represent synaptic interaction between adjacent cells, and the difference in their magnitude may be a function of the distance between the synaptic junctions and the micro-electrodes.

It may be that the slow components in the taenia coli, like the small prepotentials in embryonic heart, are not affected by polarizing currents, because the distance between the part of the membrane where they are generated and that where the micro-electrode is inserted exceeds the space constant. It is also possible that the slow potential is produced not only in a different area of the membrane but in a special structure which is not influenced by polarizing currents. Vesicles located close to the membrane might be such a structure. Another possibility is that the slow potential is an electrotonic (ephaptic, Arvanitaki, 1942) potential from adjacent cells, and it should be unaffected by polarizing currents if the resistance of the cell membrane and of the junctional membrane remain constant.

If the slow potential is a simple ephaptic potential produced by current coming from adjacent cells, it is not necessary to assume that it is produced far from the micro-electrode in order to explain its being unaffected by polarizing currents. However, it is still difficult to explain its failure to generate a spike during conditioning depolarization as seen in Fig. 3. Current coming from neighbouring cells and the polarizing current coming from the micro-

electrode should summate, the result being the sum or the difference according to the direction, so that spike generation should be affected. However, this was not seen (Fig. 3). For this reason it is more likely that the slow potential is produced far from the electrode.

(ii) External polarization. External polarization produced a quite different response pattern from that to intracellular polarization. As reported by Bülbring (1955, 1957), external polarization modified the spontaneous spike frequency. This is closely related to the membrane polarization. Hyperpolarization slowed the frequency, and finally stopped the spike activity, while depolarization increased the frequency and reduced the spike amplitude at the same time. During hyperpolarization the relation between the spike amplitude and the membrane polarization depended on the spontaneous frequency of discharge and was generally not so clear as during depolarization. When the spike frequency was high, hyperpolarization usually increased the spike amplitude together with decreased frequency. However, the study of the conducted spike evoked under quiescent condition in hypertonic solution showed that the spike amplitude was reduced by hyperpolarization. The reduction of spike size is probably explained by the decreased effectiveness in triggering the spike by local circuit current from the neighbouring cells due to the hyperpolarization of the membrane (anodal block).

Nagai & Prosser (1963b), using the cat intestinal circular muscle and stimulation by the micro-electrode, observed that the spike height was reduced both by hyperpolarization and depolarization. This result could be explained if their micro-electrodes were so big that they acted

as external electrodes, like pressure electrodes (Bortoff, 1961; Gillespie, 1962b). This interpretation also explains other results of Nagai & Prosser (1963b) which are different from those of the present experiments, such as the space constant and the time constant of the electrotonic potential produced by intracellular stimulation.

3) Spike properties

The spikes of the taenia coli show a relatively slow rate of rise about 7 V/sec) and low sensitivity to changes of the external sodium concentration (Holman, 1957, 1958; Bülbring & Kuriyama, 1963). From these observations it has been postulated that the membrane of smooth muscle may have a limited number of sodium carriers to increase the sodium influx during the rising phase of the spike (Daniel & Singh, 1958; Holman, 1958; Bülbring & Kuriyama, 1963).

It has been known that an increase of the external calcium concentration and also hyperpolarization of the membrane reduce the inactivation of the sodium-carrying system in other tissues (Hodgkin & Huxley, 1962b; Weidmann, 1955; Frankenhaeuser & Hodgkin, 1957; Frankenhaeuser, 1957). In the taenia coli, excess calcium increases the rate of rise of the spike (Holman, 1958; Bülbring & Kuriyama, 1963). Conditioning hyperpolarization has the same effect in taenia as in other tissues. When the membrane is hyperpolarized to about -90 mV the rate of rise of the spike is increased from 7 to 20 V/sec. However, even this increased rate of rise is still lower than that of many other excitable tissues, for example, in squid axon, 630 V/sec (Hodgkin & Katz, 1949); in skeletal muscle fibre, 450 V/sec (Nastuk &

Hodgkin, 1950); in heart muscle, 570 V/sec (Weidmann, 1955). Since the rate of rise is some indication of the inward Na current during the rising phase of the spike, the low rate rise of the spike in taenia indicates that smooth muscle may have not only a limited number of sodium carriers, but also fewer carriers available because they are partly inactivated in normal solution. This may be related to the low resting potential.

A low excitability of the smooth muscle is further suggested by the finding that in the majority of cells no spike could be triggered and only a few cells responded in a graded manner to intracellular stimulation. During conditioning hyperpolarization the intracellularly evoked spike was, like that of the spontaneous spike, about 50 mV, and its maximum rate of rise, about 20 V/sec. In crustacean muscle fibres, it is also difficult to trigger a spike with intracellular stimulation. The spike amplitude is relatively small (mean 63 mV), the maximum rate of rise slow (20.5 V/sec), and the spike duration at half-spike amplitude is 5.2 msec (Fatt & Katz, 1953a). These values are very similar to those of the smooth muscle fibre of the taenia coli.

The spontaneous spike generation in the taenia coli seems to be sometimes all-or-none, sometimes graded. The question arises whether the spike size depends on the number of active cells participating, or on the activity of the single cell. The second explanation is more likely because external hyperpolarization reduces the amplitude of the conducted spike (Fig. 40) with little change in its time course. If the spike size depended on electrotonic spread from neighbourning cells,

the time course of a smaller spike must be slower according to the cable properties and as seen in the passive electrotonic potential (Fig. 29). It is therefore unlikely that the size of the spike is determined by electrotonic spread from adjacent active cells, the conduction being blocked during external hyperpolarization. Moreover, when a spike is produced by intracellular stimulation it is almost always in a graded manner and its amplitude is increased by conditioning hyperpolarization applied intracellularly. The spontaneous spike is modified in the same way. The fact that very strong hyperpolarization applied intracellularly blocks the spike component of spontaneous activity also suggests that the spike is mainly due to single cell activity. However, a very small spike component and slow component are left which suggests that they may be due to electrotonic spread since it must be assumed that the surrounding cells are still active. Weak electrotonic spread of the spike component (20 msec in duration) might be due to a large capacity component of a functional bundle with a membrane time constant of 60 - 100 msec, while polarization of long duration is not affected by a capacity component. In the ephaptic synapse of the crayfish motor nerve, it was found that the spikes were more attenuated (20%) than the catelectrotonic potential (30%) (Furshpan & Potter, 1959). A similar reduction of the spike and the electrotonic spread was also observed in the ephaptic synapse of the leech ganglion (Eckert, 1963).

The electrotonic spread of the spikes from adjacent cells is probably small and slow due to the resistance and capacity components

of the muscle fibres and junctions between cells, and this passive electrotonic spread may initiate an active slow depolarization, similar to the local potential (slow component B). The size of the slow component B therefore, would depend partly on the electrical activity in neighbouring cells and partly on the responsiveness of the single cell. Thus the amplitude of the spike generated as a consequence of electrotonic spread would depend on the rate of rise and the amplitude of the slow component B.

During activity, the direct effect of electrotonic spread on the spike size is small because of the low membrane resistance during the spike. The experiment on spike collision (Fig. 43) shows this, although some interaction, which might include the indirect effect mentioned above, is detectable.

It may be concluded that the size of the spike, during the spontaneous activity in normal solution, is mainly the result of the single cell activity and only partly determined by the number (or sum of the spike activity) of participating active cells. The effect of the latter factor is mediated through the slow component B. It must be taken into account that wave-length of the conducted spike covers about 1.4 mm (14 cell lengths of 100 μ in series) if the conduction velocity is taken as 7 cm/sec and the spike duration is taken as 20 msec. In other words, more than 10 cells longitudinal direction in a function bundle are producing the spike at the same time.

Difficulties arise from the fact that the response to external stimulation is quite different from that to intracellular stimulation.

In contrast to intracellular stimulation, when external stimulating electrodes are used, the spike can be evoked in every cell in an all-or-none manner although in some cells a stimulus duration of 20 msec is required. It has also been reported that in the cat intestinal circular muscle triggering the spike by intracellular electrodes is difficult (Nagai & Prosser, 1963b) and also in cardiac muscle (Tarr & Sperelakis, 1964) although external stimulation can easily evoke a propagated spike.

There are several other differences between the responses produced by intracellular and external stimuli. No slow component is observed when a spike is triggered by intracellular stimulation, whereas a slow component is clearly seen when the spike is triggered by external stimulation. The latency of the response to intracellular stimulation is usually very short, but very long with external stimulation of threshold intensity.

The difference between the responses triggered intracellularly and externally could be due to the difference of the area activated by the stimulating current. The intracellular electrode stimulates a small area very close to the electrode where the spike component is probably produced. The external electrode activates a large area of many cells including the area where the slow component B is generated (this problem will be discussed in detail in section B in this chapter).

In the frog and crab skeletal muscles, it was found that stimulation of a small area of a single muscle fibre membrane with an extracellular micro-electrode of 2 - 4 μ diameter, produced a graded

local contraction, even when the whole fibre was able to respond to stimulation of a large area with an all-or-none twitch and propagated action potential (Huxley, 1957; Huxley & Taylor, 1955, 1958). The intracellular stimulation in the taenia may correspond to the focal stimulation in the skeletal muscle fibre.

4) Different areas in a single cell.

When one considers the observations discussed so far, it seems reasonable to postulate different membrane properties in different parts of the cell. This would explain many differences between the responses to intracellular and external stimulation e.g. that a spike cannot be triggered by intracellular stimulation in a cell which discharges stpontaneously spikes with high amplitude and which responds to external stimulation, or that intracellular polarization affects only the spike and not the slow component of the spontaneous discharge. The cell membrane may be not homogeneous but composed of areas with different membrane properties. (Terms A, B, and C are introduced for convenience).

Areas A, may be the nexuses i.e. patches of membrane very close to neighbouring cells. When an external potential field is applied longitudinally to the tissue, most of the current flows from cell to cell through these areas which are inactive and have a low electrical resistance. This assumption based mainly on the study of the electrotonic potential produced by external current application. Since the space constant (1.6 mm) is much longer than the cell-length (100 μ), the cells must be electrically interconnected. Moreover,

when the tissue is placed longitudinally between two stimulating electrodes, only the ends of the tissue are polarized but not the centre. This suggests that electrical connections between cells are made through areas of low electrical resistance remaining inactive. Since external stimulation produces the slow component and modifies its frequency, while intracellular stimulation does not, the slow component is probably generated near area A. The different effects of intracellularly applied polarization on the slow and the spike components suggest that they are produced in different areas. Therefore further areas, B and C, are postulated.

Areas B may surround A. They would produce the slow component of the electrical activity and have a long time constant. Current passing through area A might affect area B, thus initiating the slow component B.

Area C may be the remaining part of the membrane. It would produce the spike when the slow component has reached threshold level. It would have a shorter time constant than area B and might be electrically less excitable. During intracellular stimulation, most of the current would flow through area C.

These areas may not be so strictly differentiated from each other but merge gradually. It is also possible that their sizes may change when the extracellular space, especially the distance between the adjoining cell membranes, is changed by deformation of the cells, caused, for example, by applied tension or contraction in physiological conditions. The change in the extracellular space would be directly

reflected in the external electrical resistance to longitudinal current flow, and this might then modify the electrical activity.

The slow component presumably triggers the spike. Hence, on the basis of the present hypothesis, when a long current pulse is applied externally area B which has a long time constant is excited and a slow component B is produced which then generates an all-or-none spike. However, when a short current pulse is applied through an external electrode, this only stimulates area C which has a short time constant and produces a spike in graded manner.

There are many similarities between the electrical activity of the taenia and that of the muscle of Ascaris which is composed of muscle spindle, belly, arm and fingers which are interconnected with those of adjacent cells as a functional syncytium and are also connected to the nerve cord. Intracellular records from the belly show spontaneous spike potentials which are superimposed on slow waves (Jarman, 1959; Del Castillo, Morales & Sánchez, 1963; De Bell, Del Castillo & Sánchez, 1963). Electrical stimulation of the nerve cord produces a spike which is similar to the spontaneous spike, while direct stimulation of the belly with an intracellular micro-electrode produces only graded spikes. Area B in the taenia coli may correspond to the syncytial part of the arm where the spontaneous spike originates and area C to the belly and the muscle spindle in the Ascaris muscle. Acetylcholine receptors are not uniformly distributed over the surface of the Ascaris muscle cells but localized in the syncytial region (Del Castillo, De Mello & Morales, 1963) as in the end-plate of

skeletal muscle. Areas B and C in the taenia coli may also have a different chemical sensitivity.

The properties of areas B and C are also similar to those of the different membranes of two different crustacean muscle fibres studied by Dorai Raj (1964). One type of fibres (which might correspond to area B of the taenia) has a long time constant (usually at least 100 msec) and produces no spike. The other type (which might correspond to area C) has a short time constant (about 10 msec) and responds to depolarization sometimes in a graded manner and sometimes with an all-or-none spike. The fibres, which respond to direct stimulation with only a graded spike, produce a full-size spike in an all-or-none manner in response to indirect (nerve) stimulation. Another instance of non-uniformity of a muscle membrane has been reported in the grass hopper, Romalea microptera by Werman, McCann & Grundfest, (1961). Different areas in the smooth muscle can also be compared with the neurone (Freygang & Frank, 1959; Tauc, 1960; Spencer & Kandel, 1961) or the crustacean stretch receptor (Edwards & Ottoson, 1958). In these, the neurone soma is less excitable, the dendrite produces the slow potential, and the axon hillock is the most excitable area of the membrane and initiates the spike.

B) Electrical interconnection between cells.

It was suggested by Bozler (1948) that the visceral smooth muscle behaves as a functional syncytium, because it can show both monophasic and diphasic action potentials like cardiac muscle. The facts that a potential difference of 50 mV is recorded with the sucrose gap when end of the tissue is depolarized with isotonic K_2SO_4, and also the appearance of monophasic spike activity (up to 30 mV), both indicate electrical low resistance interconnections between cells (Burnstock & Straub, 1958; Burnstock & Prosser, 1960). Smooth muscles with high conduction velocity have been found to have a low longitudinal electrical resistance when measured in sucrose solution, and the spaces between cells in electron micrographs are very narrow compared with those muscles which have a low conduction velocity (Burnstock & Prosser, 1960; Prosser, Burnstock & Kahn, 1960). This indicates that a low resistance electrical connection between cells is important for spike conduction and this is consistent with the difficulty of spike generation or spike conduction in tissue strips of less than 100 μ diameter, (Burnstock & Prosser, 1960). The limiting size of the tissue strip below which the electrical activity is affected, is too large to suppose that a single cell is sufficient for excitation. The observation that conduction velocity in saline is higher than that in a high resistance medium e.g. air or oil, suggests some electrical cell-to-cell connection and local circuit current for the spike conduction (Nagai & Prosser, 1963b).

Nevertheless, all the above evidence is indirect. Sperelakis

(1962) has pointed out that it is difficult to implicate a cell-to-cell conduction mechanism only from the recorded spike shape or from the applicability of the sucrose gap method for recording electrical activity. Many cells extending across the interface of the sucrose gap might produce enough potential gradient to be recorded. The sucrose gap might work even if the resistance of the connections between adjacent cells were high, because the resistance of the extracellular pathway (sucrose solution) might be even higher than that of the cell-to-cell pathway.

1) Evidence obtained by intracellular current application.

Direct evidence for electrical cell-to-cell connection would be to apply current intracellularly and record an electrotonic potential in another cell nearby. Nagai & Prosser (1963b), obtained records in the cat circular intestinal muscle which showed that current applied to one cell affected the polarization in neighbouring cells with a space constant of 1.03 mm which is similar to that obtained with a pressure electrode. However, similar experiments (Fig. 11 - 12) performed in taenia coli of the guinea-pig showed no interaction between cells and thus were contrary to the result of Nagai & Prosser. From the similarity of their space constant to that obtained by external current application, from the effect of polarization on spike height, and from the time constant (31 msec) of the electrotonic potential, it is more likely that the micro-electrode used by Nagai & Prosser acted like an external electrode. Sperelakis & Tarr (1965) also did the same experiment in the cat circular intestinal muscle and could not get the

same results as Nagai & Prosser. According to Sperelakis & Tarr (1965), there is a sharp fall-off in current spread into adjacent cells and at an interelectrode distance of 100 μ or greater, current flow through one intracellular electrode produced only a minute potential change at the second intracellular electrode. The apparent space constant would therefore be no more than one cell length. From the differences between the responses to intra- and extracellular stimulation, from the disagreement between the indirect evidence for, and the direct evidence against electrical interconnection between cells, it may well be that conclusive results cannot be obtained by intracellular current application, probably because the current spreads in three dimensions.

2) Electrotonic potential produced by external current application.

When current is applied externally a change of the membrane polarization can be easily observed from every single cell within 2 - 3 mm distance from the stimulating electrode. This shift of the membrane potential must be the polarization of the cell membrane by the applied current for the following reasons.

(a) Anodal current produces hyperpolarization and cathodal current produces depolarization, and there is a good correlation between the membrane potential and the spike amplitude.

(b) Depolarization produces a spike at a threshold depolarization, and the spike frequency increases with increasing depolarization. Hyperpolarization blocks the spike activity; it produces an anode-break depolarization or excitation, and spike activity cannot be seen unless

it is conducted from the cathode.

(c) The peak of the spike potential remains constant even when the membrane is depolarized by the applied current, (Fig. 32) as would be expected if the membrane resistance is decreased during the spike.

(d) An increase of the external K concentration shifts the current-voltage relationship towards the current-axis as would be expected from observations in other excitable membranes.

(e) When there is no current passing through the membrane and all the current is flowing longitudinally through the tissue, there is no polarization of the membrane, as shown in the record taken from the centre portion of a preparation placed between two electrodes, one at each end (Fig. 27).

(f) The behaviour of the electrotonic potential fits the cable theory which was originally applied to the nerve fibre (Hodgkin & Rushton, 1946). (Chapters V, B and VI, C). Similar electrotonic potentials have been recorded externally in the smooth muscle of frog stomach (Shuba, 1961).

The facts that the electrotonic potential is recorded in the extra-polar region far from the stimulating electrode at a distance of many cell lengths, and that hyperpolarization is recorded near the anode and depolarization near the cathode in the intra-polar region, seems to be direct evidence for electrical cell-to-cell connections between cells.

If the maximum potential gradient produced by the propagated spike

along a single cell length (100 μ) is calculated from the conduction velocity (7 cm/sec) and the maximum rate of rise of the spike(15 V/sec), it is 21 mV/100 μ. However, if it is calculated from conduction velocity and the rate of rise of the foot of the spike near threshold (about 2 V/sec), or the rate of rise of the slow component which is just near threshold (about 1 V/sec), then the values are only 2.8 mV/100 μ and 1.4 mV/100 μ respectively. This calculation shows that the potential gradient at threshold is too small to produce sufficient change of membrane potential for excitation in a single cell, because a threshold polarization of 20 - 25 mV is necessary for producing the spike (see Figs. 18, 27, 31 and 39). However, the threshold potential gradient in producing the spike by the external current is 2 - 3 mV/100 μ which agrees with the calculated value above. This suggests that at least 10 cells in series i.e. at least 1 mm of the tissue must act as a functional bundle, in order to produce enough potential gradient for spike generation, and also that a similar mechanism is probably involved in normal spike conduction. The same calculation applied to squid giant axon (conduction velocity = 5 m/sec; maximum rate of rise = 600 V/sec) gives a similar value (maximum potential gradient = 12 mV/100 μ) to that in the smooth muscle. Many cells in taenia produce the spike almost at the same time, as shown from the wave length of the spike calculated from conduction velocity and spike duration, (A, 3 in this chapter) so that the same situation prevails during external current application as during the passage of a spike potential. Thus the effect of the activity of a single cell in the whole tissue

on adjacent cells is negligible and quite different from that of many active aggregated cells, just as different as the effect of intracellular and external stimulation.

3) Electrical connection between cells in cardiac muscle.

The electrical interaction of the cardiac muscle cells seems to be very similar to that of the smooth muscle cells. The fibres of cardiac muscle have been assumed to form a syncytium. However, recently, there is general agreement amongst electron microscopists that cardiac muscle is composed of closely-packed but discrete cells, each surrounded by a membrane (Muir, 1957a, b; Sjöstrand & Andersson-Cedergren, 1960; Ruska, 1965). If the cell membranes have a high electrical resistance, as they do in other tissues, then it is not immediately apparent how an active cell can initiate activity in its neighbours. If, on the other hand, the region of intimate cell-to-cell contact, the intercalated disc (Muir, 1957a, b), has a low resistance, the mechanism of impulse spread is the same as in nerve and skeletal muscle by local circuit current flow, and cardiac muscle will remain a syncytium from the functional point of view (Barr & Berger, 1964; Van der Kloot & Dane, 1964; Weidmann, 1960, 1965; Woodbury & Crill, 1961).

Weidmann (1952), in measuring the space constant of a long column of kid Purkinje fibre cells (which have intercalated discs), found that they behaved as a single core cable the space constant of which was 1.9 mm, when current was injected at one point. From the K^{42} diffusion experiment in sheep ventricular trabeculae, Weidmann (1960, 1965) also found a long space constant (2 mm) and concluded that the intercalated

discs had a low resistance. Woodbury & Crill (1961) obtained a relatively short space constant (130 μ)in rat atrium, but they also suggested that current can spread easily from one cell to the next via low resistance intercalated discs because the space constant is larger than the cell dimensions.

Barr & Berger (1964) and Barr, Dewey & Berger (1965) found that the action potentials jump across an inactive portion of frog atrial bundles immersed in a sucrose gap, when an appropriate shunting resistor allows current flow between the extracellular electrolyte solutions. They concluded that the interiors of cells are connected by pathways of low enough resistance to allow electrical transmission of the spike with a large safety factor. Dewey & Barr (1962, 1964) have inferred that sites of low resistance are inherent at regions of morphological fusion of the outer layers of opposing plasma membranes. It has been reported (Sjöstrand, Andersson-Cedergren & Dewey, 1958) that "tight junctions" are present at intercalated discs, however, they cover only a small fraction of the total area.

Since the measurement of the electrotonic spread of current between two cells is the most direct method to determine whether the action current can spread from one cell to another, many attempts have been made to detect electrotonic spread in adjacent cells (Woodbury & Crill, 1961; Sperelakis, Hoshiko & Berne, 1960; Tarr & Sperelakis, 1964; Lehmkuhl & Sperelakis, 1965). However, only weak electrotonic interaction was found, and therefore high resistance interconnections were assumed. This was the reason for proposing a hypothesis of chemical transmission to explain spike propagation.

Some of this disagreement between the two views about electrical connection between cardiac cells might be due to difference between species or different parts of cardiac muscle (e.g. atrium, Purkinje fibre or ventricle). The mammalian Purkinje fibre may be a cable-like preparation (Weidmann, 1952), while the wall of the rabbit ventricle may have a sheet-like membrane (Woodbury & Crill, 1961; Noble, 1962). The discrepancies could also be due to the type of electrode used (intracellular or external) and also to differences in size.

4) Interpretation of the responses produced by intracellular and external stimulation.

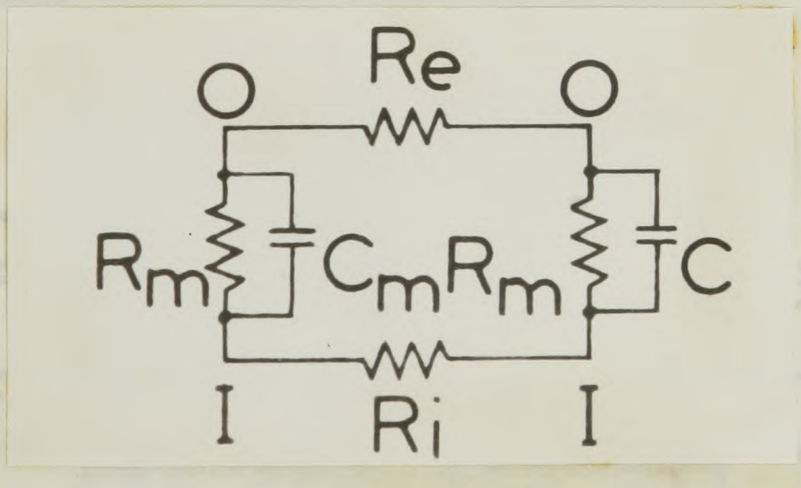
The main results may be summarized as follows: Intracellular stimulation causes no frequency modulation of the spontaneous discharge; it evokes a spike in only a few cells; the spike thus triggered has no slow components; no electrotonic spread can be detected in adjacent cells. The time constant of the response evoked by intracellular current application, using the Wheatstone bridge method, is 2.8 (2 - 4) msec. On the other hand, external stimulation produces frequency modulation of the spontaneous activity; a spike with a slow component is evoked in all cells, and electrotonic spread is easily observed. The space constant is 1.6 mm and the time constant is 60 - 100 msec.

The intensity of intracellularly applied current probably decreases very sharply with distance from the stimulating micro-electrode, because of the short space constant (0.1 - 0.2 mm) of a single muscle fibre which can be calculated from its electrical parameters and dimensions (Barr, 1961a; Sperelakis & Tarr, 1965), or because of the three-

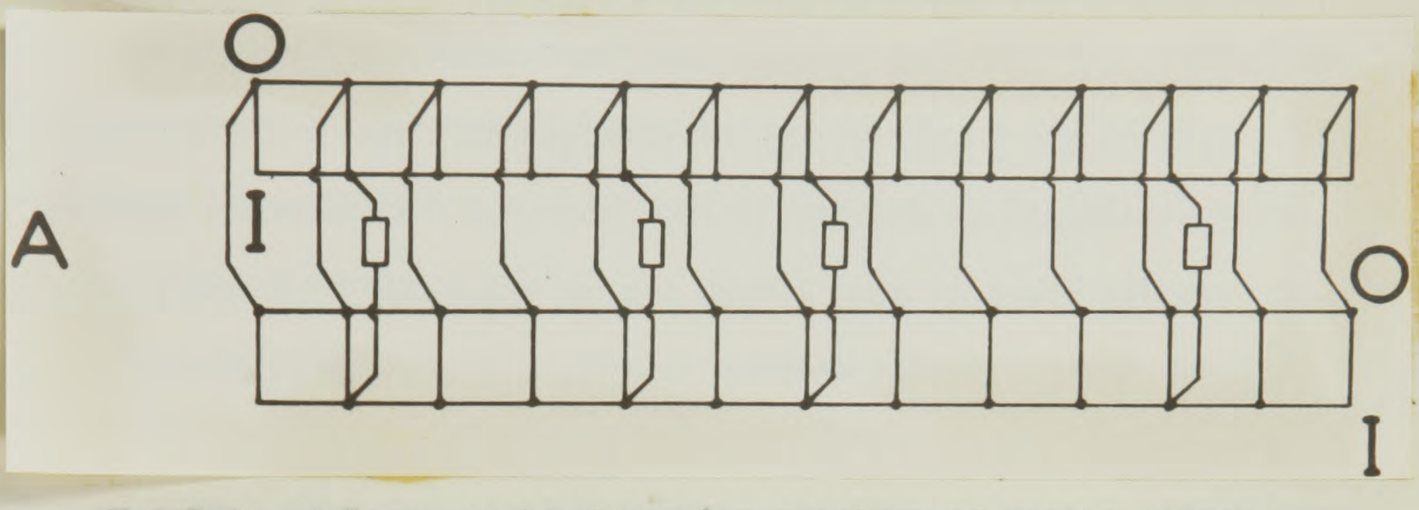
dimensional current spread through side connections to other fibres, as assumed in cardiac muscle (Woodbury & Crill, 1961; Noble, 1962). Woodbury & Crill (1961) using intracellular current application obtained a space constant of 130 μ in rat atrium, the cells of which are 15 μ in diameter.

Externally applied current, however, decays more slowly with distance because of the long space constant (1.6 mm) of the functional bundle which has cable properties and a larger diameter than a single muscle fibre. Moreover, the one-dimensional current flow along the bundle is an important factor in external stimulation.

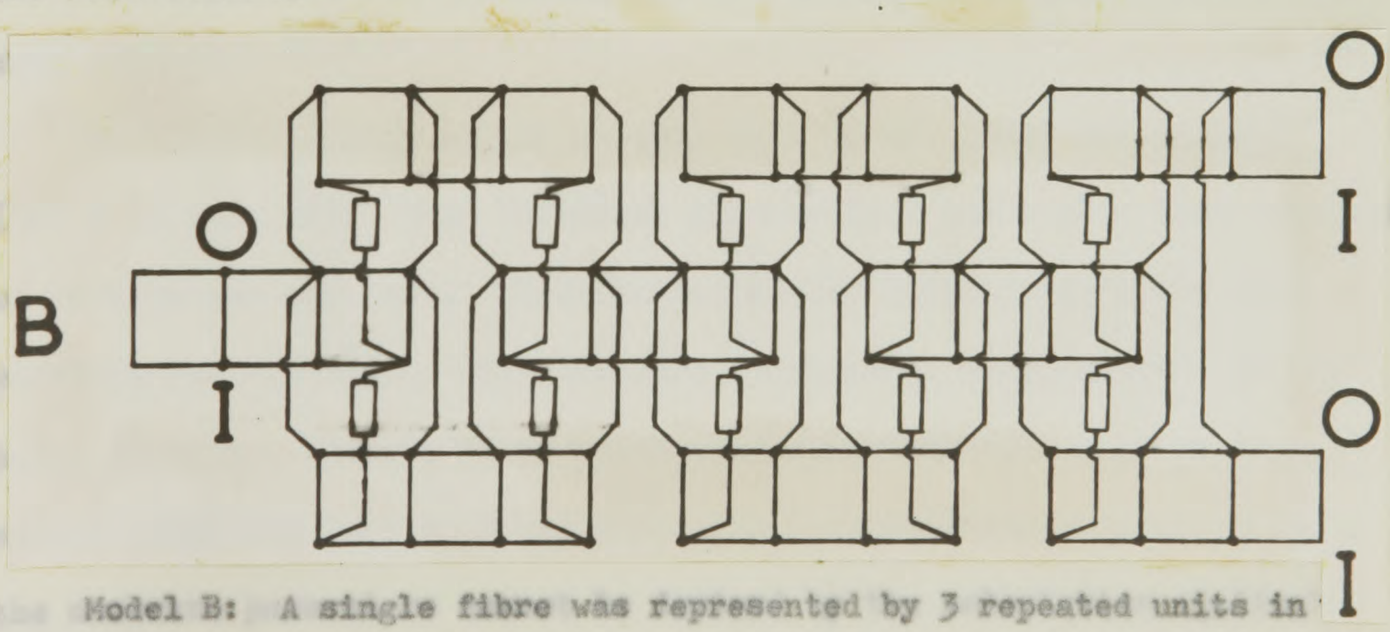
It is interesting to simulate intracellular and external stimulation with an electrical model circuit and to see how interconnections between cells affect the properties of the electrotonic potential. Two different models were constructed, using the same unit, as follows:



Re: the resistance of extracellular space, 10 KΩ; Rm: the membrane resistance, 100 KΩ; Cm: the membrane capacitance, 0.01 μF; Ri: the resistance of myoplasm, 10 KΩ.



Model A: A long single fibre (or a column of single fibres connected in series) was represented by 12 repeated units in series. The inside of some cells (point I) was connected across 10 KΩ to the inside of some side cells in an adjacent column at random, but only 4 points in one column. The outside of all cells (point O) was directly connected in parallel with the outside of the cells in the next column.



Model B: A single fibre was represented by 3 repeated units in series. The inside of the cell was connected across 10 KΩ to a side cell in the next column, at point I at each end of each cell (transverse

connection only, no longitudinal connection). The outside of all cells was connected directly in parallel as in Model A.

"Intracellular stimulation" was simulated by current application between points I and O of one cell in the centre of the model. "External stimulation" was simulated by current application between points I and O of the cells of the middle part of every column. The electrotonic potential was recorded between points I and O of all cells.

In both models it was found that, with intracellular stimulation, the effective resistance was lower, and the time constant and the space constant were shorter than those obtained by external stimulation. Though it was impossible to compare these models with the actual tissue of the taenia coli, the results were just what was expected. If the model was made to represent three dimensional connections between cells, the difference between intracellular and external stimulation would be even greater.

The time constant of the circuit used in the model was 10 msec (100 K X 0.01 μF). When "external stimulation" was used a time constant of 10 msec was obtained. With "intracellular stimulation", the time constant was 4.5 msec. When the side connections were removed, it was again 10 msec. Therefore, if intracellular stimulation is used in a muscle which has two- or three-dimensional connections between cells, the membrane parameters cannot be derived by the calculation applicable to a cable-like fibre, as theoretically analysed by George (1961).

From the above results it may be assumed that intracellular

stimulation may activate only a very small area of a single cell, while externally applied current may stimulate a much larger area and many cells. As far as physiological function is concerned, the properties studied with external stimulation may be a better representation of what happens physiologically than those studied with intracellular stimulation.

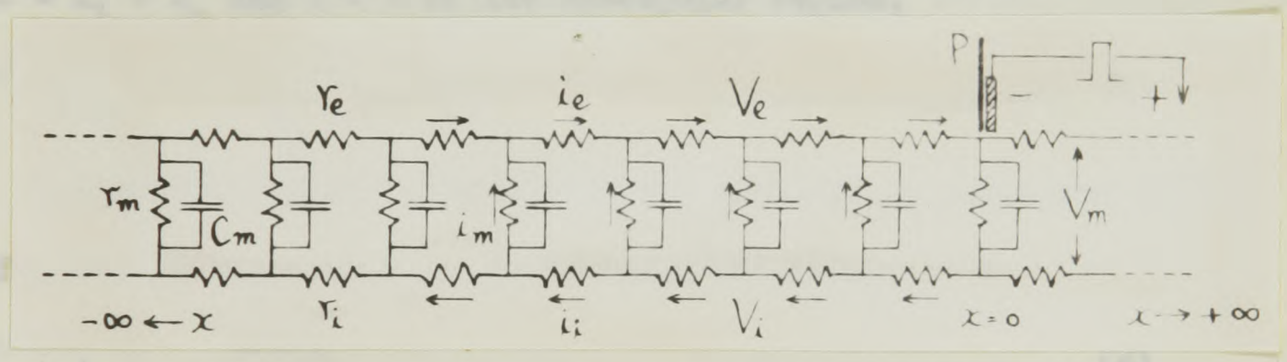
It is of interest to mention here the classical experiments on frog skeletal muscle (Jinnaka & Azuma, 1922 - 3; Davis, 1922 - 3; Watts, 1924 - 5). They showed that the chronaxie of the muscle diminished very greatly with the diminution in size of the electrodes employed, though this effect on the nerve was small. Hence, by the use of smaller and smaller electrodes the muscle chronaxie diminished much faster than that of the nerve. From these results and also by theoretical consideration, Rushton (1932a, b) concluded that only a large electrode can give the true muscle chronaxie.

C) Membrane resistance and membrane capacity.

1) Cable Theory.

It is known that the nerve fibre and most of the skeletal muscle fibres have the cable properties which were fully analyzed by Hodgkin & Rushton (1946). It seems convenient to review the cable theory here because the theory can also be applied to the smooth muscle as shown in the results.

For the recording method in which an insulating partition (P) was used (see Methods) the following circuit diagram may be considered (the resting potential is neglected in order to simplify).



As seen in the diagram, the external potential, V_e, and the internal potential, V_i, are expressed by

$$V_e = -\int_{-\infty}^{x} r_e \cdot i_e \cdot dx$$

and

$$V_i = -\int_{-\infty}^{x} r_i \cdot i_i \cdot dx$$

respectively, where

r_e is the external resistance per unit length; i_e is the external current; r_i is the internal resistance per unit length; i_i is the internal current; and x is the distance from the electrode. By differentiating the above equations,

$$\frac{\partial V_e}{\partial x} = -r_e \cdot i_e \qquad \text{..... (1)}$$

$$\frac{\partial V_i}{\partial x} = -r_i \cdot i_i \qquad \text{..... (2)}$$

The change in the membrane potential (V_m) by the applied current is the difference between the external potential and the internal potential, $V_m = V_i - V_e$. Then,

$$\frac{\partial V_m}{\partial x} = \frac{\partial V_i}{\partial x} - \frac{\partial V_e}{\partial x} = (-r_i \cdot i_i) + (r_e \cdot i_e)$$

$$= i_e (r_e + r_i) - r_i (i_i + i_e) \quad \cdots\cdots\cdots (3)$$

$$V_m = V_i - V_e = -(r_i + r_e) \int_{-\infty}^{x} i_i \cdot dx + r_e \int_{-\infty}^{x} (i_i + i_e) \cdot dx$$

$$= \frac{(r_i + r_e)}{r_i} \cdot V_i + V_e \int_{-\infty}^{x} (i_i + i_e) \cdot dx$$

As $I = i_i + i_e$ and $I = 0$ at the extrapolar region,

$$V_m = \frac{(r_i + r_e)}{r_i} \cdot V_i \quad \cdots\cdots\cdots\cdots\cdots (4)$$

The current which flows across the membrane is expressed by the difference in external current per unit distance,

$$i_m = \frac{\partial i_e}{\partial x} \quad \cdots\cdots\cdots\cdots\cdots (5)$$

On the other hand, the membrane current, i_m, is composed of ohmic current which flows through the membrane resistance and capacitative current which flows through the membrane capacity,

$$i_m = \frac{V_m}{r_m} + C_m \frac{\partial V_m}{\partial t} \quad \cdots\cdots\cdots\cdots\cdots (6)$$

From eq. (5) and eq. (6),

$$\frac{\partial i_e}{\partial x} = \frac{V_m}{r_m} + C_m \frac{\partial V_m}{\partial t} \quad \cdots\cdots\cdots\cdots\cdots (7)$$

From eq. (3)

$$\frac{\partial^2 V_m}{\partial x^2} = (r_e + r_i)\frac{\partial i_e}{\partial x} - r_i \frac{\partial I}{\partial x}$$

$$\frac{\partial i_e}{\partial x} = \frac{1}{(r_e+r_i)} \cdot \frac{\partial^2 V_m}{\partial x^2} + \frac{r_i}{(r_e+r_i)} \cdot \frac{\partial I}{\partial x} = \frac{V_m}{r_m} + C_m \frac{\partial V_m}{\partial t} \quad \cdots (8)$$

$$\left(\frac{r_m}{(r_i+r_e)}\right) \cdot \frac{\partial^2 V_m}{\partial x^2} + \frac{r_i \cdot r_m}{(r_e+r_i)} \cdot \frac{\partial I}{\partial x} = V_m + r_m \cdot C_m \frac{\partial V_m}{\partial t}$$

$$-(\lambda)^2 \frac{\partial^2 V_m}{\partial x^2} + \tau_m \frac{\partial V_m}{\partial t} + V_m = r_i (\lambda)^2 \cdot \frac{\partial I}{\partial x} \quad \cdots\cdots\cdots (9)$$

where $\lambda = \sqrt{r_m/(r_i + r_e)}$ ..... (10)

$\tau_m = r_m \cdot C_m$ (C_m: the membrane capacity per unit length) ..... (11)

Since I = 0 for the extrapolar region ($-\infty < x < 0$),

$$-(\lambda)^2 \cdot \frac{\partial^2 V_m}{\partial x^2} + \tau_m \frac{\partial V_m}{\partial t} + V_m = 0 \quad \cdots (12)$$

Eq. (12) can be solved by using Laplace transformation. The solution is

$$V_m = \frac{r_i I_o \lambda}{4}\left\{ e^X\left[1+\operatorname{erf}\left(\frac{X}{2\sqrt{T}} + \sqrt{T}\right)\right] - e^{-X}\left[1+\operatorname{erf}\left(\frac{X}{2\sqrt{T}} - \sqrt{T}\right)\right]\right\} \quad \cdots (13)$$

when current is applied at t = 0, and

$$V_m = \frac{r_i I_o \lambda}{4}\left\{ e^X\left[1-\operatorname{erf}\left(\frac{X}{2\sqrt{T}} + \sqrt{T}\right)\right] + e^{-X}\left[1+\operatorname{erf}\left(\frac{X}{2\sqrt{T}} - \sqrt{T}\right)\right]\right\} \quad \cdots (14)$$

when current is cut off at t = 0, where I_o is I at x = 0 ; X x/λ ;

$T = t/\tau_m$; and erf is the error function.

Since $\frac{r_i \lambda}{2} = \frac{r_i \sqrt{r_m/r_i}}{2} = \frac{\sqrt{r_m \cdot r_i}}{2}$ = Re (effective resistance) .. (15),

(r_e is neglected because $r_e \ll r_i$)

$R_e I_o = V_m \underset{x=0}{}{}^{t=\infty}$ (the membrane potential at the stimulating electrode and at steady state), eqs. (13) and (14) are

$$V_m = V_{m\,\substack{t=\infty \\ x=0}} \frac{1}{2}\left\{e^{x}\left[1+\mathrm{erf}\left(\frac{x}{2\sqrt{T}}+\sqrt{T}\right)\right]-e^{-x}\left[1+\mathrm{erf}\left(\frac{x}{2\sqrt{T}}-\sqrt{T}\right)\right]\right\} \quad \ldots\ldots (16)$$

and

$$V_m = V_{m\,\substack{t=\infty \\ x=0}} \frac{1}{2}\left\{e^{x}\left[1-\mathrm{erf}\left(\frac{x}{2\sqrt{T}}+\sqrt{T}\right)\right]+e^{-x}\left[1+\mathrm{erf}\left(\frac{x}{2\sqrt{T}}-\sqrt{T}\right)\right]\right\} \quad \ldots\ldots (17)$$

respectively. From eq. (16),

$$V_m = V_{m\,\substack{t=\infty \\ x=0}} \cdot e^{-x/\lambda} \quad \ldots\ldots (18)$$

for $t = \infty$; i.e. a steady state during current application. This equation is applied to get the space constant, λ.

As shown in the results (Chapter V) the behaviour of the electrotonic potential in the taenia coli agrees with that which would be expected from a tissue with cable properties. This result is very surprising because the structure of the tissue is very different from that of a simple core conductor, e.g. a nerve fibre, even if low-resistance connections between cells are taken into account.

From eq. (10), neglecting r_e,

$$\lambda = \sqrt{\frac{a}{2}} \cdot \sqrt{\frac{R_m}{R_i}} \quad \ldots\ldots (19)$$

because $r_m = R_m/2\pi a$, $r_i = R_i/\pi a^2$, where R_m is the specific membrane resistance, R_i is the specific internal resistance and a is the radius of the cable. As shown in eq. (19), the space constant, λ, is proportional to the square root of the radius of the fibre if the specific resistance of the membrane and the cytoplasm are assumed to be constant. The space constant of the smooth muscle fibre having a diameter of 5 μ should be 1/4 of that of the skeletal muscle fibre of 80 μ diameter. However, the actual value of the space constant

of the taenia coli of the guinea-pig is 1.6 mm, which is the same as that of the skeletal muscle fibre (Fatt & Katz, 1951). This could be due to a very high membrane resistance. However, it is more likely that many fibres, connected in series and in parallel, are aggregated in a bundle of large diameter. Bundles of 20 - 40 μ wide can actually be discerned through the microscope (cf. Bülbring, 1954). The trabeculae of rat atrium are also composed of cylindrical bundles about 50 μ in diameter, separated by comparatively large extracellular spaces containing capillaries (Woodbury & Crill, 1961). The cells within a bundle are closely packed, the space between cell membranes being from 200 A to 300 A. The bundles might act as functional units. If the space constant of 1.6 mm, were that of a functional bundle of 30 μ diameter, the membrane resistance of the bundle would be 2.6 times higher than that of the skeletal muscle of 80 μ diameter which has the same space constant, provided that the cable equation is applicable to the functional bundle and the specific internal resistance is the same in both muscles.

The conception of a functional bundle is also suggested by the observation that production of the spike becomes graded if longitudinal strips of less than 100 μ in width are dissected or if the stimulating electrode is less than 50 μ wide (cf. Burnstock & Prosser, 1960; Nagai & Prosser, 1963a).

If conduction is brought about by a local circuit current in a fibre with cable properties, the conduction velocity (v) is proportional to a factor (Katz, 1948)

$$v = \frac{S'}{\sqrt{2\,R'm}} \times \frac{\sqrt{\text{radius}}}{Cm\,\sqrt{Ri}}$$

where S' is a safety factor, R'm is the active membrane resistance, C_m is the specific membrane capacitance and R_i is the specific internal resistance. The conduction velocity of taenia coli is 7 cm/sec which is about 1/20 of that of skeletal muscle (1.6 m/sec, Katz, 1948). The small fibre diameter could explain the slow conduction velocity, but it was rejected for the above explanation of λ in which a large bundle diameter was assumed. However, the slow conduction velocity may also be explained by a large C_m, a low safety factor and a high active membrane resistance. The possibility of a large C_m will be discussed in the next section. The safety factor may be low because of the poorly developed Na-carrier mechanism and the slow rate of rise of the spike (Holman, 1958; Kuriyama & Tomita, 1965). Nevertheless, the above equation might still be insufficient to express all the properties of the smooth muscle because the interconnections of the fibres and the non-homogeneity of the cell membrane (see section A,4) can also modify the conduction velocity.

2) Chronaxie

The strength-duration curve can be obtained from the cable theory (Hodgkin & Rushton, 1946) as follows, if it is assumed that the critical depolarization (V_m^*) is constant with different stimulus durations. From eq. (16) of the previous section, at x = o,

$$V_{m\,x=o} = \frac{ReI}{2}\, 2 \operatorname{erf}\left(\sqrt{T}\right) = ReI \operatorname{erf}\sqrt{T} \qquad \ldots\ldots (20)$$

at threshold, $V_m = V_m^*$

$$I = \frac{Vm^*}{Re}\,\frac{1}{\operatorname{erf}\sqrt{T}} \qquad \ldots\ldots (21)$$

at rheobase $I_{t=\infty} = I'$,

$$I' = V_m^*/R_e$$

Since $I = 2I' = 2V_m^*/R_e$ at Chronaxie, i.e. pulse duration (t) at twice the intensity of the rheobase, I'. Putting this into eq. (21)

$$2\frac{Vm^*}{Re} = \frac{Vm^*}{Re}\frac{1}{\text{erf}\sqrt{T}} \quad \text{.....(22)}$$

$\text{erf}\sqrt{T} = \frac{1}{2}$

Hence, $T = t/\tau_m$

$\text{erf}\sqrt{t/\tau_m} = 0.5$ From the table of error function,

$\sqrt{t/\tau_m} = 0.49$, then $t = 0.24\,\tau_m$. Taking the time constant of 50 - 100 msec, the chronaxie (t) is calculated to be 12 - 24 msec, which agrees well with the experimental results (about 20 msec). This theoretical description can only be regarded as a rough approximation because the same time factor is considered for the development of the electrotonic potential as well as for an active process, i.e. the local potential. However, the order of magnitude of the calculated and experimentally found values seem to be substantially correct.

In table I the chronaxie values of various tissues are shown. Muscles have a longer chronaxie than nerve fibres and smooth muscles have a longer chronaxie than skeletal muscles. This suggests that the smooth muscle has a longer time constant of the membrane than other tissues, though the chronaxie is not an adequate measure of the membrane properties, for the following reasons.

If the membrane of the muscle fibre is composed of two areas with different properties, one of which has a longer time constant and produces the slow component, while the other has a shorter time constant and produces the spike component, as suggested previously, the relationship between the chronaxie and the membrane time constant may not be so simple. A shorter current pulse might trigger a spike directly (i.e.

TABLE I Chronaxie measured with external electrodes

Preparation	Chronaxie (msec)	Reference
Frog A-fibre	0.3	Erlanger & Gasser (1937)
Frog C-fibre	7	"
Frog sartorius	5-20	Schaefer, Schölmerich & Haass (1938)
Crayfish muscle	5.8	Miyamoto (1964)
Guinea-pig vas deferens	10	Burnstock & Holman (1961)
Guinea-pig taenia coli	20-30	This thesis
Cat ureter	50	Bozler (1938)
Cat intestinal circular muscle	50	Barr (1961a)
Guinea-pig ileum	80	Paton (1955)
Rabbit ureter	200	Bozler (1938)
Guinea-pig uterus	600	"

mainly by passive depolarization), while a longer current might generate a spike indirectly, initiating an active slow component between the passive depolarization and the spike. If the activity of the slow component is prominent in the smooth muscle, the chronaxie would become larger than expected from the passive properties of the muscle membrane because, when a long current pulse is applied, the slow component depolarizes the membrane further to the critical firing level acting additively to the applied current.

3) The membrane parameters (the membrane resistance and capacity).

Unfortunately, it is not possible to measure the intensity of the externally applied stimulating current which passes through the membrane because most of the current flows through the external solution and also because of the structural complexity of the tissue. Therefore no absolute values of the membrane resistance nor of the capacity can be obtained from the electrotonic potential in the present experiments. Only the time constant is a reliable value. Since this is the product of the membrane resistance and the capacity then, if one of them were estimated, the other could be calculated. The problem is how to find a correlation between the resistance or the capacity and the structure of the muscle.

i) Membrane capacity. In the frog skeletal muscle fibre, Cole & Curtis (1936) obtained a membrane capacity between the inside and outside of 1 to 2 $\mu F/cm^2$ on the basis of measurements made at frequencies around 10 kc/s. From recent measurements carried out in a similar way to those of Cole and his co-workers, it appears that

this estimate should be revised to about 2.5 $\mu F/cm^2$ (Fatt, 1964).

Using a different method, Katz (1948) carried out measurements on the frog muscle fibre which would emphasize the behaviour at very much lower frequencies around 10 c/s, and he obtained for the characteristics of the current path between inside and outside a capacity of about 6 $\mu F/cm^2$ in parallel with a resistance of about 3000 Ωcm^2. These values were confirmed in subsequent measurements in which intracellular electrodes were used (Fatt & Katz, 1951). The value computed for the capacity per unit area of muscle fibre surface seemed unreasonably high for a dielectric membrane, because the calculation from a membrane thickness of 100 A and a dielectric constant of 5 shows that the membrane capacity should be about 1 $\mu F/cm^2$. Then the possibility was raised that this might be the result of folding of the surface membrane increasing the effective area. When, however, the frog skeletal muscle fibres were examined under the electron microscope, no such folding was evident.

Following this work on the frog muscle fibre, the properties of crustacean limb muscle fibre were examined with intracellular electrodes. In the crayfish the capacity was found to be about 20 $\mu F/cm^2$ (Fatt & Ginsborg, 1958) and in some crabs about 40 $\mu F/cm^2$ (Fatt & Katz, 1953a). Table II shows comparison of the membrane capacity and resistance, together with the time constant and the fibre diameter, in various tissues. There appears to be a correlation between muscle fibre diameter and capacity per unit area of fibre surface, so that the idea suggests itself that the large capacity might arise from channels which penetrate

TABLE II Electrical membrane parameters and cell diameter.

	Capacity ($\mu F/cm^2$)	Resistance ($\Omega\ cm^2$)	Time constant (msec)	Diameter (μ)	Reference
Unmyelinated nerve fibre					
Squid: Loligo	1.1	-	-	500	(1)
" "	-	700 (400-1100)	-	500	(2)
" Sepia	1.2	9200 (1400-31000)	14.2 (8.2-18.8)	204	(3)
Lobster: Homarus	1.3	2300 (600-7000)	2.3 (0.8-5)	75	(4)
Crab: Carcinus	1.1	8000 (2000-16000)	6.8	32	(5)
Skeletal muscle					
Frog: extensor dig.IV	4.5	4000	18	45	(6)
Frog: add. magnus	6	1500	9	75	(6)
Frog: sartorius	6.1	1900	12	86-104	(7)
" "	8	4100	34.5	137	(8)
Crayfish: Astacus	20	1000	20	150	(9)
Crab: Carcinus, Portunus	42	116	4.6	180	(10)
Purkinje fibre					
Kid	12.4	2000	19.5	75	(11)
Calf, sheep	11	1200	13	75	(12)

(1) Curtis & Cole (1938), (2) Cole & Hodgkin (1939), (3) Weidmann (1951), (4) Hodgkin & Rushton (1946), (5) Hodgkin (1947), (6) Katz (1948), (7) Ishiko & Sato (1960), (8) Fatt & Katz (1951), (9) Fatt & Ginsborg (1958), (10) Fatt & Katz (1953a), (11) Weidmann (1952), (12) Coraboeuf & Weidmann (1954).

the fibre from the surface, with a large part of the measured capacity existing between the lumen of such channels and the myoplasm.

Falk & Fatt (1964) studied this problem and proposed a circuit diagram, which has two parallel paths as shown in Fig. 46, for frog skeletal and crustacean limb muscle fibres. As for the proposed location of the impedance elements, C_m and R_m, are taken to arise in a membrane at the surface of the muscle fibre. On the other hand, it is suggested that C_e and R_e arise in a system of channels which penetrate the fibre from the surface and extend through the entire cross-section. In frog muscle fibres, the most likely structure to give rise to these properties is the system which, in longitudinal section, is seen to form triads (Porter & Palade, 1957; Andersson-Cedergren, 1959; Huxley, 1964; Peachey, 1965). In the crayfish muscle fibre, C_e probably represents the capacity across the walls of channels leading from the surface to the interior while R_e may reflect the resistivity of the interior channel. According to this interpretation, the capacity of the plasma membrane, C_m, is 2.6 $\mu F/cm^2$ in frog, 3.9 $\mu F/cm^2$ in crayfish and the capacity of the tubular system, C_e, is 4.1 $\mu F/cm^2$ in frog, 17 $\mu F/cm^2$ in crayfish (Falk & Fatt, 1964). It can, therefore, be postulated that the surface plasma membrane has more or less the same capacity (1 - 4

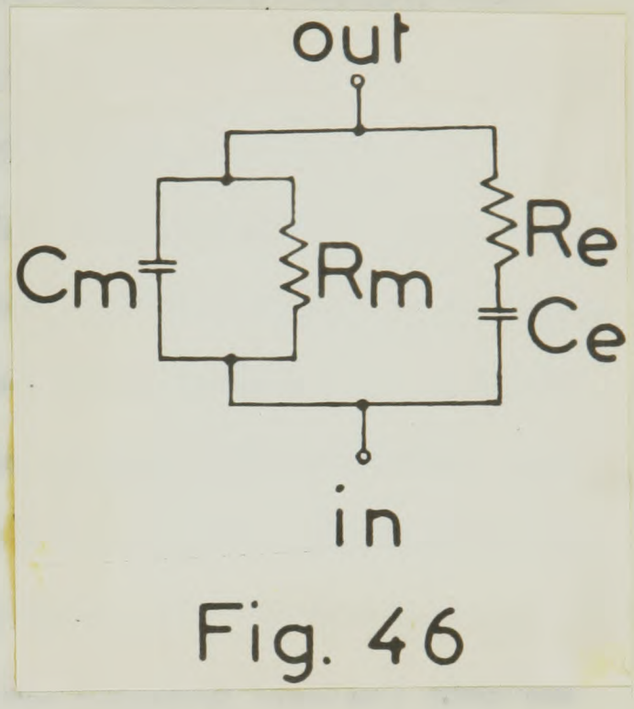


Fig. 46

$\mu F/cm^2$) in most tissues.

Fozzard (1964; 1966) presented an idea for Purkinje fibre, similar to that of Falk & Fatt (1964) for the skeletal muscle fibre. The calculation of the membrane capacity from the propagation velocity and the time course of the spike foot gives 2.4 $\mu F/cm^2$ while the square pulse method using an intracellular micro-electrode gives 12.8 $\mu F/cm^2$. Fozzard (1964; 1966) interpreted this discrepancy by assuming that it was due to the transverse sarco-tubular system. The walls of the tubules are perhaps 3/4 of the whole Purkinje fibre membrane, and the capacity of the tubular membrane is not filled by the upstroke of the propagated action current. In frog ventricle, Van der Kloot & Dane (1964) also obtained a small membrane capacity (1.2 μF) from the same calculation as Fozzard (1964; 1966). It seems worth while to point out that the internal structure of the cardiac muscle fibre is similar to the crustacean muscle fibre (Brandt, Reuben, Girardier & Grundfest, 1965; Girardier & Pollet, 1964; Nelson & Benson, 1963; Simpson & Oertelis, 1962; Girardier, 1964; Girardier, Reuben, Brandt & Grundfest, 1963).

Adrian & Peachey (1965) compared the electrical properties in the twitch fibre with those of the slow fibre from the frog iliofibularis. They found that the membrane capacity of the slow fibre (1.6 - 2.5 $\mu F/cm^2$) is 1/3 of that of the twitch fibre (6.8 $\mu F/cm^2$) and the membrane resistance of the slow fibre (29,000 Ωcm^2) is 10 times higher than that of the twitch fibre (3140 Ωcm^2). These differences are explained by the fact that the slow fibre has very poorly developed triads.

There appears to exist a critical fibre size below which no sarcoplasmic tubular system is found. In Amphioxus, the sheet-like body muscle fibre, about 1 μ in width, is lacking the sarcoplasmic reticulum (Peachey, 1961). The fibres of the turtle atrium, about 3 μ in diameter, also lack sarcoplasmic reticulum (Fawcett & Selby, 1958). The smooth muscle fibre, about 5 μ in diameter, also lacks such a system, although there are vesicles close to the membrane. Since the fibres are closely packed as a bundle and electrically interconnected, such a bundle may correspond to a single skeletal muscle fibre. The extracellular space in the smooth muscle would then correspond to the sarcoplasmic tubular system, and the single smooth muscle fibre, to the myofibril of the skeletal muscle fibre. In Fig. 47, a composite representation of the fine structure of (a) a frog skeletal muscle fibre and (b) a crayfish muscle fibre is shown. These are also similar to the cardiac muscle fibre (Nelson & Benson, 1963).

Here, the problem arises whether the smooth muscle fibre has a small membrane capacity (1 - 3 $\mu F/cm^2$) and a high resistance like the slow skeletal muscle fibre due to lack of the tubular system, or whether the smooth muscle fibres are aggregated as a functional bundle, so that the extracellular spaces in such a bundle correspond to the sarcoplasmic reticulum and triad in the frog skeletal muscle fibre, or to the transverse tubular system in the crustacean muscle fibre. There is no evidence that the properties of the cell membranes in the interior of a smooth muscle fibre bundle (which would correspond to the tubular system) are in any way different from those of the cell membranes at the

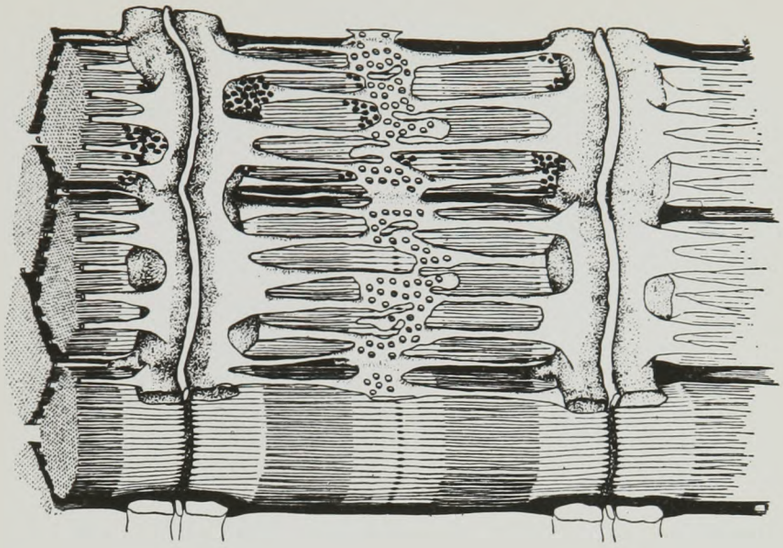


Fig. 47a. Three-dimensional reconstruction of the sarcoplasmic reticulum associated with several myofibrils in the frog skeletal muscle fibre (taken from Peachey, 1965). The endoplasmic reticulum consists of two types of enclosure which do not communicate directly with each other. One type is running alongside each myofibril, along most of its length, and forming a sheath around the myofibril, but being interrupted at the level of the Z-lines. The other type of the reticulum consists of transversely oriented tubules (the T-system) at the level of the Z-line which are continuous across the fibre with the outside. Where the terminal region of the longitudinal system of the reticulum comes into contact with the transverse system, a characteristic structure called a triad is formed.

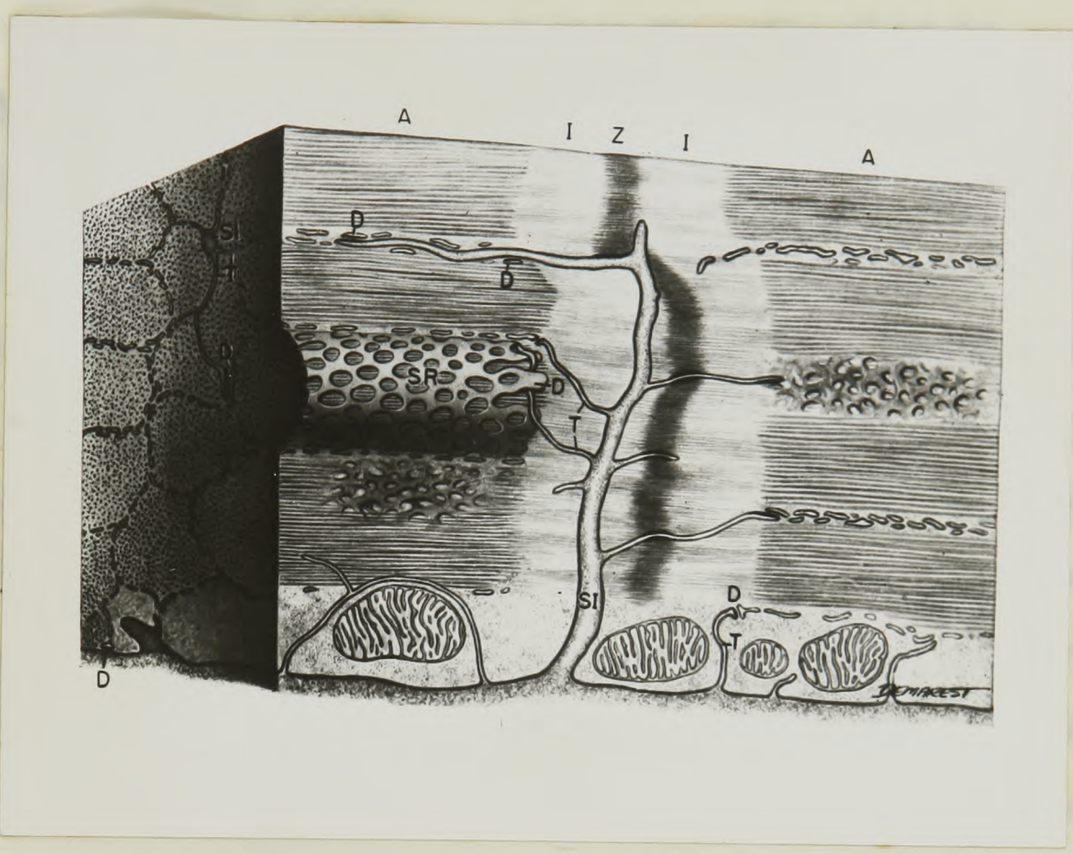


Fig. 47b. Composite representation of the fine structure of a crayfish muscle fibre (taken from Brandt, Reuben, Girardier & Grundfest, 1965). The sarcolemmal invagations (SI) form tubes about 0.2 μ in diameter which run radially into the fibre and have longitudinal side branches. Tubules (T) about 150 A in diameter arise from the SI and from the sarcolemma. The invaginations and tubules are all derived from and are continuous with the plasma membrane, forming the transverse tubular system, which is analogous with the T-system of vertebrate muscle. In the A band region each myofibril is enveloped by a fenestrated membranous covering of sarcoplasmic reticulum (SR). SR makes specialized contacts (diads, D) with T.

surface of a functional bundle (which would correspond to the plasma membrane of the skeletal muscle) nor whether the spike might be conducted along the surface of the bundle, as in a skeletal muscle fibre.

The functional bundle, as suggested before (section B in this chapter), must have a larger membrane capacity due to a larger surface area of many aggregated interconnected fibres compared with a single cylindrical fibre with the same diameter. Assuming a bundle of 30 μ diameter composed of rods of 5 μ diameter, the capacity of the bundle would be 6 times larger than that of a cylinder of the same size due to a difference of a surface area. This gives 12 $\mu F/cm^2$ for the membrane capacity if 2 $\mu F/cm^2$ is taken as the capacity of the cylinder of 30 μ in diameter. From the time constant of 60 - 100 msec, the membrane resistance of the bundle can then be calculated to be 5,000 - 8,000 $\Omega\, cm^2$.

Assuming that the membrane capacity of the single smooth muscle cell is the same as that of the slow skeletal muscle membrane (about 2 $\mu F/cm^2$), and that the time constant is that measured by external current application (60 - 100 msec), the membrane resistance of smooth muscle is calculated to be 30 - 50 X $10^3\, \Omega\, cm^2$, which is quite high compared with many other excitable membranes (see Table II), but is of the same order as that of the frog slow fibre. This high membrane resistance of the taenia coli is also compatible with the low K conductance calculated from K^{42} flux (see the next section).

However, the high membrane resistance as calculated above conflicts with the results obtained by intracellular stimulation using the

Wheatstone bridge method which show a rather low resistance 320 Ω cm^2, (Kuriyama & Tomita, 1965), although some assumptions are made in this calculation. Moreover, the hyperpolarizing response obtained by intracellular stimulation also suggests a rather low membrane resistance (Kuriyama & Tomita, 1965), if the underlying mechanism is the same as in the hyperpolarizing response obtained in KCl-depolarized membrane of squid and frog nerve (Tasaki, 1959) or in the crustacean muscle fibre which has a low membrane resistance (Reuben, Werman & Grundfest, 1961).

ii) Membrane parameters calculated from the data obtained by intracellular stimulation. Many authors (see Table III) calculated the specific membrane resistance in the smooth muscle and in the cardiac muscle by multiplying the input resistance with the calculated surface area of a cell, assuming that current spreads homogeneously all over the surface membrane. However, this assumption cannot be true even for a single cell (not considering the syncytium) because of the small diameter of the cylindrical (or double cone shaped) cell which must have a high internal longitudinal resistance. If the current density is higher at the membrane near the micro-electrode, as expected from the high internal resistance, the above calculation gives a lower value than the true value, as shown in the following examples of calculation.

(1) It is assumed that current distribution along the fibre is the same everywhere, i.e. the internal longitudinal resistance of the myoplasm is zero. The surface area of a single fibre was calculated as a double cone of 100 μ length and 5 μ maximum diameter. From the effective resistance of 40 MΩ and the surface area of 8×10^{-6} cm^2, the specific membrane resistance (R_m) is

TABLE III Membrane resistance and capacity measured with intracellular electrode

	Input resistance ($M\Omega$)	Specific resistance ($\Omega\ cm^2$)	Specific capacity ($\mu F/cm^2$)	Time constant (msec)	Dimension* (μ)	Reference
Cat: intestinal circular muscle	104	980	-	-	100 x 6 (double cone)	(1)
" " " "	70	560	56	31	100 x 5 (double cone)	(2)
" " " "	7.5	377	40	5-30	200 x 8 (cylinder)	(3)
Guinea-pig: taenia coli	40	320	10	2-3	100 x 5 (double cone)	(4)
Chick: cultured heart cell	12	480	20	9.6	100 x 15 x 5	(5)
"	7	560	-	-	200 x 15 x 5	(6)
Frog: ventricle	5.5	315	15	5-10	150 x 12 (cylinder)	(7)
Cat: ventricle	6.4	489	15	5-10	150 x 16 (cylinder)	(7)

* length x diameter (or length x width x thickness)

(1) Barr (1961), (2) Nagai & Prosser (1963), (3) Sperelakis & Tarr (1965), (4) The present thesis, (5) Sperelakis & Lehmkuhl (1964), (6) Lehmkuhl & Sperelakis (1965), (7) Tarr & Sperelakis (1964).

$R_m = 40 \times 10^6\,\Omega \times 8 \times 10^{-6}\ cm^2 = 320\ cm^2$.

(2) If it is assumed that current is distributed along the fibre as predicted from the cable theory, decaying exponentially from the stimulating electrode, and that the fibre is cylindrical, the potential change (V_m) by a current application is expressed by eq. (18) (P. 77). Since $V_m = r_m \times i_m$, where r_m = the membrane resistance per unit length, and i_m = current flowing across r_m, the following equation can be obtained from eq. (18).

$$i_m = \frac{V_{m\,t=\infty,\,x=0}}{r_m} \cdot e^{-x/\lambda} \qquad \text{.....(23)}$$

Integrating i_m from $x = -\infty$ to $x = +\infty$, the total current, I, can be expressed by eq. (24).

$$I = \int_{-\infty}^{+\infty} i_m \cdot dx = 2\int_{0}^{+\infty} \frac{V_{m\,t=\infty,\,x=0}}{r_m} \cdot e^{-x/\lambda} dx = \frac{2V_{m\,t=\infty,\,x=0}}{r_m} \cdot \lambda \qquad \text{.....(24)}$$

Putting $\lambda = \sqrt{r_m/(r_i + r_e)}$ (eq. 10) into eq. (24),

$$r_m = \left(\frac{V_{m\,t=\infty,\,x=0}}{I}\right)^2 \cdot \frac{4}{r_i + r_e}$$

From $r_m = R_m/2\pi a$, $r_i = R_i/\pi a^2$, where R_m = the specific membrane resistance, R_i = the specific internal resistance, and a = the radius of the fibre; and neglecting r_e,

$$R_m = \left(\frac{V_{m\,t=\infty,\,x=0}}{I}\right)^2 \cdot \frac{8\pi^2 a^3}{R_i} \qquad \text{.....(25)}$$

Inserting the effective resistance, $\frac{V_{m\,t=\infty,\,x=0}}{I}$, of 40 MΩ R_i of 125 Ω cm (mammalian Purkinje fibre: 105 Ω cm, Weidmann, 1952; 154 Ω cm, Coraboeuf & Weidmann, 1954) into eq. (25).

$$R_m = (40 \times 10^6\,\Omega)^2 \times 8 \times (3)^2 \times (2.5 \times 10^{-4}\,cm)^3 \times \frac{1}{125\,\Omega cm} = 14{,}400\,\Omega\,cm^2$$

(3) The cable equation for an infinitely long fibre cannot be applied to the smooth muscle fibre because the ratio of fibre length to diameter is too small (20) compared to frog skeletal muscle fibre (400). Fatt & Ginsborg (1958) used the following special equation to calculate the membrane characteristics of the crustacean muscle fibre which also has a small length to diameter ratio (65).

$$R_m = \frac{\pi^2 d^3 R^2}{4 R_i} \tanh \frac{\ell}{\lambda}$$

with λ obtained from

$$\frac{\lambda}{\ell} \coth \frac{\ell}{\lambda} = \frac{\pi d^2 R}{4 R_i \ell}$$

where d = the fibre diameter, R_i = the internal specific resistance and ℓ = the fibre length.

In their equation, the electrode is assumed to be inserted at one end of the fibre, while in smooth muscle it is probably placed in the middle part of the fibre. In this calculation, therefore, $\ell/2$ is inserted instead of ℓ in the above equation.

If the internal specific resistance (R_i) is 125 Ω cm, R_m = 1800 Ω cm^2.

None of the above calculations presents the actual electrical situation in the smooth muscle fibre which is not only relatively short but has the shape of a tapered cylinder. However, it can probably be said that calculation (1) which the most workers have employed is not reliable at all and gives a lower value than true value. Furthermore, as discussed before (P. 72), in a tissue which has two-or three-dimensional connections between cells, an intracellular current

application underestimates the effective resistance. For the same reason, the membrane time constant measured by intracellular current application is smaller than the true time constant. This is predicted from the model experiments (P.72). In the taenia coli, a time constant of 2 - 4 msec was obtained by intracellular stimulation and 60 - 100 msec by external stimulation. One of the factors which causes the larger time constant with external polarization is the increased surface area in the functional bundle as discussed before (P.86). In other tissues, no comparison has been reported between the time constants measured by intracellular and external current application.

D) Membrane properties considered from ion flux.

1) Relative permeability to K and Na, and K-conductance.

The electrical conductance of the surface membrane depends on the ease with which ions can pass through it, and is therefore directly related to the ionic flux as measured with radioactive tracers. However, a sodium flux due to the operation of an exchange diffusion mechanism of the type proposed by Ussing (1949) or a sodium pump mechanism as discussed by Hodgkin, Huxley & Katz, (1949) would not contribute to the conductance.

In general, the excitable membrane has a high degree of ion selectivity in favour of K both at rest and under conditions of imposed steady electric current flow (Brinley & Mullins, 1965). This selectivity can be expressed for resting ion fluxes by the ratio of Na permeability (P_{Na}) to K permeability (P_K) or by Na influx (m_{iNa})/external Na concentration $(Na)_o$ divided by K influx (m_{iK})/external K concentration, $(K)_o$

$$\frac{m_{iNa}/(Na)_o}{m_K/(K)_o}$$

since any electrical factor affecting influx will act equally on both ions (Keynes, 1951; 1954; Brinley & Mullins, 1965).

If the cell is first allowed to accumulate some radioactive ions, and is then put into an inactive medium, radioactivity is lost at a rate

$$dY/dt = - m_o s_i A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (26)$$

where Y is the total radioactivity of the cell, m_o is the outward ionic flux, s_i is the internal specific radioactivity (the proportion of radioactive ions to the total amount of the same ions) and A is the surface area of the cell. Y is given by

$$Y = s_i V C_i \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (27)$$

where V is the volume of the cell and C_i is the intracellular concentration of the ion. From eq. (26) and eq. (27),

$$\frac{dY}{dt} + \frac{(m_o A)}{(V \cdot Ci)} \cdot Y = 0 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (28)$$

Integrating eq. (28)

$$Y = Yo \; e^{-\frac{m_o A}{CiV} t} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (29)$$

where Yo is Y at t = 0

$$\log_e Y = \log_e Y_o - \frac{mo\ A}{CiV} t$$

$$\frac{d}{dt} (\log_e Y) = - \frac{mo}{Ci} \frac{A}{V} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (30)$$

The value of $-\frac{d}{dt} (\log_e Y)$ is termed the exchange constant, and denoted by k (Keynes & Lewis, 1951). It is the rate constant, or the reciprocal of the time constant, or 0.693/half-time. After time t the activity of the cell is proportional to e^{-kt}.

From the definition of k,

$$k = \frac{(m_o)}{(Ci)} \quad \frac{(A)}{(V)}$$

$$m_o = k. \frac{V}{A} Ci \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (31)$$

In taenia coli of the guinea-pig k = 0.0077 min^{-1} to 0.01444 min^{-1}, V/A = 1.5 μ and [K] i = 98 to 119 mM. By inserting these values into eq. (31), K efflux, m_{oK}, is calculated to be 2 - 4 pmole cm^{-2} sec^{-1} (Goodford & Hermansen, 1961). Born & Bülbring (1956) obtained a similar value for the rate constant of K efflux (0.0092 min^{-1}) from the half-time of 75 min.

Na efflux, m_{oNa}, of 33 pmole cm^{-2} sec^{-1} is obtained from the same formula using the rate constant of 0.35 min^{-1} (Half-time = 2.0 min) and the internal Na concentration, $(Na)_i$ of 37.5 mM (Buck & Goodford, 1966).

Goodford & Hermansen (1961) obtained a very high Na-efflux (200 - 300 pmole cm^{-2} sec^{-1}) from the rate constant of 1.39 min^{-1} and the internal Na concentration of 56 - 85 mM. The results by Buck & Goodford (1966) are probably more valid due to improved experimental procedure.

Since the internal concentration is roughly kept constant, the influx can be considered the same as efflux. Assuming that the whole influx is due to the influence of the concentration gradient and the potential difference across the membrane, the permeability ratio is

$$\frac{PNa}{PK} = \frac{30 \text{ pmole}}{2\text{-}4 \text{ pmole}} \times \frac{5.9 \text{ mM}}{137 \text{ mM}} = \frac{177}{274\text{-}548} = 0.32 - 0.64$$

This value is more than ten times larger than the ratio obtained in other excitable membrane as shown in Table IV. If part of K influx is due to active transport or exchange diffusion, the ratio of P_{Na}/P_K would be even greater. If the value of Na influx is taken from Goodford & Hermansen (1961), the ratio becomes 2.5-4 which is extremely high.

The interpretation of ion flux in the taenia coli must be very difficult because of the spontaneous activity, the frequency of which is 0.5-2 spikes/sec. According to the ionic theory (Hodgkin, 1951; 1958), the depolarizing phase of the action potential is brought about by Na influx and the repolarization by K efflux. If the spike in the taenia coli is produced by the same mechanism as in the nerve fibre, the total quantity of Na entering the muscle would be given by the product of the membrane action potential and the membrane capacity divided by the Faraday constant (96500 coulombs). Taking the spike as 60 mV and the membrane capacity as 2 $\mu F/cm^2$, the Na influx and K

TABLE IV Permeability ratio for Na and K, calculated from ion flux

	miK (p-mole cm^{-2} sec^{-1})	miNa (p-mole cm^{2} sec^{-1})	$(K)_o$ (mM)	$(Na)_o$ (mM)	P_{Na}/P_K	Reference
Squid axon	15	17	10	423	0.027	(1)
" "	16.7	61	9.7	458	0.077	(2)
Frog toe muscle	5	10.9	2.5	100	0.05	(3)
Rat Diaphragm	21	28	5.9	137	0.057	(4)
Guinea-pig taenia coli	2-4	30	5.9	137	0.32-0.64	(5)
" " " "	2-4	200-300	5.9	137	1-4	(6)

(1) Brinley & Mullins (1965), (2) Keynes (1951), (3) Keynes (1954), (4) Creese (1954),
(5) Buck & Goodford (1966), (6) Goodford & Hermansen (1961).

efflux during a spike would be more than 1.2 X 10^{-12} mole per spike in order to change the potential difference of the capacity (Hodgkin & Katz, 1949; Weidmann, 1952). If the measurement of K flux is reliable (2-4 X 10^{-12} mole cm^{-2} sec^{-1}), the membrane capacity must be less than 6 $\mu F/cm^2$, otherwise the calculated flux becomes larger than the measured flux. Excluding the flux due to the spike (1.2 X 10^{-2} mole cm^{-2} sec^{-1}, assuming 1 spike/sec) the permeability ratio of Na to K becomes 0.5 - 1.5.

The high P_{Na}/P_K ratio in the taenia has also been predicted from the facts that the membrane potential is much lower than K equilibrium potential and that the change of the membrane potential with the external K concentration is smaller (38 mV) than that (60 mV) theoretically expected from the K-electrode per tenfold change of the external K concentration (Kuriyama, 1963; Casteels & Kuriyama, 1966).

The permeability ratio, P_{Na}/P_K has been obtained by the following equation (Hodgkin, 1958; Hodgkin & Horowics, 1959), derived from the constant field theory when the Cl contribution to the membrane potential is neglected.

$$V = \frac{RT}{F} \log_e \frac{[K]_o + \alpha [Na]_o}{[K]_i + \alpha [Na]_i}$$

where α is the permeability ratio, P_{Na}/P_K. In squid giant axon, α is 0.01 (Hodgkin, 1958). For a single fibre of frog semitendinosus α is also 0.01 when the experiment is done in Cl-free sulphate medium (Hodgkin & Horowicz, 1959). The permeability ratio, P_{Na}/P_K obtained in this way is smaller than that calculated for the same tissue from ion flux (see Table IV). Buck & Goodford (1966) calculated P_{Na}/P_K from the following

constant field equation. In order to obtain a distribution according to the Donnan equilibrium, they assumed that some internal K, (K), and some internal Na, (Na), are sequested in a special compartment, the weight of which is W.

$$E = \frac{RT}{F}\left\{\log_e((Na)_o + \alpha\,(K)_o) - \log_e\left(\frac{(K)i-K}{W-W} + \alpha\,\frac{(Na)i-Na}{W-W}\right)\right\}$$

where E is the observed membrane potential, α is the ratio of Na to K permeability and W is the weight of the cells. They obtained the ratio α = 0.04 - 0.07 depending on the values chosen for the parameters. This α value is higher than the above value for squid giant axon or frog skeletal muscle fibre.

Geduldig (1965), using the same equation as that used by Hodgkin (1958), obtained α = 0.03 in Amphioxus muscle. He also calculated α for the frog slow muscle fibre from experimental results published by Burke & Ginsborg (1956), to be 0.042. It has been shown that the presence of a transverse tubular system is associated with a high resting K permeability in frog twitch muscle fibres (Adrian & Freygang, 1962). Geduldig (1965) assumed that the absence of the tubular system in both Amphioxus (Peachey, 1961) and frog slow muscle fibres (Peachey & Huxley, 1962) might provide a basis for the low K permeability and consequently a high α in these tissues, because The high α in taenia may be due to the same reason.

It is difficult to predict the contribution of Na to the membrane conductance without knowing more about the mechanism by which it crosses the cell membrane. Most of the Na efflux seems to be due to active transport mechanisms and some of the Na influx may be carried as

non-ionized Na, i.e. combined with some anion. Therefore, the permeability ratio of Na to K calculated from the ion flux is probably an overestimation.

It has been shown (Hodgkin, 1951; Keynes, 1954) that if K ions cross the membrane independently from one another, their distribution being decided only by the forces exerted by thermal agitation and the electric potential gradient, then for a fibre in ionic equilibrium with its surroundings (i.e. $m_i = m_o$).

$$GK = \frac{F^2}{RT} \; mk; \text{ or } mk = G_K \cdot \frac{RT}{F^2} \qquad \ldots\ldots\ldots\ldots\ldots\ldots (32)$$

where G_K is the component of the total membrane conductance due to current carried by K and m_K is the potassium flux determined with K^{42}. At 35°C the value of RT/F is 26.5 mV and RT/F^2 is therefore 2.7×10^{-7} (F=96500 coulombs/mole). The outward K flux of the taenia coli is 2 - 4 pmole cm^{-2} sec^{-1}, so that

$$GK = \frac{(2 \text{ to } 4) \times 10^{-12}}{2.7 \times 10^{-7}} = (0.74 \text{ to } 1.48) \times 10^{-5} \text{ mho cm}^{-2}$$

Table V shows the membrane conductance (and resistance) measured with the tracer method described above and also measured with the electrophysiological method. The total membrane conductance measured by electrophysiological experiment is, in all tissues, 5 - 10 times larger than the potassium conductance calculated from eq. (32), as pointed out by Keynes (1954). As seen in Table V, G_K in the guinea-pig taenia coli is very low compared with other tissues. This might suggest a high conductance for Na, or Cl, or both, if the membrane resistance in the taenia coli is similar to that in other tissues. However, this is unlikely because the effects of Na and Cl on the membrane potential and on the membrane resistance are much smaller than those of K. The

TABLE V

Comparison of membrane conductance (and resistance) measured by K flux and by electrophysiological method

	Tracer Method			Electrophysiological method		
	m_K (p-mole cm^{-2} sec^{-1})	G_K ($\mu\mho$ cm^{-2})	R_K ($K\Omega$ cm^2)	G_m (membrane conductance) ($\mu\mho$ cm^{-2})	R_m ($K\Omega$ cm^2)	
Carcinus nerve fibre	22 (1)	85 (1)	11.7	130	7.7	(2)
				306	3.3	(3)
Frog toe muscle	5 (4)	19 (4)	53	230	4.3	(3)
Frog sartorius	12 (4)	46 (4)	22	244	4.1	(5)
Rat diaphragm	21 (7)	78	13	-	-	
Guinea-pig taenia coli	2-4(8)	7.4-14.8	68-135	-	-	

(1) Keynes & Maisel (1954), (2) Hodgkin (1947), (3) Katz (1948), (4) Keynes (1954), (5) Fatt & Katz (1951), (6) Del Castillo & Machne (1954), (7) Creese (1954), (8) Goodford & Hermansen (1961).

rate constant of K efflux is also low in the smooth muscle of dog carotid artery (0.005 min^{-1}, Barr, 1961b) and in rat uterus (0.01 min^{-1}, Daniel, Sehdev & Robinson, 1962). A low G_K might be a special property of smooth muscle as mentioned before (Chapter VI C, P.86).

Since the total membrane conductance is 5 - 10 times larger than the potassium conductance in other muscles (Table V), the membrane resistance of the taenia coli may also be 1/5 - 1/10 of that calculated from G_K, i.e. 7 - 27 X $10^3 \Omega\ cm^2$. It is interesting that this value is similar to that calculated from the time constant (60 - 100 msec) of the electrotonic potential produced by external stimulation (assuming the same membrane capacity as that of the slow muscle fibre), and also to the membrane resistance of the slow fibre measured by Adrian & Peachey (1965).

An explanation for the low G_K may be possible. The smooth muscle may have a low G_K because it has no tubular system which increases G_K in skeletal muscle. According to the electrophysiological study by Adrian & Peachey (1965), the G_K of the slow muscle fibre is very low, and they explained this by the lack of the tubular system. It is also possible that the membrane of the smooth muscle cell is composed of different areas which have different K-conductances, and, moreover, the cells are electrically interconnected, as suggested from the electrophysiological studies. In both conditions the V/A would be much larger than that derived from the histological estimation, and K efflux would be underestimated. Unhomogeneous distribution of K inside the cell would also give a lower G_K than the true value, if K

is accumulated near a surface area which has a lower G_K. For example, K may be accumulated near Area B which has a high membrane resistance (a low conductance) as a result of high metabolic activity. This produces a higher membrane potential at Area B than at Area C. This potential difference produces the outward (depolarizing) current at Area B which might cause instability of the membrane potential, i.e. the generation of the slow component A. This kind of uneven distribution of K may be possible not only in a single cell but also in grouped cells. In other words, some parts of the cells in the tissue have a higher metabolic activity and accumulate more K inside than other parts of the cells, and the produced potential gradient between the two grouped cells acts as a generator potential, i.e. the slow component A.

2) Na-flux.

The influx, m_i, will be proportional to the external concentration, C_o, and will not be affected by the concentration inside the cell, C_i, if the individual ion movement is independent of the other ions (Hodgkin & Huxley, 1952_a).

$$m_i = k_i C_o \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (33)$$

where k_i is a constant which depends on the condition of the membrane and on the potential difference across it. Similarly, the efflux, m_o, is given by

$$m_o = k_o C_i \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (34)$$

where k_o is another constant. Hence

$$m_i/m_o = (k_i/k_o)(C_o/C_i) \quad \ldots\ldots\ldots\ldots\ldots \quad (35)$$

The condition for equilibrium is that $m_i = m_o$, so that

$$k_o/k_i = C_o^*/C_i$$

where C_o^* is the external concentration that would be in equilibrium with the internal concentration, under the existing value of the membrane potential, E_m.

Substituting for k_i/k_o in eq. (35)

$$m_i/m_o = C_o/C_o^* \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (36)$$

Now $C_o^*/C_i = \exp(-E_m F/RT)$, because $E_m = -RT/F \ln (C_o^*/C_i)$ and $C_o/C_i = \exp(-E_e F/RT)$, where E_e is the equilibrium potential for the ion under discussion, so that

$$C_o/C_o^* = \exp(E_m - E_e)\, F/RT$$

and the ratio of m_i to m_o is given by

$$m_i/m_o = \exp(E_m - E_e)\, F/RT \quad \ldots\ldots\ldots\ldots\ldots\ldots (37)$$

or

$$m_i/m_o = (C_o/C_i) \exp(E_m F/RT) \quad \ldots\ldots\ldots\ldots\ldots\ldots (37a)$$

If the K efflux, 2 - 4 pmole cm^{-2} sec^{-1}, represents the whole passive flux, this would be in balance with the sum of active and diffusional K influxes. According to eq. (37) or (37a), the diffusional K influx would be 1 - 2 pmole cm^{-2} sec^{-1} since $m_i/m_o = 0.5$. Taking Na influx, 30 pmole cm^{-2} sec^{-1}, as the passive flux, the diffusional Na efflux would be 0.75 pmole cm^{-2} sec^{-1} since $m_i/m_o = 40$. This indicates that most of the Na efflux is transported by an active mechanism or an exchange diffusion. As discussed before, a high proportion of Na influx may not be due to a diffusional process, so that the diffusional Na

efflux would be smaller in the same proportion.

From eq. (31)

$$m_o/(Na)_i = (V/A) \cdot k \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (38)$$

$m_o/(Na)_i$ is the term expressing the proportion of the amount of Na, which is transported across the membrane per unit area per unit time, to the internal Na concentration. Since V/A is 1.5×10^{-4} cm and k is 0.35 min^{-1} in the taenia coli, $m_o/(Na)_i$ is calculated to be 8.7×10^{-7} cm sec^{-1}. Though the rate constants of different preparations vary over a wide range, the value of $m_o/(Na)_i$ is nearly the same since V/A is taken into account, as shown in Table VI. However, in mammalian smooth muscle, cardiac muscle and motoneurone, $m_o/(Na)_i$ appears to be higher than that in the mammalian skeletal muscle and that in the cold blooded animal, except the squid axon. The higher $m_o/(Na)_i$ value means that the amount of transported Na at the same internal Na concentration is also larger. As $m_i = m_o$ in the balanced state (Conway, 1957), the large $m_o/(Na)i$ indicates that m_o as well as m_i is large.

If it is assumed that m_{oNa} is due to an active transport, the energy consumption (W) in the balanced state is expressed by the following equation (Keynes, 1951; Keynes & Maisel, 1954).

$$W = k\,(Na)_i\,F\,(E_{Na} + E_r)$$

where E_{Na} is the Na equilibrium potential and E_r is the resting potential and F is 23100 cal. V^{-1} $Mol.^{-1}$ As shown in this equation, the rate of energy consumption by the Na pump per unit volume is proportional to k. The mammalian tissue, especially smooth muscle and motoneurone, has a

TABLE VI Na Flux

	Rate constant	V/A	m_o/N_{ai}	Na_i	Temp.	Reference
	(hr^{-1})	(μ)	(10^{-7} cm sec^{-1})	(10^{-3} M/l)	(°C)	
Squid axon	0.20	136	7.6	40	18-20	(1)
Frog semitendinosus	0.67	27	4.7	9.2	17	(2)
Frog sartorius	0.81	20	4.5	12	17	(3)
Frog toe muscle	1.73	10.3	5.0	-	17	(3)
Rat diaphragm	3.8	4.3	4.5	18 - 60 (in vivo) (in vitro)	38	(4)
" "	7.3	-	-	-	38	(4)
Mouse toe muscle	4.3	-	-	-	-	(5)
Dog carotid artery	8.3	-	-	50	35-37	(6)
Rat uterus	10.4	1.8	-	-	35	(7)
Guinea-pig taenia coli	21	1.5	8.7	37.5	35	(8)
Cultured chick heart	36	1.18	11.6	12.2	37.5	(9)
Cat motoneurone*	40	4.6	51	15	37-38	(10)

* Data from the experiment in which Na was injected through micro-electrode.

(1) Hodgkin & Keynes (1956), (2) Hodgkin & Horowicz (1959), (3) Keynes (1954), (4) Creese (1954), (5) Creese & El-shafie (1964), (6) Garrahan, Villamil & Zadunaisky (1965), (7) Daniel, Sehdev & Robinson (1962), (8) Buck & Goodford (1966), (9) Burrows & Lamb (1962), (10) Ito & Oshima (1964a).

large value for k, as seen in Table VI, suggesting that much energy is consumed to pump Na out of the cell.

In the taenia coli of the guinea-pig, the energy of about 0.6 cal. g^{-1} hr^{-1} (0.00583 sec^{-1} X 37.5 X 10^{-6} M g^{-1} X 23 X 10^{3} cal. M^{-1} V^{-1} X (35 + 55) X 10^{-3} V) is necessary to move sodium against a concentration gradient of 137/37.5 and a resting potential of 55 mV (Data are taken from Buck & Goodford, 1966). The oxygen consumption of the taenia coli is about 500 mm^{3} O_2 g^{-1} hr^{-1} (Bülbring, 1953). This would correspond to a heat production of about 2.5 cal. g^{-1} hr^{-1}, if consumption of 1 mm^{3} oxygen is equivalent to a heat production of 5.05 X 10^{-3} cal. as expected from carbohydrate metabolism. This calculation shows that about 25% of the total metabolic energy is consumed by an active transport system, while the absolute energy requirement is more than ten times higher than that of other preparations of cold blooded animals.

At 4°C and 20°C the exchange of Na in the taenia coli is extremely slow, whereas at 35°C the uptake becomes very fast (Freeman-Narrod & Goodford, 1962; Buck & Goodford, 1966). This indicates the critical dependence of the Na movement on temperature, and suggests that Na movement may depend upon the supply of metabolic energy. The high vulnerability of motoneuronal potentials to asphyxia or anoxia (Lloyd, 1953; Kolmodin & Skoglund, 1959; Ito & Oshima, 1964b) and to an intracellular injection of metabolic inhibitor such as azide ions (Araki, Ito & Oscarsson, 1961; Ito & Oshima, 1964b) can also be explained by the high rate of Na extrusion consuming metabolic energy.

TABLE VII Energy requirement for Na-pump and total metabolism

	Energy for Na-pump (cal/g/hr)	% of total energy	Total metabolism (*) (cal/g/hr)	Reference
Squid axon	0.049	6	0.8	(1)
Frog skeletal muscle	0.025	10	0.15-0.2	(2)
" " "	0.051	30	0.175	(3)
Guinea-pig taenia coli	0.6	25	2.5	(4), (5)

(*) Calculated from oxygen consumption

(1) Keynes (1951), (2) Keynes & Maisel (1954), (3) Levi & Ussing (1948), (4) Buck & Goodford (1966), (5) Bulbring (1953).

In order to correlate the data obtained from the measurements of ion flux with the electrical properties of the smooth muscle, it must be clarified what percentage of the ion flux is carried by active transport or exchange diffusion, and also how much is due to the spike activity.

E) Conclusion.

The evidence presented in this thesis is consistent with the view that the muscle fibres of the taenia coli are electrically interconnected and that groups of fibres behave as a functional bundle. This arrangement makes it difficult to investigate the passive and active electrical properties of the cell membrane. The experimental results can be explained by the following hypothesis:

1) Passive properties:

It is assumed that the smooth muscle fibres of the taenia have a similar membrane resistance (about 30 $K\Omega cm^2$) and also a similar membrane capacity (about 2 $\mu F/cm^2$) to those of the slow skeletal muscle fibres. The high membrane resistance is the result of a low K-permeability, which is related to the lack of a tubular system. As a result, the ratio of Na-permeability to K-permeability is high.

The fibres of the taenia are electrically interconnected across areas of close contact which have a low electrical resistance, and the cells are aggregated forming a functional bundle.

A functional bundle of about 30 μ diameter, composed of many cells of about 5 μ diameter, has a large membrane capacity (about 12 $\mu F/cm^2$), because, compared with a cylinder of the same diameter, it has a very large surface area.

The functional bundle has cable properties if current is applied externally.

The study of the membrane properties using intracellular current

application does not give the correct values because of the three-dimensional connections between muscle fibres.

Moreover, it is possible that a single muscle membrane is composed of areas with different electrical properties and, perhaps, also different chemical sensitivity.

2) Active properties:

Many muscle fibres (a functional bundle) must participate in producing the conducted spike in response to electrical stimulation.

An intracellular micro-electrode picks up mainly the electrical activity of the single cell in which it is impaled.

The spread of a spike potential to an adjacent cell is very weak. This is probably due to the fact that the time constant of the muscle membrane is longer than the duration of the spike, and that the cell-to-cell connection acts as a barrier.

The functional mechanism of the spike propagation is, nevertheless, by means of electrotonic spread, since the functional bundle has cable properties. This mechanism may be similar to that at the ephaptic junction between neurones in some lower animals.

A single muscle fibre of the taenia has several such junctions, and the electrotonic spread from many surrounding cells must summate to generate at first an active slow component near the junction. This slow component then triggers the spike in the remaining area of the membrane.

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