

BEHAVIOUR OF THE SMOOTH MUSCLE OF THE  
CARDIOVASCULAR SYSTEM

by

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# ABSTRACT OF THESIS

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This investigation was undertaken in an attempt to study the behaviour of vascular smooth muscle at the cellular level, and in conditions as near physiological as possible. Microelectrodes were inserted into smooth muscle cells in the walls of small arteries and arterioles in the rat mesenteric circulation in the intact anaesthetized rat, and the electrical activity recorded at rest and under the influence of various physiological stimuli.

Intracellular recording gave membrane potentials that were low and variable, showing slow fluctuations at frequencies of some 9 to 12/min, and occasional action potentials which appeared to arise from the slow waves. Both these types of electrical activity were capable of being influenced by the sympathetic nervous system, as shown by the effects of stimulating the splanchnic nerves and denervating by chemical means and by cutting the nerves. However, as it is not known whether any residual nervous activity remained after denervation was attempted, it remains uncertain whether this electrical activity was generated by nervous activity or by some mechanism inherent in the smooth muscle cell itself.

Propagated action potentials could not be elicited by direct electrical stimulation of the blood vessels, and the application of a stimulating pulse across the cell membrane into the interior of the cell did not evoke an action potential, but this may have been due to technical limitations.

It was found impossible to record for long periods with an electrode inside a cell due to the small size and continuous movement of the cells, and the effects of various stimulating and inhibiting agents were therefore studied using extracellular recording methods. In general, stimulating agents such as asphyxia, electrical stimulation of the sympathetic nerves, and local application of adrenaline, noradrenaline, and vasopressin, increased the electrical activity of the muscle, and inhibiting agents such as acetylcholine and removal of the nerve supply depressed it. High concentrations of adrenaline and noradrenaline appeared to be capable of causing the muscle cells to contract without firing action potentials, but whether this type of contracture is a

physiological phenomenon remains uncertain. None of these manoeuvres had any significant effect on the frequency of the slow waves, but under the influence of stimulating agents the percentage of waves with action potentials increased, and spikes appeared in twos and threes.

It was concluded that the technical difficulties due to the small size of the smooth muscle cells relative to that of the penetrating electrode tip will limit further investigation using refined techniques until the development of methods for producing finer microelectrodes.

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## FOREWORD

In this investigation the smooth muscle of the resistance vessels of the rat mesenteric circulation was investigated by electrophysiological techniques. The aim was to study at the cellular level the behaviour of the muscle under conditions as near as possible to the normal physiological state, and to investigate the mechanisms of control by the autonomic nervous system and by agents which affect the blood pressure in an attempt to understand how these effects are brought about.

The state of knowledge concerning the electrophysiology of smooth muscle at the start of this investigation is described in the literature survey, and the development of the concept that the wide differences in the behaviour of smooth and skeletal muscle might be explained by assuming a quantitative rather than a qualitative difference in their ionic properties is stressed. It was against this background that this investigation was carried out.

TABLE OF CONTENTS

## TABLE OF CONTENTS

### PART I THE PREVIOUS LITERATURE

Introduction	1
Membrane potentials	4
Action potentials	16
Local potentials	18
Effect of calcium on membrane activity	20
Effects of transmitter substances	23
Effects of hormones	31
Vascular smooth muscle	33

### PART II METHODS

Experimental material	37
Solutions and drugs	38
Electrophysiological apparatus	40

### PART III TYPES OF ELECTRICAL ACTIVITY RECORDED

Results	
Intracellular recording	46
Membrane potentials	46
Changes in ionic environment	47
Slow waves	48
Action potentials	49
Extracellular recording	53
Discussion	
Configurations of externally-recorded potential changes	60

	Membrane potentials	61
	Slow waves	64
	Action potentials	65
<b>PART IV</b>	<b>DEGREE OF DEPENDENCE OF ELECTRICAL ACTIVITY ON NERVOUS SYSTEM</b>	
	Introduction	71
	Results	
	Effects of autonomic blocking agents	72
	Effect of cutting the splanchnic nerves	73
	Effect of asphyxia	74
	Direct electrical stimulation of the blood vessels	74
	Discussion	76
<b>PART V</b>	<b>EFFECT OF NERVOUS AND HUMORAL STIMULATION OF THE BLOOD VESSELS</b>	
	General introduction	82
	Nervous stimulation	
	Introduction	85
	Results	86
	Discussion	89
	Conclusions	93
	Humoral stimulation	
	Results	94
	Discussion	99

PART VI	GENERAL SUMMARY OF CONCLUSIONS	110
PART VII	APPENDIX	112
PART VIII	BIBLIOGRAPHY	116

An initial report of this investigation has been published (Trail, 1963).

PART 1: THE PREVIOUS LITERATURE

THE PREVIOUS LITERATUREINTRODUCTION

Initial investigations into the electrophysiology of smooth muscle using external electrodes were carried out by Bozler (reviewed in 1948) on ureter, uterus and intestine, and by Rosenblueth (1936) and Eccles and Magladery (1937) on cat pilomotor muscles and nictitating membrane. Bozler recorded action potentials similar to those which had been recorded from other excitable tissues during normal movements of the stomach and small intestine, and found that each movement was usually accompanied by a repetitive electrical discharge and Eccles and Magladery recorded all-or-nothing responses in the cat nictitating membrane associated with contractions in response to nerve stimulation. These observations conflicted with those of Rosenblueth and his collaborators who found smooth muscle to be incapable of giving all-or-nothing responses. Bozler's observations led to the conclusion that 'the activity of smooth muscle is due to the discharge of impulses which are accompanied by action potentials like those found in striated muscle and nerve', thus bringing these different tissues into line. He considered that 'the great variability of movements which is characteristic for smooth muscle can be explained by such factors as frequency and number of impulses discharged, rate of conduction, and variations in excitability'. Thus early in the history of smooth muscle was it recognised that the original idea of von Euler, Rosenblueth and others that some essential qualitative difference must exist between smooth and skeletal muscle to explain the wide differences in their



behaviour was not supported by the experimental evidence, and that in fact the different tissues probably shared the same basic properties. Bozler divided smooth muscles into two categories according to their degree of dependence on an extrinsic nerve supply - multiunit muscles, dependent on nervous influences, and similar to striated muscle in that their electrical and mechanical responses to stimulation can be analysed into changes taking place in functionally separate units responding in an all-or-nothing fashion to stimulation, and syncytial muscles, capable of myogenic activity and modified by nervous influences. The multiunit nictitating membrane muscle studied by Eccles is by no means typical of smooth muscle generally, and further investigations have been carried out mainly on the more typical spontaneously-active muscles, e.g. intestine, ureter, and uterus. It is now recognized that there is a broad spectrum of smooth muscles between Bozler's two extremes, and the position of vascular smooth muscle in this spectrum has been much debated.

With the development of the ionic hypothesis of Hodgkin and Huxley (1952), the old problem of the existence of basic similarities or differences between smooth muscle and other excitable tissues had to be approached at the cellular level. This thesis is concerned with the study of the electrophysiological properties of vascular smooth muscle by investigating the behaviour of single cells with microelectrode techniques. In this literature survey we shall see that investigation of other types of smooth muscle at the cellular level has led to a recognition of the fact that a quantitative rather than a major qualitative difference would account for the differences in properties of different tissues. This change in outlook is due to the development of apparatus and

recording techniques. With the various technical advances, better understanding of the peculiar properties of smooth muscle has resulted although much investigation remains to be carried out, and it is now realised that if some difference in the permeability of the cell membrane is assumed the characteristics of smooth muscle may be explained on the basis of the ionic theory.

## MEMBRANE POTENTIALS

### General

While external electrode studies can give valuable information about the behaviour of single cells of striated muscle and of nerve, due to the simple arrangement of their cells in selected instances, the organization of smooth muscle makes interpretation of external recordings difficult. Intracellular studies are therefore theoretically the best for the study of the electrical behaviour of smooth muscle, but application of the Ling-Gerard technique (1949) is made difficult by two main factors: (1) the cells are so small that membrane damage caused by the insertion of the electrode may reduce the membrane potential significantly; and (2) a spontaneously-active tissue is difficult to immobilize sufficiently to achieve and maintain electrode insertion.

The earliest intracellular recordings from smooth muscle were made by Bülbring from isolated guinea-pig taenia coli (Bülbring and Vaughan Williams, 1951, unpublished). Glass capillary microelectrodes, resistance not stated, were used and potentials of up to 50 mV recorded. These potentials were neither abruptly established nor well maintained, and fluctuated over a wide range; they may therefore have been recorded extracellularly.

Techniques were improved, and Bülbring and Hooton (1953) recorded potentials of up to 75 mV occurring instantaneously on penetration. These were not maintained, but decayed at varying rates. Maintained potentials in the region of 40 mV were also

## 5.

recorded and showed fluctuations which could be correlated with the spontaneous pendular movements of the tissue. Potentials could not be recorded for any length of time due to the breakage of the electrode tip by movement of the tissue, and the fluctuations observed could have been artefacts caused by change in electrode position due to the movement of the muscle. An attempt was therefore made to record from a tissue which did not exhibit spontaneous activity, and the iris of the albino rabbit was isolated for this purpose. Membrane potentials of 60 mV average, maintained for up to 10 min, were recorded in the sphincter pupillae muscle (Bulbring and Hooton, 1954). However, even in this quiescent tissue a wide scatter of potentials was observed among different cells.

Characteristic of many of these early recordings was a gradual or stepwise drop of the potential level followed by a gradual decay. The criteria applied by Nastuk and Hodgkin (1950) to frog sartorius fibres and by Kuffler and Vaughan Williams (1953) to the slow skeletal muscle fibres of the frog, viz. (a) sudden entry of the electrode, and (b) maintenance of the potential level, were not satisfied by these early 'intracellular' recordings. It is likely that these recordings of membrane potential may in fact have been extracellular and due to 'sealing' of the membrane round the relatively large electrode tip. Occlusion or bending of the electrode tip would account for the instability of the potential and for the fact that early high values (e.g. 87 mV, Bulbring and Hooton, 1953) could not be obtained after the application of the Matthews' oil-operated micromanipulator (Matthews, 1952) giving more controlled movement. The use

of microelectrodes of high resistance (35-70 meg ohms, Holman, 1958) to minimise cell injury led to the recording of steady potentials averaging 51.5 mV, showing a sudden fall as the electrode was presumed to penetrate the cell. Superimposed on the potential level were spontaneous spikes at a frequency of about 1/sec, ranging from 50 to 70 mV in amplitude and showing occasional reversal. Comparison of Holman's records with those of Bülbring (1957), who observed spikes of 1 to 8 mV depolarization using electrodes of lower resistance, indicates the difficulties involved in the interpretation of these early records.

Thus the use of high-resistance microelectrodes and the development of methods of fine control of their entry into the tissue made possible penetration of the small smooth muscle cells.

In an attempt to circumvent the difficulty of size, Woodbury and McIntyre (1954) used the hypertrophied cells of pregnant uteri. According to Stieve (1929) the diameters of the myometrial cell in the human and rabbit are: nonpregnant 2.5 to 5  $\mu$  and 3.4 to 4  $\mu$  respectively; pregnant 5 to 10  $\mu$  and 4 to 8  $\mu$  respectively. Potentials of 20 to 40 mV were obtained, characterized by an initial abrupt deflection as the electrode entered the cell, and being fairly well maintained. Similar observations were made by Greven (1955). Spontaneous action potentials were observed occasionally, and found to be only a few millivolts in amplitude. However, the introduction of the flexibly-mounted electrode (Woodbury and Brady, 1956) marked a great advance in technique. The flexible mounting allowed the electrode to ride easily with the tissue as it moved, so that the electrode could remain in the

cell for several minutes despite large movements. This technique had also the very considerable advantage of eliminating the necessity of excising and immobilizing the tissue. Using this technique, Woodbury and McIntyre (1956) recorded action potentials of the same order of magnitude as the corresponding membrane potentials with occasional overshoot. Absolute values of resting potential were still low and variable when compared with those obtained from skeletal muscle.

Electrophysiological studies on the uterus were also complicated by the regulatory effects of the ovarian hormones which determine its functional state. Use of stored tissue and lack of accurate knowledge of its physiological condition made interpretation of results difficult. Recently, however, membrane potentials have been measured with improved techniques under carefully defined endocrine conditions by Jung, (1958); Thiersch, Landa and West (1959); Goto and Csapo (1959); Marshall (1959), and Kuriyama (1961).

#### General Discussion

Measurement of membrane potential provides a starting-point for a consideration of whether electrical properties of smooth muscle are qualitatively similar to those of other excitable tissues, i. e. whether they may be predicted on the basis of the ionic hypothesis. A direct correlation between resting potential and the ratio of intracellular to extracellular potassium concentration is well and accurately established for a great variety of excitable tissues. However, many difficulties are involved in the consideration of any such relationship for

smooth muscle.

In the first place, all observers report low membrane potentials with a large scatter of individual values in smooth muscles. It is, therefore, important to establish whether this low membrane potential is a genuine property of the smooth muscle cells or an artefact due to the damaging effect of the penetrating microelectrode on the small cell, or to the difficulty of immobilization. In many of the earlier recordings, e.g. Bulbring and Hooton (1953), 'penetration' was not abrupt and potentials fell away from an initial high value, indicating damage of the cell membrane. However, in more recent work with flexible electrodes, e.g. Kuriyama (1963), on taenia coli, and Goto and Csapo (1959), Kuriyama and Csapo (1961) on uterine muscle, microelectrodes have been successfully kept inside cells for several minutes, and stable membrane potentials recorded despite considerable mechanical activity induced by trains of action potentials. Despite this stability during prolonged recording, measured values were still lower than predicted values.

The fact of stability, however, merely indicates that any injury caused by the penetrating electrode does not increase with time. Initial injury may have reduced the membrane potential, and if so the true value may be higher than that recorded. Considerable uncertainty remains therefore as to the absolute value of the resting potential. While the sucrose gap technique as used by Bulbring and her colleagues (Bulbring, Burnstock and Holman, 1958) has confirmed microelectrode observations on relative changes occurring in membrane potential of taenia coli, it does not give absolute values.



However, the fact that with improved techniques the smooth muscle membrane potentials were seen to be abruptly established and well maintained, and yet low and variable, indicates that this scatter is not merely a symptom of injury. A major difference between smooth muscles such as taenia coli and myometrium and striated muscles such as frog sartorius is that the former are capable of exhibiting spontaneous activity. The wide range of potentials recorded from such muscles could mean that the cells are not synchronously in the same functional state. If this is so it may be that 'resting' potential does not exist in the same sense as it does in frog sartorius. Thus Csapo (1956) considered that the fact that membrane potentials of about 60 mV with a scatter of approximately 20 mV were recorded in smooth muscles indicated functional inhomogeneity. Spontaneously active tissues in general have been found to have a lower membrane potential than striated and cardiac muscle, e.g. cardiac pacemaker 55 mV (Hutter and Trautwein, 1956; West, 1955).

In the uterus, measured values of membrane potential are consistently lower than theoretical values derived using the Nernst equation for the potassium equilibrium potential. Thus the calculated membrane potential of the oestrogen-dominated uterus, computed from Horvath's data on the potassium concentration outside and inside the myometrial cell, is about 82 mV, whereas the measured value is about 43 to 48 mV (Goto and Csapo, 1958, 1959) or 55 mV (Marshall, 1959), and even under progesterone domination when the membrane potential is some 10 mV greater and values up to 70 mV have been recorded, the discrepancy remains.

Daniel and Singh (1958) recorded membrane potentials of 40 to 60 mV from cat uterus, and calculated, using the Nernst equation and a conservative estimate of the ratio of internal to external potassium (22 to 24), potentials of 80 to 82 mV. The observation by Woodbury and McIntyre (1954) that uterine muscle inactivated by prolonged cooling had potentials near the potassium equilibrium potential, suggests that the low values found in more normal conditions are the potentials rather of active membranes, not membranes in the resting state.

Decreasing the conductivity of the external medium by replacing sodium chloride with sucrose did not noticeably increase the potentials recorded (Daniel and Singh, 1958), and low values have been recorded 'in vivo' as well as 'in vitro' in an artificial medium. Tip potentials (Adrian, 1956) do not explain the low values, because no considerably larger than average membrane potentials (presumably due to absence of a tip potential) were ever recorded and there was rarely any evidence of a shift of the junction potential between electrode and medium on breaking the electrode.

For all these reasons, therefore, it appears that a low membrane potential is a genuine property of smooth muscles, although the possibility of injury occurring on penetration, causing a reduction of the true potential, has not yet been excluded. Moreover, methods for determination of potassium ratios and hence of potassium equilibrium potentials are open to criticism. Determinations of extracellular space by Goodford and Hermansen (1961) on guinea-pig taenia coli yielded variable results. The histological estimate was the lowest (20%), and the

polyglucose space only slightly larger. On the other hand, inulin space was appreciably larger (33%), suggesting that this smaller molecule could penetrate spaces closed to polyglucose and too small to be observed under the electron microscope. Lithium space was larger again (40%). Uncertainty still remains as to what exactly is being measured by these various methods.

Methods used for the measurement of intracellular ion concentrations are open to very severe criticism, as several assumptions as to the distribution of the cations in the intracellular volume must be made. Thus most calculations have been based on the assumption that the ions are dissolved in the entire volume. It may be assumed alternatively that they are dissolved in the intracellular water, but it appears unlikely that no cations are associated with the dry matter of the muscle. Moreover, the possibility of the existence of more than one intracellular compartment cannot be ignored. Studies of the exchange of sodium and potassium between the taenia and a bathing fluid containing tracer isotopes was carried out, and  $^{24}\text{Na}$  exchange found to be extremely rapid, while  $^{42}\text{K}$  exchange took place at two different rates (Goodford and Hermansen, 1961). It may be that the nucleus contained the potassium which exchanged slowly, while the remaining potassium and the sodium were mainly in the cytoplasm. The cells of the taenia contain a large nucleus (and other intracellular particles) and so readily satisfy the histological requirements for a series system in which the nucleus exchanges potassium with the cytoplasm and the cytoplasm exchanges with the extracellular space. The rate of uptake of tracer sodium, on the other hand, is consistent with an uptake determined by diffusive processes

alone.

It thus appears that with present techniques 'prediction' of a 'resting potential' from observation of cation movements is open to much criticism. Apart from this, a study of the history of the subject shows that with gradually improving techniques the recorded values of membrane potential have come steadily closer to the predicted value.

However, the fact that the discrepancy between the measured and calculated values of the membrane potential can be reduced under certain conditions involving the loss of spontaneous movement indicates that the low values obtained from tissues in their normal state may indeed be genuine and due to the fact that they are not in a stable resting state. Thus, if the membrane were usually in a steady active state of high sodium permeability rather than in a resting state of low sodium permeability, then the membrane potential would be low. If this were so, then the difference between smooth and striated muscle would be a quantitative one in the normal state, the latter being truly resting, and the former more active. Experimental observations by Holman and later by Kuriyama give support to this concept.

The investigations of Holman (1958) on the guinea-pig taenia coli gave mean values of membrane potential of 51.5 mV during spontaneous activity, similar to that reported by Bülbbring (1954) and observed in other spontaneously active tissues (e.g. cardiac pacemaker 55 mV, Hutter and Trautwein, 1956; West, 1955). In the absence of spike discharge, however, the resting potential was about 70 mV, similar to that of non-spontaneously active smooth muscles, e.g. sphincter pupillae (Bülbbring and

Hooton, 1954) and salamander stomach (Greven, 1953), and close to the potassium equilibrium potential (the transmembrane potassium concentration ratio for guinea-pig taenia coli was measured by Goodford and Hermansen (1961) as 17 to 20, the intracellular potassium concentration being 119 mM, giving a calculated potassium equilibrium potential of 74 to 78 mV). These observations are in line with the theory of a 'resting' membrane with a high sodium permeability.

Holman then studied the effect of varying concentrations of potassium chloride in the bathing medium on the membrane potential of the cells of the taenia coli. The relation between membrane potential and the logarithm of the external potassium concentration differed markedly from that found for striated muscle. In smooth muscle, isotonic potassium chloride produced a depolarization of 30 mV, the maximum slope being 32 mV per tenfold change potassium concentration (Holman, 1957) or 38 mV (Kuriyama, 1963). In frog striated muscle, however, Ling and Gerard (1950) found that isotonic potassium chloride produced an 80 mV depolarization and that a tenfold change of potassium concentration resulted in a potential fall of 44 mV. Burgen and Terroux (1953) working on heart muscle recorded a 50 mV depolarization with isotonic potassium chloride and a 38 mV slope per tenfold change. If the membrane potential were only governed by the distribution of potassium ions across the membrane, a tenfold change in the external potassium concentration should give a 58 mV change in potential. The deviation of the measured membrane potential change from this theoretical value again indicates that the membrane is permeable to ions other than

potassium, e.g. sodium and chloride (Hodgkin and Katz, 1949; Adrian, 1956). However, the general criticism that alteration of the ionic composition of the bathing fluid may not be reflected by exactly corresponding alteration of the composition of the 'extracellular' fluid round the smooth muscle cells can be applied to all analyses.

Further investigations into the effect of varying potassium and other ionic concentrations were carried out by Bülbring and Kuriyama (1963), and Holman's observations confirmed. The fact that spike generation ceases when the membrane potential rises to 65 to 70 mV had previously been interpreted as indicating that the hyperpolarized quiescent membrane might correspond to the resting membrane of other tissues, and so an attempt was made to determine the relation between the membrane potential and the external potassium ( $K_0$ ) concentration when the tissue was kept quiescent by adding adrenaline. It was found that the slope of the line became steeper and slightly closer to the theoretical slope.

Previous experimental results having indicated that sodium permeability is probably much faster in taenia coli than in skeletal muscle, further experiments were carried out to compare the behaviour of the two tissues. In skeletal muscle Hodgkin and Horowicz (1959) observed the relation between membrane potential of frog skeletal muscle fibres and the logarithm of the  $K_0$  concentration in chloride-free solution, and found that whereas when  $K_0$  was greater than 10 mM the potential was close to that of a potassium electrode, in the range 1 to 10 mM  $K_0$  the membrane potential was affected by the sodium permeability constant, deviating from the Nernst equation in the manner expected from a

slight permeability to sodium. In taenia coli, Kuriyama (1963) found a similar effect up to 30 mM  $K_0$ . Moreover, in sodium-free solution the relation became more linear, and the maximum slope was increased to 44 mV per tenfold change. A similar effect was seen in excess-calcium solution, and when adrenaline was added. It may be that these both reduce sodium permeability.

The fact that chloride is also important in determining the membrane potential was shown by the effect of replacing chloride with foreign anions without changing sodium or potassium (Kuriyama, 1963). The membrane was depolarized suddenly and then reset to a new equilibrium level by such a substitution. The degree of transient depolarization was seen to be in the order sulphate bromide nitrate iodide, which fits the lyotropic series of anions better than the series based on ionic motilities. A similar phenomenon is seen in cardiac muscle and mammalian erythrocytes, but the order is reversed in skeletal muscle. It may be that in smooth muscle chloride is not merely passively distributed across the membrane as it is in skeletal muscle.



ACTION POTENTIALS

Development of the concept of a relatively high sodium permeability being a characteristic of the smooth muscle membrane led to a study of action potentials. The question of whether smooth muscle was capable of giving 'all-or-nothing' responses as in the case of skeletal muscle was disputed for some time. Although propagated action potentials were described in many smooth muscles, e.g. retractor penis of dog (Brücke, 1910), ureter (Orbeli and Brücke, 1910), rabbit oesophagus (Brücke and Inouye, 1912), the observations of Rosenblueth, Davis and Rempel (1936) and Rosenblueth (1936) suggested that smooth muscle was electrically inexcitable and incapable of conducting all-or-nothing impulses. Their experimental evidence was criticised by Eccles (1936) on the grounds that Eccles and Magladery (1937), using nictitating membrane, found that its responses to all combinations of nerve volleys and to the action of adrenaline could be explained in terms of all-or-nothing responses of individual units. Thus, once again, early statement of a major qualitative difference between smooth muscle and other excitable tissue was invalidated by improvement in experimental approach and technique.

In a few years of intracellular recording, improvement of techniques changed the picture considerably from that of spikes a few millivolts in amplitude (Bulbring, 1954) to spikes with an overshoot often exceeding 15 mV (Holman, 1958). Holman found that these spikes were abolished in sodium-free solution, in accordance with the ionic hypothesis of Hodgkin and Huxley (1952). However, spikes of normal amplitude were recorded in 20 mM Na, and this

was at first taken as evidence that the spike mechanism in smooth muscle was basically different from that in most excitable tissues. The idea that the membrane of the taenia might have only a limited number of sodium carriers was put forward by Holman (1958), and this was consistent with the observation that the rate of rise of normal spikes was relatively slow. However, as pointed out by Bülbring and Kuriyama (1963), if the sodium permeability is in fact high in the resting state in smooth muscle, any change in extracellular sodium will be followed by a corresponding change in intracellular sodium. Thus the ratio of internal to external sodium concentrations will not change, and so, by the Nernst equation, the overshoot should not change. The above observations therefore are not incompatible with the ionic theory, as was at first thought, granted an initially high sodium permeability in the spontaneously active cell membrane.

Action potentials recorded from the uterus (Kuriyama, 1961) rarely showed any overshoot, although a theoretical overshoot of 13 mV would be predicted from measurements of internal and external Na concentrations (Daniel, 1958). This discrepancy was considered by Kuriyama (1961) to be due possibly to the effect of hormones on the membrane structure, changing its permeability to sodium and chloride ions.

LOCAL POTENTIALS

Study of the configuration of the spike showed that, in general, it is preceded by a prepotential of very variable size. This slow prepotential closely resembled an ephaptic or junctional potential (Búlbring, Burnstock and Holman, 1958; Goto and Woodbury, 1958; Thiersch, Landa and West, 1959; Goto, Kuriyama and Abe, 1960; Burnstock and Prosser, 1960), and was found to be apparently specific to the muscle-muscle junction. The question of the origin of this slow potential remains unsolved, although it has been much studied.

Separation of the two components of the action potential - slow potential and spike - was observed when extracellular electrical stimulation was applied to an area remote from the recording electrode (uterus - Goto, Kuriyama and Abe, 1961). In this situation, gradual strengthening of the stimulus caused first the appearance of a slow potential, and next a spike potential when threshold level was exceeded. This suggests that the slow potential is not an artefact due to movement of the electrode or to injury, but a true prepotential, as does the fact that the prepotential seen in spontaneous activity in taenia coli preparations is affected in different directions by calcium and magnesium - excess magnesium increasing its size and excess calcium decreasing it.

The fact that it is unaffected by atropine indicates that it is not a junctional potential due to acetylcholine, and as adrenaline and the sympathetic supply to the intestine are inhibitory the prepotential can not be an adrenergic junction potential. Moreover, these prepotentials are quite unlike the junction potentials of

Burnstock and Holman (1961, 1962), again suggesting that they are not of a chemical nature.

The observations of Bozler (1938) are relevant to this problem. He concluded that conduction of excitation in ureter, uterus and intestine was muscular and occurred independently of nervous elements. As both the motor nerves to the uterus and the sympathetic supply to the intestine are inhibitory and there is no excitatory nerve supply to the uterus, it appears that the automaticity of these tissues resides either in the muscle fibres themselves or in the nerve plexuses in their walls. That the prepotential is not due to electrotonic spread of excitation from an adjacent cell is indicated by the fact that it is affected by changes in ionic composition which have no effect on the action potentials themselves, e.g. changes in calcium and magnesium. Thus the slow potential might represent an excitation transmission process. If it is a characteristic of a pacemaker, every smooth muscle cell appears to have this quality potentially (Bülbring, 1957; Sperelakis and Prosser, 1959; Daniel and Singh, 1958; Kuriyama and Csapo, 1961).

The finding by Bülbring and Kuriyama (1963) that the local potential is closely related to the external sodium concentration, showing increased amplitude and duration in the presence of excess, and disappearing in absence of sodium, suggests the possibility that it may represent an intrinsic sodium-dependent mechanism on which the automaticity of the smooth muscle depends. However, whether the origin of this is electrical or chemical still remains uncertain, although the observations described above suggest that it is related to some electrical transmission process.

EFFECT OF CALCIUM ON MEMBRANE ACTIVITY

In order to elucidate further the mechanism of spike generation in smooth muscle, the effect of varying calcium concentrations was studied (Holman, 1958; Bülbring and Kuriyama, 1963) and compared with similar observations on other excitable tissues (Frankenhaeuser and Hodgkin, 1957). Kuriyama varied the calcium concentration from 0.25 mM to 15 mM and found that membrane potential was increased by excess calcium and decreased by calcium deficiency, while action potentials showed a change in amplitude corresponding to the membrane potential change. In general, excess calcium increased the spike threshold and thus decreased spike frequency.

Bülbring and Kuriyama found that the local potential was suppressed by excess calcium, which appeared to inhibit the sodium current. The shape of the spike thus came to resemble more closely that of skeletal muscle. This effect of calcium on the subthreshold change of sodium permeability could be explained on the basis that there are in the smooth muscle membrane sodium channels unoccupied by calcium at normal concentrations (Frankenhaeuser and Hodgkin, 1957), and any increase in calcium concentration would increase the number of sodium channels occupied by calcium (and thus decrease the sodium permeability of the 'resting' membrane so increasing the membrane potential) and so more calcium would need to be removed from the membrane to expose the number of sites required for the sodium current to exceed the potassium current. Thus the threshold for setting up of the spike would be increased, but once activity had been

sufficient to reach this level the regenerative depolarization of the spike would release the sodium channels occupied by the excess calcium and made available by its removal during depolarization. Thus both the amplitude and rate of rise of the spike would be increased. Skeletal muscle, cardiac muscle, and vertebrate nerve fibres exposed to excess calcium do not, however, show an increase in rising phase and maximum rate of rise of spike, presumably because all the sodium channels are occupied by calcium at normal concentrations. Thus if such membranes were previously depolarized by voltage clamp or by low calcium in the resting state, the effect of excess calcium would presumably be the same as it is in the taenia, and thus the differences between these different tissues would be merely quantitative.

In general, it is seen that changes in calcium concentration and changes in membrane potential have similar effects on the systems which allow sodium and potassium to move through the membrane during the spike in smooth muscle as in other excitable tissues. It has been suggested (Frankenhaeuser and Hodgkin, 1957) that the increase in permeability underlying electrical activity occurs because depolarization removes calcium ions from sites in the membrane, possibly 'sodium channels'. Thus nerve is more excitable in low calcium because sodium conductance is affected such that a smaller depolarization is required to increase the sodium conductance to a given size, sodium inactivation is reduced, and the delay occurring before a rise in potassium conductance is shortened. In smooth muscle, excess calcium has been found to inhibit sodium current, so that the threshold for excitation is increased and the local potential is

suppressed, indicating again a basic similarity between smooth muscle and other excitable tissues.

This work leads to the conclusion that the low membrane potential of smooth muscle and its instability might be due to a relatively high sodium permeability resulting from a poor calcium adsorption at the cell membrane.



EFFECTS OF TRANSMITTER SUBSTANCES

One of the major gaps in the understanding of the mechanism of control of the body by the autonomic nervous system is at the cellular effector level. Precise information as to the nature of the response of smooth muscle cells is thus required.

The concept of chemical transmission from nerve to muscle was apparently first put forward by Elliott (1904) who, on the basis of the similarities in action of adrenaline and sympathetic nerve activity (whether excitatory or inhibitory), suggested the possibility that the sympathetic nerve impulse brought about its effect by liberating adrenaline at the target cell. The idea was extended to the parasympathetic system by Dixon and Hamill (1909) who compared the action of parasympathetic nerves with that of muscarine, and the observations of Dale (1914) and Loewi (1921) indicated that the sympathetic transmitter was an adrenaline-like substance, while that of the parasympathetic system was acetylcholine. Cannon's review (1933) advanced strong evidence for an adrenaline-like substance, and the transmitter substance was later identified (in mammals) as noradrenaline (von Euler, 1946).

The investigations of Burnstock and Holman (1961) using the isolated hypogastric nerve-vas deferens preparation of the guinea-pig showed that the mechanism of the transmission of excitation from sympathetic nerve to smooth muscle does not differ fundamentally from transmission at other neuroeffector junctions, stimulation of the effector nerve producing depolarization of the post-junctional membrane. They found too (Burnstock and

Holman, 1961) that a spontaneous discharge of small potentials, similar to that seen in other innervated muscles, could be recorded from this preparation, and that stimulation of the hypogastric nerve gave rise to junction potentials which appeared to be compounded of many of the spontaneous potentials.

They also observed that both the rate of the small potential discharge and the amplitude of the junction potentials were reduced in denervated and reserpine-treated preparations. The noradrenaline content of sympathetically-innervated organs of reserpine-treated animals is reduced by about 90% (Muscholl and Vogt, 1958), and so the reduction by 90% of the rate of appearance of spontaneous small potentials and of the amplitude of the junction potentials treated in the same way indicates that a catechol amine such as noradrenaline is responsible for the appearance of these potentials.

An investigation into the effects of adrenaline and acetylcholine must therefore be carried out before the normal behaviour of smooth muscle can be understood.

### Acetylcholine

Acetylcholine has for long been implicated as the excitatory transmitter at the neuromuscular junction in skeletal muscle (Dale, Feldberg and Vogt, 1936; Brown, Dale and Feldberg, 1936; Brown, 1937) and as the inhibitory transmitter in the heart (Dale, 1937). Bülbbring (1955, 1957) observed that ACh had an excitatory effect on the guinea-pig taenia coli, causing depolarization of the membrane, increase in spike frequency, and prolongation of spike duration, chiefly by delay in repolarization, i.e., it caused changes

in the opposite direction to those in heart muscle (also spontaneously active) where inhibition and increase of membrane potential occur.

The problem of the mode of action of acetylcholine has been much studied both in heart and in skeletal muscle, but as yet similar techniques, e.g. ion injection, measurement of membrane resistance, have not, for technical reasons, been applied to smooth muscle cells.

Experiments of Howell and Duke on the heart (1908) indicated some mobilization of potassium ions under the influence of acetylcholine, and study of the excitatory action of acetylcholine on the neuromuscular junction led to the development of the concept that a change in ionic permeability occurred (Fatt and Katz, 1951). A similar hypothesis for the action of acetylcholine and related substances on cardiac muscle fibres - that they act by increasing permeability to potassium ions - was proposed by Burgen and Terroux (1953). Against the background of the ionic theory of electrical activity in nerve and muscle (Hodgkin, 1951) this hypothesis could account satisfactorily for all features observed during vagal stimulation or the application of acetylcholine to auricular and pacemaker fibres (Hoffman and Suckling, 1953; West et al., 1956; del Castillo and Katz, 1957; Hutter and Trautwein, 1956). The idea received further support from measurements of membrane resistance during the action of acetylcholine (Trautwein et al., 1956; Trautwein and Dudel, 1958).

Hutter (1961) and his associates, using radioactive isotopes to measure ion movements, investigated the effect of acetylcholine

on the heart, and found striking increase in the rate of influx and of efflux of potassium, but no significant changes in movements of  $^{36}\text{Cl}$  or  $^{82}\text{Br}$ . It was also known that the presence of small anions was not essential for the arrest of the heart by acetylcholine. It was, therefore, concluded that acetylcholine inhibits the heart by causing a great and specific increase in potassium permeability.

This mode of action of acetylcholine is different from that at the motor end-plate where it is excitatory. There, it appears to have a non-specific effect on the permeability of the cell membrane, tending to drive the membrane potential, irrespective of its initial level, towards the liquid junction potential of 10 to 20 mV, interior negative (del Castillo and Katz, 1954). Denervation is known to lead to expansion of the acetylcholine-sensitive area over the entire cell membrane (Axelsson and Thesleff, 1957; Thesleff, 1959, 1960), and again a non-specific increase in ionic permeability is indicated.

In the uterus, (Kuriyama, 1961), acetylcholine caused excitation -depolarization and sudden increase in spike frequency. High concentrations caused contraction followed by contracture (which was not accompanied by action potentials), which may be compared with the contracture caused by excess acetylcholine at the neuromuscular junction. Analysis of this effect has not yet been carried out.

Difficulties involved in the application of these various techniques to smooth muscle cells have not yet been overcome. Born and Bulbring (1956) observed an increase in the rates of potassium influx and efflux in taenia coli under the influence of

acetylcholine, and the fact that it depolarizes the cell membrane in sodium-free solution (indicating increase in potassium permeability) confirms this. The problem of whether this permeability change is a specific one (as in cardiac muscle) or a non-specific one still remains to be solved, although the observation that acetylcholine tends to reset the membrane potential to an equilibrium level of 15 to 25 mV, interior negative (Bülbring and Kuriyama, 1963 a) indicates that the change may be non-specific. Measurement of membrane resistance would be a direct way to show whether or not there was a simple 'short-circuiting' of the membrane as in the motor end-plate.

#### Adrenaline

The accelerating effect of sympathetic stimulation and of adrenaline on the heart has long been recognized, as has its stimulating action on certain smooth muscle, a.g. that of the vascular system, and the nictitating membrane. The fact that the smooth muscle of the gut is inhibited is also well known, and several theories have been put forward to account for these two opposing effects.

Cannon (1933, 1934) and Rosenblueth (1932, 1935, 1936) proposed that the transmitter substance must combine with a receptive substance in the effector cells to form a 'sympathin' before it could exert its effect. They also postulated the existence of two 'sympathins' to account for the dual effect, but Eccles (1936) in his review criticised the experimental evidence for this, finding that the transmitter substance is similar in all parts of the sympathetic system, and considering that the transmitter acts

directly on the muscle fibres.

Experiments on the heart (Hutter and Trautwein, 1955, 1956; West et al., 1956) showed that sympathetic acceleration was due to a faster depolarization of the membrane during diastole. This suggested the possibility that adrenaline might act exactly in the reverse way to acetylcholine, i. e. by decreasing the permeability to potassium. However, no evidence for this could be obtained in experiments using  $^{42}\text{K}$ .

It was also observed that while adrenaline normally caused contraction of nictitating membrane accompanied by a rhythmic impulse discharge, an excess set up a prolonged contracture of the muscle even though the rhythmic production of impulses has ceased. This effect is very similar to that of acetylcholine on the motor end-plate, where high concentrations produce contractures. This result suggests that, in the nictitating membrane, adrenaline may have a direct action on the membrane permeability properties, causing excitation.

The effect of adrenaline on the spontaneously active taenia coli is, on the other hand, known to be inhibitory, and Bulbring (1955, 1957) found that it caused hyperpolarization, decrease or disappearance of spikes, and reduction of their duration. It thus appears that, while adrenaline causes non-spontaneously active smooth muscles (e.g. nictitating membrane) to show rhythmic activity, in those situations tested it exerts a stabilizing effect on intestinal muscle which is normally spontaneously active. In the post-partum rat myometrium (Kuriyama, 1961) adrenaline induced slight hyper-polarization and decrease in spike frequency, i. e. it has an inhibitory effect on this spontaneously active muscle also.

An hypothesis to account for the dual action of adrenaline, developed by Bülbring, proposes that its inhibitory effect is due to a 'metabolic' action, while its excitatory effect is due to a direct action on membrane permeability.

The hyperpolarizing, stabilizing effect of adrenaline has been found to require the presence of sodium and of calcium in the external medium, but not of potassium. It thus appears that some effect on the electrogenic extrusion of sodium is involved, as evidence for an accelerated rate of active sodium extrusion in the presence of adrenaline has been obtained in work with radioactive tracers (Bülbring and Goodford, 1962). Also, simultaneous measurement of biochemical and biophysical changes occurring under the influence of adrenaline (Axelsson, Bueding and Bülbring, 1961) has shown increase in phosphorylase activity at the same time as the hyperpolarization. It has therefore been suggested that an increased supply of energy produced by adrenaline is utilized by processes in the cell membrane to stabilize the membrane. Adrenaline appears to affect chiefly the sodium conductance during the active and resting state of the membrane, modifying the movement of sodium across the membrane (Bülbring and Kuriyama, 1963 a).

In smooth muscles such as nictitating membrane which are, in the absence of nervous stimulation, quiescent, the membrane potential is presumably normally stable, and an increased supply of metabolic energy could not increase that stability. Adrenaline would, therefore, only exert a direct stimulating action, as would be expected of an excitatory transmitter. Analysis of the excitatory effect of adrenaline on the cell membrane has not yet

been reported, but it is presumed to have a permeability effect similar to that of acetylcholine at the neuromuscular junction.



EFFECTS OF HORMONES

Recently, much work has been done on the smooth muscle of the uterus, studying its electrical properties at the cellular level under carefully defined endocrine conditions. This pioneer work into the question of how cellular functions are regulated by hormones is still in its very early stages, but it appears that the changes in excitability, spontaneous activity, pharmacological reactivity, and mechanical response occurring in the uterus following hormone treatment may be accounted for by the effects of the various hormones on the membrane and action potentials of the cells.

Thus, Jung (1960) observed that oestrogenic substances induced hyperpolarization and appearance of spontaneous activity in the immature rat uterus (which apparently has a membrane potential below the threshold value for excitability), and Goto and Csapo (1959) found that the membrane potential of the rabbit uterus was increased by oestrogens and still further increased by the simultaneous administration of progesterone.

Kuriyama and Csapo (1961a) found that treatment with progesterone 'in vivo' increased the threshold and stability of the membrane. They observed that block of propagation preceded abolition of spontaneous activity, and that hyperpolarization of the membrane developed gradually thereafter. 'In vitro' progesterone treatment suppressed both spontaneous and oxytocin-induced activity, and the membrane became slightly hyperpolarized. This explained the observation of Csapo (1956a) that 'in vitro' progesterone treatment reduces or suspends the excitability and

mechanical activity of the uterus.

The various observations by Goto and Csapo (1959), Marshall (1959), Kuriyama and Csapo (1961a), Marshall and Csapo (1961), and Kuriyama (1961a) provide some basis for an understanding of the action of progesterone on the cellular level. The main role of progesterone in the uterus is believed to be that of maintaining pregnancy until delivery (Csapo, 1959). Experimental observations indicate that it may do so by increasing the stability of the myometrial membrane, thus increasing the threshold to stimulus, causing hyperpolarization, blocking propagation, and reducing sensitivity to circulating compounds.

The mechanism of action of oxytocin has been investigated using electrophysiological techniques by Goto (1960) and Kuriyama (1961a). In general, it has been found that administration of oxytocin leads to an increase in activity, the frequency and number of impulses discharged being increased. The effective concentration is determined by the endocrine condition of the uterus (Csapo, 1959), and microelectrode studies showed that the uterus of the rat was most sensitive on the last day of gestation and post-partum. These observations are in line with the fact that progesterone exerts a blocking action on the propagated activity of the uterus, and that this progesterone block must be withdrawn before the uterus can respond to oxytocin. Electrophysiological studies have thus provided some explanation for the observed hormone effects on the uterus at the cellular level, although the mechanisms of their action still remain unknown.

VASCULAR SMOOTH MUSCLE

As the foregoing review shows, the study of various smooth muscles, particularly those of the intestine and uterus, using electrophysiological techniques has added greatly to the understanding of basic contractile processes. However, as recently as 1963 at a Symposium on Vascular Smooth Muscle was it stated that 'concepts concerning the contractile process in vascular smooth muscle must, for the most part, be borrowed by wishful analogy from more easily studied tissues'. The last few years have seen considerable developments in the study of membrane phenomena in this muscle.

Studies of the electrical activity of the smooth muscle of the vascular system were first reported by Funaki (1958, 1960, 1961) who investigated the blood vessels of the tongue and skin of the lateral abdomen of the frog.

Resting membrane potentials with a mean value of 40.5 mV were recorded in the muscle of the tongue blood vessel walls, i. e. much smaller than membrane potentials recorded from skeletal muscle. The membrane potential of cutaneous vascular smooth muscle was even lower, averaging about 25 mV. In most cases levels of the membrane potential were stable for a long time, and only very rarely were any fluctuations observed.

Single action potentials were recorded from tongue vessels in response to single induction shocks. These were about 60 mV in amplitude and 200 msec in duration, although some recorded from the region of the bifurcation of small vessels were only 50 msec long. They tended to be simple in shape, similar to

skeletal muscle action potentials, but some were 'complex' with variable 'humps'. Usually they did not follow the 'all-or-none' law, but varied with the strength of the stimulus and hence may not be conventional action potentials.

Records of spontaneous activity were obtained only from the cutaneous vessels. These action potentials had an overshoot of about 10 mV and a time-course of about 200 msec, and appeared to consist of an initial slow potential and a large simple spike. The slow potential was rather similar to the prepotential of other smooth muscles (taenia coli, uterus, etc.).

In view of the initial slow potential, it was considered that the action potential recorded was probably due to conducted excitation. This type of action potential had a quite different shape from that evoked by a single electrical stimulus presumed to be applied to the same muscle fibre, and whereas the amplitude of the induced action potentials varied with the stimulus strength, the spontaneous spikes appeared to be all of the same size.

Membrane potentials have also been recorded from muscle cells of isolated segments of the aorta and inferior vena cava of the turtle (Roddie, 1962). Potentials of about 40 mV were recorded, and action potentials were seen in association with rhythmical contractions of the segments. In the aorta, the action potential consisted of a spike of depolarization followed by a plateau of repolarization lasting 5 to 40 secs. In the veins repolarization was much more rapid.

Little investigation into the effects of chemicals and hormones on vascular smooth muscle has as yet been carried out. Funaki (1958) found that acetylcholine caused a positive after-

potential in electrically induced spikes in the vessels of the frog's tongue, and that larger amounts abolished the spike discharge - i.e. acetylcholine appears to have an inhibitory action on this smooth muscle as it has on the heart, rather than an excitatory effect similar to that on spontaneously active smooth muscle. On the other hand, Roddie (1962) found that both adrenaline and acetylcholine increased the frequency of spontaneous action potentials in the turtle aorta.

Whilst comparative work provides useful information for an understanding of the basic mechanisms of activity, of more immediate interest to the physiologist is a knowledge of the behaviour of mammalian tissues in their normal environment, influenced by the normal direct and reflex control mechanisms. For these reasons, this investigation was undertaken in an attempt to examine membrane phenomena in the smooth muscle which forms a large part of the walls of mammalian small arteries and arterioles, and determines the peripheral resistance to blood flow. The small vessels in the mesenteric circulation in the region of the small intestine were found easily accessible and surrounded by relatively little connective tissue, and the studies of Chambers and Zweifach (1944) showed that these vessels are highly responsive to various stimulating agents. The experimental conditions were as near physiological as possible in an anaesthetized animal, circulation and innervation being intact and surgical interference reduced to a minimum.

The effect of acetylcholine and of catechol amines was studied, as an understanding of this is necessary for an understanding of normal function, as the regulation of blood pressure and formation

of interstitial fluid depend to a great extent on the control of arteriolar (and venular) smooth muscle by the autonomic nervous system. The sympathetic system is particularly involved in the control of the tone of this smooth muscle, but as yet the mechanism of this control is unknown.

PART 2: METHODS

METHODSEXPERIMENTAL MATERIAL

Male and female rats, age 3 to 6 months and weight 150 to 250 g were anaesthetized with intraperitoneal sodium penotobarbital (Nembutal), 5 mg/100 g body weight. Additional anaesthetic, if required after opening the abdominal cavity, was administered intramuscularly.

The trachea was cannulated and a small midline incision made in the abdominal wall, the animal placed on its side and a loop of small intestine drawn out. The mesentery was spread over the floor of a 'Perspex' box and the gut fitted into a horseshoe-shaped groove. A lid with a corresponding groove was placed on top, and Krebs solution dripped on to the tissues through polythene tubing inserted into the lid at a rate (adjustable) sufficient to maintain the temperature at 35 to 39°C. Electrodes, both recording and for drug application, were inserted through a hole in the lid. When required, the splanchnic nerves and adrenal vein were exposed through incisions made about 1 cm lateral to the dorsal mid-line and extending downwards from the lower ribs. A silver earth plate was attached to the floor of the 'Perspex' box immediately in contact with the mesentery.

This whole system - animal and box containing gut loop - was mounted on a table placed on the platform of a Zeiss sliding micromanipulator. Manipulation of electrodes was carried out under binocular microscopic control (Zeiss Stereomicroscope I), magnification being variable from 6 to 80 times.



SOLUTIONS AND DRUGS

The tissues were kept warm and moist with Krebs solution, the temperature being kept within the range 35 to 39°C. The composition of the Krebs solution (mM) was; Na 137.4, K 5.9, Mg 1.2, Ca 2.5, Cl 134,  $H_2PO_4$  1.2,  $HCO_3$  15.5. Sodium-deficient solutions were prepared by replacing the sodium with lithium. Excess potassium solutions were prepared by adding solid potassium chloride. Gelatin (10 g/l) was added to the solution to prevent oedema and petechial haemorrhages (Chambers and Zweifach, 1944).

Drugs for intravenous injection were diluted to the appropriate concentrations with 0.9% (w/v) saline, and injected into the tail vein of the rat. Drugs for topical application were diluted with the Krebs fluid and dropped on to the preparation through a glass microelectrode with a broken off tip. This electrode was mounted on the Zeiss sliding manipulator, thus allowing the drug to be applied to a very small area of blood vessel close to the recording electrode. Drugs were applied a few drops at a time, and allowed to be washed away by the Krebs solution which was dripped on at a very slow constant rate. It was found extremely difficult to keep an electrode inside a cell for the prolonged period of time required for these drug studies, especially when stimulating drugs were applied, and for this reason extracellular recording methods were used in most experiments. Thus relative changes in membrane potential were observed, but absolute values could not be measured.

The chemicals used were adrenaline B.P. (Burroughs

Wellcome & Co.), L-noradrenaline bitartrate (L. Light & Co.), acetylcholine chloride (Roche), tetraethylammonium chloride (TEA - Parke, Davis & Co.), dihydroergotamine methanesulphonate (DHE 45 - Sandoz), and synthetic oxytocin (Syntocinon- Sandoz), vasopressin B.P. (Pitressin - Parke, Davis & Co.). Stock solutions were made up the day before use and diluted as required. Only one chemical was applied to a single preparation to avoid the possibility of a response being modified by a change in sensitivity induced by the previous administration of another agent, and where different concentrations of a chemical were used, a period of several hours was allowed to elapse between successive administrations. Solid adrenaline was dissolved in normal saline and equal weights of ascorbic acid (BDH) added to prevent oxidation.

ELECTROPHYSIOLOGICAL APPARATUSMicroelectrodes:

Glass microelectrodes of the type introduced by Ling and Gerard (1949) were used, and in order to record from moving tissues were mounted flexibly (Woodbury and Brady, 1956).

Preparation

Borosilicate glass tubing ('Pyrex'), outside diameter approximately 2 mm and inside 1 mm, was used. 40 to 50 mm lengths were pulled out to about 70 mm by hand in the flame of a micro-bunsen, and mounted vertically in the pulling machine so that the drawn-out region passed through the heating coil.

The electrode pulling machine (circuit diagram in Figure 1) was based on the design of Alexander and Nastuk (1953). The pulling force was supplied by a solenoid and plunger arrangement, and the glass tube was clamped rigidly and passed through a nickel chrome heating loop *H* of 4.5 mm diameter. Power was supplied from a 24 V D.C. supply to the Solenoid *Sol*, and from the mains through a transformer and rheostat *Rh* to the heater (2 to 3 V).

At first, while the glass was being heated, the solenoid exerted a small force due to the small current from the 24 V power supply passing through the series resistor *R* plus the winding resistance of the solenoid. As the glass softened it extended and broke the contacts *C*, so releasing a relay *Rly* which in turn a) released the switch *S2* switching off the heater current, and b) made the switch *S1*, short-circuiting *R* and thus increasing the current through the solenoid. The glass was pulled out rapidly

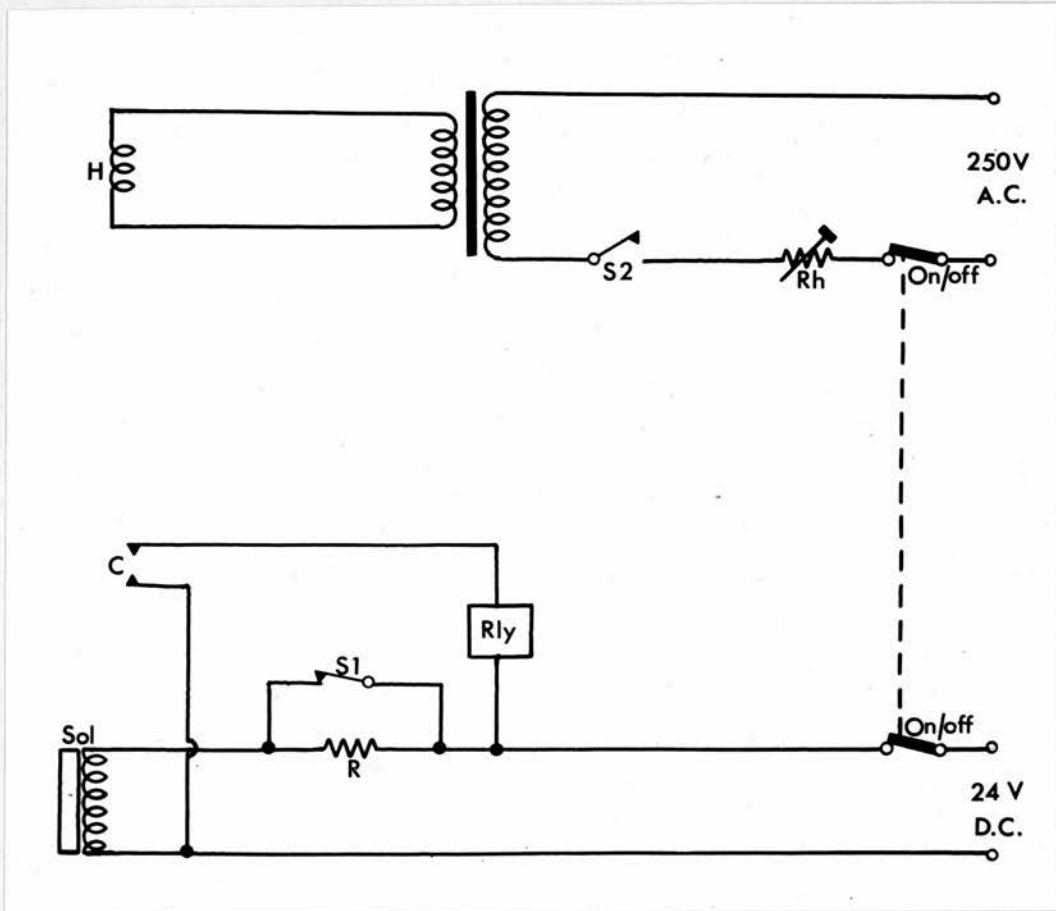


Fig. 1. Circuit diagram of electrode pulling machine.  
 H, heating coil; Sol, solenoid; Rly, relay operating  
 switches S1 and S2; R, series resistance; Rh, rheostat.

by the increased solenoid force, which was maintained until the supplies were disconnected. This continuing solenoid pull together with the presence of a rubber collar round the plunger helped to prevent breakage of the electrode tips by vibration. This machine allowed variation of the heater current (by the rheostat Rh) and also of the point at which the contacts C were broken.

Immediately after pulling, the electrodes were placed in methyl alcohol and examined under 40 times magnification. Those electrodes in which the alcohol penetrated up the stem from the tip by more than  $100\mu$  were rejected.

Method of filling:

The selected electrodes were mounted, tips downwards, round the perimeter of a 'Perspex' cylinder with its base rounded off to prevent formation of large bubbles on its surface during boiling, and the whole placed in a flask of methyl alcohol. The pressure was reduced to approximately 100 mm Hg or until the alcohol boiled gently, and boiling continued until the electrodes were filled. They could then be stored in methyl alcohol for up to eight weeks.

Three or four days before required, the electrodes were placed in distilled water for a few minutes and then transferred to concentrated potassium chloride solution (3M) and left to fill by diffusion. Once in the potassium chloride they could not be kept for longer than 3 to 4 weeks as the tips tended to break and deposits appeared. Before use, the electrodes were washed with distilled water to remove all external potassium chloride.

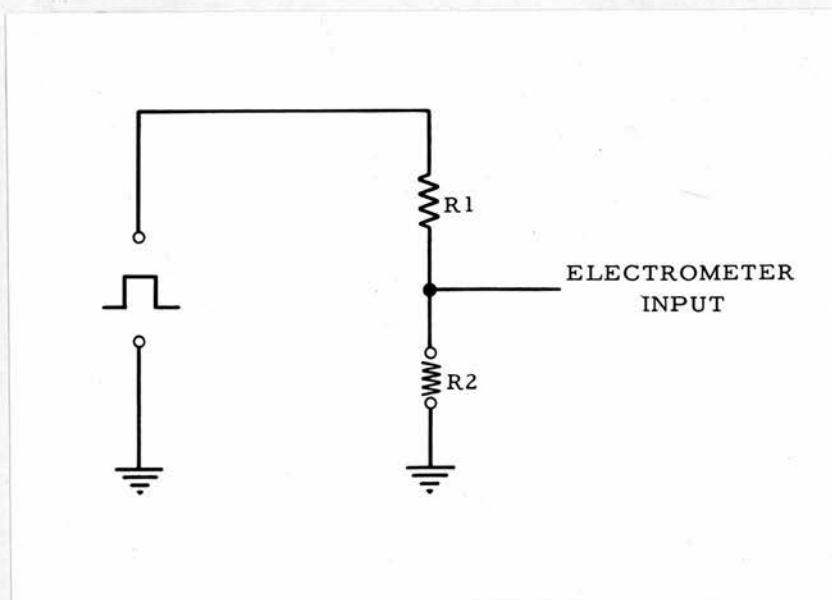
Mounting the microelectrodes:

Recordings were made with flexibly-mounted electrodes (Woodbury and Brady, 1956). The two-stage method of pulling enabled the last few mm at the tip of the electrodes to be broken off without destroying the tips. This tip plus the last few mm of shaft was suspended from fine silver wire,  $50\mu$  diameter and insulated with Diamel, and connected through a short length of coaxial cable to the grid of the electrometer valve of the Bak Wide Band Electrometer. As the stray capacitance to earth of the input lead would act as a high-frequency shunt, the lead was kept short and screened, and the screening connected to the cathode of the electrometer valve. This increased the high-frequency response, and shielded the input lead from interference. The coaxial cable was held in a clamp mounted rigidly on one platform of the Zeiss micromanipulator.

Measuring the electrode resistance:

The system used is shown in Figure 2. The Bak Wide Band Electrometer was designed to allow a pulse to be applied through a series impedance (R 1) to the grid of the electrometer valve. A 50 V pulse (from a Tektronix Type 161 Pulse Generator) was applied and the size of the output pulse, i. e. deflection on the oscilloscope screen, noted. Known resistances were inserted between the grid and earth (R 2) to allow calibration of the output pulse size. The electrode to be tested was then mounted in this position (R 2) and its resistance thus measured directly.





**Fig. 2.** Diagram of the method used for measuring the resistance of the microelectrodes.  $R_1$ , series impedance;  $R_2$ , microelectrode or calibrating resistance.

The Recording System:

Figure 3 is a block diagram of the stimulating and recording systems. The signal from the microelectrode was fed through a short insulated lead to the electrometer valve of the wide band electrometer. The electrometer functioned primarily as a cathode-follower, converting the high impedance input from the electrode to a low impedance output, with unity gain, which was then fed directly to a conventional high-gain amplifier. The electrometer valve was mounted close to the preparation so that interference could be minimized by using a very short input lead. Provision was made for measurement of grid current (by switching a  $10^9 \Omega$  resistance between grid and earth) and for adjustment of grid current to nearly zero (less than  $10^{-12}$  amps) as required for biological reasons (any grid current would flow through the electrode and through it into the cell and would cause marked changes in potential due to the small size of the cell). The instrument also allowed adjustment of input and output resistance balance to minimize distortion of the signal.

The output from the electrometer was displayed on one beam of a dual-beam oscilloscope (Tektronix Type 565). The second beam could be split to allow a time signal from a crystal controlled frequency source (Venner) and a signal from the stimulator to be displayed on the screen.

When recordings were made with two microelectrodes simultaneously the above arrangement for the input was duplicated, each signal being fed through an electrometer to one beam of the oscilloscope.



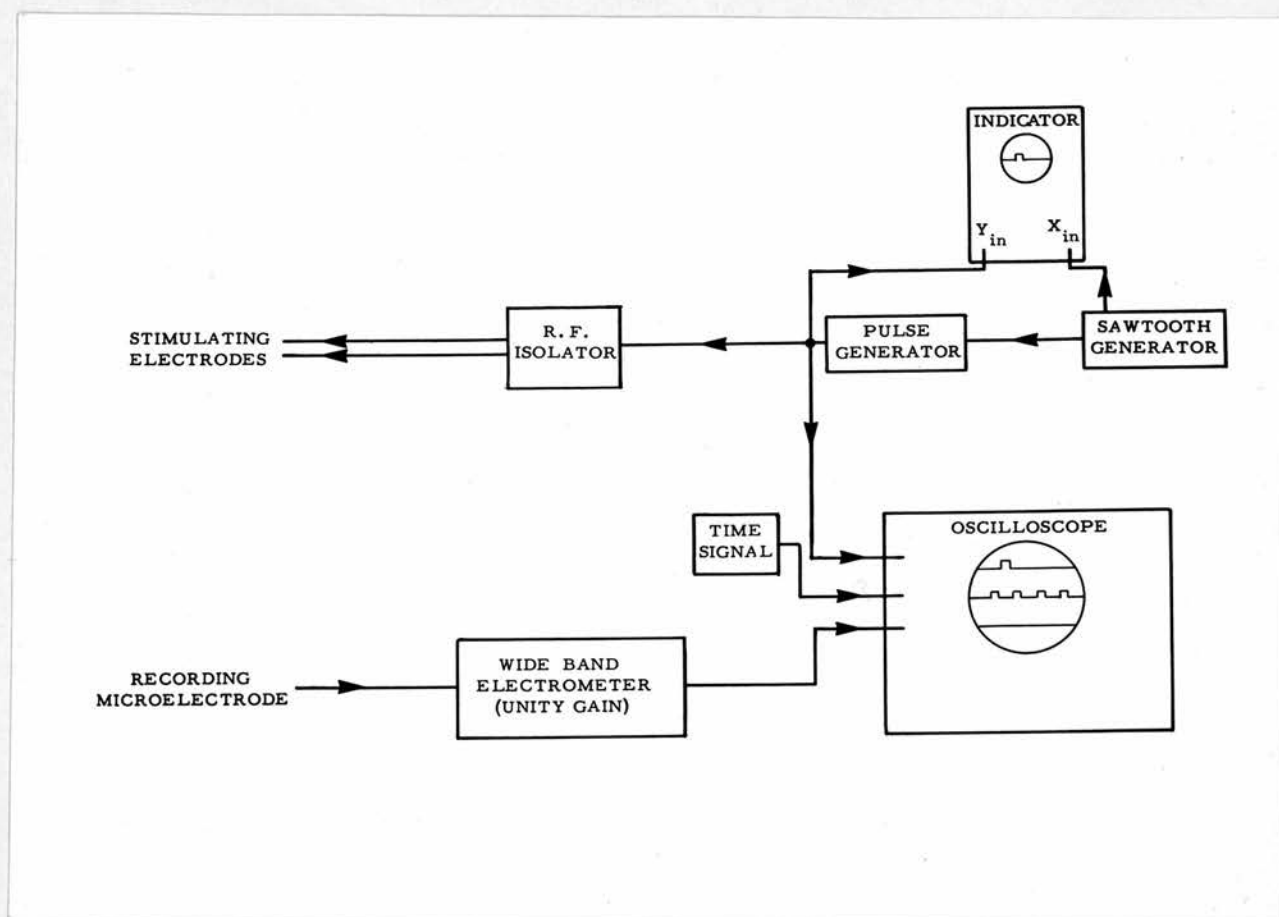


Fig. 3. Block diagram of electrophysiological apparatus.

Stimulating Circuit:

The Tektronix 160-Series instruments were used to generate the stimulating pulse. The pulse generator (Type 161) provided rectangular pulses variable in amplitude and duration, and was triggered by a sawtooth waveform supplied by the waveform generator (Type 162). The pulse was shown on an indicator unit (Type 360), the sweep voltage being provided by the sawtooth generator. This pulse was fed to the tissue through an R. F. isolator (American Electronics Laboratories) that isolated the pulse from the earth.

For stimulating the splanchnic nerve silver wire electrodes were used, but for local stimulation of the blood vessels themselves a metal concentric electrode was used, the current pulse flowing between the tip of a fine insulated wire, pushed down the barrel of a hypodermic needle, and the needle itself.

Wheatstone Bridge Circuit:

Attempts were made to stimulate the smooth muscle cells by passing a current across the cell membrane. This was done by using the method developed by Araki and Otani (1955) and modified by Frank and Fuortes (1956) who used one electrode for both stimulating and recording, and employed a bridge circuit for balancing out the potential difference developed across the microelectrode resistance by the stimulating current. The circuit used is shown in Figure 4; a  $20\text{ K}\Omega$  variable resistance (Fluke Vernier Potentiometer Type 20 A) is built into the Bak Electrometer and a  $10^9\ \Omega$  resistor is included in the probe unit. As shown in the diagram, when the bridge was balanced the

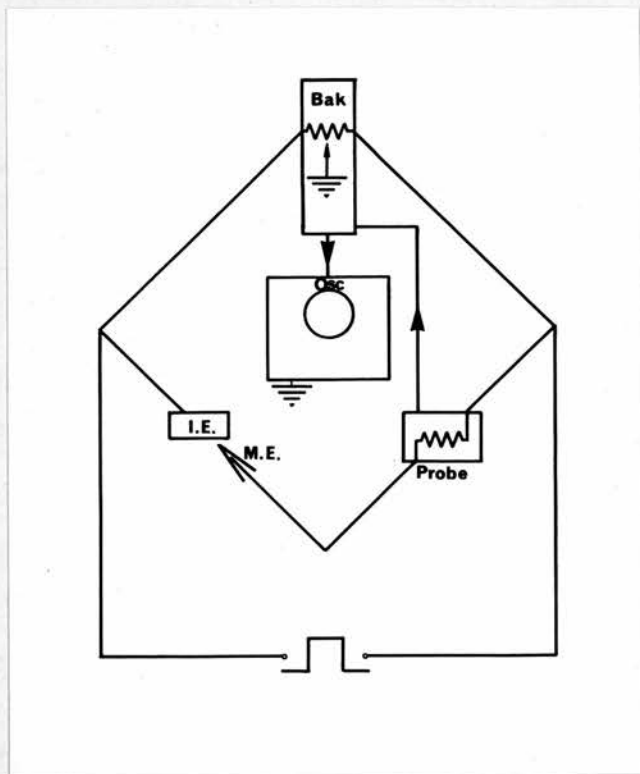


Fig. 4. Diagram of the Wheatstone bridge circuit. M.E., microelectrode; I.E., indifferent electrode; Bak, electrometer with 20 K $\Omega$  potentiometer; Osc, dual-beam oscilloscope; Probe, probe unit of Bak electrometer.

stimulating current was divided between the resistance of the microelectrode (ME), cell, fluid etc., and the resistor in the probe. The presence of this high resistance caused a drastic reduction in the size of the stimulating current, but was so high compared to the resistance of the microelectrode-cell-fluid combination that it ensured that the current being supplied to the cell was as near constant as possible. The range of currents that could be applied was similar to that found to stimulate intestinal smooth muscle by Nagai and Prosser (1963) and Kuriyama and Tomita (1964).

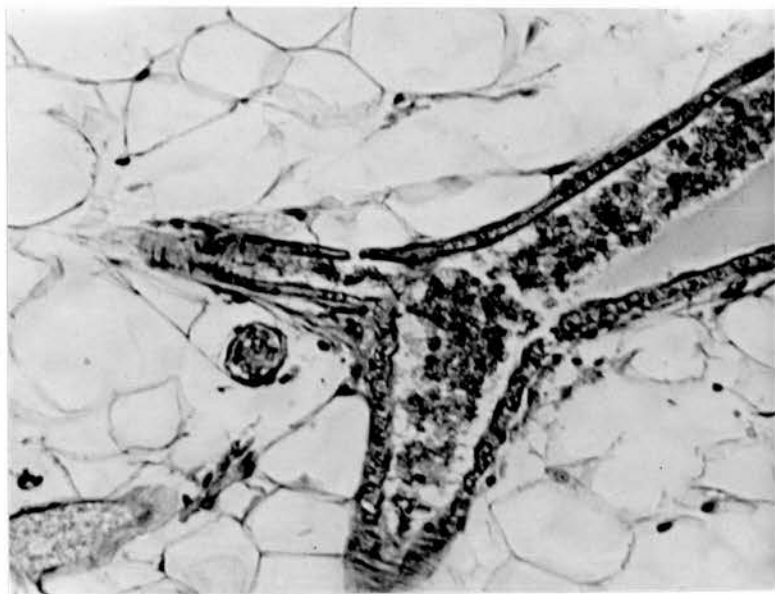
PART 3: TYPES OF ELECTRICAL ACTIVITY RECORDED

TYPES OF ELECTRICAL ACTIVITY RECORDEDRESULTSIntracellular Recording:

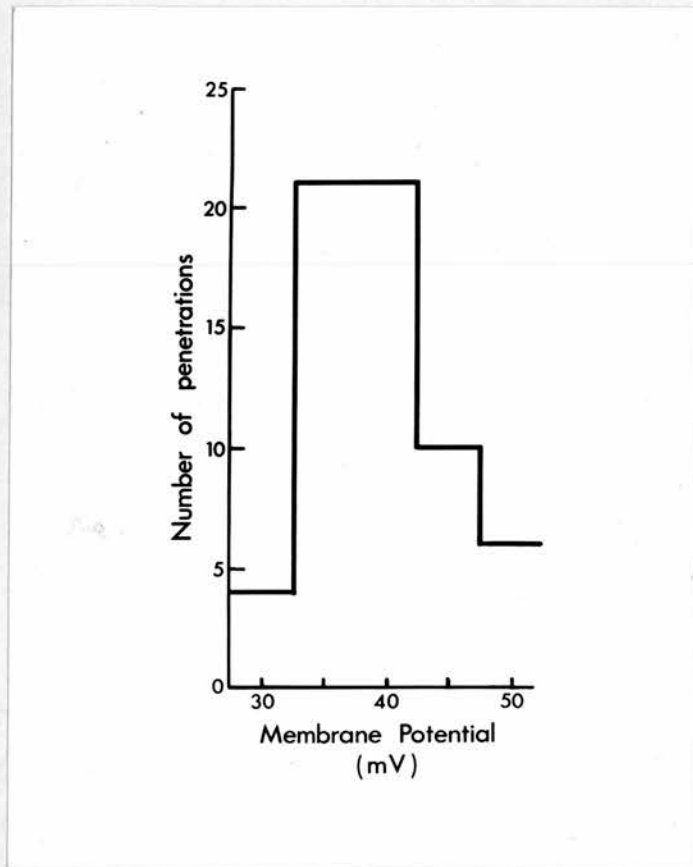
Small arterioles of the rat mesenteric circulation, 80 to 150 $\mu$  diameter, were selected and the microelectrode tip inserted along the line of the vessel and at an angle of approximately 45°. Occasional observations were made on venules of similar size. Penetration was found difficult due to the small size and frequent movements of the cells, but was found easier where the vessels bifurcated. At these regions a larger area of vessel was available for observation, and there appeared to be less movement of the vessel away from the penetrating microelectrode. Histological examination showed a condensation of connective tissue around the bifurcation, as shown in the section in Figure 5. Here, the connective tissue seen between the arms of the Y formed by the dividing vessel would tend to immobilize the vessel. The technique employed in these experiments was to bring the electrode tip into contact with the exterior of the blood vessel, tap the table, and if this resulted in a potential drop of at least 30 mV it was assumed that the electrode tip had penetrated the cell membrane.

Membrane potentials:

The potential recorded on penetrating a cell was invariably fluctuating, and the maximum level of polarization was taken as the 'resting' potential. This varied from 30 to 50 mV, mean



**Fig. 5.** Section of dividing rat mesenteric arteriole. H. & E. stained. 280x.



**Fig. 6. Distribution of values of membrane potential recorded from rat mesenteric vessels.**



39.4 mV  $\pm$  5.5 (S.D.) for n=62. Figure 6 shows the distribution of recorded values. In general these potentials were not well maintained, showing either a gradual decline presumably due to leakage through the damaged membrane, or falling abruptly due to blockage or breakage of the electrode tips. High membrane potentials approaching 50 mV were invariably recorded from resting cells in deeply anaesthetized animals, while lower values were in the main recorded from more active cells. This is shown in Table 1; the values given were obtained from 12 different cells in 6 different animals, each potential being abruptly established and maintained for periods of 2 to 5 min, and the maximum level of polarization measured. Membrane potentials tended to be higher in an inactive cell than in an active preparation, but there was considerable variability.

Figure 7 shows recordings made from two cells as the electrode sank through the first cell into another in a deeper layer of the vessel wall. This situation could be avoided by raising the electrode slightly after it had penetrated a cell to prevent it sinking further under its own weight.

#### Changes in ionic environment:

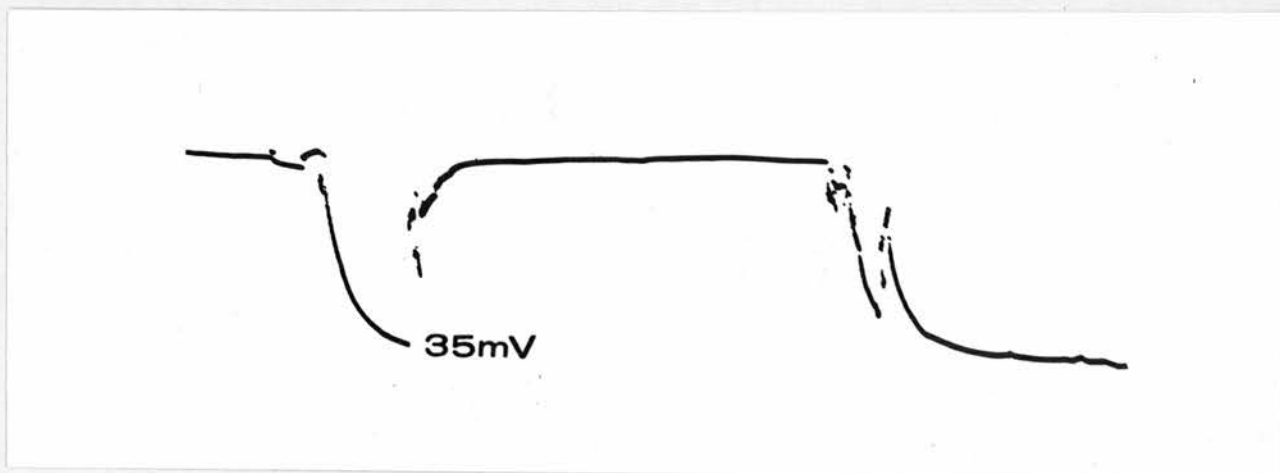
Variation of the potassium concentration in the solution flowing over the tissue altered the values of the membrane potential. Solid potassium chloride was added to the solution in amounts sufficient to double or treble the potassium concentration, and measurements made 10 to 15 min after addition. In an experiment where one good electrode was used to record membrane potentials at different potassium concentrations and an attempt

The continuing flow of blood through the vessel may have washed away and diluted the applied solution.

TABLE 1.

Effect of anaesthesia on electrical activity  
of vascular smooth muscle cells

DEEP ANAESTHESIA (no action potentials)		MODERATE ANAESTHESIA (some action potentials)	
Membrane Potential mV	Wave Amplitude mV	Membrane Potential mV	Wave Amplitude mV
	Mean $\pm$ S.E. (n=12)		Mean $\pm$ S.E. (n=12)
50	4 $\pm$ 0.3	43	11 $\pm$ 0.3
50	5 $\pm$ 0.3	39	11 $\pm$ 0.3
45	2 $\pm$ 0.3	35	8 $\pm$ 0.6
40	2 $\pm$ 0.3	32	9 $\pm$ 0.3
35	2 $\pm$ 0.3	30	10 $\pm$ 0.3
35	3 $\pm$ 0.2	30	6 $\pm$ 0.3
Mean $\pm$ S.E.			
43 $\pm$ 3	3 $\pm$ 0.1	35 $\pm$ 2	9 $\pm$ 0.2



**Fig. 7. Changes in potential observed on insertion of a microelectrode into the wall of a mesenteric arteriole.**

had been made to remove the peritoneum, it was found that only large changes in concentration caused significant alterations in membrane potential. Thus with normal potassium concentration the membrane potential (mean of 10 penetrations  $\pm$  S.D.) was  $35 \text{ mV} \pm 3$ ; with twice normal potassium, the membrane potential averaged  $30 \text{ mV} \pm 3$ , while with three times normal potassium the average was  $27 \text{ mV} \pm 3$ . As there was no way of determining how much of the excess potassium was actually reaching the muscle cells through the peritoneum and adventitia, no attempt was made to establish a quantitative relationship between membrane potential and external potassium concentration.

Reduction of the sodium content of the bathing fluid in another animal by substituting the sodium with lithium did not have any significant effect on the membrane potential until all the sodium had been replaced, when the membrane was depolarized from  $40 \text{ mV}$  to  $25 \text{ mV}$  (mean of 10 penetrations, standard deviations in each case being  $\pm 3$ ). Again there was no way of establishing the true external composition for the cells concerned.

#### Slow waves:

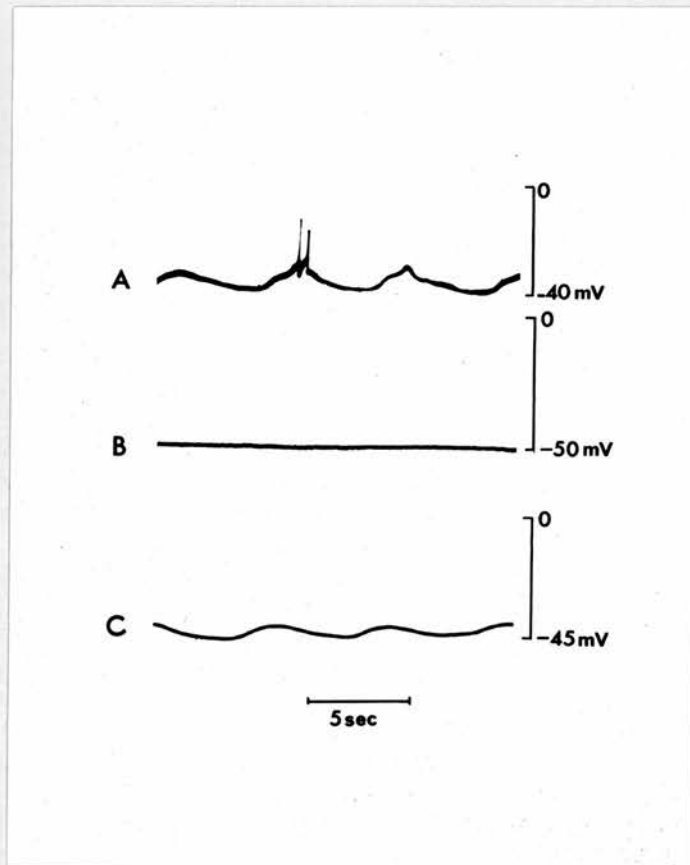
The membrane potentials recorded on penetration were not constant but showed continuous rhythmic fluctuations with a period varying from 4.5 to 7.8 sec in different animals. These slow waves varied considerably in size - from less than 1 to 14 mV - and could under certain conditions give rise to action potentials. These were superimposed on the slow waves, and did not appear to affect either their frequency or their amplitude. Slow waves could be recorded from every cell, and their amplitude

varied considerably in different cells and in the same cell from time to time. Figure 8 shows intracellular recordings from three different cells. In 8 A, a record taken from a highly active cell in a moderately anaesthetized animal, the waves are large (5 to 9 mV) and action potentials appear frequently. In 8 B, a record taken from a deeply anaesthetized animal, the membrane appeared to be stable at a high level of polarization (50 mV). In 8 C, from the same animal as 8 B but some 5 sec after the tracheal cannula had been closed, the slow waves are fairly large (4 to 7 mV), maximum membrane polarization is 45 mV, and there are no action potentials. The durations of the slow waves (measured between the points of maximum polarization) varied only slightly and in an apparently random fashion in any one cell. Thus in Figure 8 A wave durations were 5.7 and 5.9 sec; in 8 C, in a different animal they were 5.6 and 5.8 sec.

As shown in Figure 8, the amplitude of the slow waves appeared to be affected by the level of anaesthesia. Reference to Table 1 shows a tendency for the membrane to be stable at a high membrane potential with small waves in the deeply anaesthetized animal, but more labile at a lower potential with relatively large waves when under lighter anaesthesia.

#### Action potentials:

As well as the above fluctuations in membrane potential, action potentials were recorded. They were first observed in lightly anaesthetized animals without any external stimulus, as in Figure 8 A. It should be noted that this 'spontaneous' activity was recorded from the muscle 'in vivo' with circulation



**Fig. 8.** Intracellular recordings from smooth muscle cells in the walls of rat mesenteric arterioles.

A moderately anaesthetized animal,

B deeply anaesthetized animal,

C same animal as in B, but 5 sec after closure of the tracheal cannula.

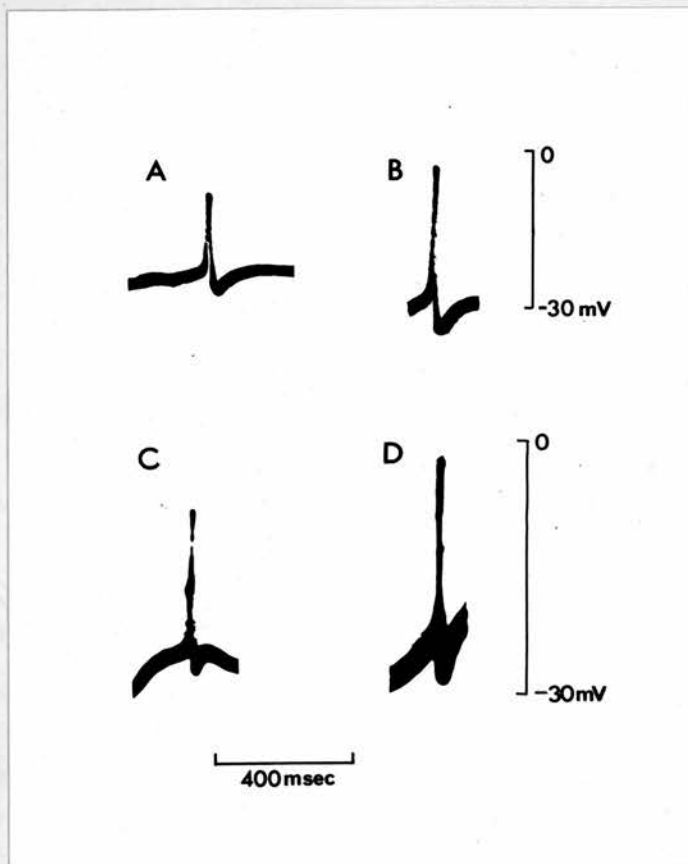
and innervation intact, the cells being under the influence of several nervous and humoral factors. The action potentials recorded in response to external stimulation, nervous or humoral, were similar in shape to those occurring 'spontaneously'.

Figure 9 shows representative action potentials recorded from different animals under different conditions. Figure 9 A was recorded from an arteriole and 9 B from a venule in the absence of any externally-applied stimuli; 9 C and 9 D were recorded from arterioles after stimulation with adrenaline ( $10^{-6}$  g/ml) and through the splanchnic nerve respectively. The action potentials were similar in shape in all cases.

Activity was rhythmic, the spikes arising apparently from the slow waves, when the depolarization of the wave reached a threshold value. In some cases the wave would give rise to a pair of action potentials - in any one cell this depended on the level of depolarization reached by the wave. In Figure 10 A two spikes arose from a wave, the first at 29 mV, the second at 24 mV, the amplitude of the wave being some 30% of the maximum membrane polarization; in 10 B a single spike arose at 28 mV from a wave with amplitude 20% of the 'resting' potential in the same cell.

Figure 11 shows the activity recorded from a different cell in the same animal with the same electrode. The first wave gave rise to two spikes and had an amplitude 17.5% of the 'resting' potential; the second wave with a lower amplitude (15%) gave rise to one spike; no spikes arose from the third wave, amplitude 12.5%; the fourth wave with an amplitude of 20% again gave rise to two spikes; while the fifth wave, amplitude 13%, had a small





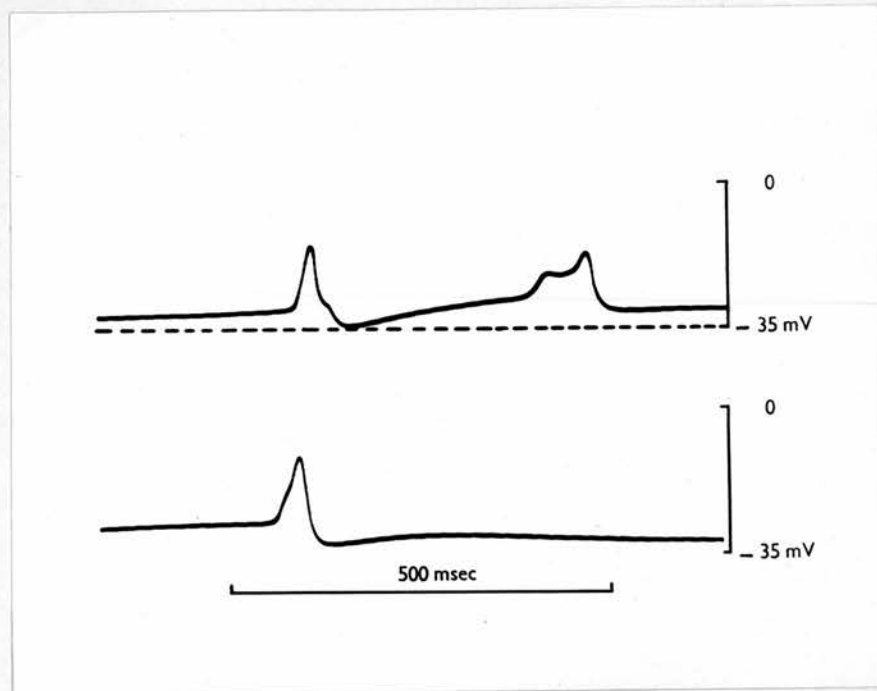
**Fig. 9.** Action potentials recorded with intracellular electrodes.

A from an arteriole, and B from a venule, in the absence of external stimulation;

C from an arteriole 30 sec after applying adrenaline ( $10^{-6}$  g/ml);

D from an arteriole 10 sec after stimulation of the right splanchnic nerve.





**Fig. 10.** Action potential spikes recorded intracellularly from the wall of a mesenteric arteriole 120  $\mu$  diameter in the rat. Upper trace, two spikes arising from a potential wave of amplitude greater than 30% of the membrane potential; lower trace, a single spike on the succeeding wave which had an amplitude that was 20% of the membrane potential.

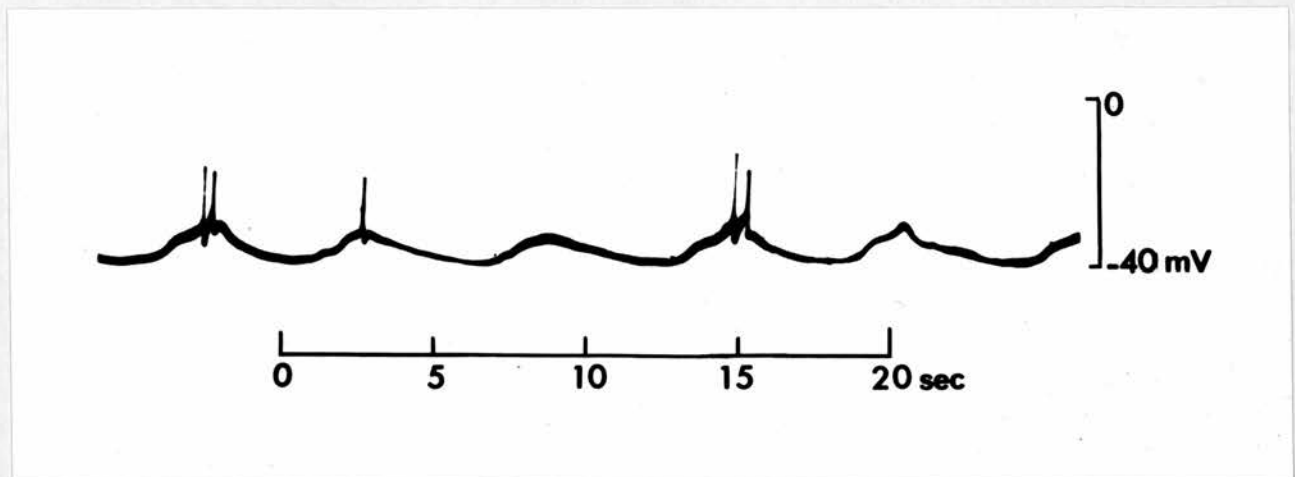


Fig. 11. Recording of transmembrane potentials in the smooth muscle in the wall of a mesenteric arteriole of a rat under moderate Nembutal anaesthesia.

hump superimposed on it. Comparison of this with Figure 10 shows that the presence or absence of spikes could not be related accurately to the membrane potential or size of the slow waves in different cells.

However, the level of depolarization reached before a spike could arise was comparable in the two cases illustrated. Depolarization to 29 to 31 mV was required before a spike would arise, although the second of a pair of action potentials tended to take off at a higher level of depolarization, 24 to 28 mV.

Comparison of the values of membrane potential at which action potentials arose in different animals was not possible as different electrodes were used and the amount of cell damage, the size of tip potentials, and other factors were variable.

Closer examination of the shape of the action potentials recorded showed several characteristic features - a slow initial depolarization leading into a faster rise, a variable amplitude and duration, a repolarization rate greater than the rate of depolarization, and a marked positive after-potential best seen in Figure 10. For ease of description, the action potential is divided into the spike component, i. e. the large, brief depolarization - repolarization cycle, and the after-potential, i. e. the small, slowly declining potential change following the spike. An analysis of the characteristics of the action potential is given in Table 2.

In most cases the foot of the spike was rounded, the depolarization rate being as low as 0.3 V/sec, making a definite inflection on the rising phase. Where this initial phase was slow, as in the second of the pairs of action potentials in Figures 10 and



TABLE 2.

Characteristics of action potentials of vascular smooth muscle

	Mean	Range	S. D.	No. of obsvns.
<u>Spike</u>				
Amplitude	22 mV	15-35 mV	6	13
Duration - total	46 msec	40-50 msec	5	13
at 60% spike height	19 msec	15-20 msec	2	13
Rate of depolarization	0.9 V/sec	0.7-1.3 V/sec	0.2	8
Rate of repolarization	1.4 V/sec	1.2-1.9 V/sec	0.2	8
<u>Slow potential</u>				
Amplitude	6 mV	4-8 mV	1	13
Rate of depolarization	0.4 V/sec	0.3-0.6 V/sec	0.1	8
<u>After-potential</u>				
Amplitude	6 mV	4-8 mV	1	13
Duration	118 msec	100-150 msec	5	13

11, a higher level of depolarization was reached before the faster depolarization of the spike started. Occasionally when a wave did not quite reach the threshold for activity, e.g. the fourth wave in Figure 11, a small depolarization appeared on the summit, apparently corresponding to the initial slow phase of the spike. It had a long duration - 60 to 100 msec - difficult to measure accurately as the end of the slow potential tended to merge into the slow wave. The rate of depolarization was similar to that of the initial phase of a spike with a marked inflection on its rising phase.

The total duration of the spike was variable - 40 to 50 msec, mean  $46 \text{ msec} \pm 5$  (S. D.) - but the duration measured at 60% of the spike height, i. e. without the slow component, was much shorter and less variable - 15 to 20 msec, mean  $19 \text{ msec} \pm 2$  (S. D.). The spike potential of these smooth muscle cells thus appeared to have two components - an initial slow phase and a larger fast phase. The fact that the standard deviation of the mean was larger where total spike duration was measured suggested that the slow component was more variable in character than the fast component.

The amplitude of the spike potential was also variable. Only very rarely was a spike with reversal of membrane potential recorded and then only immediately after penetration of a cell. The true value of spike amplitude could not be established due to the uncertainties in resting potential measurement, but recorded values ranged from 15 to 35 mV, mean  $22 \text{ mV} \pm 6$  (S. D.). These low values were at least partly due to the damage caused by the penetrating electrode.

The spike was invariably followed by a long positive after-potential, duration 100 to 150 msec, mean 118 msec  $\pm$  5 (S. D.), and amplitude 4 to 8 mV, mean 6 mV  $\pm$  1 (S. D.). The duration was rather difficult to estimate exactly as the tail merged into the slow wave.

The rate of repolarization of the spike was greater than the rate of depolarization - 1.4 V/sec mean as compared with 0.9 V/sec.

When two spikes arose from one wave, they were always separated by at least 150 msec and frequently 300 to 400 msec. The second spike originated from a more depolarized membrane potential than the first, and its slow component was invariably more pronounced. On one occasion a burst of very small spikes (8 to 15 mV amplitude) lasting several seconds was observed arising from a potential of 20 mV, possibly due to injury of the cell by the impaling electrode. The maximum frequency was 8/sec.

#### Extracellular Recording:

Recordings were made with an extracellular electrode of tip diameter 1 to 5 $\mu$  in contact with the outside of the blood vessel. Again, slow waves and spikes were recorded. A recording made with this method is shown in Figure 12 C and may be compared with an intracellular recording in Figure 12 A. This shows that the polarity of the slow waves was reversed on penetrating the cell membrane, the action potentials arising from the point corresponding to the point of greatest depolarization, and the ratio of slow wave amplitude to action potential amplitude was markedly different.

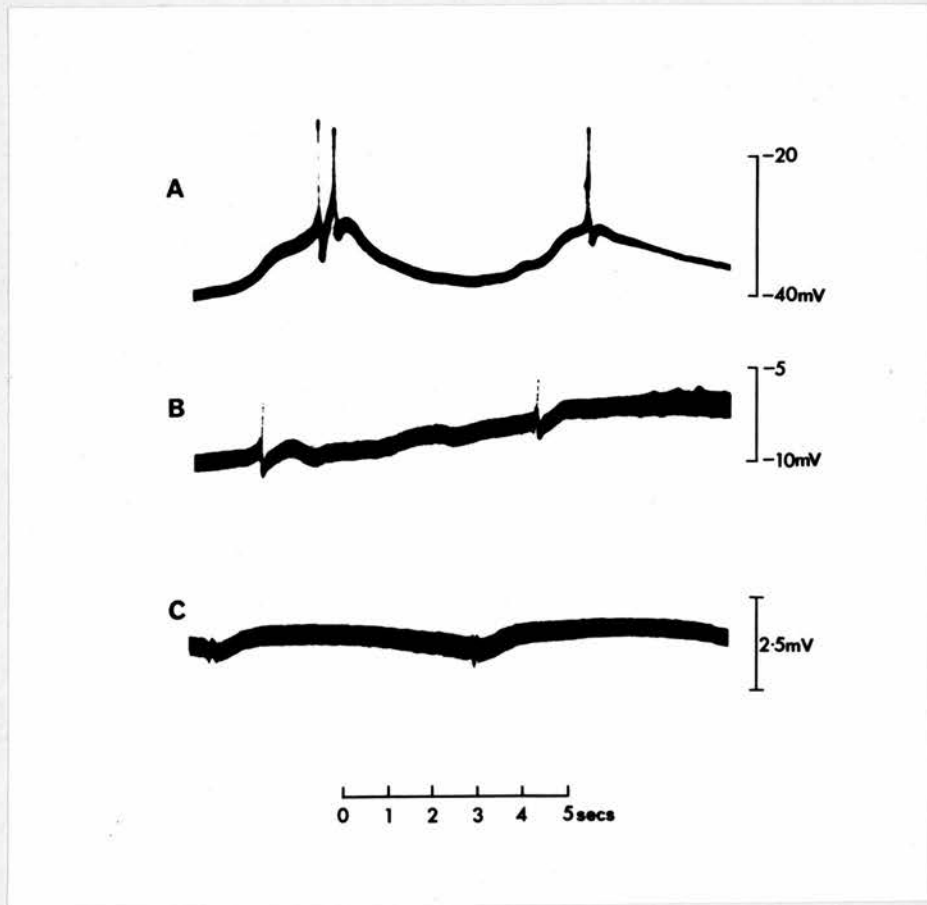


Fig. 12. Potential changes observed with three different methods of recording from rat mesenteric arterioles. A, intracellular; B, 'pressure extracellular'; C, extracellular.



Figure 13 shows recordings made with extracellular electrodes under different conditions. 13 A and C show the electrical activity recorded before and 10 sec after adrenaline ( $10^{-6}$  g/ml) had been dropped on to the preparation; in 13 A the waves were small and only occasional action potentials were observed, while in 13 C the waves were larger and the spikes more frequent. In 13 B, obtained from a different animal, the right splanchnic nerve had been stimulated, and waves of amplitude up to 3 mV were seen with action potentials occurring singly or in groups of two or three.

The duration of the slow waves was found to be remarkably constant in any one animal, but varied in different animals. This is shown in Table 3 which gives the results of a statistical analysis of the durations of slow waves recorded from three different animals under different conditions. The range of values obtained from different animals, 5.2 to 7.2 sec, was considerably greater than the ranges obtained in individual animals, e.g. 5.7 to 6.0 sec in animal B. The amplitude was considerably more variable, from 0 to 8 mV under varying conditions.

Whereas in intracellular records there was a clear distinction in the level of depolarization reached by waves giving rise to action potentials and waves which did not, in the extracellular records during periods of activity there was not always such a distinction. This can be seen in Figure 13 B where a group of three action potentials (marked with an arrow) arose from the smallest wave. However, Table 4 shows that there was a distinction between average slow wave amplitudes before and



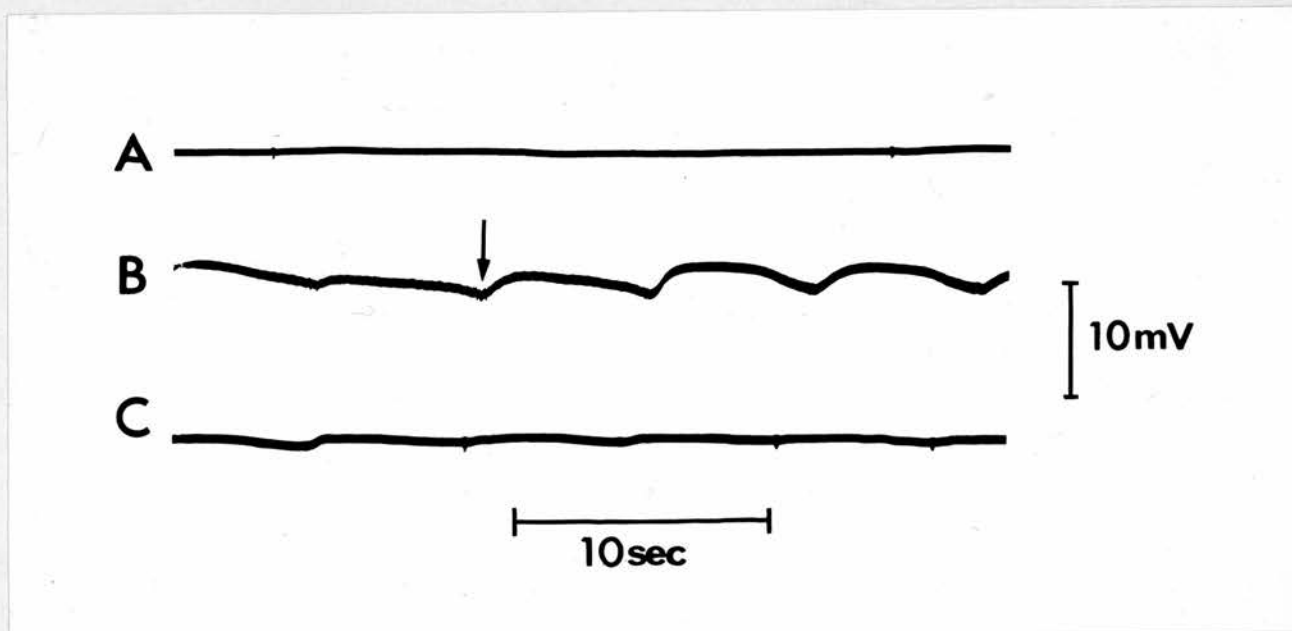


Fig. 13. Recordings made with extracellular electrodes in contact with the wall of mesenteric arterioles in the rat.

A, 'spontaneous' activity; B, after splanchnic nerve stimulation; C, after adrenaline ( $10^{-6}$  g/ml).

TABLE 3.

Duration of slow waves recorded in vascular smooth muscle cells  
under different conditions

	Mean secs	Range secs	S. D.	No. of obsvns.
<u>Animal A</u>				
Control	5.7	5.2-5.9	0.2	15
After adrenaline ( $10^{-6}$ g/ml)	5.7	5.2-5.9	0.2	15
<u>Animal B</u>				
Control	5.7	5.1-6.3	0.4	15
During asphyxia	6.0	5.5-6.3	0.3	12
<u>Animal C</u>				
Control	6.8	6.2-7.2	0.3	29
After nerve stimulation	6.7	6.6-6.8	0.1	12

after stimulation by asphyxia, adrenaline, and through the splanchnic nerve, and that in most cases this was accompanied by increased firing of action potentials. Thus Figure 13 B shows the effect of stimulating the peripheral cut end of the right splanchnic nerve. Before stimulation, no spikes or waves had been seen; after stimulation waves and spikes appeared. Figure 13 A and C show the marked increase in both waves and spikes caused by adrenaline ( $10^{-6}$  g/ml).

It would appear therefore that the relatively large wave amplitudes recorded with extracellular electrodes during periods of spontaneous spike activity or in response to stimulation arose, at least in part, from greater depolarizations occurring in the cells in the field of the electrode, this being reflected in the occurrence of action potentials in some of these cells although these might not appear in the recording. Any interpretation of changes in wave amplitude in extracellular records must be limited by the fact that these reflect the summed currents from many cells and are affected by the degree of synchrony as well as by the voltage at individual cells.

Extracellularly-recorded action potentials were extremely variable in shape and size. In general they were triphasic in the form positive, negative, positive, although the initial and final positive phases were occasionally absent, the relatively large negative phase being invariably present. The amplitude of the action potentials was in general less than the amplitude of the slow waves, again indicating that the external electrode was recording the summed slow potential changes from many different cells, while recording the action potentials of the cell

TABLE 4.

Characteristics of slow waves recorded from vascular  
smooth muscle (extracellular recording)

	Amplitude		Duration		No. of obsvns.
	Mean mV	S. D.	Mean secs	S. D.	
<u>Animal A</u>					
Control	0.2	0.1	5.1	0.3	41
After adrenaline ( $10^{-6}$ g/ml)	4.1	0.1	4.8	0.8	18
<u>Animal B</u>					
Control	2.6	0.6	5.7	0.4	15
During asphyxia	4.0	0.8	6.0	0.3	12
<u>Animal C</u>					
Control	0.2	0.1	6.7	0.1	12
After nerve stimulation	1.3	0.3	6.7	0.1	12
<u>Animal D</u>					
Control	0.7	0.8	5.7	0.6	47
After noradrenaline ( $10^{-6}$ g/ml)	2.2	0.8	5.7	0.7	18

immediately in contact.

Occasionally the electrode appeared to be in close contact with two cells, and recorded two rhythmically-occurring spikes bearing a variable amplitude and time relationship to each other, one being larger than the other presumably due to electrode position. Thus the action potential spikes of adjacent cells were not occurring exactly in phase, but the time of discharge of individual cells varied about a common mean.

Thus the conclusion might be drawn that the slow wave allows the cells in a given area of vessel wall to become excitable more or less simultaneously, so providing a method of coordination of the activity of the smooth muscle in the vessel walls. Whether this slow wave is driven by some mechanism inherent in the muscle cells themselves or is controlled by external influences is not yet known, and will be discussed later.

A different type of extracellular recording was made by pressing the microelectrode against the blood vessel wall until marked dimpling of the surface could be seen. When this was done, the potential level being recorded altered in a negative direction, returning to the original level after the pressure was released. Figure 14 shows an example of records obtained using this recording method - when the electrode (approximately  $1\mu$  tip diameter) was pushed against the blood vessel the potential level fell in a negative direction by 18 mV and the small action potentials were positive-going.

The action potentials recorded in this 'pressure extracellular' manner were similar in shape and polarity to those recorded intracellularly, but smaller and more variable, and the slow

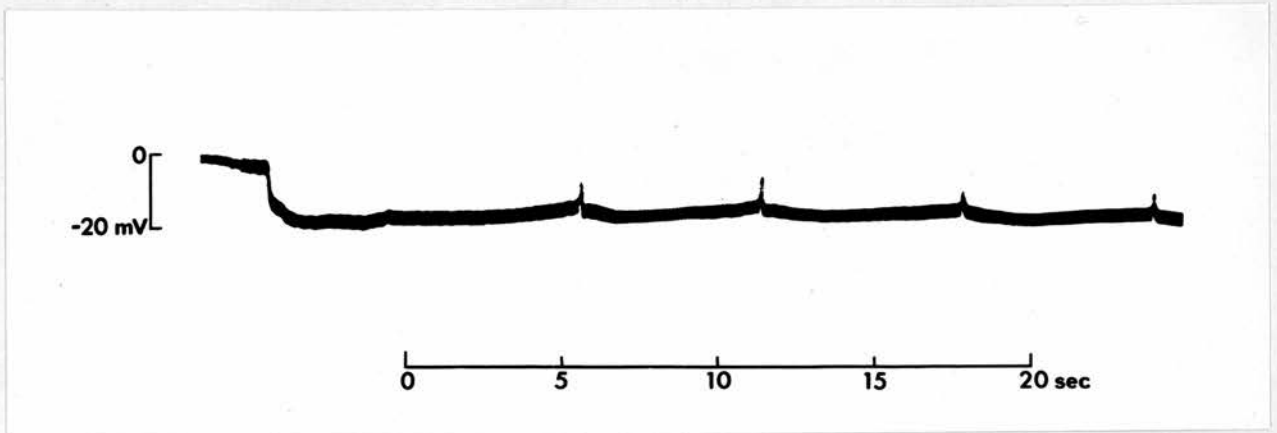


Fig. 14. Record obtained by pressing electrode against wall of rat mesenteric arteriole.

wave amplitude was much smaller than that of the spike. Table 5 compares the ratio of spike amplitude to wave amplitude obtained with the three different methods of recording. The values tabulated for 'pressure extracellular' recording were obtained with electrodes, tip diameters less than 1 to  $5\mu$ , which were observed to dimple the surface of the vessel, and those tabulated for intracellular recording were obtained with 5 electrodes, resistances 50 to  $80 M\Omega$ , showing an abrupt fall of potential on penetration and action potentials 15 mV or more in amplitude. The ratio of spike to wave amplitude was similar in intracellular and 'pressure extracellular' recordings, where the spikes were some three times the size of the waves, while with the external electrode just in contact with the vessel the reverse was true. These observations supported the conclusion that intracellular and 'pressure extracellular' electrodes recorded the potential changes from individual cells while an external electrode recorded the summed slow potentials occurring in its field, but only the action potential of the cell in contact.

Figure 12 shows records obtained by pressing an electrode against the outside of a vessel and gradually releasing the pressure. The potential fell (in a negative direction) by 12 mV when the pressure was applied, and in 12 B was gradually returning to the zero line. The action potentials, 3 mV amplitude, were positive-going. In 12 C, some 30 sec later, the potential was fluctuating about zero, and the typical small triphasic action potentials arose from the lowest part of the

TABLE 5.

Ratio of spike amplitude to wave amplitude in vascular  
smooth muscle using different recording methods

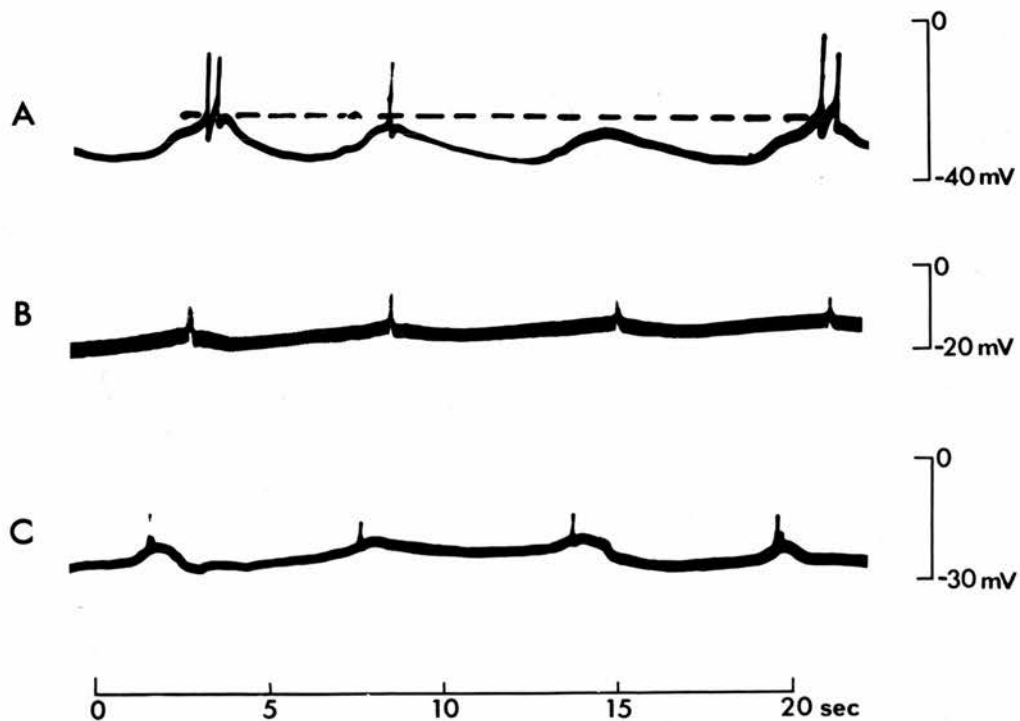
Recording Conditions	Spike Amplitude S mV	Wave Amplitude W mV	Ratio S/W
Intracellular	15 - 30	4 - 10	2.5/1 - 5/1 av. 3.5/1
Extracellular	0.8 - 1.2	0.8 - 4.2	1/1 - 1/3 av. 1/2.5
'Pressure extra- :cellular'	2.8 - 9.0	0.8 - 4.0	2.5/1 - 4/1 av. 3/1



slow waves. Figure 12 A is a record obtained with an intracellular electrode. The polarity of the waves recorded with the 'pressure extracellular' method was thus the reverse of that in the normal extracellular recording, and the same as that in an intracellular record.

Figure 15 shows that it might be difficult to decide when true intracellular potential changes were being recorded. Figure 15 A was considered to be an intracellular record, as penetration of the cell had been signalled by an abrupt drop in the potential level to a maximum of 38 mV, and the action potentials recorded were relatively large (14 to 29 mV). The recording in Figure 15 B was made with an electrode of tip diameter 1 to 2 $\mu$  dimpling the vessel wall; small (4 to 8 mV) positive-going spikes arose from the least negative parts of the slow waves, which were smaller and more irregular than those recorded intracellularly. In Figure 15 C the maximum potential level was low (28 mV) but had been reached in a step-wise fashion, the slow waves were very irregular, and the action potentials were smaller and more variable (3 to 8 mV) than in A; this recording might therefore have been extracellular, or intracellular where the membrane had been damaged severely. As microscopic observation of the movement of the electrode had shown an initial dimpling of the vessel which was then released, the electrode was assumed to have penetrated the cell membrane with difficulty and the potential changes across the membrane distorted by the degree of cell damage.

Figure 16 compares an action potential recorded with an intracellular electrode (16 A) with one recorded with an external



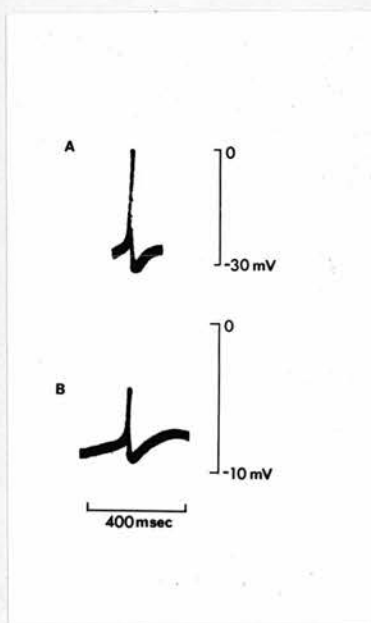
**Fig. 15.** Potential changes recorded from smooth muscle cells of rat mesenteric arterioles.

**A,** with an intracellular electrode;

**B,** with an extracellular electrode pressing against the vessel;

**C,** with an intracellular electrode with broken tip.

electrode dimpling the surface of the vessel (16 B). The externally-recorded potential shows similar features - a spike potential with an initial slow component and a positive after-potential of long duration - but is considerably smaller (4 mV as compared with 26 mV).



**Fig. 16. Action potentials recorded with two different methods.**

**A, intracellular ;**

**B, 'pressure extracellular'.**

DISCUSSIONConfigurations of externally-recorded potential changes:

When the recording electrode was placed on the surface of the blood vessel, the fact that the active muscle cells were surrounded by interstitial fluid and other cells meant that the electrode was recording potentials at a point in a volume conductor with respect to an indifferent electrode so far away that its potential was negligible. The current generated by the propagating impulse was distributed in the conducting medium according to the laws governing the flow of current in a volume conductor (Lorente de No, 1947).

The nature of the external field produced by a travelling impulse has been summarised by Lloyd (1947) - as an impulse approaches a point, that point acts as a source; as the impulse reaches that point, it acts as a sink; as the impulse recedes from that point, it again acts as a source. Thus an action potential travelling under the recording electrode will appear triphasic in the form positive, negative, positive, the relative size and duration of each phase varying according to the electrode position; action potentials initiated under or near the electrode and travelling away from it will appear diphasic in the form negative, positive; while an action potential travelling towards the electrode but not reaching it will appear diphasic in the form positive, negative.

When an extracellular electrode was pressed against the blood vessel wall, the potential level altered in a negative

direction, and the action potentials recorded were similar in shape and polarity to those recorded intracellularly. It would appear (Bortoff, 1961; Gillespie, 1962a and personal communication) that the region under the electrode was now depolarized, the electrode being effectively in contact with the interior of the cell. The polarity of the potential changes recorded would thus be the same as those recorded with an intracellular electrode, but the amplitude would be smaller and more variable due to the unknown resistance of the depolarized membrane. In addition, as there was an unknown external shunt resistance between the electrode tip and the indifferent electrode through which changes in external potential field appeared, the membrane resistance required to be made as low as possible compared with this shunt - i. e. the electrode tip must dimple the vessel surface sufficiently to 'seal' in the membrane - and thus the potential changes recorded would be distorted as little as possible by the external field appearing through the shunt resistance.

#### Membrane potentials:

The membrane potentials recorded were similar in size and variability - 30 to 50 mV, mean 39.4 mV - to those recorded in other mammalian vascular muscle - 26 to 59 mV, mean 38.7 mV in guinea pig mesenteric artery (Speden, 1964);  $33 \pm 1.3$  mV (mean  $\pm$  S.E.) in isolated rabbit anterior mesenteric vein (Cuthbert et al., 1964; Cuthbert and Sutter, 1964); and 30 to 65 mV in isolated rat portal vein (Funaki and Bohr, 1964). These in turn were similar to those recorded from other types of

smooth muscle showing spontaneous activity, as described in the literature survey. It must be emphasised that the preparation used in this series of experiments was an intact animal, the arterioles and venules from which recordings were made having their circulation and innervation intact. Thus any comparison of the results obtained with those obtained from isolated vessels must take into consideration the different influences acting in each case, and the extent to which removal of the tissue from the body leads to deterioration.

Microscopical examination (Rhodin, 1962; Grigor'eva, 1962) has shown that the smooth muscle cells in the walls of small arteries and arterioles are almost circularly arranged, and in the highly relaxed state have a diameter of 1.5 to 2.5 $\mu$  and length of 20 to 60 $\mu$ . Thus penetration of the cell membrane by an electrode 0.25 $\mu$  tip diameter was likely to cause a large amount of damage, and the membrane might not seal completely around the electrode. The size of the damaged region relative to the surface area of these small cells might be so large as to allow an appreciable shunting of the membrane voltage, making the measured values considerably smaller than the true values. In addition, leakage of electrolyte from the electrode might alter the membrane potentials in small cells (Coombs et al., 1955).

Further errors tending to reduce the membrane potentials arose from the development of diffusion potentials due to the different mobilities of the potassium and chloride ions in the microelectrode; this error was minimized by using a high concentration of potassium chloride solution (3M). A much larger source of error was the presence of 'tip potentials'

(Adrian, 1956) which are very pronounced in the small diameter, high resistance microelectrodes required to penetrate small cells. These potentials are due to exaggerated differences in potassium and chloride ion mobilities caused possibly by development of charge on the glass of the microelectrode or by plugging of the electrode tip.

Thus values obtained for membrane potentials were subject to the limitations imposed by these possible sources of error.

As was described in the Introduction, the conclusion was reached for spontaneously active smooth muscles that the low membrane potential was associated with a high membrane permeability to sodium compared to that in skeletal muscle. Investigations on ionic distribution and movement using radioactive isotopes would be required before applying this conclusion to vascular smooth muscle, together with an analysis of the effects of alterations in ionic composition on the shape of the action potential. The relatively small effect of change in potassium concentration of the bathing fluid on the membrane potential might indicate that this is not governed merely by the distribution of potassium ions across the membrane. Moreover the fact that the membrane potentials recorded were similar in size and variability to those recorded from other active smooth muscle cells, and that under deep anaesthesia the potential was higher and more stable indicated that the low values recorded in an active cell might indicate a steady active state of high sodium permeability rather than a resting state of low sodium permeability.



Slow Waves:

That these slow, rhythmic potential changes were not movement artefacts arising from the pulsing of the vessels but represented an electrical event occurring across the cell membrane was indicated by the following lines of evidence:

- 1) The polarity of the slow wave was reversed as the recording electrode penetrated the cell membrane.
- 2) Intracellularly-recorded action potentials were three times the amplitude of the slow waves, while extracellularly-recorded spikes were in general smaller than the waves, indicating that the external electrode was recording the algebraic sum of waves from a mass of cells but action potentials from one cell at a time. This point could be clarified by recording differentially from two extracellular electrodes fairly close together to discriminate the spikes recorded from individual cells from the waves being recorded from every cell.
- 3) Slow waves could be recorded from every active cell, but did not appear to be exactly synchronous.
- 4) The action potentials always occurred rhythmically at an equivalent point in every slow wave.
- 5) In intracellular records there was a close relation between the level of depolarization reached by the waves and the presence or absence of action potentials.

Slow waves have been recorded from various types of muscle and nerve, vertebrate and invertebrate, as described by Burnstock et al., (1963). Characteristic of the longitudinal

- 6) Slow waves could be recorded after occlusion of the blood vessel, and 10 min after cessation of the heart.

muscles of the gastrointestinal tract are sinusoidal slow waves which initiate firing of action potentials either singly or in groups provided a critical level of depolarization is reached (Bulbring et al., 1958; Gillespie, 1962). The mechanism underlying the slow waves and hence the spontaneous activity of smooth muscles has not yet been established. Daniel and his co-workers (Daniel et al., 1960) showed that slow waves were not due to stretch or mechanical strain, as they could be recorded in the gastrointestinal tract in the absence of contractile activity after administration of atropine or nicotine, nor were they dependent on an extrinsic nerve supply as they were recorded from isolated muscle and after inhibition of ganglionic transmission by nicotine or hexamethonium. The recording of rhythmic spontaneous activity from nerve-free chick amnion (Prosser and Rafferty, 1956; Cuthbert, 1962) supported the view that slow waves did not originate from nervous influences, although these might have a modifying effect.

#### Action Potentials:

Action potentials occurred both in the absence of external stimulation and in response to nervous and humoral stimulation. They were similar in shape in all cases, and differed from action potentials in skeletal muscle in several ways:

- 1) The foot of the action potential was rounded, and in some cases there was a definite inflection on the rising phase, and it was found that slow waves that did not quite reach the threshold for a complete action potential might have superimposed a small slow potential change apparently corresponding to this first part

of the action potential. It was observed that the appearance of this slow potential was related to the level of depolarization reached by the slow waves, and that it in turn required to reach a critical level of depolarization before a spike would occur (this threshold appeared to be higher for the second spike of a pair than for the first or single spike possibly due to some refractiveness in the cell membrane) making it unlikely that the slow potential was an artefact produced by movement of the electrode or a passive reflection of activity in an adjacent cell spreading electrotonically. The slow potential thus appears to reflect some change occurring in the cell penetrated and associated with the action potential mechanism. Several possible explanations for this slow potential should be considered:

a) It may be concerned with a transmission process, either from (i) an adjacent muscle cell (by electrotonic spread, contraction of the adjacent cell stretching and causing depolarization, or by chemical transmission) - similar to the muscle-muscle junction potential described in uterine muscle by Goto et al., (1960); or from (ii) a nerve fibre - an excitatory junction potential as described for longitudinal muscle of rabbit colon (Gillespie, 1961), rabbit bladder (Ursillo, 1961), guinea-pig vas deferens (Burnstock and Holman, 1961) and dog retractor penis (Orlov, 1962).

b) It may be due to electrotonic spread of the process of initiation of the action potential occurring in some other part of the cell. Thus in the mammalian motoneurone (Eccles, 1957) the spike in the motoneurone body is preceded by and normally

arises from a spike in the initial segment, while in crustacean stretch receptors (Eyzaguirre and Kuffler, 1955) the generator potential induced in the dendrites by stretch secondarily, by electrotonic spread, reduces the membrane potential in the cell soma initiating an action potential if threshold is reached.

c) It may be a pacemaker potential, as described for uterine muscle (Marshall, 1962).

An initial step to investigate the origin of this slow potential would be to study the effects of different transmitter chemicals and blocking agents on the shape of the action potential. Investigation of the effects of various ions is also required to elucidate the mechanism.

2) The amplitude of the action potential was low and variable, reversal of membrane potential being seen very rarely. This may have been due to technical difficulties, for example in establishing the true value of the membrane potential, and the unknown amount of damage done to the cell might have caused a shunting of some of the potential difference across the membrane.

3) The duration of the action potential was considerably longer than that of skeletal muscle, but within the wide range observed in other smooth muscles (5 msec in guinea-pig vas deferens to 35 msec in rat pregnant uterus, measured at 50% spike height; 19 msec at 60% spike height in the present investigation).

4) The maximum rate of depolarization (0.9 V/sec) was considerably slower than that for striated muscle, and slower than in other smooth muscles (e.g. 7.6 V/sec in guinea-pig taenia coli; Bulbring and Kuriyama, 1963). Again, the unknown amount

of leakage round the electrode might have had a considerable effect. The repolarization phase had a faster maximum rate (1.4 V/sec) - this was noted in taenia coli by Bülbring and Kuriyama (1963) and by Gillespie (1962 and personal communication) in rabbit colon, and may be an important difference between the action potentials of smooth and skeletal muscles. Thus the slow rate of depolarization in smooth muscle might indicate that a limited number of sodium carriers are available, possibly due to inactivation by the low membrane potential, and the faster rate of repolarization might indicate a higher rate of potassium efflux than of sodium influx. There is as yet no evidence on which to build a working hypothesis.

5) The large positive after-potential was similar to that recorded from other smooth muscles e.g. taenia coli (Bülbring and Kuriyama, 1963) and uterus (Marshall, 1959). It probably represents the increased potassium permeability of the falling phase of the action potential driving the membrane closer to the potassium equilibrium potential.

Further investigation into the mechanism of the action potential is required. Thus investigations of the effects of changes in ionic environment on the shape of the action potential, of ion movements using radioactive tracers, and of ion currents using the voltage-clamp technique of Hodgkin and his co-workers (Hodgkin et al., 1952) should be made. Considerable technical difficulties due to the small size of the cells may be foreseen and may prevent the application of these techniques.

These electrical recordings may be compared with those

made for other vascular smooth muscles - in frogs (Funaki, 1958, 1960, 1961), turtles (Roddie, 1962), rats (Trail, 1963; Cuthbert et al., 1964; Cuthbert and Sutter, 1964; Funaki and Bohr, 1964), sheep (Keatinge and Richardson, 1963) and guinea-pigs (Speden, 1964). In the non-mammals, the activity recorded by Funaki has been described in the literature survey, and Roddie recorded plateau-type action potentials, often with a small prepotential, in turtle aorta and inferior vena cava, rather similar to cardiac action potentials. Plateau-type action potentials have also been recorded from earthworm blood vessel (Kuriyama et al., 1960) and with the sucrose-gap technique from sheep carotid artery (Keatinge and Richardson, 1963).

In mammalian vascular tissue, microelectrode studies on isolated rabbit anterior mesenteric vein (Cuthbert et al., 1964; Cuthbert and Sutter, 1964) gave membrane potentials averaging 33 mV, and action potentials of 19 mV with variable prepotentials and positive after-potentials. Their records of membrane potential showed slow waves which were not, however, described in the text. Funaki and Bohr (1964) using isolated rat portal vein recorded slow waves, amplitude some 20 mV and duration 5 to 10 sec, and action potentials of 8 to 30 mV with no overshoot lasting 50 to 100 msec. Using intact guinea pigs and recording from small mesenteric arteries, Speden (1964) observed action potentials, amplitude 17 to 42 mV and duration 22 to 107 msec at 50% spike height, occasionally overshooting and with fast repolarization and marked positive after-potentials. He recorded slow waves (duration not stated) which he attributed on the basis of their rhythmicity to movement artefacts arising from the

pulsing of the vessel. In each case, action potentials occurred at intervals, either singly or in small groups.

Thus in both the intact animal and the isolated preparation membrane potentials and action potentials of similar size and shape were recorded in mammals. The next step in this investigation was stimulated by the fact that in certain smooth muscles, e. g. ureter and intestine, motility and tone are based on myogenic activity, and aimed at studying the degree of dependence of the activity recorded on the innervation.



PART 4: DEGREE OF DEPENDENCE OF ELECTRICAL  
ACTIVITY ON NERVOUS SYSTEM



DEGREE OF DEPENDENCE OF ELECTRICAL ACTIVITY  
ON NERVOUS SYSTEM

INTRODUCTION

The electrical activity recorded in the small resistance vessels of the rat mesenteric circulation consisted of slow waves and action potentials; the frequency of the slow waves was very similar to that of Traube waves in blood pressure which were found by Iggo and Vogt (1960) to reflect a rhythmical efferent discharge of impulses in the preganglionic fibres of the cervical sympathetic of the cat, used as a measure of central sympathetic activity. This consideration led to the investigation into the degree of dependence of the electrical activity on nervous influences. Interruption of nervous pathways was attempted by the injection of autonomic blocking agents - the ganglionic blocking agent tetraethylammonium (TEA) and the peripheral adrenergic blocking agent dihydroergotamine (DHE) in dosages previously found to effect adequate blocking (Lloyd and Pickford, 1961). Surgical interference with the autonomic nervous system by cutting the splanchnic nerves was also attempted. The sympathetic nervous system was also excited by closing the tracheal cannula temporarily - this was shown by Adrian et al., (1932) and Iggo and Vogt (1960) to cause an increase in sympathetic efferent outflow. The effects of direct stimulation of the preganglionic sympathetic fibres of the splanchnic nerve will be described later.

RESULTSEffects of autonomic blocking agents:

Tetraethylammonium (TEA) (4 to 8 mg) was administered intravenously, and recordings made at intervals after the injection was washed in. In general, in an active preparation action potentials ceased during the first five minutes, and the average wave amplitude decreased. Occasionally in a preparation showing no spike activity there was no significant effect on the wave amplitude.

The results of one experiment where recordings were made for two hours after injection are shown in Figures 17 and 18. The graph in Figure 17 shows that spike activity ceased within the first five minutes, and reappeared two hours later. The slow wave frequency remained unchanged (10/sec) but the amplitude fell off markedly during the first 30 minutes from the average value before injection of 8.4 mV to so small as to be under the noise level of the electrode 35 minutes after injection, and then recovered slowly towards the original level. The records in Figure 18 show that the membrane potential was apparently lower 30 minutes after injection than during the period before injection, and in the final recording the potential was low, the waves irregular, and the action potentials variable. The same microelectrode was used for the entire experiment, and thus repeated penetrations might have caused breakage of the tip or blockage with cell protoplasm, so that the final recording could have been distorted to an unknown extent by leakage round the electrode through damaged membrane. Thus the small and gradual

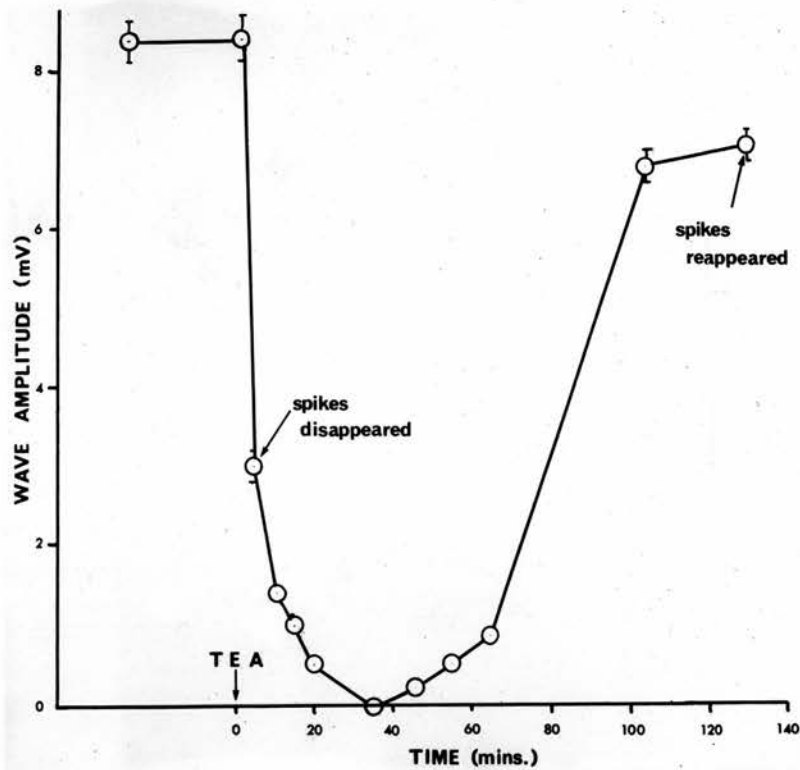
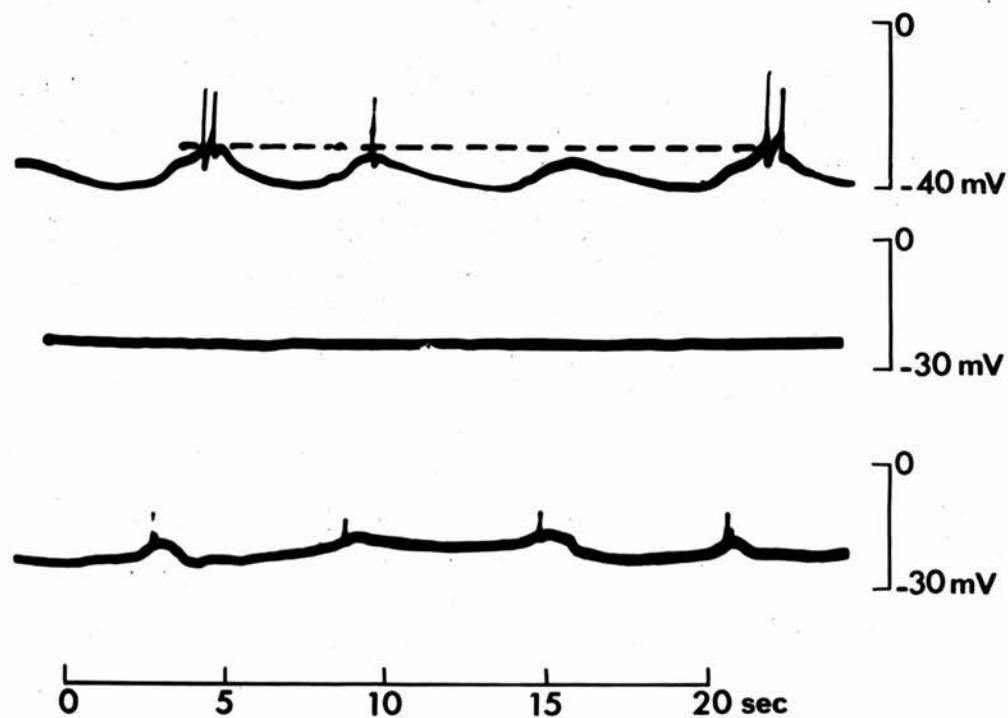


Fig. 17. Graph, derived from intracellular records, showing the effect of i. v. TEA on the smooth muscle cells in the walls of rat mesenteric vessels. Each point represents the mean of 12 observations from 3 cells at the indicated times in 1 animal; the vertical lines represent  $2 \times S.E.$  (the S. E. s of the points in the trough of the graph lie within the diameter of the circles). The differences between the means of the values obtained 0-80 min after injection were significant (Student t-test). The i. v. injection of TEA to similar rats generally caused an initial sharp fall in blood pressure (from about 125 to 90 mm Hg) and rise to about 100 mm Hg within 5-10 min, followed by a gradual rise to about 115 mm Hg after 2 hours.



**Fig. 18.** Transmembrane potential changes recorded from smooth muscle cells in the walls of rat mesenteric vessels, upper trace, before, middle trace, 30 min after, and lower trace, 2 hr after, i. v. injection of TEA.

depolarization that apparently occurred after T E A injection might have been due to damage of the recording electrode. The low membrane potentials in the recordings made  $1\frac{1}{2}$  to 2 hours after injection (25 to 28 mV) were undoubtedly due, in part at least, to membrane damage or blocking of the electrode. However, as the membrane potential fell gradually from the control of 39 mV to 35 mV ten minutes after injection, 30 mV after 20 min and 26 mV after 35 min, the possibility exists that the blocking drug caused a gradual depolarization of the muscle cells.

In general, injection of T E A was followed by temporary cessation of action potentials and decreased slow wave amplitude, and possibly some depolarization of the cell membrane.

Dihydroergotamine (DHE) (0.1 to 0.3 mg) was injected intravenously and recordings made at intervals thereafter. In this case electrical activity, both spikes and waves, ceased during the first five minutes after injection. Insufficient penetrations were made to determine whether or not any change in membrane potential occurred.

#### Effect of cutting the splanchnic nerves:

The greater splanchnic nerves were cut as they emerged from the psoas muscles and passed towards the kidneys; activity in the muscle cells was reduced - wave amplitude decreased (from  $4.0 \text{ mV} \pm 0.1$  to  $2.6 \text{ mV} \pm 0.4$ , means  $\pm$  S.E.,  $n=20$ ), spike frequency decreased, and the vessels dilated. The effect was much smaller than that produced by injecting autonomic blocking agents. This method would not result in complete denervation of the

smooth muscle cells as the lesser splanchnic nerve might have been left intact, and contributed some fibres through the coeliac and superior mesenteric plexuses to the walls of the blood vessels.

#### Effect of asphyxia:

There was no difference between the activity recorded from animals with chronic respiratory disease and that recorded from normal healthy animals. Some rats were subjected to acute partial asphyxiation by blocking the tracheal cannula. It was found difficult to keep an electrode inside a cell during asphyxiation as the movements of the animal generally broke the tip; extra-cellular recordings were therefore made. The slow waves invariably increased in amplitude without altering in frequency. The values recorded from one animal are shown in Table 4, where the wave amplitude increased from a control mean of 2.6 mV to 4.0 mV during asphyxiation. Average values obtained from seven different animals showed an increase in wave amplitude from 2.4 mV to 3.8 mV, with no significant change in wave duration. Figure 8 B and C show recordings made with an intracellular electrode before and after closure of the tracheal cannula; in 8 B the animal was deeply anaesthetized and the membrane potential stable at 50 mV, while in 8 C some 5 sec after closure of the cannula the maximum membrane polarization had fallen to 45 mV, and large slow waves had appeared.

#### Direct electrical stimulation of the blood vessels:

Attempts were made to stimulate the blood vessels directly using the syringe needle stimulator and positioning the recording

electrode 1 to 10 mm distant. Shocks of up to 50 V amplitude and duration up to 100 msec were applied, but no conducted action potentials were recorded. Stimulation was also applied through silver wire electrodes so allowing a larger number of cells to be stimulated, and recordings made 10 to 40 mm distant. Again, no action potentials were recorded, although localized muscle contraction could be observed microscopically.

Stimulation was also attempted by the application of transmembrane current through the recording microelectrode. The Wheatstone bridge circuit was used as described, the resistances being chosen so that currents of up to  $5 \times 10^{-8}$  amps could be applied (threshold for stimulating visceral smooth muscle was found to be approximately  $10^{-10}$  to  $10^{-9}$  amps by Nagai and Prosser (1963) and Kuriyama and Tomita (1964)). No action potentials were recorded.



DISCUSSION

The apparent inability to conduct a response to electrical stimulation might be a property of the smooth muscle or might be due to the stimulus parameters being unsuitable. Thus, Roddie (1962) observed responses conducted over 2 cm in turtle aorta and inferior vena cava when stimulated through an external microelectrode; pulses of 0.7 mA current strength and 100 msec duration produced a response, but the most suitable parameters were 0.4 mA and 2 seconds.

Conducted responses have been observed in other types of smooth muscle. Bülbring et al. (1958) recorded conducted responses in taenia coli when a large number of cells were stimulated; stimulation of a small number of cells caused a response conducted only over a few cell lengths. Burnstock and Prosser (1960) found that pig oesophagus, guinea-pig taenia coli, cat longitudinal and circular intestinal muscle, dog retractor penis, and rat ureter all gave conducted responses when stimulated electrically, minimum stimulus duration varying from 1 to 90 msec at maximum strength; they could, however, record no conducted action potentials from pig carotid artery or renal vein, or from cat nictitating membrane. Further investigations using different parameters of electrical stimulation are required before conclusions can be drawn as to the ability of the muscle of the small resistance blood vessels to conduct.

The unresponsiveness to intracellular stimulation with currents up to  $5 \times 10^{-8}$  amps again might be physiological or due to technical limitations. Nagai and Prosser (1963) using cat



circular intestinal muscle found that passage of transmembrane current caused membrane instability and occasional spikes at a threshold of  $0.2 \times 10^{-9}$  amps. Kuriyama and Tomita (1964) could record action potentials in response to depolarizing currents of  $10^{-10}$  to  $10^{-8}$  amps, but only in some 10 to 20% of cells in guinea-pig taenia coli (personal communication). On this basis it would be necessary to pass current into a large number of cells to determine whether any are excitable, and the apparatus used in these experiments would require modification to allow larger currents to be passed.

In the experiments using chemical means of interfering with the autonomic nervous system it was generally found that such interference resulted in a marked diminution of the electrical activity of the smooth muscle. Simultaneous recording of blood pressure would be required to establish whether or not the changes in electrical activity were paralleling pressure changes. However, the effects of TEA and DHE on rat blood pressure have frequently been studied (e.g. Lloyd and Pickford, 1961). The immediate response to TEA was found to be variable - a rise, a fall, or no change - probably depending on the dosage and speed of injection. An initial fall was invariably followed by a gradual rise and stabilization at a level only slightly lower than the original level, maintained for 4 to 5 hours. Thus the effect on electrical activity did not parallel the blood pressure effect, again indicating that the slow wave was not a mechanical artefact produced by pulsation of the vessels. The effect of DHE on blood pressure was to cause an initial abrupt fall followed by a return to slightly below the original level.

Recordings of sympathetic ganglionic activity and post-ganglionic discharge would be required to determine the extent and duration of the ganglion block produced by TEA. Pharmacological investigation has indicated (Ginsborg, personal communication) that the dosages of ganglion-blocking agents commonly used do not in fact stop the activity of all ganglion cells. This point requires further investigation before it can be decided whether or not the return of the electrical activity of the smooth muscle cells is in fact due to recovery of nervous activity or to some generating mechanism in the muscle cells recovering from an initial depression evoked by the abrupt removal of nervous influences.

Thus while it may be concluded that under the conditions of experiment the autonomic nervous system had a profound effect on the activity of the smooth muscle cells, these experiments provide no justifiable basis for deciding whether or not the cells are capable of generating electrical activity independently of nervous influences.

The problem of autonomic nervous control of vascular smooth muscle has been investigated recently from the anatomical viewpoint by, for example, Rhodin (1962) and Grigor'eva (1962). Grigor'eva, with light microscopy and methylene blue and silver staining, observed a dense plexus of non-myelinated fibres ending directly on the smooth muscle cells in both major arteries and smaller muscular vessels; the electron microscopic studies of Rhodin, using lead hydroxide or uranyl acetate staining, revealed no evidence of nerve fibres amongst the smooth muscle cells. These conflicting observations make it impossible to state

histologically whether vascular smooth muscle represents a multiple-unit system, extensively innervated and organized into motor units as is the case with the smooth muscle of the nictitating membrane, or a syncytial system, with the individual fibres in functional, if not protoplasmic, continuity with each other, as in visceral smooth muscle.

Physiological observations have produced equally conflicting results, but have indicated that the smooth muscle of the vascular bed does not form a homogeneous system. Thus Burnstock and Prosser (1960a) could record no conducted action potentials nor any response to stretch from the pig carotid artery or renal vein, and electron microscopic studies (Prosser, Burnstock and Kahn, 1960) showed that the muscle cells were widely separated by connective tissue. There is, however, physiological evidence that the smooth muscle in the peripheral arteries is capable of myogenic contractions - thus stretch of a resistance vessel by an elevation of its distending pressure has been found to cause an increased active contraction of the smooth muscle (Sparks and Bohr, 1962; Gordon and Nogueira, 1962).

These observations led to the view expressed by Bader (1963) that the muscle of the large elastic arteries formed a multiunit system, while that of the small muscular arteries in the periphery formed a syncytial system. In connection with this, it may be noted that two types of muscles have been described in the vascular wall - the tension muscles of Benninghoff (1927) which are connected to elastic fibres and membranes using them as tendons, and the ring muscles (Fischer, 1951) which are connected with each other. The smooth muscle of the aorta and

pulmonary artery is almost entirely composed of tension muscles, while the peripheral muscular vessels have ring muscles helically in the walls.

Folkow (1964) also concluded that the muscle of the large elastic vessels was multiunit in nature and that of the smaller muscular vessels syncytial. He further modified this hypothesis on the basis of histological observations showing that adrenergic nerve fibres apparently reach the outer surface of small arteries but do not penetrate within the muscle layer (Rhodin, 1962; Falck, 1962); and suggested that there might be an inner sheath of 'visceral' smooth muscle enclosed in an outer sheath of innervated 'multiunit' muscle. This ingenious theory extended the concept of functional differentiation within the vascular bed to differentiation within individual vessels.

Other investigations have produced conflicting results. Thus electrical activity has been recorded from isolated strips of vascular tissue - from turtle aorta and inferior vena cava (Roddie, 1962), from rat portal vein (Funaki and Bohr, 1964), and from anterior mesenteric veins of rabbit, rat, guinea-pig, and baboon (Cuthbert et al., 1964; Cuthbert and Sutter, 1964). Speden (1964) recorded activity from guinea-pig mesenteric arteries 'in situ' and found that splanchnic nerve stimulation evoked junction potentials which facilitated with each other to trigger action potentials, and that these action potentials were markedly different in shape from those discharged spontaneously; this led to the conclusion that the muscle was multiunit in type, every cell being innervated, but might also be capable of myogenic activity. In the present investigation on the resistance vessels

of the rat mesenteric circulation, junction potentials were not recorded in response to nerve stimulation (this might have been due to the technique employed) and the response appeared to be a prolonged enhancement of the basic activity, the action potentials being unchanged in shape.

In view of these conflicting observations, it would be dangerous to draw conclusions as to the multiunit or syncytial nature of the smooth muscle. In the vessels investigated under the conditions of experiment, the muscle appears at least to be strongly influenced by the sympathetic nervous system.

Smooth muscles form a broad spectrum of functional types, from those completely dependent on their innervation e. g. nictitating membrane to those with inherent rhythmicity e. g. intestinal muscle. It is possible that some types of smooth muscle occupy a middle position in this range, being capable of myogenic activity but normally being strongly influenced by nervous activity.

PART 5: EFFECT OF NERVOUS AND HUMORAL STIMULATION  
OF THE BLOOD VESSELS

EFFECT OF NERVOUS AND HUMORAL STIMULATION OF THE  
BLOOD VESSELS

General Introduction:

Until recent years, the responses of vascular smooth muscle in the mesenteric circulation to nervous and humoral stimulation were studied indirectly using a variety of different techniques, all of which gave results which are difficult to interpret due to the limitations of the methods (see Grim, 1963 for references).

Two methods involving direct observation have been used. The rat mesoappendix preparation of Chambers and Zweifach (1944) has been used for observing changes in arteriole diameter in response to a variety of agents (Bohr et al., 1955), and histological examinations of quick-frozen sections of rat gastric mucosa (Schnitzlein, 1957) have been made. However, although these methods showed changes in vessel diameter, they cannot be interpreted as indicating active changes in the smooth muscle.

Many different methods, both direct and indirect, have been used to observe changes in blood flow. Indirect methods involved measurement of plethysmographic volume of intestinal segments (e.g. Burn and Hutcheon, 1949), weight measurements (e.g. MacLean et al., 1956), and thermoelectric techniques (Grayson, 1951). Such methods produced conflicting results - for example, MacLean found that adrenaline caused a decrease in intestinal weight, which he interpreted as due to a decrease in blood flow caused by a vasoconstrictor effect, whereas Burn and Hutcheon found that adrenaline increased the intestinal volume, interpreted

by them as indicating a vasodilator effect. Direct measurements of blood flow have been carried out by many observers (e. g. Burton-Opitz, 1912), and combined with pressure recordings to allow calculation of vascular resistance (e. g. Deal and Green, 1956). All these techniques gave information about the behaviour of the mesenteric circulation as a whole, a complex situation further complicated by the use of intact animals in most cases. Interpretation of results is thus even more difficult, as the blood vessels are influenced by several uncontrolled variables, including humoral and nervous agents and purely passive effects. Moreover, respiratory movements, both thoracic and abdominal, would cause alterations in intestinal blood flow, as would variations in the motility and tonicity of the intestinal muscle itself. Thus the conflicting results with adrenaline might be explained by the fact that its relaxing effect on the intestinal smooth muscle might decrease the transmural pressure in the blood vessels sufficiently to overcome the direct vasoconstrictor effect, so resulting in an increase in intestinal volume rather than a decrease.

These considerations led several investigators (e. g. Bean and Sidky, 1958) to use isolated perfused preparations, measuring blood flow and pressures, and intestinal motility and tone, in an attempt to separate the blood flow changes caused passively by intestinal activity from those caused by alterations in the blood vessels themselves. However, perfusion pressures tend to be abnormally low in these preparations, and their results must be considered within this limitation.

Folkow and his co-workers (Folkow et al., 1963) evolved a



technique for study of the splanchnic vasculature, eliminating extrinsic influences as far as possible, and measuring simultaneously resistance changes in both pre- and post-capillary sections, capacitance changes, and net movement of fluid across the capillary bed. This allows behaviour of different parts of the vascular bed to be studied, but still does not give information about the behaviour of the smooth muscle.

Interpreted with due regard to their limitations, these studies indicate that administration of catechol amines, adrenaline and noradrenaline produces vasoconstriction in the intestine.

NERVOUS STIMULATION:Introduction:

Investigations of the effects of nerve stimulation on vascular smooth muscle have yielded conflicting results, and have been very difficult to interpret. Methods used for recording vascular responses have been highly inaccurate - e.g. Bulbring and Burn (1936) found that splanchnic nerve stimulation resulted in an increase in plethysmographic volume of an intestinal loop, and concluded that the nerve carried vasodilator fibres; Folkow et al., (1948) obtained a similar response in the ergotaminized cat, but concluded that this was due to variations in peripheral vascular resistance caused by relaxation of intestinal muscle. However, even where the actual vascular response is being measured, this will give very little information as to the mechanism of action of the controlling neurons, as these responses are sluggish and do not reflect the behaviour of individual neurons. Moreover, interpretation of the effect of stimulating preganglionic nerve fibres (as in the splanchnic nerve) must take into account the effect of the ganglion cells themselves. Recordings by Bronk (e.g. Bronk, 1939) from postganglionic sympathetic fibres while stimulating preganglionic nerves indicate the occurrence of overlap, subliminal fringe, occlusion and recruitment within the ganglia, although they had no evidence to show how the presumed integrative activities worked. This evidence that transmission across ganglia does not simply involve a presynaptic nerve impulse giving rise to a brief release of transmitter and brief

action of a single ganglion cell is supported by direct observations on ganglion cells (Laporte and Lorente de Nó, 1950; R. M. Eccles, 1952) showing that preganglionic volleys evoke a complex series of potential waves in ganglion cells. Application of results obtained by such methods to the normal situation is further complicated by the fact that all observations have been made on the anaesthetised animal so that nervous compensatory mechanisms active in the conscious animal may be absent or distorted.

It has been found that the physiological discharge frequencies in sympathetic nerves are very low. Bronk et al., (1936) recording the thoracic cardiovascular outflow in the cat found a 'tonic' discharge frequency of 2 to 3/sec, increasing during asphyxia to 10 to 15/sec with maximal vasoconstrictor and cardioaccelerator responses at this frequency. Iggo and Vogt (1960) recorded efferent discharge from fine strands of the cat cervical sympathetic (preganglionic fibres) and found a low frequency resting discharge, often less than 1/sec, with a maximum rate of 30/sec and then maintained only for a few impulses. Folkow et al., (1964) found that stimulation of the cat splanchnic nerve gave definite vascular responses at frequencies of 1/sec or less, 'normal' constrictor tone at 1 to 2/sec, and maximal effects at 4 to 10/sec; however, these responses to synchronous volleys of impulses sent down the nerves cannot be compared to the physiological situation where the sympathetic outflow is intermittent and asynchronous in nature.

#### Results:

Stimulation of the cut peripheral end of the right splanchnic

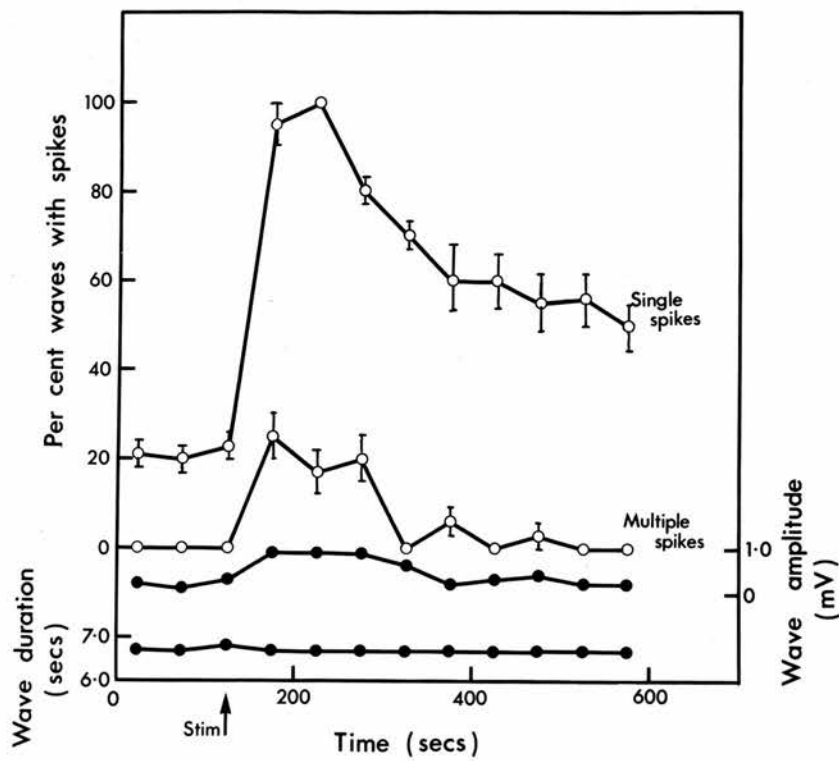
nerve with brief trains of pulses evoked a very marked and complex response in the vascular smooth muscle. It was found impossible to keep an electrode inside a cell to record the response, as contraction of the whole vessel occurred either dislodging the electrode or breaking the tip. Recording was therefore done with an electrode of 1 to 3 $\mu$  diameter pressed close to the vessel. The types of electrical activity recorded, slow waves and action potentials, have already been described.

The stimulus frequencies used (5/sec and 10/sec) were considered to be within the 'physiological range'; stimulus strengths (5 and 10 V) were chosen to give a marked but not extreme vasoconstriction; and pulse duration was 1 to 2 msec.

The results are shown in Figures 19 to 25.

It was found that short bursts of stimuli, lasting 3 to 5 sec, caused an immediate and prolonged increase in the muscle activity; however, the duration of the slow waves did not change. There was an immediate increase in the percentage of waves giving rise to spikes, both single and in pairs, and with strong stimulation spikes occasionally occurred in groups of three during the first 100 sec. Figure 19 shows the effect of a 5 sec burst of 5 V shocks at 10/sec. The number of action potentials (19 B) reached maximum in the first 50 sec, and gradually declined towards the resting level during the next 250 sec. Waves with two spikes (19 A) occurred in the first 150 sec, and only rarely after this. The amplitude of the slow waves increased from 0.2 to 0.9 mV during the 150 sec after stimulation, and then returned to normal during the next 100 sec. The duration of the slow waves remained unchanged.

A



B

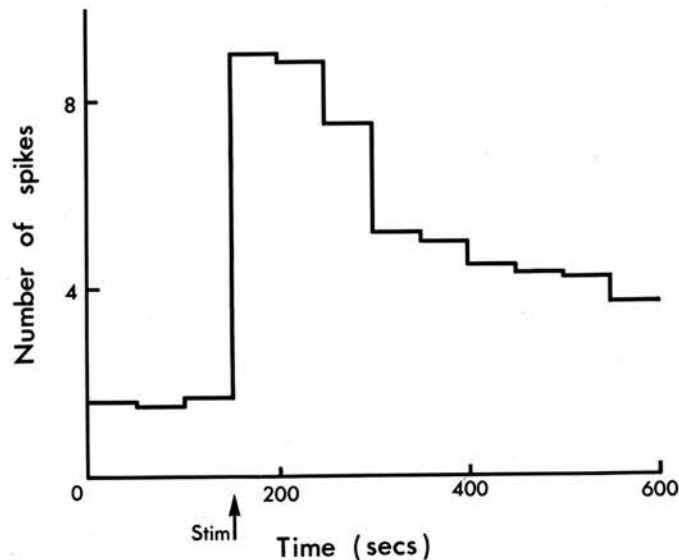
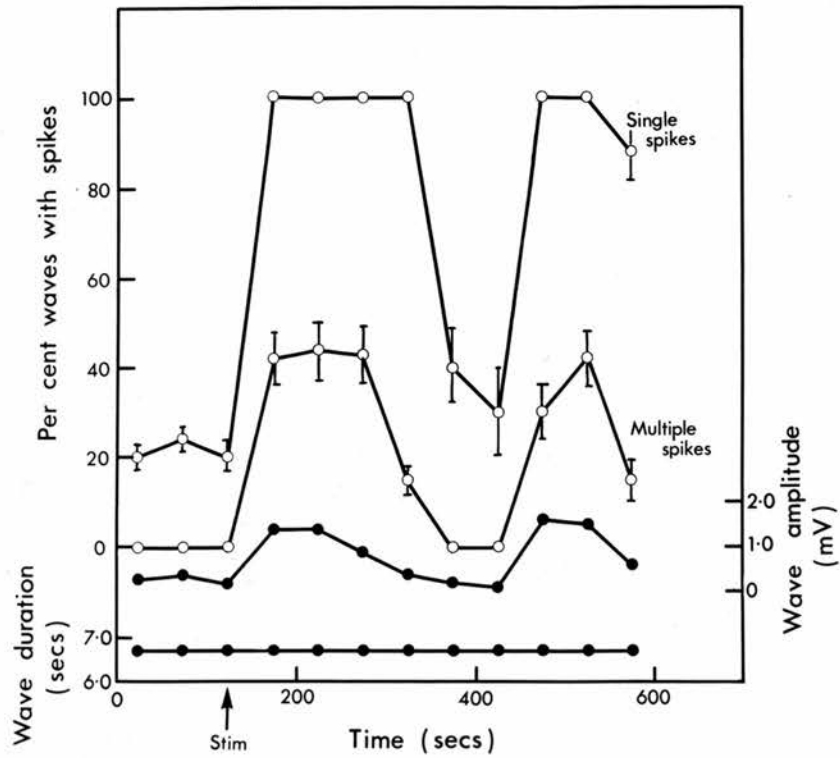
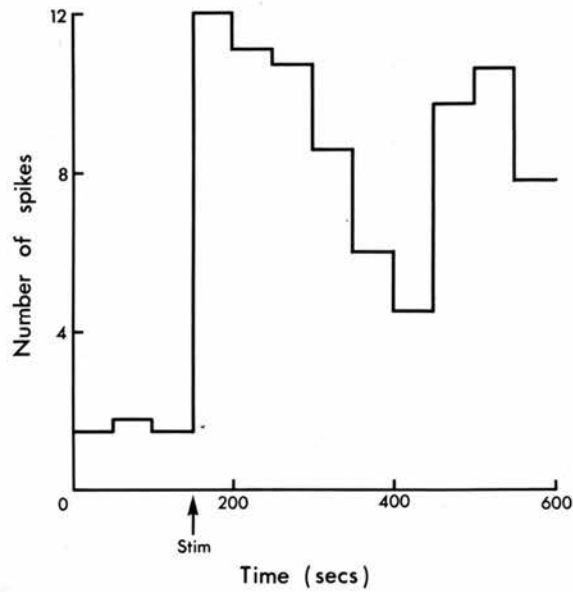


Fig. 19. Graphs, derived from extracellular records, showing the response of the smooth muscle cells to stimulation of the right splanchnic nerve with a 5 sec burst of 5 V shocks at 10/sec. Measurements were made over 50 sec periods, and the results obtained from 3 animals (4 observations each) pooled. Each point represents the mean, and the vertical lines represent  $2 \times S.E.$  A, the percentage of waves with spikes (open circles), and the amplitude and duration of the slow waves (filled circles); B, the total number of spikes. (The slow wave duration remained constant, see text).

After a 5 sec burst of 10 V shocks at 10/sec a dual effect was observed, as shown in Figure 20. Action potential discharge (20 B) increased markedly during the first 150 to 200 sec, then declined rapidly to rise again to a second peak after 300 sec, falling off again after 400 sec. The effect on wave amplitude and on the appearance of multiple spikes followed a similar time course (20 A). In an attempt to establish whether the release of catechol amines by the adrenal medulla stimulated through its splanchnic preganglionic nerve supply was involved in this response, four experiments were carried out with the right adrenal vein occluded. Figure 21 shows the average response, and it would appear that this had one peak and a slow decay. However, the scatter of individual observations was so great in each case that the difference between the responses with or without the right adrenal vein was not significant (significance of differences of means established using the Student t-test for small samples, arriving at a value of about 0.05 for P, which cannot be regarded as being significant).

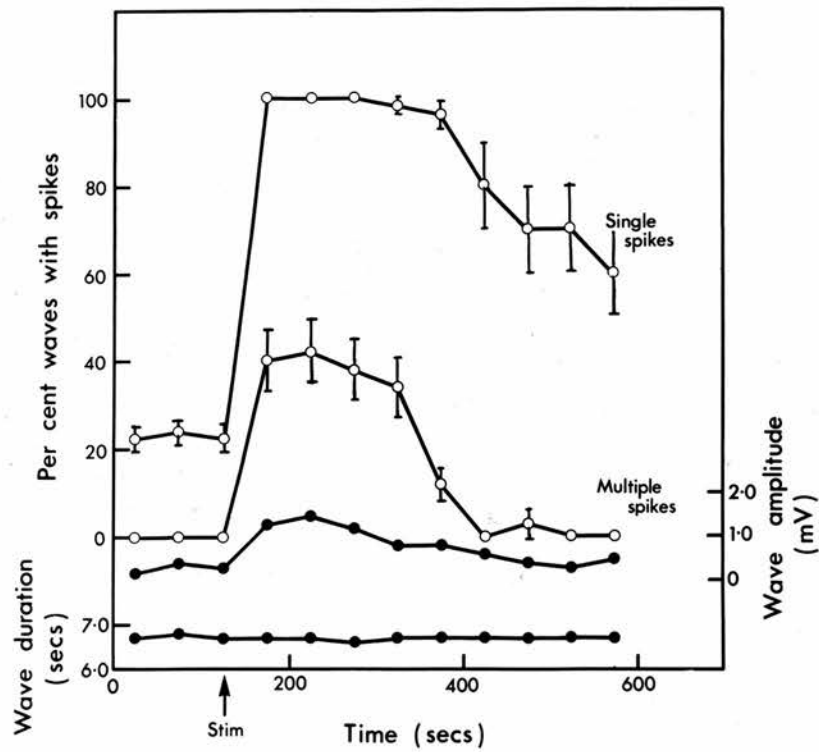
When an initial train of 10 V shocks was followed after 150 sec by a second series, the number of action potentials discharged increased in response to the second train of stimuli, due to the appearance of more triplets of spikes. Figure 22 A shows that the percentage of waves giving rise to spikes remained at a high level after the second stimulation, but the appearance of more triplets is indicated in Figure 22 B where the actual number of spikes is plotted. Figure 23 compares the responses to two trains of stimuli with the response to one of the same strength and frequency.

**A****B**

**Fig. 20.** Graphs showing changes in electrical activity in vascular smooth muscle in response to stimulation of the right splanchnic nerve with a 5 sec burst of 10 V shocks at 10/sec. Response was plotted as in Fig. 19.



A



B

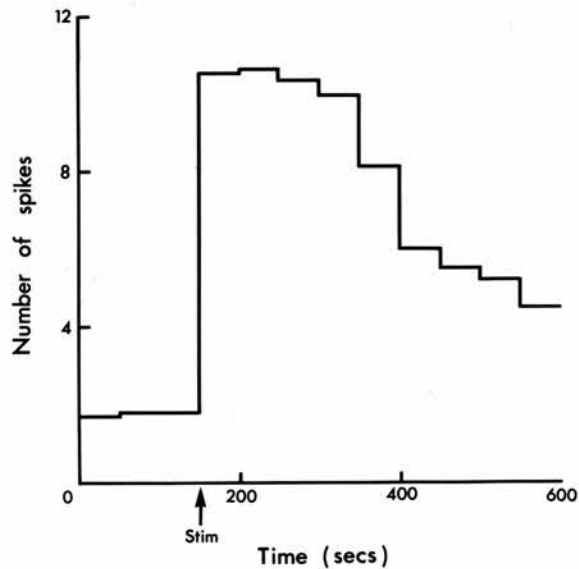
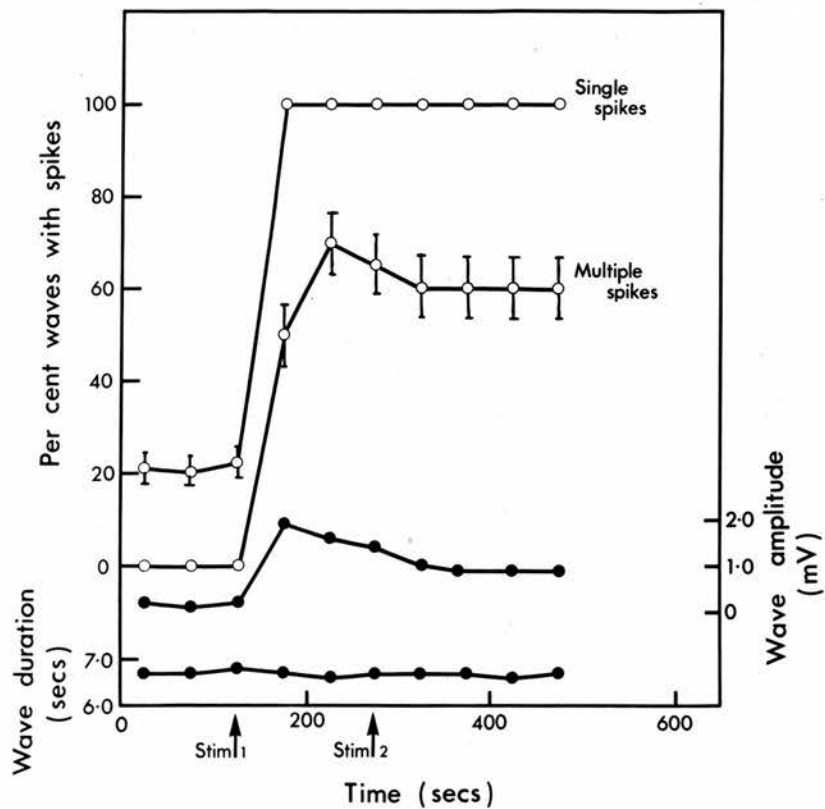
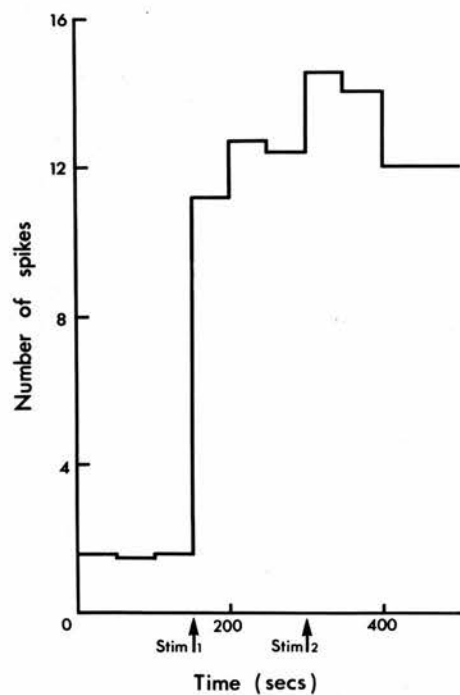


Fig. 21. Graphs showing changes in electrical activity in vascular smooth muscle in response to stimulation of the right splanchnic nerve with a 5 sec burst of 10 V shocks at 10/sec. The right adrenal vein was occluded. Response was plotted as in Fig. 19.



**A****B**

**Fig. 22.** Graphs showing changes in electrical activity in vascular smooth muscle in response to stimulation of the right splanchnic nerve with two 5 sec bursts of 10 V shocks at 10/sec. Response plotted as in Fig. 19.

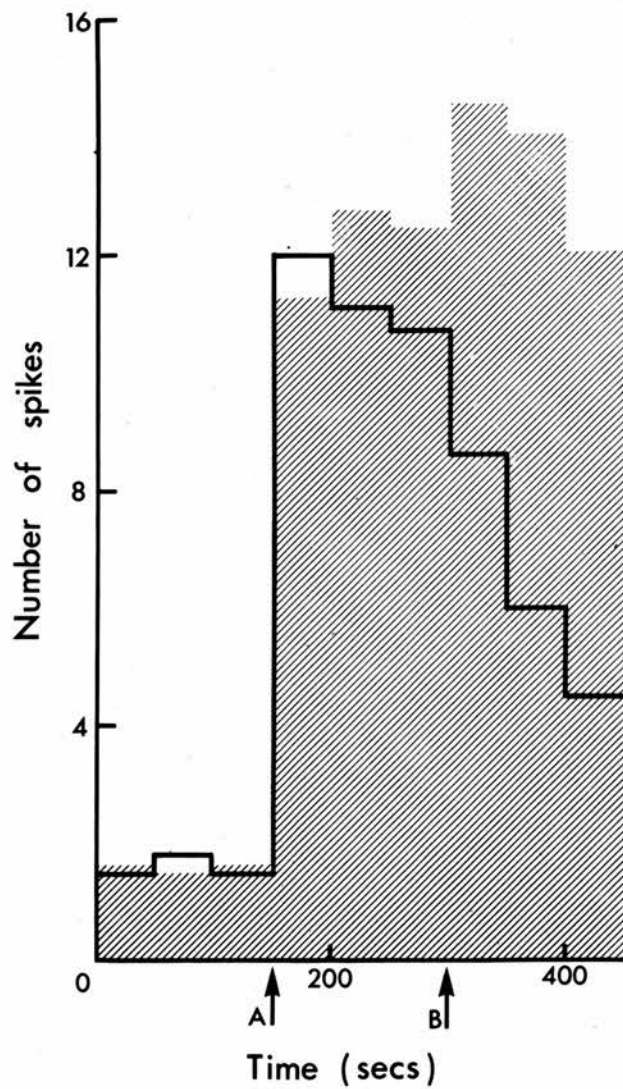


Fig. 23. Plot comparing the number of action potentials recorded from vascular smooth muscle in response to a single burst (heavy line) and two bursts (hatched area) of 10 V shocks at 10/sec.

Figures 24 and 25 summarize the effects of different strengths and frequencies of stimulation on the number of spikes recorded. Figure 24 shows that increasing the stimulus strength increased the response - 12 spikes occurred in the first 50 sec after a burst of 10 V stimuli compared to 9 after stimulation for the same period of time and at the same frequency but at 15 V - and the responses appeared to decay at corresponding rates. In Figure 25 stimulation at 10/sec evoked 12 spikes in the first 50 sec, while stimulation at 5/sec evoked almost 11.

Thus at a given stimulus frequency increasing the strength of stimulation increased the response, and changing the frequency at a constant stimulus strength had a similar effect. However, the initial response to 10 V stimulation at 5/sec was greater than that to 5 V at 10/sec - the possible significance of this will be discussed later.

#### Discussion:

A brief train of stimuli appeared to evoke a very marked and prolonged effect in the vascular smooth muscle cells. The response was apparently an enhancement of the electrical activity already present - an increase in amplitude of the slow waves without any change in their frequency, and the appearance or increase in frequency of action potentials.

All cells from which recordings were made showed increased activity in response to nerve stimulation, indicating that every cell was capable of being influenced by nervous effects. However, this does not necessarily imply that every muscle cell was

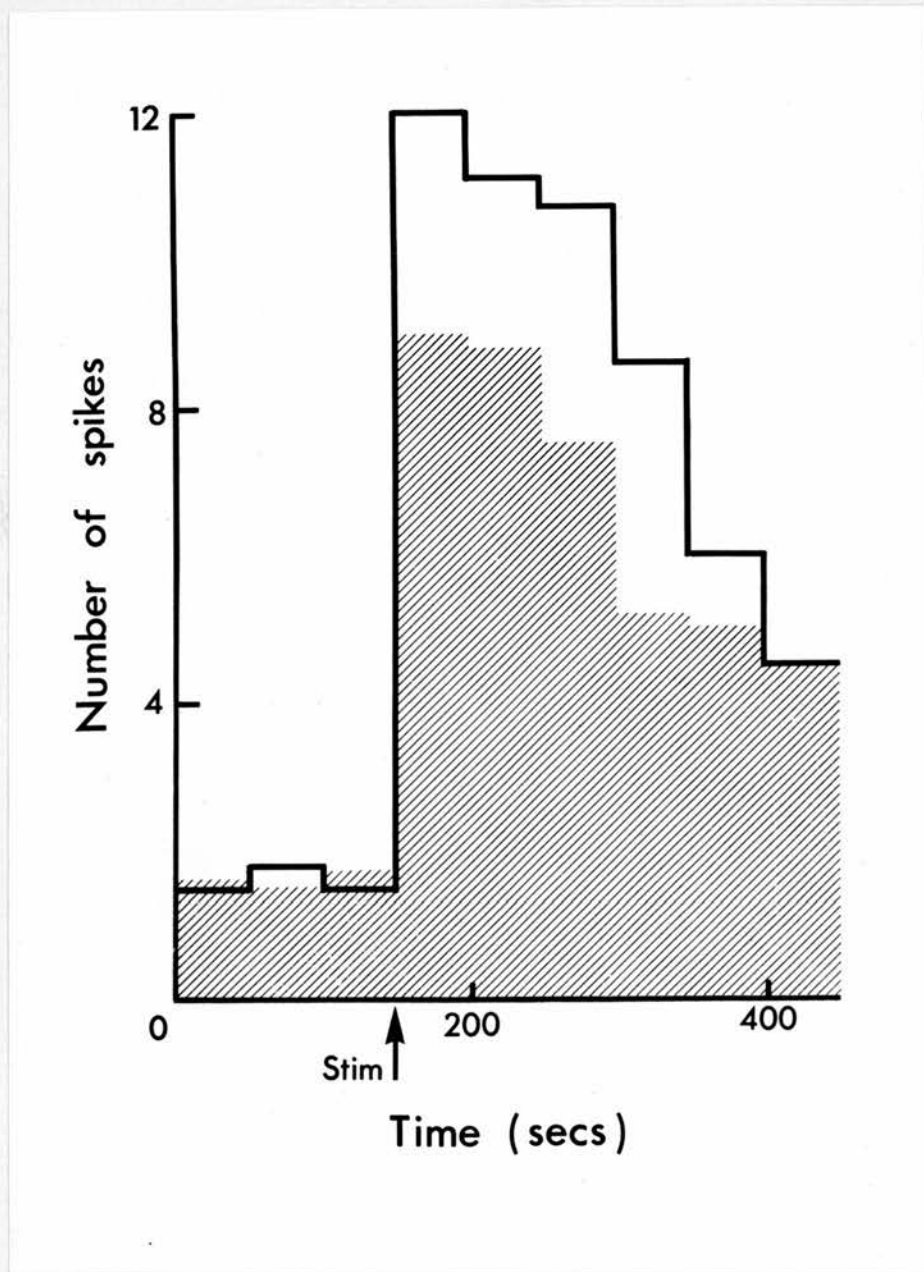


Fig. 24. Plot comparing the number of action potentials recorded from vascular smooth muscle in response to 5 sec bursts of 10/sec shocks, at strengths of 10 V (heavy line) and 5 V (hatched area).

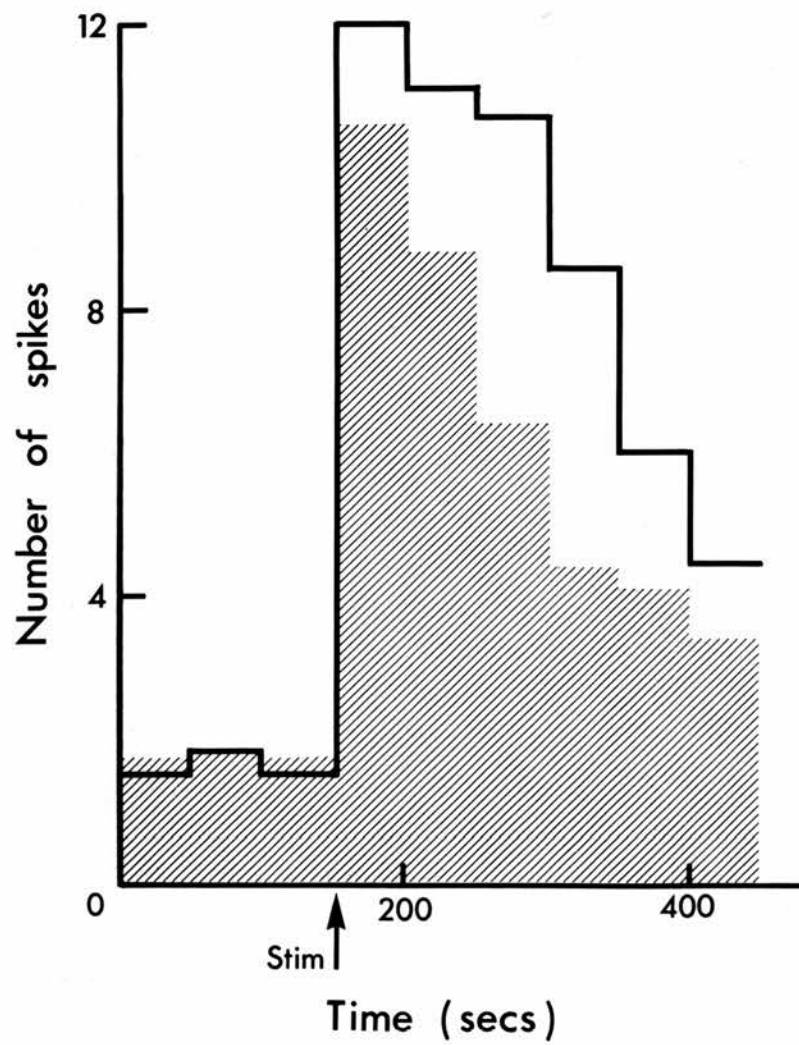


Fig. 25. Plot comparing the number of action potentials recorded from vascular smooth muscle in response to 5 sec bursts of 10 V shocks, at frequencies of 10/sec (heavy line) and 5/sec (hatched area).

directly innervated as the influence might be indirect, transmitter released from one neuron affecting several cells, or some cells being stimulated by spread of excitation from adjacent cells. As already described, histological studies have produced conflicting results on this problem of innervation.

In connection with this, a more detailed study of the effects of single shocks on the muscle needs to be made. In the present series of experiments it was found impossible to keep an electrode inside an already active cell during stimulation of the nerves, and no junction potentials were observed. Excitatory junction potentials (EJPs) have been recorded with intracellular electrodes from the smooth muscle of the dog retractor penis in response to stimulation of its sympathetic nerve (Orlov, 1962); from the guinea-pig vas deferens in response to stimulation of the hypogastric nerve (Burnstock and Holman, 1961; Kuriyama, 1963; Ferry, 1963) and from small mesenteric arteries of the guinea-pig in response to stimulation of the splanchnic nerve (Speden, 1964). In all these cases nerve stimulation gives rise to EJPs which are capable of facilitating with each other until the membrane is sufficiently depolarized to initiate an action potential and contraction.

Che Su and his co-workers (Che Su et al., 1964), recording intracellularly from the isolated rabbit pulmonary artery while stimulating its sympathetic supply, have reported that stimulation evoked no action potentials and only occasional variable fluctuations in membrane potential; however, insufficient information was given to enable a critical assessment of these results to be made.



The finding that removing the influence of the adrenal medulla by tying off the adrenal vein made little difference to the response is in line with the observation of Celander (1954) that the vascular changes produced by sympathetic nerve stimulation were 10 to 20 times greater than those elicited purely by the adrenal catechol amines secreted as a result of a corresponding stimulation of the splanchnic nerve fibres supplying the adrenal gland. This, together with the findings of Folkow (Folkow et al., 1964) that the pattern of responses of the various sections of the intestinal vascular bed to splanchnic nerve stimulation was the same in the presence or absence of adrenal medullary secretion, suggests that vasomotor control from the central nervous system may be dominated by the neural component of the sympathetico-adrenal system. It is, therefore, possible that the direct effect of the splanchnic nerves on the smooth muscle is much more significant in central vasomotor control of the mesenteric circulation than is the indirect effect of catechol amines secreted by the adrenal medulla.

Interpretation of the effects of varying strength and frequency of preganglionic stimulation is difficult due to the organization of the ganglia. The increased response of individual cells due to increased stimulus strength may occur as a result of an increased frequency of discharge of previously active post-ganglionic fibres, or of discharge of a larger number of post-ganglionic fibres which would indicate that single cells might be influenced by more than one nerve fibre. Investigations of the effects of postganglionic nerve stimulation would be required to clarify this point. However, the fact that the initial response to

10 V stimulation at 5/sec (10.6 spikes in the first 50 sec - Figure 25) was greater than that to 5 V stimulation at 10/sec (9 spikes in the first 50 sec - Figure 24) might indicate that increasing the strength of preganglionic stimulation altered not only the frequency of postganglionic discharge but also the number of active fibres, and that each smooth muscle cell was capable of being influenced by more than one postganglionic nerve fibre.

Any interpretation of these complex responses to brief trains of stimuli applied to the preganglionic fibres of the splanchnic nerve must be limited by the fact that the smooth muscle cells were under the influence of the untouched left splanchnic nerve, of the lesser splanchnic nerves, and of various humoral agents including the secretions of the adrenal medulla. Thus the cells might be affected reflexly from lumbar afferents in the region of stimulation and from receptors in the splanchnic region.

Moreover, the stimuli were applied to preganglionic nerve fibres, and the functional organization of the ganglia might have exerted a considerable modifying influence. Thus, due to the fact that many preganglionic fibres may converge on one ganglion cell, an increase in stimulus strength with activation of a larger number of preganglionic fibres may lead not only to an increase in the number of active postganglionic fibres, but also to an increased impulse frequency in already active fibres. On the other hand, increasing the frequency of preganglionic stimulation may lead to the activation of a greater number of ganglion cells by recruitment from the subliminal fringe as well as increasing the discharge frequency of active cells. In addition, the functional organization at the cellular level is such that the arrival of a single impulse at



the terminal of a preganglionic nerve fibre has a complex action on the ganglion cell. The complex potential waves recorded in response to preganglionic stimulation (R. M. Eccles, 1955) have been further investigated by R. M. Eccles and Libet (1961) using a number of selective blocking agents. They postulate that the acetylcholine released at the preganglionic terminals acts at two receptor sites on the ganglion cell, and in addition acts on chromaffin cells present in the ganglion, releasing adrenaline which then acts at a third receptor site on the ganglion cell.

Thus when a preganglionic nerve is stimulated electrically a volley of impulses will reach the ganglion simultaneously (a highly artificial situation) and may be reorganized considerably while crossing the ganglionic synapses.

#### Conclusions:

It would appear that preganglionic sympathetic stimulation enhances the tonic activity of the smooth muscle, the duration of the effect long outlasting the duration of the stimulus. The magnitude of the response could be altered by increasing the frequency or strength of the stimulus, and there were indications that this was accompanied, although not paralleled, by an increase in frequency and number of active postganglionic nerve fibres. With the parameters of stimulation used, the responses did not appear to involve the adrenal component of the sympathetico-adrenal system.

## HUMORAL STIMULATION

### Results:

#### The action of adrenaline:

##### a) Intravenous injection

When injected into the tail vein of the rat in a dose (0.1 to 0.5  $\mu$ g) sufficient to produce a marked rise in blood pressure adrenaline did not produce a single repeatable effect on the muscle of the mesenteric vessels. In the main, there was no significant change in membrane activity, although occasionally spike frequency was either enhanced or depressed for a short period of time. Thus in one experiment where the muscle cells had been inactive, sending off one spike in some thirty to forty waves, injection of adrenaline was followed after about 20 seconds by a group of four waves each giving rise to a spike, after which the muscle reverted to its previous state of inactivity. No visible constriction of the vessel was seen, and no significant change in the duration of the slow wave occurred (t-test).

##### b) Local application

The diluted solutions were applied to the exterior of the blood vessels through a microelectrode with the tip broken off, and the Krebs fluid was dropped on at a very slow rate to prevent the adrenaline being washed away rapidly. It was found necessary to bring the tip of the electrode containing the adrenaline solution very close to the recording electrode before any response could be recorded. Adrenaline was dropped on to the blood vessel in four concentrations -  $10^{-9}$ ,  $10^{-8}$ ,  $10^{-6}$ , and  $10^{-3}$  g/ml, and a period of

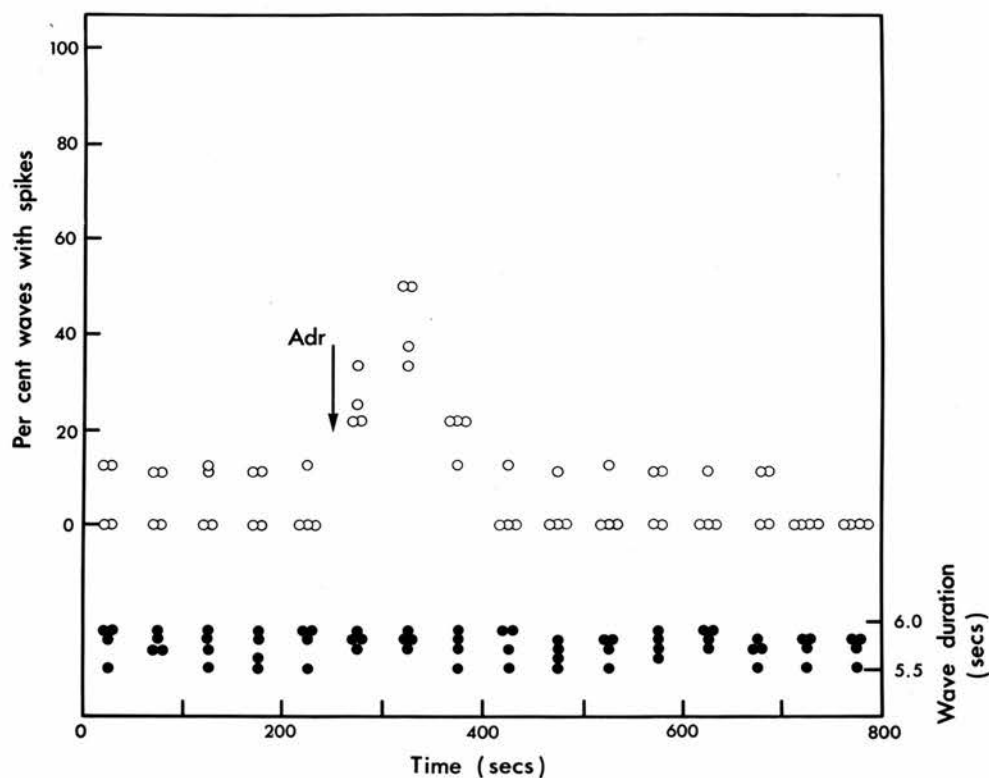
several hours allowed to elapse between successive administrations. In each case the animal was deeply anaesthetized, and very infrequent, if any, action potentials were recorded. The results are shown in Figures 26 to 28.

In no experiment did the  $10^{-9}$  solution cause any increase in activity or visible constriction. Threshold concentration for an electrical effect was found to lie between  $10^{-9}$  and  $10^{-8}$  g/ml, although no constriction was observed. The effect of a  $10^{-8}$  solution (results obtained from four different animals) is seen in Figure 26. Spike activity rose during the 100 sec after application, and then fell rapidly back to normal during the period 100 to 150 sec. Closer examination of the first 50 sec period showed that there was a latency of 20 to 30 sec before the effect started. The duration of the slow waves remained unchanged.

Application of a  $10^{-6}$  solution caused visible constriction of the vessel and a very great increase in action potential discharge after a latency of 10 to 30 sec, from a baseline of 5.4% active waves to a maximum with every wave giving rise to spikes in the period of 100 to 150 sec after application (Figure 27). This again rapidly fell to the original level at the end of the period 150 to 200 sec. At this concentration the adrenaline also caused the appearance of pairs of spikes (Figure 27 A). About 25% of the waves in the first 50 sec gave rise to two spikes, reaching a maximum of 50% in the period 100 to 150 sec.

Observations on the effect of the  $10^{-3}$  g adrenaline/ml solution were unexpected. The vessels were observed to constrict vigorously, but only rarely was spike activity recorded. On one occasion two spikes occurred about 10 to 20 sec after the adrenaline

A



B

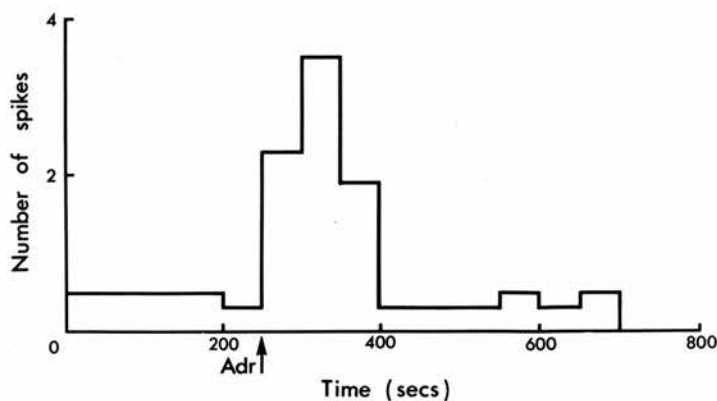
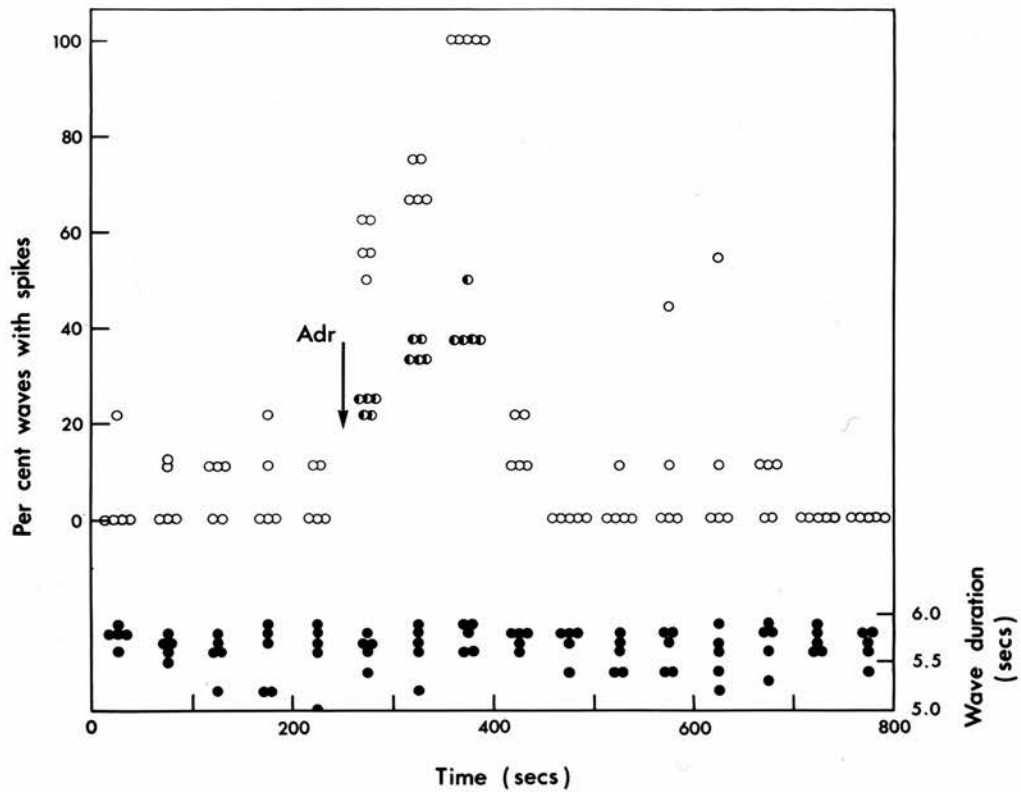


Fig. 26. Graphs, derived from extracellular records, showing the response of vascular smooth muscle to the application of adrenaline ( $10^{-8}$  g/ml). Measurements were made over 50 sec periods. A, the percentage of waves with spikes (open circles) and the duration of the slow waves (filled circles) - each circle represents measurements made from one animal; B, a histogram showing the total number of spikes, obtained by pooling the results from 5 different animals and plotting the mean values.

A



B

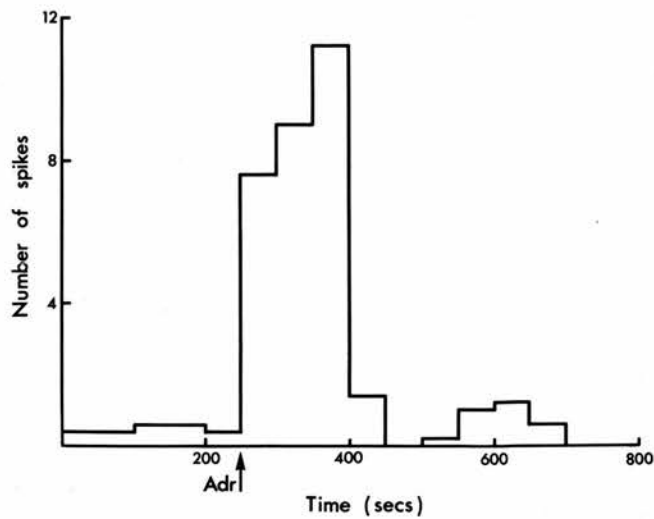


Fig. 27. Graphs showing the response of vascular smooth muscle to the application of adrenaline ( $10^{-6}$  g/ml). A, the percentage of waves with single spikes (open circles) and double spikes (half-filled circles), and the duration of the slow waves (filled circles); B, the total number of spikes. Individual observations are plotted.

application, but no further spike activity was recorded. No consistent effect on wave amplitude was recorded. Interpretation of this result must take into consideration the fact that although the recording microelectrode was lowered in an attempt to maintain contact with the constricting vessel, the decrease in size of the smooth muscle cells may have broken this contact.

One observation with the electrode dimpling the surface of the vessel showed a slow depolarization in a previously steady base-line. This may reflect a membrane depolarization. Further experiments will be required to establish whether or not the electrode maintained contact with the cells.

In all cases, even at the point of maximal stimulation, the spikes arose at fairly regular intervals of 5.0 to 5.9 sec. Statistical investigation of this inter-spike distance, or duration of the slow wave in the potential record, showed no significant difference between rest and at the peak of activity. Detailed investigation of activity within the first 50 sec period showed no significant change in wave duration (Student t-test).

Figure 28 summarizes the effects of varying concentrations of adrenaline on the spike activity, and shows that the response to the  $10^{-6}$  solution (mean of 5 experiments) was much greater than, although lasting for about the same time as, the response to the  $10^{-8}$  solution (mean of 4 experiments), while the  $10^{-9}$  solution (mean of 5 experiments) was ineffective.

The action of noradrenaline:

The response to intravenous L-noradrenaline was variable.

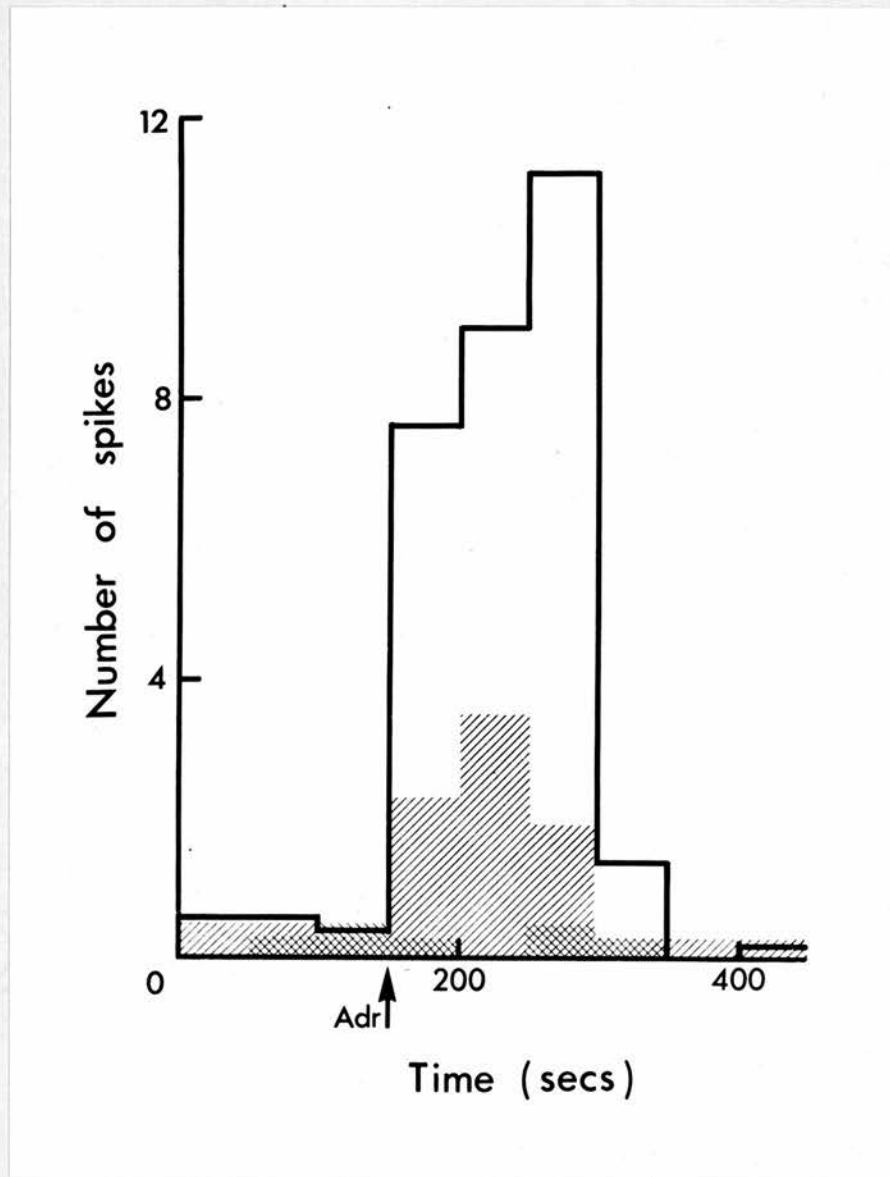


Fig. 28. Plot comparing the number of action potentials recorded from vascular smooth muscle in response to adrenaline in three concentrations,  $10^{-6}$  g/ml (heavy line),  $10^{-8}$  g/ml (hatched area), and  $10^{-9}$  g/ml (cross-hatched area).



In general, locally-applied noradrenaline had a similar effect to adrenaline, although apparently extending over a wider area, causing increased electrical activity and constriction of the vessels. The threshold concentration was rather variable -  $10^{-10}$  to  $10^{-8}$  g/ml - but at  $10^{-6}$  g/ml there was visible vasoconstriction and marked increase in the discharge of action potentials, as shown in Figure 29. As with adrenaline, some waves gave rise to two spikes at the higher concentrations, although the wave duration remained unaltered. Figure 30 compares the total number of action potentials recorded in response to  $10^{-6}$  and  $10^{-9}$  g noradrenaline/ml solutions (mean of 5 experiments in each case) - the  $10^{-9}$  solution had no effect, but the  $10^{-6}$  solution increased the discharge of spikes, from 0.5 in 50 sec previously to 10.5 in the period 50 to 100 sec after application. As with adrenaline, with high concentrations ( $10^{-3}$  g/ml) no spikes were recorded although marked vasoconstriction could be observed.

#### The action of acetylcholine:

No consistent effect was observed after intravenous injection. With local application, no significant effect was obtained until a concentration of  $10^{-4}$  or  $10^{-3}$  g/ml was applied. All spike activity was abolished after a latency of 10 to 30 sec. In deeply anaesthetized animals where there were no 'spontaneous' action potentials, acetylcholine had no measurable effect on the potentials recorded extracellularly. There was no indication as to whether or not any change in membrane potential occurred.



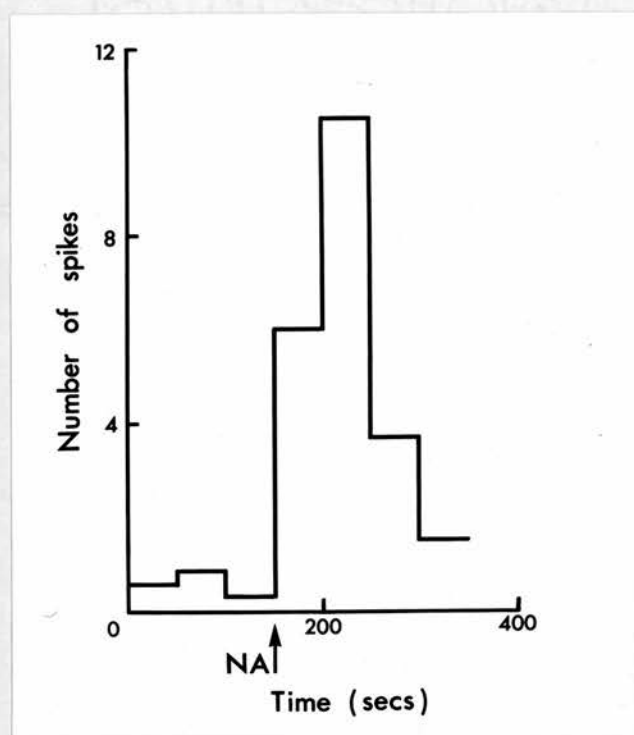
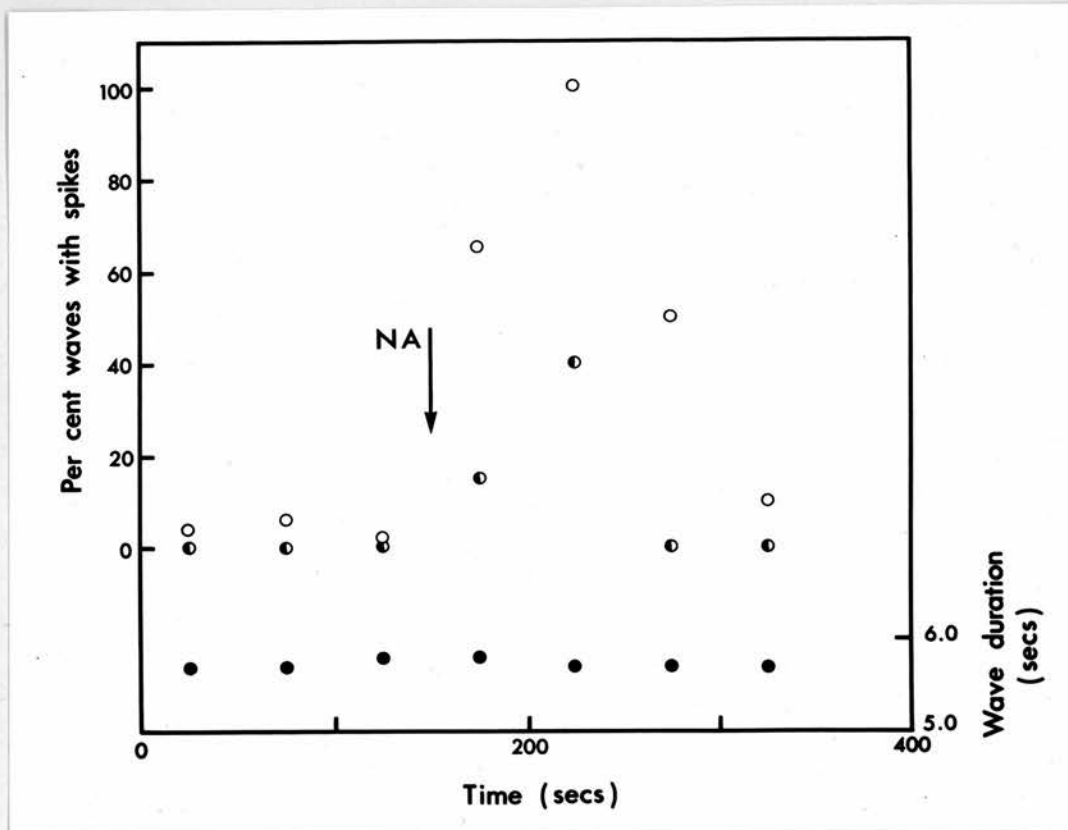


Fig. 29. Graphs showing the response of vascular smooth muscle to the application of noradrenaline ( $10^{-6}$  g/ml). A, the percentage of waves with single spikes (open circles) and multiple spikes (half-filled circles), and the duration of the slow waves (filled circles); B, the total number of spikes. Each point represents the mean of several observations.

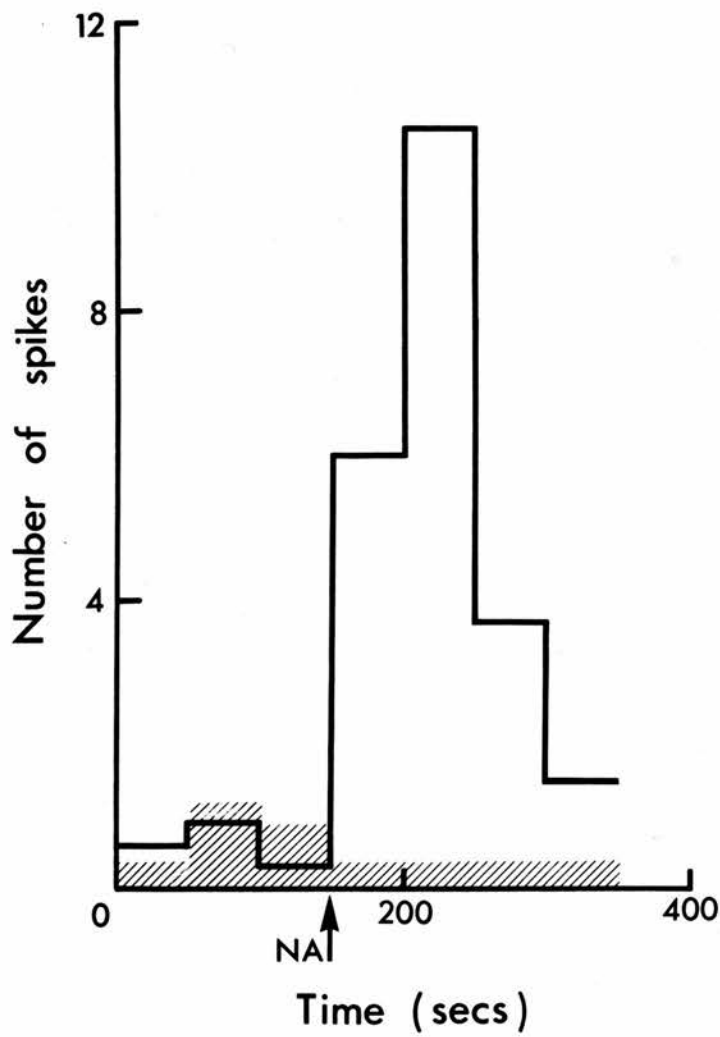


Fig. 30. Plot comparing the number of action potentials recorded from vascular smooth muscle in response to noradrenaline in two concentrations,  $10^{-6}$  g/ml (heavy line) and  $10^{-9}$  g/ml (hatched area).

The action of vasopressin:

Local application of vasopressin (0.25 m-u/ml) caused marked constriction of the arterioles accompanied by increased firing of action potentials. This lasted for several minutes, falling off gradually while the drug was being washed away. A detailed investigation was not carried out.

Summary:

These experiments on the local application of drugs showed that:

- 1) the threshold concentration of adrenaline was  $10^{-9}$  to  $10^{-8}$  g/ml, that of noradrenaline  $10^{-10}$  to  $10^{-8}$  g/ml for the vessels studied;
- 2) increasing the concentration from  $10^{-9}$  to  $10^{-6}$  g/ml increased the strength and duration of the effect, but at high concentrations ( $10^{-3}$  g/ml) no spikes could be recorded;
- 3) the duration of the slow waves remained unchanged, but their average amplitude increased (see Table 4);
- 4) threshold for acetylcholine was  $10^{-4}$  to  $10^{-3}$  g/ml, when spikes and waves were both reduced;
- 5) vasopressin caused vasoconstriction accompanied by an increase in action potential discharge, maintained until the drug was washed off.

## Discussion

### General:

The results obtained in this investigation must be interpreted with care. Information is obtained only from the cell penetrated, or with the extracellular method from the cell immediately below the electrode tip. It cannot therefore be assumed that all cells behave in a similar fashion. Moreover, the results should be restricted to the species of animal and type of vessel investigated under the conditions of experiment - it will be shown later that different results may be obtained from different vascular tissues. It is possible that sensitivity to various agents e.g. circulating adrenaline may vary in different parts of the vascular bed. Alterations in the arteriovenous shunts would affect blood flow and pressure without there being any active changes in the resistance vessels - for example, bradykinin is thought to constrict the arteriovenous shunts, so driving more blood through the arterioles and capillaries without any active change in arteriole diameter (Lim, personal communication).

Interpretation is further limited by the methods required to apply the drugs to the muscle. They were either injected intravenously, in which case they traversed the circulation before reaching the recording site so that the direct effects were complicated by reflex effects, heart effects, etc., or were dropped on to the outside of the vessel, reaching the muscle by a highly unphysiological route. In the case of local application, the drug must penetrate the peritoneum before acting on the smooth muscle, and thus any changes in permeability of the peritoneum may alter

the vascular responses. This factor could not be controlled. As in all experiments on the intact animal, the level of anaesthesia, the extent to which the vessels were under the control of humoral and nervous influences, and many other factors, could not be controlled accurately.

The actions of adrenaline, noradrenaline, and acetylcholine:

Some general points about the action of these neuro-humoral agents may be discussed first.

1) The area from which the effect of locally-applied drugs could be recorded varied quite considerably. With adrenaline, the recording electrode had to be within millimeters of the point of application, but with noradrenaline and acetylcholine the effect appeared to be more widespread. In this connection we may note the findings (Lutz et al., 1950) that the contractile response to locally-applied adrenaline when applied with methyl green was localized to the smooth muscle within the field of diffusion of methyl green whereas the dilator response to acetylcholine was more extensive.

2) When these drugs were given intravenously, no consistent effect could be recorded. This must be considered in the light of the fact that intravenous injection of adrenaline and noradrenaline will have direct cardiovascular effects causing a rise in blood pressure which will increase baroreceptor discharge, leading to decreased vasoconstrictor discharge and hence reflex fall in arteriolar peripheral resistance and venous tone which will tend to offset the causative pressure rise. The reverse will apply

with acetylcholine.

3) The thresholds found for locally-applied adrenaline and noradrenaline ( $10^{-10}$  to  $10^{-8}$  g/ml) indicate that electrical recording is considerably more sensitive than direct observation methods (e.g. Lutz et al., 1950; Bohr et al., 1958; found thresholds of  $10^{-6}$  g/ml for similar vessels).

The action of adrenaline:

That adrenaline causes constriction of the vessels accompanied by increased electrical activity is confirmed by other workers using microelectrode and sucrose-gap techniques. With microelectrodes, Roddie (1962) found that adrenaline ( $10^{-6}$  to  $10^{-4}$  g/ml) caused contraction of strips of turtle artery and vein, accompanied by increased frequency of action potentials; Funaki and Bohr (1964) found that  $10^{-9}$  to  $5 \times 10^{-8}$  adrenaline caused contraction of rat portal vein strips, increased spike frequency and progressively depolarized the cells. Using the sucrose-gap technique on sheep common carotid artery, Keatinge (1963 and 1964) showed adrenaline-induced depolarization and spikes accompanying contraction, and Cuthbert (personal communication) recorded tension development associated with action potentials in isolated mammalian veins.

In the present studies, although dilute adrenaline increased the electrical activity, more concentrated solutions apparently caused vigorous vasoconstriction, but no recordable spikes. As already mentioned, the possibility exists that this lack of activity is apparent and not real, the muscle cells contracting to such an

extent as to break electrical contact with the interior of the recording microelectrode. However, in view of unpublished observations by Cuthbert and the studies of Headings et al., (1960) on electrolyte movements, the possibility that contraction in vascular smooth muscle may be induced by some means other than action potentials cannot be dismissed.

Cuthbert (personal communication) using the sucrose-gap technique with isolated mammalian veins, found that strong solutions of adrenaline caused considerable tension development without any detectable electrical change, while dilute solutions increased the electrical activity initially, although later tension development was not associated with any detectable action potentials or membrane depolarization. Headings stimulated rings of dog carotid artery electrically and with adrenaline ( $10^{-6}$  g/ml) and subjected the tissue to electrolyte analysis; he found that although there was a marked change in electrolyte composition after electrical stimulation, adrenaline caused tension development without electrolyte movement. This rather crude experiment was taken to indicate that the adrenaline-induced contraction was not accompanied by action potentials. In this connection too we may note the observations of Keatinge (1963) and Waugh (1962) that the muscle of sheep common carotid and dog mesenteric arteries, like intestinal smooth muscle (Singh and Acharya, 1957; Evans et al., 1958) gives mechanical responses to drugs, without any detectable electrical changes, when in potassium-rich solutions ('potassium contractures'). Moreover, Eccles and Magladery (1937) demonstrated that adrenaline-stimulated nictitating membrane contracted initially due to impulses in the motor units, but



maintained contraction in the absence of impulses (adrenaline contractures).

The various methods used are not sensitive enough to exclude the possibility that membrane depolarization to a certain critical level is required for initiation of contractures. Further investigations using voltage clamp techniques would be required to determine whether or not the contractile mechanism can be activated without any electrical change occurring at the membrane. The mode of action of an agent causing contraction without membrane depolarization could still involve the electrical state of the membrane, as it might cause the potential to activate contraction at values which normally do not produce tension. In this case a large hyperpolarization of the membrane would be expected to prevent contraction, and if contraction still continued it would indicate that the stimulating agent was acting independently of the membrane potential.

Thus at this stage the possibility cannot be excluded that vascular smooth muscle may contract by more than one mechanism:

1. Activation of the contractile mechanism by the ionic processes constituting the action potential. These processes appear to involve calcium at two stages (Schatzmann, 1964):

a) at the level of the membrane, where it seems to increase the availability of the sodium transfer system, and,

b) in the coupling of excitation to contraction, as in the absence of external calcium the contractile response to single action potentials declines (Axelsson and Bülbring, 1959; Axelsson, 1961).



2. Activation of the contractile mechanism by means other than the normal action potential. Calcium also appears to be involved in this, as Durbin and Jenkinson (1961) and Edman and Schild (1963) showed that depolarized smooth muscle stops contracting in response to drugs in the absence of external calcium.

According to current theories, excitation may either increase the Ca-permeability of the membrane allowing Ca to move into the cell, or may release Ca from some cellular site of high Ca-concentration into the free intracellular space. More detailed investigations using physiological, histological, and biochemical techniques require to be performed to determine whether there is calcium movement across the membrane or within the cell, and whether there are in fact any intracellular calcium-storing structures as has been suggested for the sarcoplasmic reticulum (Schatzmann, 1964).

#### The action of noradrenaline:

These observations on the effect of noradrenaline on the small arterioles of the rat mesenteric circulation indicate a vasoconstrictor effect accompanied by increased rhythmic discharge of action potentials, except for strong solutions when no spikes could be recorded. Keatinge (1964) found that noradrenaline behaved very like adrenaline, causing contraction accompanied by depolarization and spike discharge in normal solutions (see also Roddie, 1962), and without any electrical change in potassium-rich solutions. Che Su et al., (1964) using isolated strips of pulmonary artery could, however, record no change in membrane potential during contraction caused by  $10^{-6}$  and  $10^{-7}$

noradrenaline. Thus again the possibility of contraction occurring in different vessels under different circumstances by means other than the firing of spikes must be considered.

The action of acetylcholine:

It was found that relatively high concentrations of acetylcholine ( $10^{-4}$ ,  $10^{-3}$ ) dilated the vessels and abolished the spike discharge. This is in agreement with observations on frog vessels (Funaki, 1960). In turtle arterial and venous strips, Roddie (1962) found that acetylcholine in high concentrations stimulated contraction and spikes, whereas low concentrations either reduced spike discharge or had no effect.

Funaki and Bohr (1964) made the interesting observations that acetylcholine administered to an isolated rat portal vein preparation with low membrane potentials (20 mV) hyperpolarized the membrane and initiated rhythmic discharge of action potentials. Burn and Rand (1958) showed that vagal stimulation could excite quiescent rabbit atria apparently because the resting potential was too low for pacemaker potentials to be generated, and the hyperpolarization caused by the ACh increased the membrane potential to a level from which pacemaker potentials could arise thus initiating contraction.

These two observations indicate that there is not only a threshold for action potential discharge, but a level of membrane potential below which no action potentials can occur. It would thus be of great interest to investigate the effect of ACh on depolarized vascular smooth muscle 'in situ'.

The action of vasopressin:

The concentration of vasopressin used (0.25 m-u/ml) was that found by Lloyd (1959) to cause constriction of arterioles and capillaries when applied topically to the rat mesoappendix preparation of Chambers and Zweifach (1944). It now appears that this constriction is associated with an increased electrical activity of the cells.

General considerations:

One important point that has become clear in the discussion is that in the present state of knowledge any generalisations on the mode of action of various stimulating agents on vascular smooth muscle cannot be justified. Application of results obtained must be limited to the particular material and conditions of experiment.

Thus these observations on the intact animal, recording from blood vessels with circulation and innervation intact, show that the mechanism of stimulation is in general an enhancement of the type of activity already present - i. e. an increase in the number of spikes appearing rhythmically in ones, twos or threes.

Although the mechanical response could not be recorded under these experimental conditions, it is probable that the increased number of action potentials was associated with the contractile response. In general, increased electrical activity accompanied constriction of the arterioles, except with high concentrations of adrenaline and noradrenaline.

Brown and Gillespie (1957) found that unphysiologically high rates of sympathetic stimulation were required before demonstrable amounts of noradrenaline were obtained from the venous

blood from the spleen, indicating that any circulating catechol amines 'in vivo' must be derived from the adrenal medulla and not from overflow from nerve terminals. As already described, there is evidence that under physiological conditions vasomotor control is exerted mainly by a direct effect of the sympathetic system on the smooth muscle of the vessel wall, when action potentials and contraction appear to be associated. In emergencies the secretions of the adrenal medulla will increase, but the concentrations involved may be such that contraction is still associated with action potentials, at least in its initial phase as shown with the more dilute solutions in these experiments. The contractions observed with local application of relatively high concentrations of adrenaline and noradrenaline may be unphysiological. However, until a technique is developed for recording tension changes in either single cells or very small numbers of cells while recording electrical changes, accurate correlation of the mechanical and the electrical responses cannot be made.

Further investigation on the effects of various blocking agents is required in connection with the hypothetical receptor sites in arterioles suggested by Ahlquist (1948) and extended by Green and Kepchar (1959). On this theory adrenergic blocking agents would be expected to abolish the electrical response to splanchnic stimulation and noradrenaline, and convert the increase in activity produced by adrenaline to a decrease. However, the classification of receptor sites into  $\alpha$  and  $\beta$  types is purely descriptive, based on empirically observed responses of different tissues to the catechol amines and it is not known what or where the hypothetical receptors are. Thus it may be that the initial interaction between

hormone and receptor may be similar in all cases, the specificity of the response pattern being built into the cell. It is possible that the metabolic characteristics of the cell at any one time might determine the type of response, and whether or not a blocking agent could work effectively.

Celander and Folkow (1951) found no indication of a cholinergic innervation of mesenteric vessels; in this investigation acetylcholine was found to cause vasodilatation accompanied by increased electrical activity, but no attempt was made to investigate the possibility of a dilator response to splanchnic nerve stimulation being unmasked after blocking the constrictor response.

The failure to record junction potentials might indicate that the effect of nerve stimulation enhancing the 'tonic' activity of the cells is exerted by a diffusion of transmitter over an area of cells or by a few innervated cells influencing non-innervated cells. Further investigation is required before conclusions can be drawn as to the extent of innervation of these smooth muscle cells. Unfortunately, vascular smooth muscle cells are so small that further technical developments will be necessary before such refined techniques as micro-electrophoretic injection, as used by Katz and others (del Castillo and Katz, 1955) for the investigation of neuromuscular synapses in skeletal muscle, may be employed. Transmission across the nerve-muscle junction has been thoroughly studied by applying the transmitter substance, acetylcholine, and recording the response intracellularly. del Castillo and Katz (1955) have injected acetylcholine into the muscle cells and found it ineffective, concluding that the acetylcholine receptor sites must be exclusively located on the outer surface of the end-

:plate membrane, and that this membrane is so impermeable to acetylcholine that these external receptors are not accessible to internally-injected acetylcholine. This type of investigation would provide information necessary for the understanding of the mechanism of the control of vascular smooth muscle.

PART 6: GENERAL SUMMARY OF CONCLUSIONS

GENERAL SUMMARY OF CONCLUSIONS

This investigation has shown that the smooth muscle in the walls of small arteries and arterioles of the rat mesenteric circulation exhibits two types of electrical activity - slow waves and action potentials - which appear to be closely related. This activity is very strongly influenced by the autonomic nervous system, but whether it is generated by nervous activity or by some mechanism inherent in the smooth muscle cell remains uncertain. Whilst direct comparison with other tissues is not admissible, the fact that slow waves have been recorded in nerve-free chick amnion (Prosser and Rafferty, 1956; Cuthbert, 1962) indicates the possibility that the slow waves might be generated in a manner analogous to the slow diastolic depolarization in cardiac muscle by some mechanism inherent in the muscle cell membrane. The frequency at which they are generated might be imposed by nervous influences, or might be a characteristic of the cell themselves, possibly involving progressive and rhythmic changes in sodium and potassium conductances controlled by the metabolism of the cell.

In general, stimulating agents such as asphyxia, electrical stimulation of the sympathetic nerves, and local application of adrenaline, noradrenaline and vasopressin, appear to increase the basic electrical activity of the muscle cells, and inhibiting agents such as acetylcholine and removal of the nerve supply depress it. There is evidence that high concentrations of adrenaline and noradrenaline are capable of causing the muscle to contract without firing action potentials, but whether this type of contracture is a



111.

physiological phenomenon remains uncertain.

Possible lines of future research have been discussed, but it would appear that technical difficulties due to the small size of the cells relative to that of the penetrating electrode tip will limit the application of refined techniques, e.g. voltage-clamp and intracellular injection of various agents, until the development of methods for producing finer microelectrodes.

PART 7: APPENDIX

APPENDIXFROGS:

Initial investigations were carried out on frogs.

Methods:

The tongue of a pithed frog was spread out and examined under a binocular dissecting microscope, small blood vessels of about 50 to 150 $\mu$  diameter selected, and the connective tissue dissected off. Microelectrodes of 30 to 70 M $\Omega$  resistance were used and inserted into the smooth muscle cells as previously described. Vessels in the skin of the lateral abdomen were similarly examined. The preparations were kept irrigated with a Clark's Ringer solution at 20 $^{\circ}$ C. (composition (mM); NaCl 111, KCl 19, CaCl<sub>2</sub> 1.1, NaHCO<sub>3</sub> 2.4, NaH<sub>2</sub>PO<sub>4</sub> 0.083).

Single shocks were applied (from a pulse generator through an isolation transformer) to the blood vessel wall through a second glass capillary which could be manipulated into position close to the recording electrode. Stimulus artefacts were large, and a system involving a second microelectrode (used as an indifferent electrode) connected to the grid of the second cathode follower valve was set up so that potential changes were amplified differentially.

Results:

It was found easier to penetrate smooth muscle cells in the

walls of small blood vessels in the frog than in the rat, and large, stable membrane potentials were recorded in the frog.

a) Tongue vessels:

Membrane potentials recorded showed a bimodal distribution according to size:

1) below 30 mV - these were neither abruptly established nor well maintained. When these were found, measurement of the resistance of the microelectrode showed that it was fluctuating, indicating the possibility of obstruction. Also, in many cases marked dimpling of the cells and bending of the electrode were seen. Hence these potentials were considered to be 'contact' effects, possibly due to obstruction of the electrode tip.

2) 55 to 75 mV - these were abruptly established and well maintained, remaining steady in some cases for 5 min or more. Recordings were made from 60 muscle fibres in blood vessels of the tongue, and averaged  $64.7 \text{ mV} \pm 0.2$  (S.D.). Values of less than 55 mV were discarded as these were usually obtained with electrodes of slightly lower resistance which gave low values of resting potential when tested on the frog sartorius muscle.

Only in about 10% of cases could action potentials be recorded in response to the application of single shocks. Spike potentials of about 60 mV amplitude, with occasional overshoot, and 100 to 200 msec duration were recorded. An example is shown in Figure A 1.

b) Cutaneous vessels:

Resting potentials recorded from the vessels of the skin of

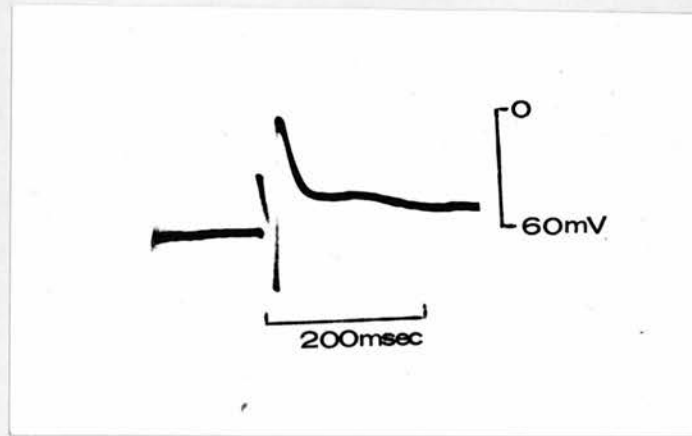


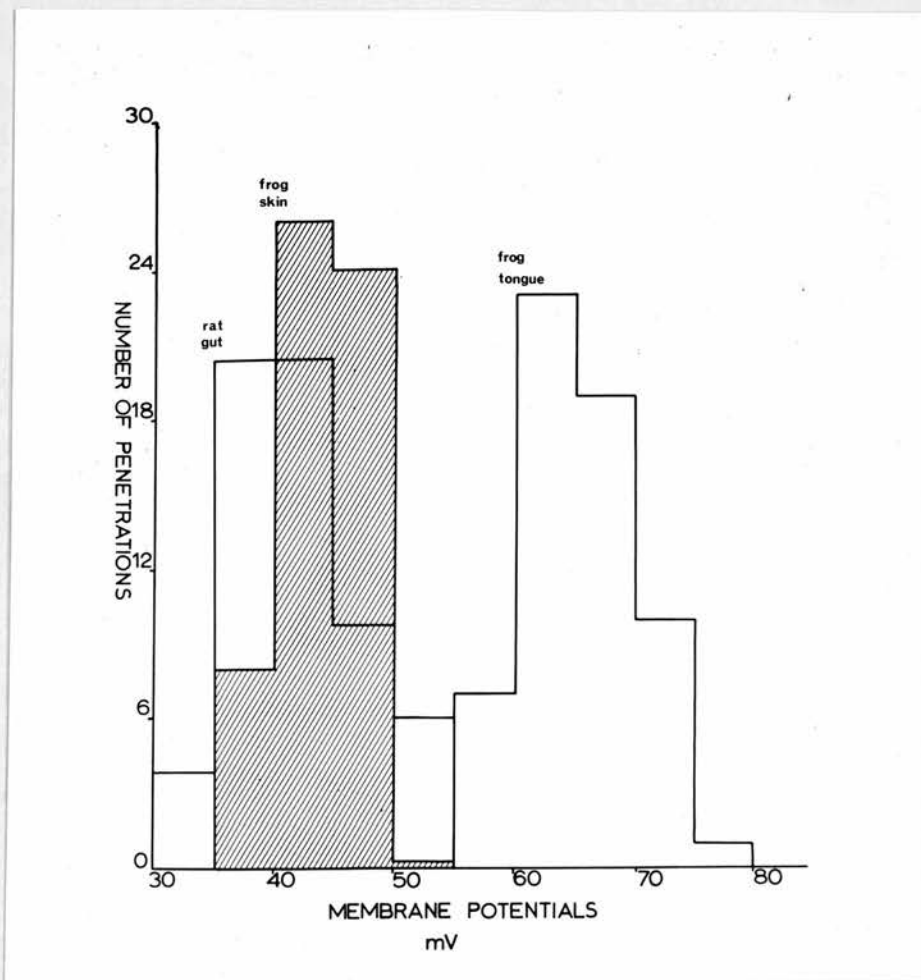
Fig. A1. Action potential recorded from a smooth muscle cell in the wall of an arteriole in the frog tongue, in response to electrical stimulation of the vessel.

the lateral abdomen showed a similar bimodal distribution. Membrane potentials were lower than those recorded from the tongue vessels, ranging from 35 to 50 mV and averaging  $43.6 \text{ mV} \pm 0.2 \text{ (S.D.)}$  for  $n=30$ . Again electrodes giving membrane potentials of less than 35 mV tended to be of rather lower resistance, and were discarded. No action potentials were recorded, nor could they be elicited by electrical stimulation.

#### Discussion:

This investigation was carried out in an attempt to confirm the observations of Funaki (1958, 1960, 1961). The average membrane potentials here recorded were rather higher than Funaki reports, 60 mV compared with 40.5 mV in the tongue vessels, and 45 mV compared with 25 mV in the cutaneous vessels. This may be partly accounted for by the fact that the electrodes used in the present work were of higher resistance ( $70 \text{ M}\Omega$  compared with 30 to 50  $\text{M}\Omega$ ).

The distribution of membrane potentials recorded from frog tongue and skin vessels are compared with the potentials recorded from rat gut vessels in Figure A 2. It may be noted that the membrane potentials recorded from rat gut vessels, found to show considerable 'spontaneous' activity, were distributed around a lower mean than the potentials recorded from frog skin vessels, found by Funaki to exhibit occasional 'spontaneous' activity, which were in turn lower than those observed in frog tongue vessels which did not show any spontaneous activity.



**Fig. A2. Frequency distribution of membrane potentials recorded from vascular smooth muscle in different sites.**

MICE:

Several observations were also made on the mesenteric arterioles of the mouse. Membrane potentials similar in amplitude to those observed in similar vessels in the rat were recorded, and slow waves, period 5 to 7 sec, were observed in lightly anaesthetized preparations.



PART 8: BIBLIOGRAPHY

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