Investigations of the Electrical Properties of Cardiac Muscle Fibres with the Aid of Intracellular Double-Barrelled Electrodes

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ABSTRACT Current has been passed through the cell membrane of muscle fibres of the isolated rabbit right ventricle with the aid of intracellular double-barrelled microelectrodes. Two types of muscle fibres were distinguished which are called P and V fibres. The relation between the intensity of a hyperpolarising current applied during the rising phase and the maximum amplitude of the action potential was different in these fibres. For P fibres the relation was essentially linear over most of the range of currents used. For V fibres the change in maximum action potential amplitude was either negligible or did not appear until a certain value of hyperpolarising current was reached. This behaviour of V fibres can be understood if a drop in polarisation resistance occurs during the rising phase and is of such short duration that the polarisation resistance has returned to its resting value before the crest of the action potential is reached. P fibres have an estimated mean resting polarisation resistance of (106 ± 13) K ohms, and a rheobase current strength of (0.08 ± 0.02) μa. In V fibres the resting polarisation resistance was (47 ± 29) K ohms and the rheobase current strength (0.47 ± 0.28) μa.

INTRODUCTION

Weidmann has carried out a detailed investigation of the electrical properties of cardiac Purkinje fibres. He found that a profound fall in membrane resistivity occurred during the depolarisation phase of the Purkinje fibre action potential (Weidmann, 1951). The only investigations related to ventricular fibres have been confined mainly to the estimation of the changes in membrane resistance during the course of the repolarisation phase (Coraboeuf, Zacouto, Gargouil, and Laplaud, 1958).

The results presented in this paper form part of an investigation and comparison of the electrical behaviour of muscle fibers in the rabbit ventricular

muscle and comprise findings related to membrane polarisation resistance during rest and activity, the current threshold, and membrane time constant. A preliminary account of some of the experiments has already appeared (Johnson, Robertson, and Tille, 1958).

APPARATUS AND METHODS

Rabbits were killed by a blow on the neck, and the heart excised. The right ventricular wall was removed and pinned out, endocardial surface uppermost, on a polythene slab which lay on the floor of an organ bath. This bath was made of silver and was irrigated with Krebs-Hensleit solution aerated with 95 per cent O_2 and 5 per cent CO_2. It was maintained at a constant temperature (38°C. ± 0.05). The temperature of the muscle was recorded continuously by a thermistor in contact with its under-surface. The tissue was stimulated externally at a constant rate (3.4/sec.) *via* two silver electrodes situated 2 mm. apart on the periphery of the preparation.

Microelectrodes Double-barrelled microelectrodes were drawn by means of a modified version of a machine described by Alexander and Nastuk (1953) from double bore pyrex tubing which was prepared by hand. The electrodes were filled with 3 M KCl by boiling under reduced pressure. The tip sizes were less than 0.5 μ and the resistances ranged between 10 and 50 MΩ. Resistive leakage between the two barrels at the blunt end was reduced by treating this end with a 2 per cent solution of Wacker siliconol AK350 in carbon tetrachloride. Electrical connections to the stimulating and recording barrels were made by chlorided and bright silver wire respectively.

Experimental Arrangement Fig. 1 shows the apparatus in block diagram. A Grass stimulator provided a pulse which was used to stimulate the tissue externally *via* a stimulation isolation unit and to trigger the time base of the main CRO (tektronix 532 equipped with beam-splitting unit 53/54C). The stimulator also provided a pulse which eventually caused a current pulse to be applied to the stimulating barrel of the microelectrode from the output of a constant current generator. The magnitude, duration, and timing of the pulse were controlled by the waveform and pulse generators (tektronix Series 160). A Shackman autocamera (Mk3) was used to photograph the screen of the main CRO. It was switched on by a pulse from the output of the scaler (1:2, 4, 8) and was then switched off by a pulse derived from the Grass stimulator. This arrangement allowed the film to be exposed for the duration of the sweep in which current was passed, and of the sweep immediately preceding. The first derivative of the action potential was obtained from the output of the feedback differentiator.

The Recording Input Stage This was a type of electrometer circuit described by Murray (1958) wherein both anode and cathode of an input electrometer tube are made to follow closely the input signal. Very high input impedance is obtained with extreme linearity over a large input voltage range. The input stage had the following specifications (without microelectrode): input capacitance $<10^{-4}$ $\mu\mu$f.; input resistance $>10^{18}$ Ω; permissible input voltage swing, ±25 volts; grid current $<10^{-16}$ A;

drift, ±2 mv./hour; rise time (10 per cent limits), 1 μsec.; output impedance, 100 Ω; gain 0.99996. The electrometer was capable of accepting the voltages incurred during the experimental procedures and thus the elimination of voltage artefacts could be carried out at a more convenient point in the ensuing circuitry.

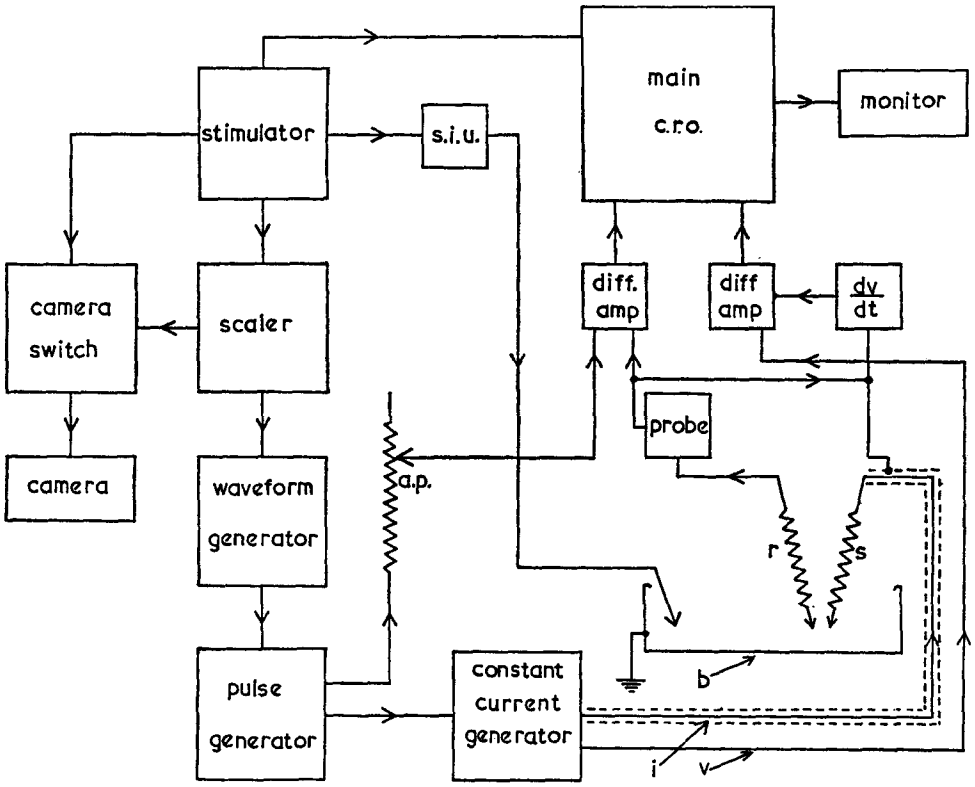


FIGURE 1. The experimental apparatus in block diagram. *b*, the organ bath; *i*, the screened cable from the output of the constant current generator to the stimulating barrel of the microelectrode, *s*; *v*, the lead carrying the voltage signal (proportional to current flowing through the stimulating circuit) to one input of one of the differential amplifiers (diff. amp); $\frac{dv}{dt}$, the feedback differentiator; probe, the floating grid electrometer input stage which received the signal from the recording barrel of the microelectrode (*r*); s.i.u., stimulus isolation unit; *a.p.*, rheostat which controlled the amplitude of the antiphase injection signal applied to the input of the second differential amplifier (diff. amp); monitor, monitoring oscilloscope.

The Constant Current Generator In order to ensure constant current conditions during the passage of current through the stimulating barrel, the usual procedure is to insert a high resistance in series with the microelectrode. With microelectrodes having resistances ranging from 10 to 50 M Ω a series resistor of the order of 1000 M Ω would be needed. As we use currents as high as 2×10^{-6} A, the high voltage necessary would be both dangerous and impractical.

A constant current generator was designed (Murray, personal communication) which had an effective output impedance of the order of 1000 M Ω and an available voltage output of ±50 volts. The rise time (10 per cent limits) of the current pulse was close to 20 μsec. The output of the generator was coupled to the microelectrode by a cable, the screening of which was driven from the output of the electrometer input stage. This procedure, together with the high source impedance of the generator effectively isolated the capacitance between the two barrels of the microelectrode from ground and hence prevented the attachment of the current source to the stimulating barrel from reducing the speed of response of the recording channel. With the tip of the microelectrode just immersed in the bath liquid and the stimulating lead not connected, a voltage step applied between the organ bath and ground had a rise time of approximately 2 μsec. This figure was not significantly increased by connecting the cable from the current generator to the stimulating barrel.

The current generator was activated by pulses derived from the pulse generator. It also provided a voltage signal proportional to the current flowing through the external load. This load, however, was complex for it consisted not only of the resistive component of the stimulating barrel but also of the capacitance of the stimulating cable and microelectrode to ground. It is therefore apparent that at the beginning of a current pulse, most of the current would flow into these capacitances. As it was not possible to prevent this, constant current conditions through the resistive element of the barrel would not have been established for at least twice the time constant of microelectrode resistance and the capacitances of the cable and stimulating barrel. This would have been less than 1 msec. under our particular experimental conditions.

Recording Artefacts The resistive coupling between the two barrels of a double-barrelled microelectrode represents the resistance common to both the recording and stimulating circuits at the tip of the microelectrode (Coombs, Eccles, and Fatt, 1955) and is dependent on the proximity of the two barrel openings and the resistivity of the medium in which the tip is situated. The usual means of eliminating the voltage artefacts which appear in the recording channel when current is passed is to neutralise them by suitable antiphase injection at some point in the recording circuit (Coombs, Curtis, and Eccles, 1959). When this is achieved with the tip outside the cell any voltage change recorded during the passing of current when the tip is in the cell can be attributed to the now added cell component. This assumes, however, that the resistivity of the medium inside the cell is the same as outside. If the amplitude of the artefacts recorded outside the cell without antiphase injection is small, as would be the case when small currents (10^{-9} A) are used or when the coupling resistance is low, the error introduced by small changes in resistivity may not be very great. However, as the extracellular artefacts corresponding to currents of up to 2×10^{-6} A were of the order of a volt, even small changes in resistivity would produce serious errors in our estimates of changes of membrane potential. Therefore, the antiphase injection which we employed was merely a D.C. shift necessary to maintain the action potential on the screen during current flow. Direct determination of the changes in membrane potential produced by the current was not possible. An indirect method which we used is described in the relevant section of the results.

Criteria Employed for Ensuring the Intracellular Location of the Electrode Tip The tip of the microelectrode was microscopically examined before use and the two barrels

were seen to terminate within 0.5 μ of each other. It was therefore virtually impossible for one tip to be inside whilst the other was outside the cell.

A penetration was considered satisfactory if the following criteria were met: (*a*) The appearance of an abrupt potential drop within a few microns of advancement of the electrode tip and the subsequent recording of monophasic action potentials. The maximum amplitude of the action potentials was not less than 90 mv. and did not vary more than 2 to 4 mv. throughout the period of the experiment. (*b*) Vertical movements of the electrode tip of 5 to 10 μ did not affect the action potential form and amplitude nor the effects of current flow upon it.

Extracellular Potential Changes The magnitude of the extracellular electric field is proportional to the radial current density and the specific resistance of the external solution. This field leads to a slight distortion of the observed membrane potential change (Fatt and Katz, 1951). The extracellular potentials which we recorded with the tip just outside the cell were diphasic. They never exceeded 3 mv. and in the majority of cases were less than 1 mv.

RESULTS

From the results which follow, the fibres found in the ventricular muscle mass can be divided into two main groups. These two groups differ in at least three respects: action potential shape, rheobase current strength, and behaviour of the depolarisation phase of their action potentials during current flow. Because the majority of the results included in one of the groups was obtained from fibres situated in the visible Purkinje network we refer to these fibres as P fibres and those from the other group as V fibres.

Definitions

(*a*) The resting membrane potential existing in the absence of polarising current will be called the normal resting potential. Resting potential will in general refer to cases in which this potential is artificially altered by polarising current.

(*b*) The membrane potential will be said to be increased if it becomes more negative with respect to the outside fluid.

(*c*) The slope of the relationship between the amplitude of the action potential and the applied current will be called positive when the amplitude is increasing as the value of hyperpolarising current is increasing.

1. *Action Potential Forms*

All the fibres in the group referred to as P fibres had action potentials of a particular form. This form was characterised by a spiked rising phase followed in most cases by a dip and hump. An example of the characteristic shape of

these fibres is shown in Fig. 2 together with examples of the shapes of V fibres. These shapes were, however, merely used as a guide to the selection of a fibre for experiment. As will be seen, the final classification into the two groups was made on other grounds, namely rheobase current strength, and particularly the behaviour during current flow.

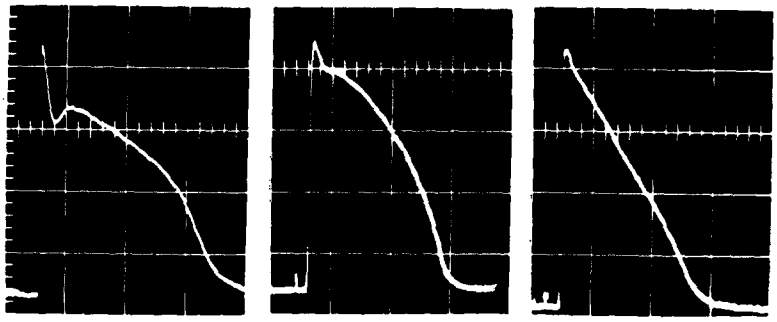


FIGURE 2. Typical action potential forms. *a*, P fibre action potential; *b*, *c*, V fibre action potentials; ordinate, 25 mv./large division; abscissae, 50 msec./large division.

2. *Resting Polarisation Resistance*

Rectangular current pulses of 100 msec. duration were passed through one barrel of the microelectrode, the onset of the pulse being arranged to begin approximately 80 msec. before the time of arrival of every 4th or 8th propagated action potential. This allowed sufficient time for charging of the membrane capacitance and for any inactivation or reactivation of the excitatory mechanism to be fully established. The maximum time required for such a steady state to be established was determined experimentally by varying the time of onset of the current pulse prior to the action potential. It was always found to be less than 10 msec.

We have attempted to determine the resting polarisation resistance (Weidmann, 1952), that is to say, the ratio of the change in resting membrane potential in volts produced by a given current (at an infinite time after the start of the current and at zero distance from its site of application) and the current strength in amps. The method which we employed is that previously described by Frank and Fuortes (1956). The maximum amplitude of the action potential which occurred during current flow was plotted against the current; the amplitude of the preceding action potential was plotted as a control.

The first derivative of the action potential was recorded as it provided a simple means of revealing changes in the rate of depolarisation which would

not have been evident with the sweep velocities used. We have not attempted to relate the maximum value of the first derivative to inward ionic current for this relationship is no longer clear when current is being passed through the microelectrode.

P FIBRES

The results from 41 out of a total of 101 fibres examined fall into this group. These results can be best described by reference to Fig. 3 which incorporates in generalised form the more important features of the relationship between the action potential amplitude and applied current. The relationship can be

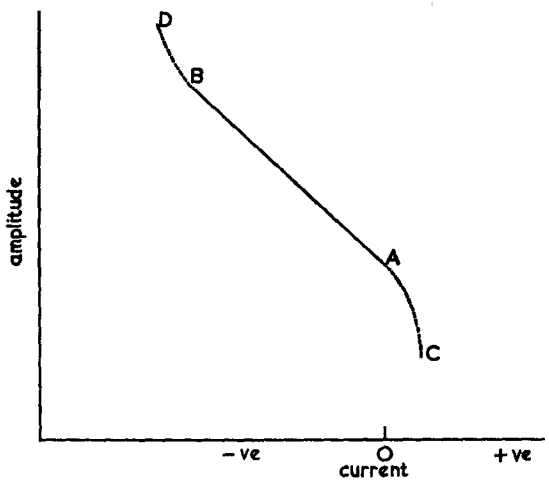


FIGURE 3. Curve showing the important features of the relationship in P fibres between amplitude of the action potential and applied current; (+*ve*, depolarising, −*ve*, hyperpolarising). The significance of points A, B, C, D is discussed in the text.

divided into a linear portion A–B and two non-linear ones A–C and B–D. The value of current corresponding to the point A was either zero or very small and negative. The region A–B where the action potential amplitude increased linearly with increasing values of applied hyperpolarising current extended to action potential amplitudes of up to, and often exceeding 160 mv.

An example of the relationship between current and maximum amplitude of the action potential of a P fibre is illustrated in Fig. 4. The accompanying changes in maximum rate of depolarisation are also shown in the figure. Typical examples of the records from which such results were obtained are shown in Fig. 5.

The extent to which we were able to follow the relationship for negative currents was limited either by the current-carrying capabilities of the microelectrode in use or by the dislodgment of the microelectrode from the cell. In the few cases in which we were able to increase the height of the action po-

tential to values in the vicinity of 180 mv. (corresponding to the region B–D in Fig. 3) the relationship became non-linear, the height of the action potential increasing more steeply with current, indicating an increase in the resting polarisation resistance. There appears to be a limit to the increase in amplitude produced by hyperpolarising current. The maximum amplitudes which we obtained in our experiments in P fibres never exceeded 190 mv. This corresponded to a hyperpolarisation of the resting membrane of approximately 90 mv. In fibres in which we were able to increase the amplitude to this value, further increases of hyperpolarising current resulted in a negative

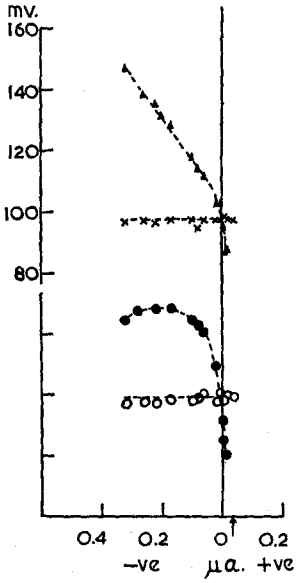


FIGURE 4. The relationship between curren and the maximum amplitude and maximum rate of depolarisation of a P fibre action potential. ▲, the maximum amplitude in millivolts and ●, the maximum rate of depolarisation (in arbitrary units) are plotted against current in microamperes (*−ve* hyperpolarising, *+ve* depolarising). The abscissa does not apply to the corresponding control values for the maximum amplitude (X) and maximum rate of depolarisation (○) which are also included in the graph. The horizontal interrupted lines indicate their approximate mean values. The arrow ↑ marks the value of rheobase current. The amplitude of the action potential increases linearly with increasing values of *−ve* current: the sloping interrupted line in this instance has a slope of 110K ohms.

slope in the relationship between current and the action potential amplitude. This behaviour is illustrated in Fig. 6.

Analysis We shall now consider the curve in Fig. 3 in more detail. There are two possible ways in which the action potential amplitude can be increased by hyperpolarising current: (*a*) the resting resistance is higher than the impedance[1] at the crest so that the voltage drop produced by the current will be greater in the resting state than at the crest and when hyperpolarising current is used the increase in the action potential amplitude will be equal to the difference between the IR drop on the resting state and the voltage drop at the crest; (*b*) the current and/or the polarisation produced by it has a direct

[1] At the crest of the action potential the voltage displacement produced by the current, although dependent on the polarisation resistance at this time, is also dependent on the time required to recharge the membrane capacitance after a drop in polarisation resistance (see Discussion). We therefore use the term impedance, as distinct from resistance, to describe the relationship between voltage displacement and current at the crest.

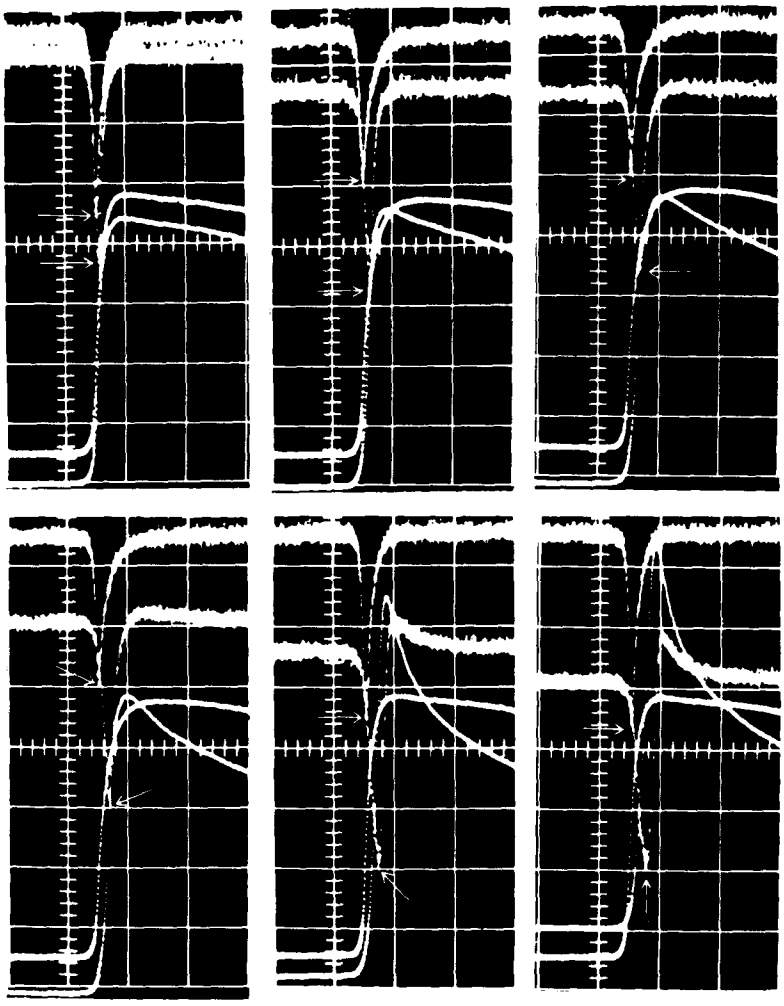


FIGURE 5. Typical experimental records of the effect of negative (hyperpolarising) current on the rising phase of a P fibre action potential. In each record the upper two traces show the first derivative of the rising phase before (upper of the two) and during (lower of the two) current flow. The magnitude of the current is proportional to the vertical displacement of these two traces (1 large division = 2.5×10^{-7}A). The maximum amplitudes of the first derivatives are indicated by the arrows. The lower two traces in all but the lower right hand figure show the rising phase of an action potential occurring before current flow (corresponding to the upper of the two base lines to the left of each figure) and during current flow (the lower of the two). Time, 1 large division = 2 msec.; voltage, 1 large division = 25 mv. In the lower right hand figure the upper of the base lines belongs to the action potential occurring during current flow.

In each figure the vertical displacement of the two lower traces does not signify the magnitude of the polarisation produced by the current. The interruptions in the upstroke of the action potential are the result of the beam switching and occur at intervals of 10 μsec.

effect on an "excitatory mechanism," that is, the flow of negative current and/or the hyperpolarisation produced by it in the resting state has altered the state of the excitatory mechanism in such a way that the fibre is now capable of attaining a higher overshoot.

Several situations could arise depending on the nature of the excitatory mechanism. In one, if the excitatory mechanism is a regenerative increase in the permeability to sodium ions and its initial state depends on the resting potential (Hodgkin and Huxley, 1952), the activation of this mechanism

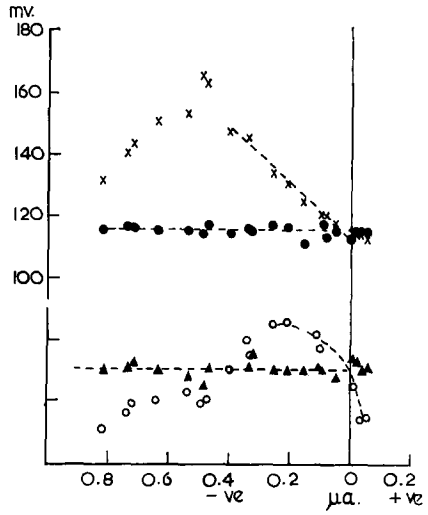


FIGURE 6. The relationship between current and the amplitude and maximum rate of depolarisation of a P fibre action potential. The values for maximum amplitude of the action potential in millivolts, ×; and the maximum rate of depolarisation ○ (in arbitrary units) during current flow are plotted against current in microamperes (−*ve*, hyperpolarising; +*ve*, depolarising). The abscissa does not apply to the corresponding control values for the maximum amplitude, ●, and the maximum rate of depolarisation, ▲, which are also included in the graph. The horizontal interrupted lines indicate their approximate mean values. The amplitude of the action potential increased in a linear manner (sloping interrupted line) with increasing −*ve* currents until a value in the vicinity of 0.4 μa. was reached. Beyond this point non-linear behaviour became evident.

must result in a decrease in membrane resistivity during the rising phase of the action potential and, (unless the action of the excitatory mechanism ceased some time before the crest of the action potential was attained; see Discussion), it must also result in a lower impedance at the crest. Thus in this situation some part at least of the increase in the amplitude of the action potential must be the difference between the IR drop at the base and the voltage drop at the crest of the action potential.

The second possible situation would arise if the excitatory mechanism were in such a state that the flow of hyperpolarising current and the accompanying hyperpolarisation had no effect upon it. In this situation the increase in ampli-

tude of the action potential would be equal to the difference in the IR drop at the base and the voltage drop at the crest, and the slope of the relationship between action potential amplitude and applied current could be then taken as being equal to the difference between the resistance at the base and the impedance at the crest.

Thus there are two possible situations: (*a*) either the increase in amplitude with hyperpolarising current is solely the difference between the IR drop at the base and the voltage drop at the crest of the action potential, or (*b*) it is the sum of the difference in IR and voltage drops and an increase in overshoot.

It has been shown by Weidmann (1955) that in Purkinje fibres the increase in overshoot obtained by increasing the resting potential reaches a limiting value for resting potentials little greater than the normal resting potential (90 mv.). As this limiting value is approached the relationship between resting potential and overshoot is non-linear. This behaviour is identical to that found by Hodgkin and Huxley (1952) in squid axon and this was taken by Weidmann (1955) as strong evidence for the existence of a sodium carrier mechanism in Purkinje fibres of the type proposed by Hodgkin and Huxley (1952).

Thus we would expect the increase in action potential amplitude to be composed of a term proportional to the current intensity and a second term more or less non-linear for resting potentials below and slightly above the normal resting potential and tending to zero for resting potentials considerably higher than the normal resting potential. The first term corresponds to the difference between the IR drop at the base and the voltage drop at the crest, and the second term to the increase in overshoot caused by the altered initial state of the excitatory mechanism. The relationship between the action potential amplitude and applied hyperpolarising current should therefore exhibit a non-linear and relatively steep portion for low values and a less steep linear portion for higher values of current. The slope of the linear portion would then be equal to the difference between the resting polarisation resistance and the impedance at the crest. These two portions correspond to the two possible situations (*b*) and (*a*) described above. Our results clearly demonstrate the existence of these two situations. In referring to Fig. 3 the non-linear region A–C can now be seen to represent the situation (*b*) and the linear region A–B, the situation (*a*) in which the increase in amplitude is solely the difference between the IR drop at the base and the voltage drop at the crest. The non-linearity is confined, as one would expect from Weidmann's results, mainly to the region of positive current, that is to resting potentials below the normal resting value. The slopes obtained from the experimental results corresponding to the region A–B in Fig. 3 can therefore be expressed as the difference between the polarisation resistance at the base and the impedance at the crest of the action potential. The slopes of this portion of the relation-

TABLE I

P FIBRES

Normal action potential amplitude	Polarisation resistance	Rheobase current
mv.	*kΩ*	*μa*
90		0.01
94	150	0.03
90		0.01
98	105	0.04
93	130	0.045
102		0.06
105	85	0.065
92		0.04
104	125	0.015
97	160	0.04
97	155	0.035
94		0.08
100		0.01
98	100	0.06
103		0.05
97	82	0.085
94		0.07
97		0.07
92		0.06
102	80	
94	95	
99	90	
100	90	
93	180	
99	120	0.05
99	60	
100	170	
106	240	
113	100	0.08
111	100	0.12
108	95	0.11
112	122	0.085
114	80	0.17
109	130	0.08
107	95	0.10
94	56	0.18
96	65	0.12
91	65	0.09
96	79	0.14
97	165	
102	70	
102	67	
100		0.16
91	120	0.10
103	94	0.15
95	138	0.17
100		0.11
94	122	0.09
91	60	0.07

TABLE I (*concluded*)

Normal action potential amplitude	Polarisation resistance	Rheobase current
mv.	*kΩ*	*μa*
95	60	0.1
91		0.06
102		0.09
107	65	
105	75	0.13
100	90	0.10
96		0.08
92		0.04
Total No. 57	41	45
Mean 98.9	106	0.08
S.D. 6.2	13	0.02

ship from P fibres ranged from 56 to 240 KΩ with a mean of 106 KΩ S.D. 13 (41). The separate observations are given in Table I.

V FIBRES

The relationship which we found between the maximum amplitude of the action potential and current in 60 V fibres can be divided into two classes.

In the first class which included 39 out of the total of 60 fibres, the amplitude of the action potential remained constant for all values of negative current used. However, the current was not without effect on these fibres. Changes in the time relationship and maximum value of the rate of depolarisation were observed, corresponding to changes in shape of the depolarisation phase. As the negative current was increased the maximum rate of depolarisation increased up to a certain value and then remained approximately constant whilst the point at which it occurred moved progressively up the rising phase. An illustration which is typical of such changes is shown in Fig. 7, and an example of the relationship between current and the maximum rate of rise and amplitude of a V fibre of this class is given in Fig. 8.

In the second class of fibres, similar results were obtained up to a certain value of negative current. The changes in the time relationship and amplitude of the maximum rate of depolarisation were indistinguishable from those of the first group. However, with higher negative currents the amplitude of the action potential increased linearly with current and a spike developed at the crest of the action potential. The amplitude of this spike corresponded to the increase in amplitude of the action potential. The slopes of this portion of the relationship were constant with values ranging from 14 to 118 KΩ, mean value 47 KΩ S.D. 29 (19). This slope occurred in 21 out of 60 of our results from V fibres (see Table II). An example of the relationship between current and action potential amplitude and maximum rate of depolarisation for this class is shown in Fig. 9 and an illustration of the experimental records of this

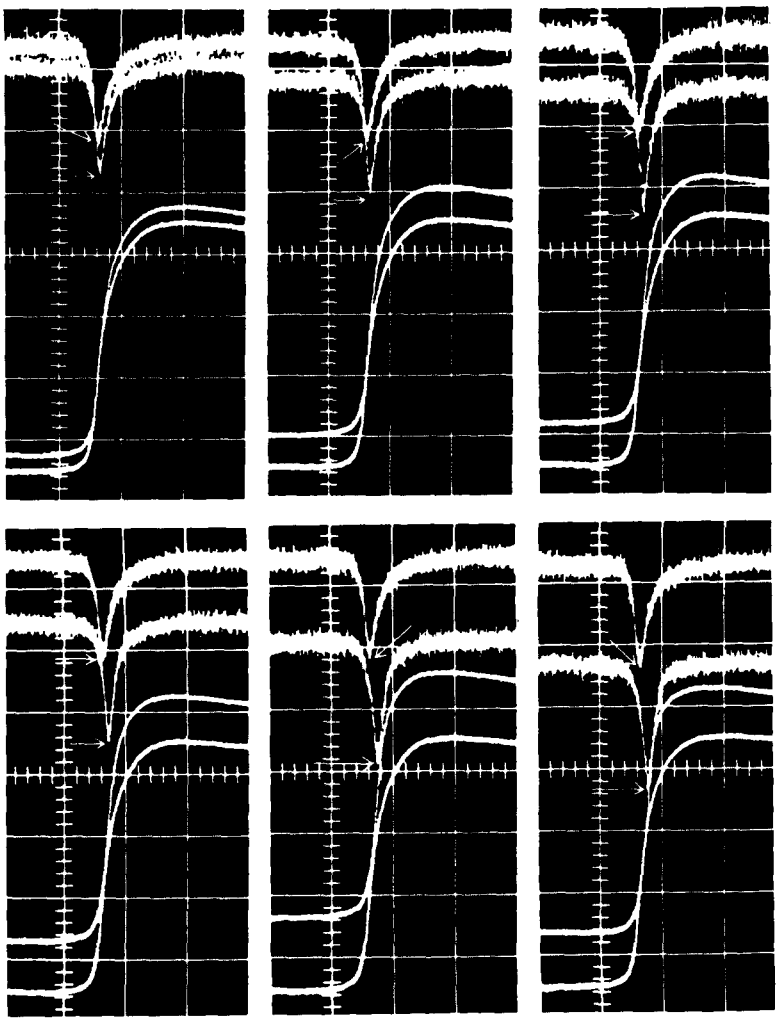


FIGURE 7. Typical experimental records of the effect of negative (hyperpolarising) current on the rising phase of a V fibre. In each record the upper two traces show the first derivative of the rising phase before (upper of the two traces) and during (lower of the two traces) current flow. 1 large division = 100 v. sec.$^{-1}$. The magnitude of the current is proportional to the vertical displacement of these two traces (1 large division = 5×10^{-7}A). The maximum amplitudes of the first derivatives are indicated by the arrows. The lower two traces show the rising phase of an action potential occurring before current flow (corresponding to the lower of the two base lines to the left of each picture) and during (upper of the two) current flow. Time, 1 large division = 2 msec.: voltage scale, 1 large division = 25 mv. The vertical displacement of these two lower traces does not signify the magnitude of the polarisation produced by the current. Although the maximum amplitude of this V fibre action potential is unaffected by the current, its maximum rate of rise and shape is noticeably affected.

behaviour is shown in Fig. 10. It is clear from the results from *both* classes of V fibres that as the negative current is increased (at least up to a certain value), the crest and base of the action potential must experience the same voltage displacement and the current must have no effect on the overshoot for the height of the action potential to remain unchanged.

Analysis The difference in behaviour between the two classes of V fibres can be explained in the following way: Consider a situation in which the polarisation resistance is very low at a point in the rising phase of the

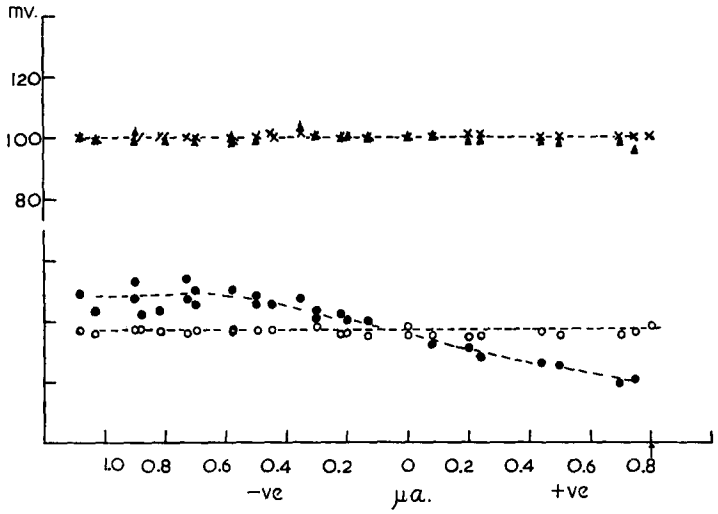


FIGURE 8. The relationship between current and the maximum amplitude and maximum rate of depolarisation of a V fibre action potential. Scales and symbols are the same as in Fig. 4. Although the amplitude of the action potential is not affected by current flow, the maximum rate of depolarisation is.

action potential and increases rapidly to its normal resting value as the crest is approached. The application of constant hyperpolarising current during the rising phase of the action potential will cause a voltage displacement, the magnitude of which at any time will be related to the polarisation resistance at that time. It is obvious that the point of lowest resistance will suffer the least voltage displacement. As the current is increased, the higher resistance portions of the rising phase will be increasingly displaced. Because the point of lowest resistance suffers the least voltage displacement its position relative to the rest of the rising phase will change and it will *appear* to move up the rising phase. Eventually, at a certain value of current it will occupy the crest of the action potential. Thus for all higher values of current the crest of the action potential will suffer no further voltage displacement and the relationship between action potential amplitude and applied current will assume a positive slope. Since the resistance drop is very brief an IR displacement equal

TABLE II

V FIBRES

Normal action potential amplitude	Polarisation resistance	Rheobase current
mv.	*kΩ*	*μa*
92	No slope	
94	75	0.24
90		0.44
100		0.24
90		0.75
97	22	0.24
91		0.42
90	S	0.33
97		0.17
98	S	
90	No slope	0.56
90	" "	0.28
95	25	
105		0.50
105	No slope	
105	" "	
101	" "	0.38
112	" "	
106		0.17
105	26	0.70
107	No slope	0.84
97	70	
102	No slope	
93	" "	0.35
110	" "	0.45
100	" "	0.44
97	56	0.45
103	No slope	0.46
101		0.14
100	100	
103	No slope	
91	13.5	0.50
91	No slope	1.35
111	" "	
112	" "	
102		0.54
99	No slope	
105	46	
101	19.5	
101	33.3	
101	118	
110	37	
110	33	
101	No slope	0.10
98	" "	
101	" "	
99	" "	
100	" "	
100	" "	0.19

In two fibers (denoted by the letter S) the value of the slope is not given because the number of points was insufficient.

TABLE II (*concluded*)

	Normal action potential amplitude	Polarisation resistance	Rheobase current
	mv.	*kΩ*	*μa*
	100	No slope	
	106	" "	1.05
	106	" "	0.17
	105	" "	0.48
	101		0.24
	95	53	0.34
	91	No slope	
	99	" "	
	93	" "	
	95	18	0.47
	99	No slope	0.66
	99	" "	0.84
	98	" "	0.75
	104	77	0.21
	97	No slope	
	95	" "	
	106		1.05
	91	No slope	
	98	" "	
	102	44	
	92	No slope	
	105	30	
Total No.	71	21 with slopes 39 no "	37
All fibers	99.7		
Mean No slope	99.8	47	0.47
With slope	99.7		
All fibers	5.9		
S.D. No slope	6.2	29	0.28
With slope	5.6		

to the IR displacement of the resting membrane will be rapidly reestablished, transforming the crest of the action potential into a brief spike.

The experimental results obtained from the second class of V fibres support the hypothesis that a brief drop in polarisation resistance occurs during the rising phase of the action potential. A spike and a positive slope in the relationship between action potential amplitude and applied current are obtained only with currents higher than a certain value. As in P fibres the magnitude of this slope can be taken as the difference between polarisation resistance in the resting state and the impedance at the crest of the action potential.

It is reasonable to assume that in the first class, in which the amplitude remained unaffected for all values of current used, the voltage displacement of the crest of the action potential was not sufficient to achieve the state where

the crest contains the lowest resistance. This could have been caused by the effect of one or more of the following factors: (*a*) the fall and recovery of membrane resistivity occurring at an earlier part of the depolarisation phase, (*b*) a lower resting polarisation resistance, and (*c*) a lower upper limit of the negative currents used. The possibility that the resting polarisation resistance was so low that the voltage drop produced by the current was too small to be observed can be discounted, for in every case we observed a change in the time relationship and magnitude of the rate of depolarisation. Such changes could not have occurred unless measurable changes in resting potential had

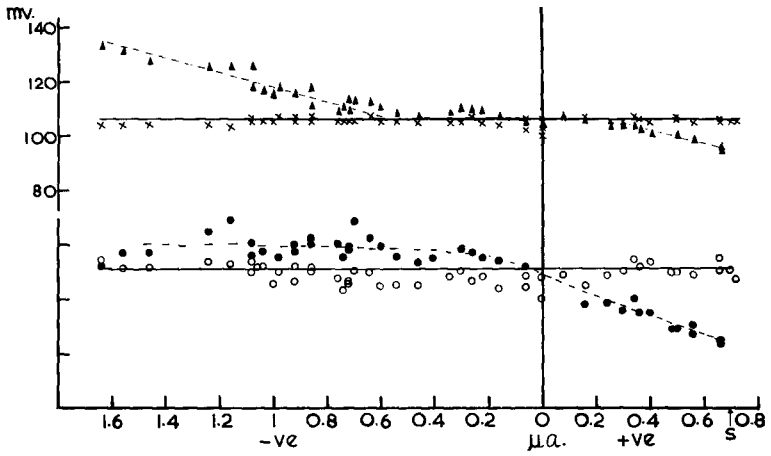


FIGURE 9. The relationship between current and the maximum amplitude and maximum rate of depolarisation of a V fibre action potential. Scales and symbols are the same as in Fig. 4. The amplitude of the action potential is unaffected by $-ve$ current up to a certain value beyond which it increases linearly with current: the sloping interrupted line in this instance has a slope of 26 K ohms.

been produced. The point to be emphasised is that the drop in polarisation resistance in V fibres must be extremely brief, for the membrane capacitance must be fully recharged by the applied current at the time the crest of the action potential is reached in order that the displacement may be the same at both the crest and base of the action potential. The question whether this drop in polarisation resistance can be said to be briefer than that in P fibres will be considered in the Discussion.

3. *Comparison of Normal Action Potential Amplitudes*

The mean normal amplitudes of action potentials included in the P group and V group are 98.9 mv. (6.2 S.D.) and 99.7 mv. (5.9 S.D.) respectively. The

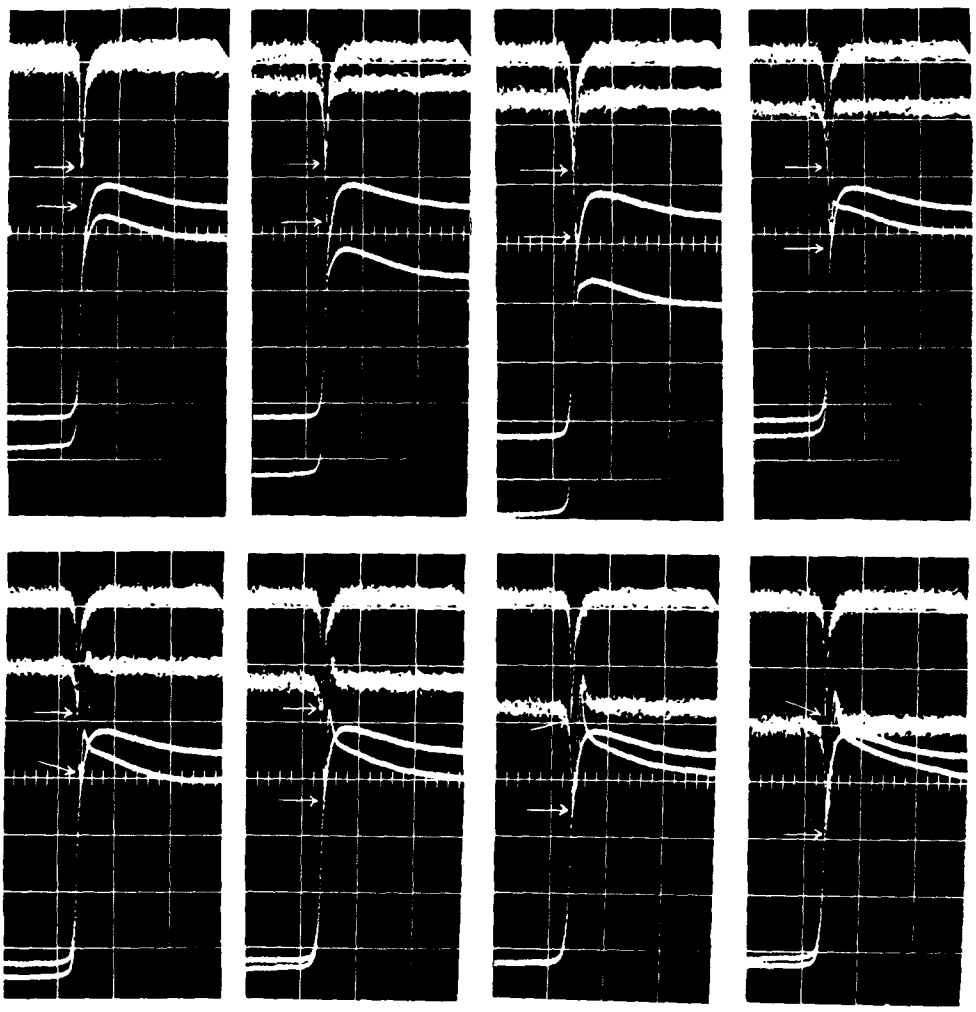


FIGURE 10. Typical experimental records of the effect of negative (hyperpolarising) current on the rising phase of a V fibre action potential. In each record the upper two traces show the first derivative of the rising phase before (upper of the two) and during (lower of the two) current flow. The magnitude of the current is proportional to the vertical displacement of these two traces (1 large division = 5×10^{-7}A). The maximum amplitudes of the first derivatives are indicated by the arrows. The lower two traces in each figure show the rising phase of the action potential occurring before current flow (corresponding to the upper of the two base lines to the left of each figure) and during current flow (the lower of the two). Time, 1 large division = 5 msec.; voltage, 1 large division = 25 mv.

mean amplitudes of the two classes of V fibres, that is to say those with a slope and those without, are 99.8 mv. (6.2 S.D.) and 99.7 mv. (5.6 S.D.) respectively. The separate measurements are given in Tables I and II. The greatest normal action potential amplitude which we have ever recorded in rabbit

ventricle was 115 mv. The amplitude of the action potential is a better index of the state of the fibre than is the value of its resting potential for the amplitude depends on both resting potential and overshoot. Therefore the possibility that the differences in behaviour among fibres in the P group and the two classes of V fibres could be due to differences in the state of the fibres, can be discounted.

4. *Rheobase Current Strength*

One of the striking differences between P and V fibres was found to be in rheobase current strength. Depolarising current pulses of 100 msec. duration were applied *via* the stimulating barrel 80 msec. before the onset of every fourth or eighth propagated action potential. The minimal current strength required to initiate an extra action potential was recorded as the rheobase. The mean of the values from 45 P fibres was 0.08 μa. (0.02 S.D.) and from 38 V fibres was 0.47 μa. (0.28 S.D.). The separate measurements are given in Tables I and II. It would appear that part of this difference is either the result of a higher threshold or a greater area needed to be depolarised to initiate an action potential in V fibres, as the polarisation resistance of P fibres is only about twice that of V fibres.

5. *Strength-Duration Curve*

The relationship between the strength and duration of a just threshold current (applied *via* one barrel of an intracellular electrode) can theoretically be fitted by an equation of the form

$$I_{rh} = I(1 - e^{-t/\tau})$$

in which I_{rh} is the rheobase current, I the minimal current required to stimulate, and t its duration. The errors associated with taking the constant, τ, as an estimate of the membrane time constant have been discussed by Frank and Fuortes (1956) and Coombs, Curtis, and Eccles (1959). These errors are associated with such events as accommodation and subthreshold activity.

The values of τ were obtained from the slope of the line relating $\ln \frac{I}{I - I_{rh}}$ to t. The values of τ from 14 P fibres ranged from 2.5 to 5 msec. Similar measurements in V fibres gave values of τ close to 1 msec. However, in V fibres these values are of questionable significance for the following reasons: (*a*) the current pulses through the tip of the microelectrode had a rise time of less than 1 msec., the exact value depending on the resistance and

capacitance to ground of the stimulating circuit. This rise time could, at the short pulse durations required in V fibres, seriously increase the final estimate of membrane time constant; (*b*) the current strengths required to plot a strength-duration curve in V fibres frequently exceeded the current-carrying capabilities of the microelectrodes and hence our measurements were limited to V fibres which had relatively low values of rheobase.

DISCUSSION

Polarisation Resistance

RESTING

If we assume that in the situation when a positive slope is obtained in the relationship between action potential amplitude and applied current, the impedance at the crest of the action potential is small compared with the resting polarisation resistance, the values of the polarisation resistance given in Tables I and II can be taken as being approximately equal to the resting polarisation resistance. The scatter of the values for the polarisation resistance within each of the groups need not necessarily reflect differences in membrane resistivity of the fibres for some variation can be expected in view of the syncytial arrangement of the fibres of ventricular tissue. The polarisation resistance is related to the effective area of membrane seen by the microelectrode and this would depend on the architecture of the interior of the fibre in the immediate vicinity of the microelectrode. As the fibre is not a simple cable but as a first approximation resembles a multibranched cable, this architecture would be expected to vary considerably with the site of penetration of the microelectrode. Consequently some variation would be expected in the strength of current required to evoke a given hyperpolarisation at different sites in the muscle. Differences in fibre diameter would also contribute to the scatter of the polarisation resistance values in each group. The ratio of approximately two times between the means of the polarisation resistances found for P and V fibres, though possibly due to a true difference in membrane resistivity, may in part be the result of a difference in frequency of branching in the two types of fibres.

TIME COURSE OF RESISTANCE CHANGES

The equation used by Frank and Fuortes (1956) for determining the resting resistance of motoneurons was as follows:—

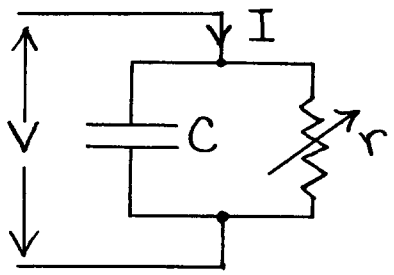
$$\frac{dE_s}{dI} = R_r - R_s \qquad (1)$$

in which E_s is the height of the action potential during current flow, I the applied current, R_s the polarisation resistance at the crest of the action potential, and R_r the resting polarisation resistance. This equation takes no account of the time required to recharge the membrane capacitance after the drop in resistance. Although this was of no consequence in their experiments, it is of considerable importance when applying it to the time course of polarisation resistance changes.

Consider the extreme case in which the polarisation resistance returns instantaneously to its original resting value at some point during the depolarisation phase. The IR_r displacement would then be reestablished at a rate determined by the time constant of the membrane. It is obvious that under such conditions a period equal to several time constants of the membrane must elapse before the IR_r potential can be fully established. This means that, if the time constant of the membrane is greater than the duration of the rising phase, no significant voltage displacement of the crest of the action potential can occur.

We have attempted to deduce the time course of potential change produced by constant current, when the resistance returns from zero to its original value at a certain rate. For the sake of simplicity we have assumed that the resistance returns exponentially to its resting value and that the membrane capacitance remains constant.

The equivalent circuit of the membrane component seen by the microelectrode can be approximated by the simple circuit



The relationship between the voltage V and the time t when $r = R(1 - e^{-t/\tau})$ and I and C are constant, is given by the following equation:

$$\frac{dV}{dt} + \frac{V}{RC(1 - e^{-t/\tau})} = \frac{I}{C} \tag{2}$$

This equation was solved numerically for us on an electronic computer and the solutions are shown graphically in Fig. 11.

It can be seen from Fig. 11 that when $\tau \approx RC$ the curve lies between those of $V = IR(1 - e^{-t/RC})$ and $V = IR(1 - e^{-t/2RC})$. When $\tau \ll RC$ the curve follows to a first approximation that of $V = IR(1 - e^{-t/RC})$.

As the time constants of P fibres are of the order of 4 msec. and the duration of the depolarisation phase in P fibres is close to 1 msec., it can be concluded that the crest of the action potential would not suffer any significant voltage displacement by the current even though the resistance may have returned by that time to normal. Although it is possible, by the method we used, to determine whether a drop in resistance occurs during the depolarisation phase, it is not possible to determine its exact time of occurrence or its duration.

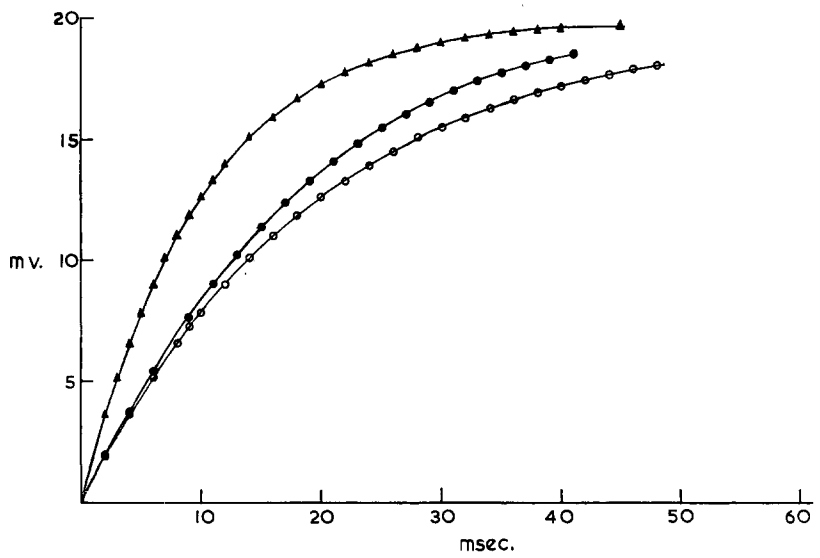


FIGURE 11. ●——● Graph of the numerical solution of the equation

$$\frac{dV}{dt} + \frac{V}{RC(1 - e^{-t/\tau})} = \frac{I}{C}$$

for $R = 100$ K; $C = 0.1$ μf; $I = 2 \times 10^{-7}$A; $\tau = 10$ msec. ▲——▲ and ○——○ are exponential curves with equations

$$V = RI(1 - e^{-t/RC})$$

and $$V = RI(1 - e^{-2(t/RC)})$$ respectively.

Though these conclusions do not affect estimates of resting polarisation resistance, they do, however, invalidate estimates of the ratio of resting to crest resistance of Purkinje fibres (Weidmann, 1951, 1957). The time course of membrane resistance changes during the repolarisation phase of Purkinje fibres should also be reconsidered. These fibres have a membrane time constant of approximately 20 msec. (Weidmann, 1952) and the technique which was used to determine the membrane resistance would be affected in the same way as the one used in the present experiments. Weidmann's conclusion was (Fig. 7 of Weidmann, 1957) that the membrane resistance increases

progressively throughout the plateau of the action potential. He took the voltage displacement at the end of a 40 msec. constant current pulse to be proportional to the polarisation resistance at that time. However, he did not appear to take into account the following facts: (*a*) It can be seen from equation (2) that the time course of charging of the membrane capacitance while the membrane resistance is changing depends on both the value of membrane resistance and its rate of change, and that the potential does not change with an exponential time course. (*b*) The fact that a threefold increase in membrane resistance was reported to occur during the repolarisation phase means that the time constant of the membrane increased to approximately 60 msec. Because of the time taken to charge the membrane capacitance, changes in polarisation resistance are accompanied by slower changes in the potential drop produced by constant current. The ability of Weidmann's method to follow changes in polarisation resistance is limited by the value of membrane time constant. For instance an instantaneous change in resistance to three times the resting value would be recorded as an exponential increase in potential with a time constant of 60 msec. This would place the occurrence of the increase in membrane resistance earlier in the repolarisation phase than he reported. Indeed it would be extremely difficult to follow the time course of the membrane resistance changes in these fibres because of the high value of the membrane time constant. A fibre such as a V fibre with its short membrane time constant would be the more suitable.

A more definite conclusion can be reached about the duration of the drop in polarisation resistance in V fibres. We can assume that a drop in resistance occurs at some stage of the depolarisation phase; this assumption is based on the appearance of the positive slope in some of these fibres and the rapidity of the potential change during depolarisation. The fact that both the crest and the base of the action potential suffer the same displacement by moderate values of hyperpolarising current must mean that by the time the crest is reached the membrane resistivity has been at its resting value for a period equal to several time constants of the membrane. From equation (2), when $\tau \approx RC$, the "time constant" of the potential change during the application of constant current is approximately equal to $\tau + RC$. Taking 2 msec. as a typical value for the duration of the depolarisation phase of the V fibre action potential, this places an upper limit on $\tau + RC$ of 2 msec. at the most. This supports the conclusion that the time constant of V fibres is indeed less than 1 msec.

If our conclusions are correct in that the duration of the drop in membrane resistivity is less than the duration of the depolarisation phase, we are faced with the situation in which in the terminal phase of depolarisation the membrane resistivity has returned to its resting value. On the basis of the sodium theory the fibre can continue to depolarise only if the membrane remains

dominantly permeable to sodium ions. Since the membrane resistivity at this time is normal this dominance could only be maintained by a drop in permeability of the membrane to potassium and other ions. If such a drop in permeability occurred towards the end of the transient increase to sodium ions the ratio of the sodium permeability to potassium permeability could remain high and depolarisation could continue with a membrane resistivity not far different from its resting value.

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