RESTING POTENTIAL
AND ELECTRICAL PROPERTIES OF FROG SLOW MUSCLE
FIBRES. EFFECT OF DIFFERENT EXTERNAL SOLUTIONS

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SUMMARY

1. The electrical properties of frog slow muscle fibres were investigated with intracellular micropipettes to determine their characteristic length (λ), specific membrane resistance (R_m) and specific membrane capacitance.

2. The value of λ was about 1 cm in fibres of 1·2 cm length. The 'short cable model' was used to calculate R_m. Its mean value was $1·12 \times 10^5$ ohm cm², about 10–20 times larger than the value for twitch fibres. The mean value for C_m was $3·24 \times 10^{-6}$ F/cm².

3. Resting potentials measured immediately after penetration with a single micropipette were about -80 mV. Lower values can be attributed to the effects of damage or leakage produced by micropipette insertion.

4. Changes in external K concentration produced changes in the initially recorded resting potentials which follow the constant field theory using a ratio of Na:K permeabilities $P_{Na}/P_K = 0·02$. Changes in external Cl concentration produced little or no change in the resting potential or membrane resistance, indicating a low Cl permeability.

5. In agreement with previous work, slow fibres showed a time-dependent decrease in resistance ('delayed rectification') for membrane potentials more positive than -60 mV. 'Anomalous rectification' observed in twitch fibres was not seen in slow fibres. In high external K concentrations the resistance of slow fibres is almost unaffected by changes in membrane potential.

6. Increasing the concentration of external Ca (up to isotonic) has two distinct effects on slow fibres. It increases R_m up to ten times, and it improves the stability of trans-membrane recordings, probably by reducing the leakage due to micropipette penetrations. Magnesium does not appear to have either of these effects.

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INTRODUCTION

It is well established that two distinct groups of extrafusal muscle fibres are present in the frog: 'twitch' muscle fibres and 'slow' muscle fibres (Tasaki & Mizutani, 1944; Kuffler & Vaughan Williams, 1953a, b). They differ in their innervation, electrical properties, contraction characteristics and structure (Burke & Ginsborg, 1956; Peachey & Huxley, 1962; Adrian & Peachey, 1965; Page, 1965; Nasledov, Zachar & Zacharova, 1966; Lannergren, 1967; Peachey, 1968).

The intracellular recording of slow junctional potentials (s.j.p.s) after nerve stimulation is normally used to identify slow fibres. Because we wished to identify slow fibres in denervated muscles, we have made a further study of the electrical properties of normal slow fibres. In addition to providing reliable electrical criteria that enable one to identify slow fibres, our results extend previous investigations on the 'cable properties' of the fibres (Adrian & Peachey, 1965), the rectifying properties of the membrane (Burke & Ginsborg, 1956; Oomura & Tomita, 1960a, b) and the effects of changes in external solution on the resting potential (Kiessling, 1960). The effect of different external solutions on the membrane resistance and rectifying properties of the slow muscle fibres membrane was also studied.

METHODS

The experiments were made on the piriformis, iliofibularis and extensor longus digiti IV (EDL IV) muscles of male English frogs (*R. temporaria*) during the winter and spring 1967/68.

Conventional techniques for intracellular recording and stimulation were used. Micropipettes were pulled from calibrated serological pipettes (Dispo, 20 microlitres). The use of such pipettes was suggested by Dr G. Blackman, and gave a dependable supply of high resistance micropipettes. Recording pipettes were filled with 3 mM-KCl, and were selected to have resistances of 20–60 MΩ and tip potentials of −5 mV or less. Such micropipettes were generally stable and little affected by the various external solutions used.

Current-passing micropipettes were filled with 2 M-K citrate. A symmetrical assembly of saturated KCl bridges was used to minimize liquid–junction potentials when changing solutions (Fig. 1). Current micropipettes were connected via a chlorided silver wire to a 500 or 1000 MΩ resistance leading to a pulse generator in parallel with a d.c. voltage source. The current was recorded as a voltage drop across a 500 kΩ resistance connecting the bath to ground (Fig. 1). Single muscle fibres were generally impaled under visual control in the middle of their length with the recording and current micropipettes separated by 50–200 μ. Where the cable properties were studied, the current micropipette was inserted either at the end or in the middle of the muscle fibre, and two recording micropipettes were placed, one 50–200 μ and the other 1–3 mm from the current micropipette.

In the iliofibularis muscle slow fibres occurred throughout the tonus bundle (Sommerkamp, 1928), but locating them was a matter of trial and error. The connective tissue surrounding the muscle always presented difficulties and we usually found only two to six slow fibres in more than forty penetrations per muscle. In the EDL IV muscle, slow fibres were very easily damaged during dissection since they were located along the external border and cutaneous

face of the muscle. Connective tissue was sometimes troublesome, but it seemed that fibres with a prominent striation pattern (using a compound microscope) were usually slow fibres and this helped in locating them.

The piriformis muscle (see p. 185, Ecker & Wiedersheim, 1896) proved to be a superior preparation for work on the slow fibres, for a number of reasons. When dissected the muscle could be flattened to allow good viewing of fibres. The connective tissue surrounding the muscle was soft and loose. In an average preparation of two paired muscles up to twenty slow fibres could be found, usually along the internal border of the cutaneous face, and recognizable by their prominent striation pattern. The slow fibres seemed to be large (about 80 μ in diameter), and could be followed for some distance over the muscle's surface. For these reasons the results reported here were obtained primarily from studies of the
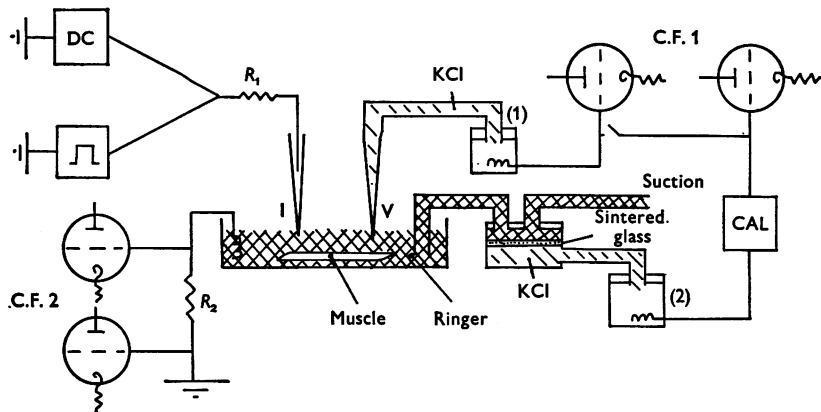

Fig. 1. Diagram of apparatus. Recording micropipette (V) was connected to a calomel half cell (1) via a 3 M-KCl bridge. The Ringer bath was connected via a fluid bridge to a reservoir where it made contact through fine porosity sintered glass with 3 M-KCl and then with a second calomel half cell (2). The voltage was recorded by a cathode follower (C.F. 1). Current was injected through R_1 (500 or 1000 MΩ) in series with a current micropipette (I) and was recorded as a voltage drop on R_2 (500 kΩ) by a second cathode follower (C.F. 2).

TABLE 1. Composition of the bath solutions used, given in mM/l.

Ringer	NaCl	KCl	CaCl$_2$	Tris Cl
	115	2·5	1·8	2·5
		K solutions		
Isotonic KCl		KCl	CaCl$_2$	Tris Cl
		117·5	1·8	2·5
Isotonic K$_2$SO$_4$*	K$_2$SO$_4$	CaSO$_4$	NaH$_2$PO$_4$	Na$_2$HPO$_4$
	95	9	0·43	1·08
(K).(Cl) = 310 mM^2		KCH$_3$SO$_3$	CaCl$_2$	Tris CH$_3$SO$_3$
		86	1·8	34
		Other solutions		
Isotonic MgCl$_2$		KCl	MgCl$_2$	Tris Cl
		2·5	80	2·5
Isotonic CaCl$_2$		KCl	CaCl$_2$	Tris Cl
		2·5	80	2·5
Low Cl	NaCH$_3$SO$_4$	KCl	CaCl$_2$	Tris Cl
	115	2·5	1·8	2·5

* Hodgkin & Horowicz (1959) equivalent to K (167 mM/l.) and Ca (1 mM/l.).

piriformis muscle. The other muscles were used early on in the study, and were re-examined to confirm results in the piriformis.

Solutions. Table 1 gives the composition of the different solutions used. Isotonic stock solutions of differing ions were mixed, or were added to Ringer, to make up the required test solutions. All solutions were buffered to pH 7·3 with Tris (hydroxymethyl) aminomethane chloride (Sigma), and stored at 4° C. The temperature of the bath was kept at 4–7° C by a Peltier cooling unit attached to the experimental chamber.

RESULTS

Identification of slow muscle fibres

While surveying the mechanical responses elicited in different muscles by solutions with high potassium, it was found that the piriformis muscle developed a maintained contracture of about 1–2 g. Such a maintained

TABLE 2. Correlation between electrical characteristics and innervation of twitch (t) and slow (s) muscle fibres in the piriformis muscle

No. of fibres	Nerve stimulation		Action potentials	Effective resistance (V_0/I_0) ($\times 10^6 \, \Omega$)	Time constant (msec)
	Delay (msec)	Multi-innervation			
16 (t)	3.95 ± 1.10	—	+	< 1.5	< 30
12 (s)	21.20 ± 1.04	+	—	6.22 ± 1.44	341 ± 88

Values are expressed as mean ± S.D. The time constant of slow muscle fibres was estimated according to the short cable model, assuming $R_1 = 250$ cm, $d = 8 \times 10^{-3}$ cm and $L = 1.2$ cm. Action potentials were tested by intracellular stimulation (Temp. 5° C).

contracture is usually attributed to the presence of slow muscle fibres (Kuffler & Vaughan Williams, 1953b). The presence of slow fibres in the piriformis muscle was then confirmed by intracellular recording from single muscle fibres. It was immediately seen that there were multiple-innervated fibres with long latency s.j.p.s presenting the electrical properties of slow fibres (see Table 2). It may be helpful at this point to summarize the electrical properties we found useful for identifying slow fibres, keeping in mind that the summary is to a large extent the result of work discussed later in this article, although it is also dependent on previous work (e.g. Burke & Ginsborg, 1956; Adrian & Peachey, 1965; Stefani & Steinbach, 1968).

(i) Effective resistance (resistance between the inside and the outside, written henceforth V_0/I_0) over 2 MΩ, reflecting high values of specific membrane resistance (R_m); (ii) time constant over 100 msec; (iii) absence of action potentials (but this is significant only if the muscle fibre is not in cathodic depression); (iv) 5–10 times increase in V_0/I_0 in high Ca external solutions. A low value of resting potential (E_{RP}) or the presence of delayed rectification, were not reliable indicators of slow fibres, since they can be encountered in damaged twitch muscle fibres.

Measurements of characteristic length (λ), *specific membrane resistance* (R_m) *and specific membrane capacitance* (C_m)

The theory applied in this section has been described by Hodgkin & Rushton (1946) and the notation follows that of Katz (1948). The modification for the short cable model has been described by Weidmann (1952). The experiments described here were made on dissected bundles of about ten muscle fibres in order to obtain better viewing for an approximate measure of the diameter of the muscle fibres.
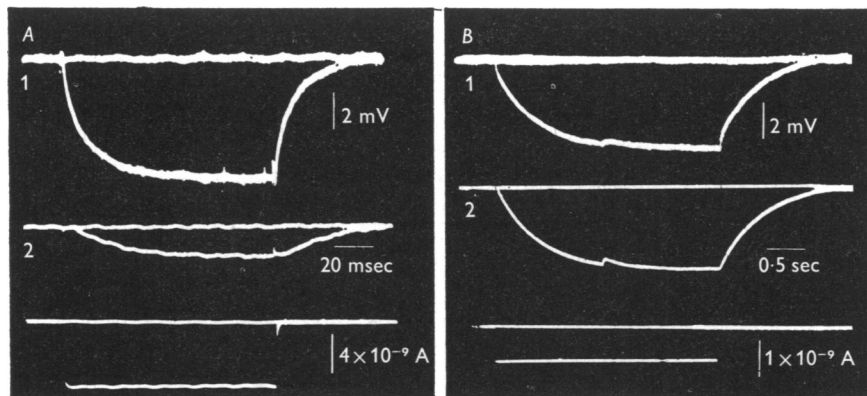

Fig. 2. Simultaneous records of V_o and V_x in twitch and slow muscle fibres with the current micropipette in the middle of the fibres. A: twitch muscle fibre, in 1, $x = 0$; in 2, $x = 0.23$ cm. B: slow muscle fibre; in 1, $x = 0$; in 2, $x = 0.30$ cm. Note the different time course of the pulses as $x = 0$ in twitch (A, 1) and slow (B, 1) muscle fibres, and the small attenuation with distance in the slow muscle fibre (B, 2).

One current-passing micropipette and two recording micropipettes were inserted into single fibres selected for study. As stated in Methods, the current micropipette was inserted either at the end or in the middle of the fibre. A long square pulse of current (I_0) was passed through the current micropipette and the resulting change in transmembrane potential was simultaneously recorded at the near recording micropipette ($V_{0, t}$) and the distant recording micropipette ($V_{x, t}$) (Fig. 2). Steady values of $V_{0, t}$ and $V_{x, t}$ produced by I_0 are denoted by V_0 and V_x respectively. The resting potentials (E_{RP}) of slow fibres recorded immediately after the insertion of the first micropipette were about -80 mV, but fell off rapidly to -60 to -70 mV (see Fig. 4A, next section). The insertion of the current micropipette invariably produced a further drop of E_{RP}. Thus in all slow fibres hyperpolarizing current was passed through the current micropipette to drive the membrane potential (E_m) to a value of about -80 mV, roughly corresponding to the E_{RP} recorded immediately after the first penetration. At this membrane potential V_0 was linearly proportional

to I_0; hence in our measurements delayed rectification does not occur and the membrane resistance can be considered linear.

For twitch muscle, previous authors have used the infinite cable model (e.g. Katz, 1948; Fatt & Katz, 1951). For slow muscle fibres the short cable model is necessary, since the length of the fibres is about 1·2 cm and the apparent value of λ, if calculated according to the infinite cable model, is about 3 cm. For the short cable model the following relations hold (Weidmann, 1952):

$$\text{at} \quad x = 0, \quad V_0/I_0 = r_1\lambda \coth (L/\lambda), \tag{1}$$

$$\text{at} \quad x \neq 0, \quad V_x/V_0 = \cosh \left(\frac{L-x}{\lambda}\right)\bigg/\cosh (L/\lambda), \tag{2}$$

where the current micropipette is inserted at the end of the muscle fibre, x is the distance between the current and recording micropipettes, L is the length of the fibre, r_1 the internal resistance of the fibre per unit length (Ω/cm), $\lambda = (r_m/r_i)^{\frac{1}{2}}$ and r_m is the transverse membrane resistance x unit length ($\Omega \times \text{cm}$).

When the current micropipette is inserted in the middle of the fibre, L is replaced by 0·5L, and the right-hand side in e.g. (1) should be multiplied by 0·5. The short cable equations are necessary when L is less than about 2λ; for example, if $L/\lambda = 2$, with the current electrode at the end, and using the semi-infinite cable equation, $r_1\lambda$ would be over-estimated by a factor of 1·037. When $L \gg \lambda$ the short cable eqns. (1) and (2) are transformed into the semi-infinite cable eqns. $V_0/I_0 = r_1\lambda$ (3) and $V_x/V_0 = \exp (-x/\lambda)$. (4)

Equation (2) was used to obtain the values of λ for slow fibres given in Table 3. From each value of λ and V_0/I_0 one can obtain r_1 from eqn. (1). Since $\lambda = (r_m/r_i)^{\frac{1}{2}}$ a value of r_m is also obtained. To calculate R_m, one needs to know the diameter of the fibre or to calculate it from $d = (4R_1/\pi r_1)^{\frac{1}{2}}$, since $R_m = \pi d r_m$. We assumed $R_i = 250 \, \Omega$ cm (Katz, 1948; Fatt & Katz, 1951) and then compared the calculated diameter with the diameter estimated from visual measurement. The mean diameter measured in slow muscle fibres $7·5 \pm 0·57 \times 10^{-3}$ cm ($\pm$ S.E.) was not significantly different from the mean diameter calculated $8·3 \pm 0·77 \times 10^{-3}$ cm ($\pm$ S.E.).

Table 3 presents the results for nine slow fibres and six twitch fibres. The infinite cable equations were used to calculate R_m and λ for the twitch fibres. Qualitatively, the difference in λ, and thus in R_m, in fast and slow fibres can be seen in Fig. 2.

Having obtained R_m it was then necessary to calculate the time constant (τ_m), in order to find C_m, since $\tau_m = R_m . C_m$. For the infinite cable model, the time taken for $V_{0,t}$ to reach 0·84 of its steady value (V_0) is a measurement of the τ_m. In a spherical core conductor the equivalent value of $V_{0,t}/V_0$ is 0·63. The appropriate values of the $V_{0,t}/V_0$ for the short cable model lie between these two extremes, and can be estimated by assuming total

TABLE 3. Electrical characteristics of slow and twitch muscle fibres in the piriformis muscle

Slow muscle fibres were always polarized to a membrane potential (E_m) close to the initially recordable resting potential. The values shown were obtained from analysis of hypolarizing pulses 2–5 mV in amplitude. Similar values were obtained with 15–25 mV pulses. The current micropipette was always located in the middle of twitch muscle fibres, and in the middle or at one end of slow muscle fibres. Diameters were calculated assuming $R_i = 250\ \Omega$ cm in both fibre types. Values, corrected according to the short cable equation, of the time constant (τ_m) and of the specific membrane capacitance (C_m), are given for slow fibres assuming $d = 8 \times 10^{-3}$ cm and $L = 1\cdot2$ cm. Values at the bottom of each column are expressed as mean ± S.D.

Slow muscle fibres

Membrane potential (E_m) (mV)	Position current micro-pipette	Effective resistance (V_o/I_o) (×10⁶ Ω)	Length constant (λ) (= cm)	Diameter measured (cm ×10⁻³)	Diameter calculated (cm ×10⁻³)	Time constant (τ_m) (msec)	Corrected time constant for short cable (msec)	Specific membrane resistance (R_m) (×10⁵ Ω cm²)	Specific membrane capacitance (C_m) (μF/cm²)	Specific membrane capacitance corrected for short cable (C_m) (μF/cm²) Ω
−80	Middle	5·55	1·23	—	8·2	935	545	1·80	5·19	3·03
−80	Middle	4·70	0·97	—	7·3	620	385	1·28	4·84	3·00
−80	Middle	2·66	0·76	7	8·2	310	208	0·69	4·47	3·00
−80	Middle	4·66	0·75	7	6·2	510	300	0·92	5·56	3·28
−80	Middle	4·64	0·71	7	6·0	545	360	0·85	6·45	4·25
−75	End	2·60	0·85	8	10·1	465	268	0·60	7·18	3·95
−80	End	6·80	1·30	7	9·1	845	395	1·83	4·62	2·26
−80	End	3·91	1·05	9	10·0	640	320	1·06	6·03	2·91
−81	End	4·52	1·00	7·5	9·2	730	370	1·09	6·70	3·50
—	—	4·44 ± 1·35	0·96 ± 0·2	7·5 ± 1·5	8·3 ± 2·3	623 ± 57	351 ± 89	1·12 ± 0·43	5·72 ± 1·26	3·24 ± 0·59

Twitch muscle fibres

Resting potential (E_{RP}) (mV)	Position current micropipette	Effective resistance (V_o/I_o) (×10⁶ Ω)	Length constant (λ) (cm)	Diameter calculated (×10⁻³ cm)	Time constant (msec)	Specific membrane resistance (R_m) (×10³ Ω cm²)	Specific membrane capacitance (μF/cm²)
84	Middle	0·370	0·208	9·45	24	4·620	5·20
83	Middle	0·565	0·202	9·10	20	4·500	4·45
83	Middle	0·390	0·195	7·40	19	5·110	3·72
87	Middle	0·835	0·195	6·00	24	6·221	3·86
84	Middle	0·500	0·204	8·00	21	5·149	4·09
83	Middle	0·795	0·216	6·60	25	7·050	3·54
—	—	0·575 ± 0·057	0·203 ± 0·014	7·76 ± 0·13	22·2 ± 2·5	5·430 ± 0·89	4·14 ± 0·58

electrical reflexion from the ends of the short cable and generating responses by the graphical superimposition of known responses in the infinite cable model (Fig. 3).

Table 3 shows the uncorrected and corrected values of τ_m for the short cable model. From the measurement of τ_m, knowing R_m, we can calculate C_m.
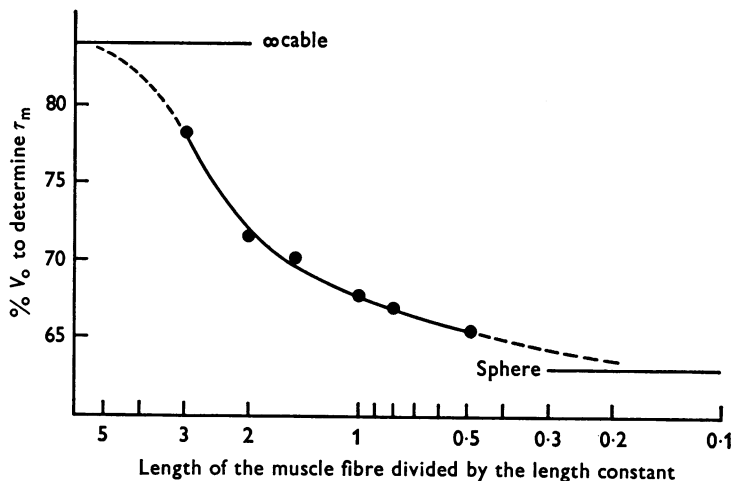

Fig. 3. Theoretical relation between the length of a muscle fibre divided by its length constant and the percentage of the final value of V_0 (during a current step) that is reached when $t = \tau_m$. Values range from 63 % in the very short (special) case to 84 % in the infinite cable.

The calculated mean value of R_m for nine slow fibres was $1\cdot12 \pm 0\cdot43 \times 10^5$ $\Omega\,cm^2$ (mean $\pm$ s.d.). Although V_x/V_0 was not determined in the slow fibres of the iliofibularis muscle, in one experiment eight fibres examined gave a value of V_0/I_0 of $8\cdot13 \pm 2\cdot1 \times 10^6\,\Omega$; other experiments gave comparable values. Since the diameter of the iliofibularis slow muscle fibre is probably less than that of a piriformis slow fibre (Adrian & Peachey, 1965) the value of R_m for iliofibularis slow fibres is probably no larger than that for piriformis slow fibres.

The mean calculated value of C_m for the piriformis slow fibres was $3\cdot24 \pm 0\cdot59 \times 10^{-6}$ F/cm². The value for twitch muscle fibres in the piriformis was $4\cdot14 \pm 0\cdot58 \times 10^{-6}$ F/cm². The C_m calculated for slow fibres was less than that for twitch fibres, but quite clearly greater than 1×10^{-6} F/cm² expected for a simple cylindrical cell.

Measurement of resting potential

The high specific membrane resistance of slow muscle fibres greatly increases the difficulty of obtaining reliable measures of resting potential.

Ideally, a micropipette should penetrate suddenly a cell surface, the cell membrane should seal around the micropipette, and a steady potential difference (E_{RP}) should be recorded relative to the bath. This was not usually the case in our experiments with slow fibres. Penetrations of fibres which were later identified as slow fibres fell into three categories: (i) when using unselected micropipettes, or when working with preparations with abundant connective tissue, penetrations were slow and the values of E_{RP} measured were -55 to -70 mV; (ii) when micropipettes were carefully
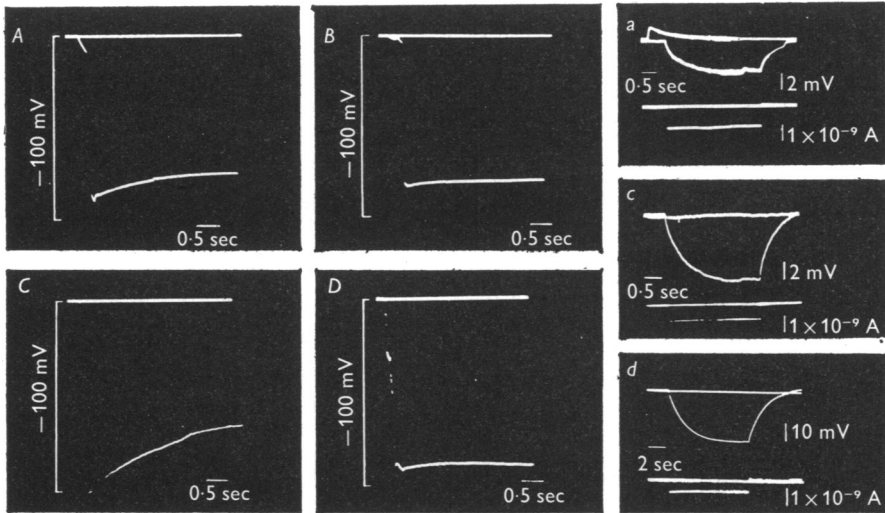

Fig. 4. Records of penetrations and effective resistance (V_0/I_0) in slow muscle fibres in Ringer (A, a; B), in isotonic MgCl$_2$ (C, c) and in isotonic CaCl$_2$ (D, d). In A the initial recorded resting potential (E_{RP}) falls to a steady value; (a) shows V_0/I_0 in the same fibre (note miniature end-plate potential at the beginning of base line). In B the E_{RP} was stable. In C (isotonic MgCl$_2$), the E_{RP} fell to a steady value; c shows V_0/I_0 in the same fibre. In D (isotonic CaCl$_2$) the initial E_{RP} was stable, even when the penetration was not good; (d) shows V_0/I_0 in the same fibre (note very slow time course of V_0).

selected, penetrations were abrupt, the value of E_{RP} reached an initial value of about -80 mV, but fell to -50 to -70 mV within a few seconds (Figs. 4A and 5A); (iii) in a few cases, when penetrations were abrupt, the measured value of E_{RP} stayed at about -80 mV until the second electrode was introduced (Fig. 4B). In previous work, the whole range of E_{RP} from -55 to -85 mV has been regarded as representative of slow fibres. We are inclined to believe that only the initial deflexions measured in 'good' penetrations represent the real values of resting potential, and even then only approximately. High values of E_{RP} were obtained only with micropipettes of both high resistance and low tip potential, and when the tip potential did not appreciably change in low Cl or high K solutions. In the

iliofibularis muscle, the initial resting potential was $-79\cdot7 \pm 2\cdot1$ mV (mean $\pm$ S.D., determined in eight fibres of one preparation), which is not significantly different from the values obtained in the piriformis muscle ($81\cdot3 \pm 3\cdot0$, the mean of twenty-four fibres in three preparations, Fig. 5, Tables 4, 5).

The effects of changes in external solution upon resting potential and resistance

Our initial attempts to record from a single slow fibre continuously during solution changes were frustrated by incidental damage to the fibre membrane. The following procedure was then used to obtain more reliable estimates of E_{RP} as a function of external ionic composition. Initial and steady levels of E_{RP} were recorded in the starting solution (usually Ringer, Table 1). A second micropipette was inserted, and responses to square pulses of current were recorded at various levels of E_m. Each fibre was classed as twitch or slow by using the criteria mentioned on page 385 (in practice, slow fibres are seldom confused with fast fibres because of their strikingly slow response to applied current). Several fibres were investigated in this way, then the solution was changed and time allowed for equilibration. Some of the fibres initially penetrated were re-examined, and several additional fibres were penetrated for the first time. The 10–20 slow fibres available in a single preparation of two piriformis muscles made this procedure possible.

(a) *Effect of changes in external K.* Figure 5 is a plot of steady and initial values of E_{RP} for twitch and slow fibres as a function of external potassium concentration $[K]_o$. The values for twitch fibres, in good agreement with previously reported results, fall along the theoretical curve using eqn. (4) of Hodgkin & Horowicz (1959), and assuming a permeability ratio $P_{Na}/P_K = 0\cdot01$ ($K_{int} = 140$ mM, $Na_{int} = 15$ mM). If one considers the initial values of E_{RP} for slow fibres in this experiment to represent the actual resting potentials of the fibres ($-82 \pm 3\cdot3$ mV), the results suggest a P_{Na}/P_K for slow fibres of about $0\cdot02$. If one considers only the steady values of E_{RP} the results are similar to those obtained by Kiessling (1960) recording from the same slow fibre during solution change. Note that the post-penetration fall-off in E_{RP} decreases with increasing external K, as one would expect if the fall-off reflected damage resulting in an ionic leak shunting the normal cell membrane.

As expected, the values of V_0/I_0 measured in both twitch and slow fibres decreased as external K was raised (Fig. 6). In normal Ringer ($[K]_o = 2\cdot5$ mM) all slow fibres studied showed a time-dependent decrease in resistance when E_m was above -60 mV (Fig. 6). This is the delayed rectification described previously (Burke & Ginsborg, 1956).

In normal Ringer twitch fibres showed the phenomenon of 'anomalous

rectification': a decrease in membrane resistance with hyperpolarizing pulses (Adrian & Freygang, 1962). This phenomenon was previously described in high external K (Katz, 1949). Contrary to twitch fibres, we found that slow muscle fibres behaved in a linear way in high $[K]_o$ (Figs. 6 and 7). This agrees with findings of Oomura & Tomita (1960a, b) in experiments in which voltage clamps were applied to slow muscle fibres. This
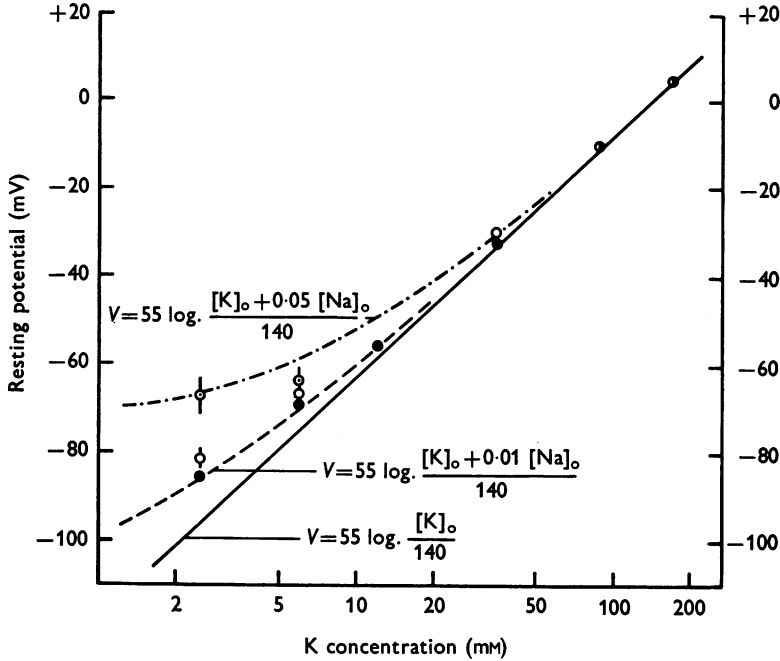

Fig. 5. Relation between testing potential (E_{RP}) and log external K concentration in twitch and slow muscle fibres. Each point represents the average data of eight to twenty-two determinations with the s.d. shown as a bar. Filled circles (●) are values for twitch muscle fibres; open circles (○) are values of the initial E_{RP} in slow muscle fibres and circles with dots (⊙) the steady E_{RP}. Half-filled circles (◑) represent identical values for twitch and slow muscle fibres. Up to 30 mM-$[K]_o$, NaCl was isotonically replaced by KCl. In 86 mM-$[K]_o$ the product $[K]_o \times [Cl]_o$ was maintained at 310 mM². The right-hand point in the K scale was obtained using a solution containing 190 mM-$[K]_o$ and corrected for the activity coefficient (Hodgkin & Horowicz, 1959, soln. H).

simple behaviour is noteworthy precisely because so few excitable cells are comparatively simple (Grundfest, 1966).

(*b*) *Effect of changes in external Cl⁻*. In several experiments piriformis muscles were kept in low Cl solution containing 25 mM-Cl⁻ for 1–2 hr before beginning micropipette impalements. Fibres were also examined 30–60 min after a change to Ringer. In agreement with previous results, the twitch

fibres showed the expected increase in resistance (V_0/I_0) in low Cl (Hutter & Noble, 1960). Slow fibres showed a slight decrease or no change in resistance between Ringer and low Cl⁻ solution (Table 4). E_{RP} was essentially the
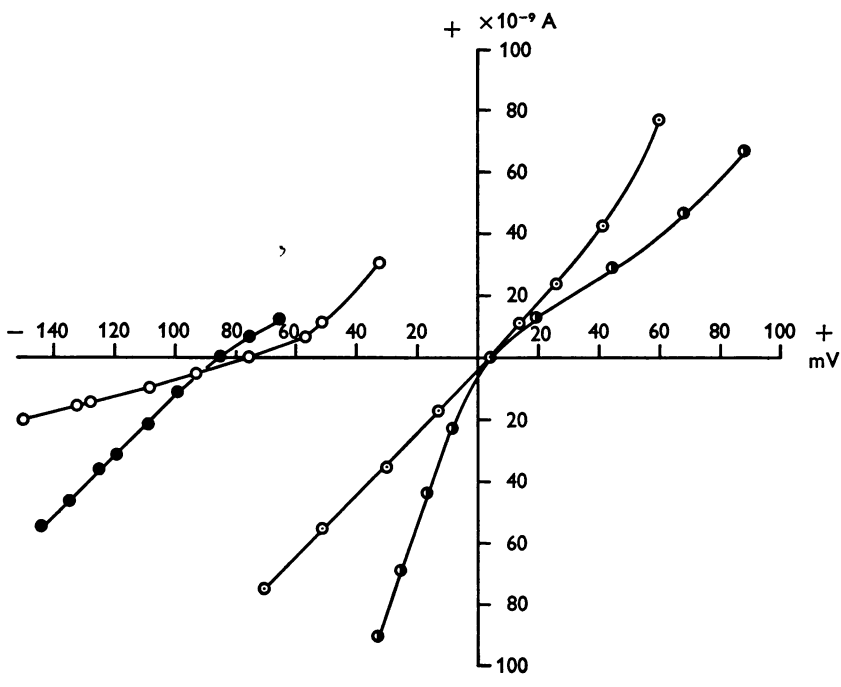

Fig. 6. Current–voltage relation at the current micropipette in slow and twitch muscle fibres in Ringer (○, slow and ●, twitch) and in isotonic K_2SO_4 (⊙, slow and ◑ twitch). Note that the slow muscle fibre did not show anomalous rectification in isotonic K_2SO_4 (⊙).
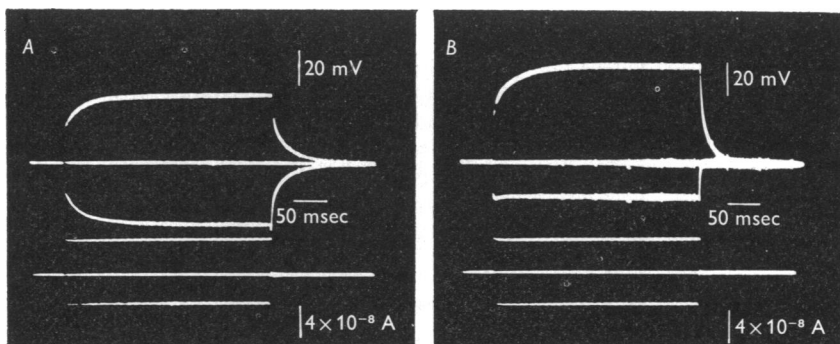

Fig. 7. Records of potential changes in inward and outward currents in slow (A) and twitch (B) muscle fibres in isotonic K_2SO_4. ($E_{RP} + 4$ mV for both fibres; inward current shows downward deflexion.) Note symmetry of the voltage response in both directions in (A) (slow muscle fibre). On the other hand, in (B) (twitch muscle fibre) the voltage response to outward current is larger (anomalous rectification).

same in both solutions and both types of fibres. These results indicate little or no participation of Cl^- in the membrane resting conductance.

(c) *Effects of changes in external Ca and Mg.* When the external concentration of calcium $[Ca]_0$ was raised, a large increase was observed in the effective resistance (V_0/I_0) of slow fibres, but only a slight increase in twitch muscle fibres (Fig. 4D; Table 5). In spite of the large increase of V_0/I_0 as

TABLE 4. Effect of low chloride Ringer on slow (s) and twitch (t) muscle fibres

Solution	No. of fibres	E_{RP} initial (mV)	E_{RP} steady (mV)	V_0/I_0 ($\times 10^6 \Omega$)	τ_m (msec)
Low Cl	10 (t)	80.2 ± 3.4	—	2.36 ± 0.84	31.4 ± 5.0
	6 (s)	76.2 ± 4.2	62.0 ± 5.5	3.97 ± 0.59	387 ± 71
Ringer	9 (t)	88.2 ± 1.4	—	1.15 ± 0.53	21.5 ± 6.0
	7 (s)	81.4 ± 1.5	63.0 ± 3.7	4.83 ± 1.18	229 ± 63

The preparation was equilibrated in 25 mM-Cl, the rest being replaced by CH_3SO_4; it was later examined in Ringer. The steady value of the resting potential (E_{RP}) in twitch muscle fibres was the same as the initial value. The time constant (τ_m) was estimated according to the infinite cable eqn. for twitch muscle fibres and to the short cable equation for slow muscle fibres, assuming $R_i = 250 \Omega$ cm, $d = 8 \times 10^{-3}$ cm and $L = 1.2$ cm. Values given are mean $\pm$ S.D.

TABLE 5. Effect of calcium on slow (s) and twitch (t) muscle fibres

Ca concentration (mM)	No. of fibres	E_{RP} initial (mV)	E_{RP} steady (mV)	V_0/I_0 ($\times 10^6 \Omega$)	τ_m (msec)
1.8	10 (t)	82.8 ± 2.4	—	0.860 ± 0.056	21.1 ± 2.5
	3 (s)	80.6 ± 2.2	69.3 ± 2.1	6.203 ± 0.337	558 ± 222
9.0	4 (t)	87.2 ± 1.0	—	0.907 ± 0.091	28.3 ± 3.1
	5 (s)	81.8 ± 3.0	71.6 ± 4.9	10.30 ± 4.00	1926 ± 300
20	5 (t)	88.6 ± 2.1	—	0.965 ± 0.077	34.3 ± 3.8
	5 (s)	81.8 ± 6.8	77.0 ± 7.1	25.30 ± 5.32	3502 ± 380
40	7 (t)	92.4 ± 2.8	—	1.224 ± 0.141	39.9 ± 6.1
	3 (s)	83.3 ± 4.2	83.1 ± 3.1	23.83 ± 2.35	3160 ± 80

The preparation was bathed in solutions of increasing Ca concentration allowing 30 min for equilibration after each change. Procedure of measurement the same as in Table 4. Values given are mean $\pm$ S.D.

$[Ca]_0$ was increased, slow fibres also appeared to become less susceptible to micropipette damage, i.e. impalements were more stable and the post-penetration fall-off in E_{RP} was practically abolished in 40–80 mM-CaCl$_2$ (Fig. 4D): this suggests an improved sealing of the membrane around the micropipette insertion under the influence of high $[Ca]_0$. Moreover, when the $[Ca]_0$ was raised in *damaged* slow fibres, a dramatic increase of E_{RP} to a value approaching the normal was usually observed (Stefani & Steinbach, 1968). The resting potential increased slightly in both twitch and slow fibres as $[Ca]_0$ was raised (Table 5).

The increase in V_0/I_0 produced by high external [Ca] in slow fibres was

about sixfold. If the fibres were considered to be an infinite cable, this would imply an increase in R_m by about 36-fold. However, as the specific membrane resistance of a 'short cable' increases, the electrical characteristics of the fibre approach those of a sphere. To illustrate the validity of this assertion, values of V_0/I_0 measured in various external Ca solutions have been distributed along the theoretical plot of V_0/I_0 versus R_m for a short cable (continuous line), $d = 8 \times 10^{-3}$ cm, $L = 1 \cdot 2$ cm, $R_i = 250 \ \Omega$ cm (Fig. 8). The interrupted lines show theoretical plots for the infinite
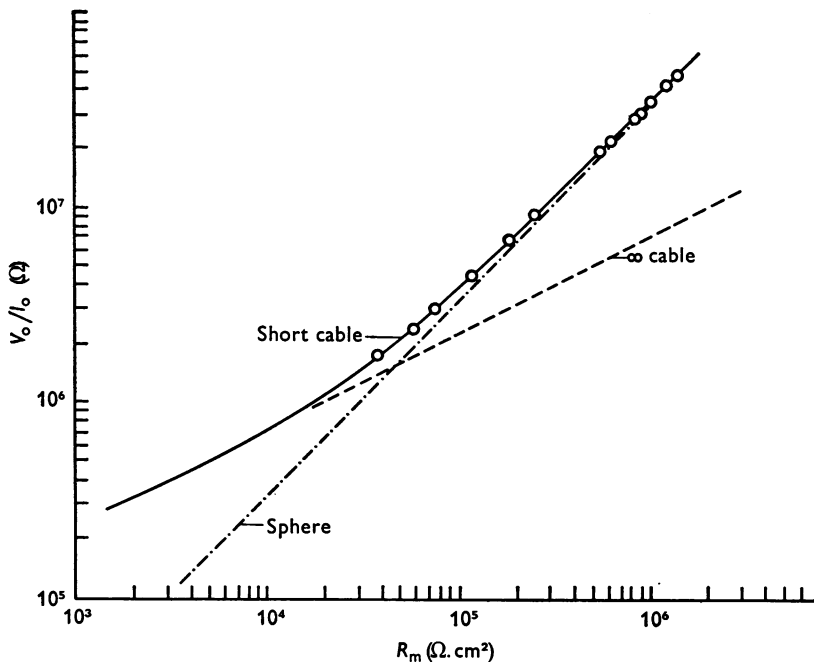

Fig. 8. Theoretical relation on log log plot between effective resistance (V_0/I_0) and specific membrane resistance (R_m) for the infinite cable (interrupted line); the short cable (continuous line) and sphere (interrupted and pointed line). The following values have been assumed: $R_i = 250 \ \Omega$ cm; $d = 8 \times 10^{-3}$ cm; $L = 1 \cdot 2$ cm; for the sphere, $d_s^2 = d \times L$ of short cable. Observed values of V_0/I_0 have been placed on the short cable curve and values of R_m were read on the abscissa.

cable and the interrupted pointed lines those for the sphere, diameter d_s, with $d_s^2 = L \cdot d$. From the values of R_m predicted for each value of V_0/I_0, and from measurements of the τ_m, C_m for each fibre was calculated. If the fibres behaved as short cables, the mean value of C_m should be close to that calculated from earlier measurements in Ringer (Table 3). In fact there was a close agreement (C_m estimated for fibres with values of V_0/I_0 between 2×10^6 and $10^8 \ \Omega$ was $3 \cdot 50 \pm 0 \cdot 98 \times 10^{-6}$ F/cm^2), especially considering the oversimplifying assumption of uniform fibre diameter. If the values of

V_0/I_0 were assumed to lie along the plot for the infinite cable model, clearly aberrant values of $C_m (0{\cdot}4{-}0{\cdot}02 \times 10^{-6}$ F/cm$^2)$ were calculated. Thus (from Fig. 8) an increase of a factor of 6 in V_0/I_0 corresponds to only a tenfold increase in R_m of the slow fibres.

The effects of Ca on R_m increased with time. To demonstrate this, a preparation was soaked overnight in a solution containing 9 mM-Ca at 5° C (10 % isotonic CaCl$_2$, 90 % Ringer). V_0/I_0 measured the next day exceeded those measured 1 hr after immersion of a fresh preparation in

TABLE 6. Effect of isotonic calcium and isotonic magnesium solutions
on slow muscle fibres

Solution	No. of fibres	E_{RP} initial (mV)	E_{RP} steady (mV)	V_0/I_0 ($\times 10^{-6}\ \Omega$)	τ_m (msec)
Isotonic MgCl$_2$	4	$95{\cdot}0 \pm 4{\cdot}4$	$77{\cdot}5 \pm 10{\cdot}0$	$11{\cdot}38 \pm 2{\cdot}80$	986 ± 380
Isotonic CaCl$_2$	6	$87{\cdot}3 \pm 4{\cdot}5$	$87{\cdot}7 \pm 2{\cdot}8$	$35{\cdot}36 \pm 9{\cdot}54$	1500 ± 110

The preparation was first immersed in isotonic MgCl$_2$ and then in isotonic CaCl$_2$. Procedure of measurement was the same as in Table 4. The measurements in isotonic CaCl$_2$ are subject to 5–10 % attenuation of the input resistance load on the cathode follower (about 80 MΩ total). Values given are mean $\pm$ S.D.

TABLE 7. Effect of prolonged soaking in Ringer containing 9 mM-CaCl$_2$
on slow (s) and twitch (t) muscle fibres

Solutions	No. of fibres	E_{RP} initial (mV)	E_{RP} steady (mV)	V_0/I_0 ($\times 10^6\ \Omega$)	τ_m (msec)
9 mM-CaCl$_2$	10 (t)	$89{\cdot}5 \pm 2{\cdot}0$	—	$1{\cdot}08 \pm 0{\cdot}48$	$27{\cdot}4 \pm 4{\cdot}8$
	6 (s)	$78{\cdot}4 \pm 2{\cdot}1$	$67{\cdot}0 \pm 4{\cdot}0$	$57{\cdot}75 \pm 5{\cdot}85$	2206 ± 220
Isotonic CaCl$_2$	12 (t)	$95{\cdot}9 \pm 1{\cdot}7$	—	$1{\cdot}33 \pm 0{\cdot}86$	$46{\cdot}9 \pm 10{\cdot}5$
	7 (s)	$81{\cdot}2 \pm 6{\cdot}4$	$82{\cdot}0 \pm 5{\cdot}6$	$88{\cdot}98 \pm 21{\cdot}5$	9642 ± 1350

The muscle was left overnight in Ringer with 9 mM-CaCl$_2$ at 5° C, and was examined next morning. Then it was equilibrated in isotonic CaCl$_2$ and re-examined. Procedure of measurement same as in Table 4. The measurements in slow muscle fibres are subject to 5–10 % attenuation because of the input resistance load on the cathode follower (80–130 MΩ). Values given are mean $\pm$ S.D.

isotonic CaCl$_2$ (Tables 6, 7). Thus the values in Table 5, measured after only 30 min of equilibration, are probably low estimates. The penetrations were not improved after the long treatment with [Ca]$_o$ = 9 mM. Addition of isotonic CaCl$_2$ to this preparation produced an immediate twofold increase in V_0/I_0 and stable E_{RP} were obtained, reducing the fall-off of E_{RP} to practically zero. This twofold increase in V_0/I_0 may be interpreted as a result of an improved sealing around the micropipette penetrations in isotonic CaCl$_2$. It may be concluded that Ca has at least two effects on slow muscle fibres: it increases the membrane resistance and improves the micropipette penetrations. These two effects can be partially dissociated by leaving the preparation overnight in Ringer with [Ca] = 9 mM, since

V_0/I_0 is considerably increased, with no apparent improvement in the impalements.

Isotonic $MgCl_2$ produced at most a doubling in V_0/I_0 and virtually no improvement in post-penetration fall-off (Fig. 3C; Table 6). The effects of Mg did not seem to increase with time.

DISCUSSION

In previous studies of fibre types in frog skeletal muscles, slow fibres were selected on a basis of electrical responses to nerve stimulation (Kuffler & Vaughan Williams, 1953a; Burke & Ginsborg, 1956). The identification of fibres was sometimes confirmed by electron micrographs of thin sections through marked fibres (Adrian & Peachey, 1965). The population of fibres that we have called slow fibres have properties which, as far as we have examined them electrically, are identical to those of slow fibres previously studied.

Slow muscle fibres have a specific membrane resistance (R_m) about twenty times greater than twitch muscle fibres, and a specific membrane capacitance (C_m) smaller than that measured in twitch muscle fibres, but definitely larger than that expected for a simple cylindrical cell (Katz, 1949; Fatt & Katz, 1951). These measurements are subject to errors, since it was necessary either to assume a value of specific internal resistance (R_i) or to calculate it from the measured diameter of the muscle fibres. A fair agreement was found between the measured diameter and the one calculated by assuming $R_i = 250$ Ω cm (Bozler & Cole, 1935; Katz, 1949), suggesting that this assumption is likely to be valid. Moreover, direct measurement of R_i in twitch muscle fibres at different temperatures gave a value of about 300 Ω cm at 6° C (Tamashige, 1950). Our measurements of R_m for slow muscle fibres are somewhat higher than the values obtained by Adrian & Peachey (1965). However, considering the difficulties in obtaining good measurements of R_m in slow muscle fibres because of their high membrane resistance, we do not think that the difference in the results is very significant. Moreover, for the same reasons the membrane resistance and the resting potential measurements we obtained may be underestimates.

Falk & Fatt (1964) have attributed the large membrane capacitance measured in sartorius muscle fibres to the capacity of the walls of the transverse tubular (T) system (Andersson-Cedergren, 1959; Peachey, 1965), which would be in parallel with the capacity of the surface membrane.

Recent ultrastructural studies reported the presence of the transverse tubular system in slow muscle fibres of the frog (Page, 1965). However, in the slow muscle fibres the relative amount of the transverse tubular system is somewhat less than in twitch muscle fibres (Page, 1965). Our

estimate of membrane capacitance is within the limits estimated by Adrian & Peachey (1965), and consistent with the idea that slow muscle fibres have a somewhat smaller amount of transverse tubular system compared with twitch muscle fibres.

The present results suggest that slow fibres normally have higher resting potentials than reported previously (Kuffler & Vaughan Williams, 1953a; Oomura & Tomita, 1960a, b; Kiessling, 1960). Slow fibres have high values of V_0/I_0, and therefore the resting potential recorded should be very sensitive to the shunt produced around the micropipette insertion. In order to assess the magnitude of the shunt, the resistance was calculated from an equivalent circuit. In some penetrations the initially recorded resting potentials of about 80 mV decayed to a steady level around 65 mV, suggesting a progressive shunt that attained a final value of about 20 MΩ. However, when stable penetrations were obtained with little loss of E_{RP}, the calculated shunt resistance had much higher values. Although such a shunt would produce a significant error in the determination of the E_{RP}, it would produce a less noticeable attenuation of about 20 % in the V_0/I_0 measured. The same shunt introduced in a twitch fibre membrane would have a negligible effect on either V_0/I_0 or E_{RP}, because of the lower V_0/I_0 in such fibres (Nastuk & Hodgkin, 1950; Fatt & Katz, 1951).

Kiessling (1960) measured resting potentials in iliofibularis slow fibres as a function of the external sodium [Na]$_0$ and potassium [K]$_0$ concentration, and concluded that the changes of *mean values* of resting potential did not fit in a simple way the predictions of the constant field equation (Goldmann, 1943; Hodgkin & Katz, 1949). Specifically, increasing [K]$_0$ from 0·5 to 10·0 mM slightly affected the value of the E_{RP} recorded, as one would have expected if P_{Na}/P_K was large. However, the effect of reducing [Na]$_0$ did not agree with the hypothesis. We obtained the same results when considering the steady level as the real value of E_{RP}. But, for the reasons stated before, if one considers the initial deflexion after the penetration as the real value of the E_{RP} in slow fibres, the behaviour of the membrane in various [K]$_0$ is in agreement with a simple version of the constant field equation (Hodgkin & Horowicz, 1959) assuming a value of $P_{Na}/P_K = 0·02$. That is, our results suggest that resting sodium permeability in slow fibres is low, as it is in twitch fibres. Reduction of the external Cl concentration does not significantly change the value of the resistance in slow muscle fibres. This indicates that resting Cl permeability in slow fibres is also low, unlike that in twitch fibres. All these results suggest that slow muscle fibres are principally permeable to K ions. With R_m for slow fibres $\simeq 1 \times 10^5 \ \Omega \ cm^2$, the specific membrane conductance (G) is $\simeq 10 \ \mu mho/cm^2$, and if the whole of this is due to K conductance, G_K is about five to ten times smaller than the values obtained for twitch

muscle fibres (Hodgkin & Horowicz, 1959). If the resting potassium permeability is calculated from the constant field equation (Hodgkin & Katz, 1949, eqn. 6.0) a value of $\simeq 1 \times 10^{-7}$ cm/sec is obtained which is also about five to ten times less than the value for twitch muscle fibres (Hodgkin & Horowicz, 1959).

One of the most striking observations in the present study was the large increase of the membrane resistance of the slow fibres when the external Ca concentration was raised. The mechanism underlying the change remains unknown, but since the slow fibre membrane appears to be permeable mainly to K, it may be that Ca acts by reducing K permeability. In contrast to slow fibres, the membrane resistance of twitch muscle fibres was only slightly increased by raising external Ca. Perhaps a similar effect on the K permeability occurs in twitch fibres, but is masked by the Cl shunt (Hodgkin & Horowicz, 1959; Hutter & Noble, 1960) which is known to be unaltered by increasing the external Ca concentration up to 20 mM (Adrian & Freygang, 1962; Hutter & Warner, 1967). A possibly related finding is that in the gall-bladder epithelium an increase in external Ca reduces the conductance to cations, while it increases that of anions (Wright & Diamond, 1968).

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