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THE EFFECT OF DIAMETER ON THE ELECTRICAL CONSTANTS OF FROG SKELETAL MUSCLE FIBRES

BY A. L. HODGKIN AND S. NAKAJIMA*
From the Physiological Laboratory, University of Cambridge, CB2 3EG

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SUMMARY

1. Electrical constants were determined on isolated single fibres or on fibres from bundles from frog's twitch muscles by analysing the low frequency cable properties.

2. The sarcoplasmic conductivity (G_i) was 5·9 mmho/cm at 20° C, and its temperature coefficient (Q_{10}) was 1·37.

3. The Q_{10} of the membrane conductance (G_M) was 1·49, and that of the membrane capacity (C_M) was 1·02.

4. C_M increases with diameter (D) in an approximately linear manner: the values were 4·6 μF/cm^2 at $D = 50\ \mu$, and 8·5 μF/cm^2 at $D = 130\ \mu$.

5. G_M also increases with diameter, being 0·21 mmho/cm^2 at $D = 50\ \mu$ and 0·37 mmho/cm^2 at $D = 130\ \mu$.

6. These results suggest that the transverse tubular system contributes substantially to the values of low frequency capacity and conductance measured at the surface membrane.

INTRODUCTION

The main aim of the present study is to obtain further information about the electrical characteristics of the transverse tubular system (T-system) of amphibian skeletal muscle. Such information is important because the transverse tubules play an essential part in the activation of muscle, and their electrical properties determine the speed and extent of current spread into the interior of the fibre. The method of investigation is to see how parameters such as the membrane capacity or conductance vary with fibre diameter. It is customary to treat a muscle fibre in the same way as a nerve fibre, and to express the results of cable analysis in terms of the surface area. If the capacity resides wholly in the surface membrane, the apparent capacity per unit area of surface (C_M) should be independent of

* Wellcome Trust Fellow. Present address: Department of Biological Sciences, Purdue University, Lafayette, Indiana, U.S.A.

diameter. On the other hand, if the capacity is largely tubular in origin, C_M should increase linearly with fibre diameter, provided that current spreads into the middle of the fibre. It turns out that C_M is 4·6 μF/cm^2 in a fibre of diameter 50 μ, and 8·5 μF/cm^2 in one of diameter 130 μ. This result indicates that the tubules contribute substantially to the capacity, and is in qualitative agreement with those obtained by other methods. From an analysis of the frequency dependence of the impedance, Falk & Fatt (1964) assigned 2·6 μF/cm^2 to the surface membrane and 4·1 μF/cm^2 to the tubules. Gage & Eisenberg (1969) found a capacity of 2·1 μF/cm^2 in detubulated fibres as compared with 5–12 μF/cm^2 in normal fibres.

A secondary objective was to obtain a more reliable figure for the sarcoplasmic conductivity G_i at different temperatures. This was necessary because previous authors disagree about the temperature coefficient of G_i, and, to a lesser extent, about its absolute magnitude at room temperature. Thus Tamasige (1950) reported a Q_{10} of 2 for G_i whereas del Castillo & Machne (1953) considered that 1·3 was appropriate to their measurements. Previous estimates of G_i at 20° C are: 3·8 or 5·0 mmho/cm, Katz (1948); 7·7, Tamasige (1950); 5·3, Fatt (1964). Our conclusion is that G_i is 5·9 mmho/cm at 20° C and that it has a Q_{10} of 1·37.

Some of the results were described in a letter to Nature (Nakajima & Hodgkin, 1970).

SYMBOLS AND DEFINITIONS

I_0 current flowing through electrode.

V_m change in potential difference across surface membrane.

x distance along the fibre from the current electrode.

l_1, l_2 distance between the current electrode and each end of the fibre.

r_i internal resistance per unit length of fibre (Ω/cm).

r_m membrane resistance × unit length of fibre (Ω cm).

D fibre diameter.

G_i specific conductivity of interior of fibre (mho/cm).

$$G_i^{-1} = R_i = \tfrac{1}{4}\pi D^2 r_i. \tag{1}$$

G_M apparent membrane conductance per unit area at low frequency, referred to the surface

$$G_M^{-1} = R_M = \pi D r_m. \tag{2}$$

C_M apparent membrane capacity per unit area at low frequency, referred to the surface.

λ length constant of fibre $\lambda = \sqrt{(r_m/r_i)}$. (3)

τ_M time constant of membrane $\tau_M = C_M/G_M$. (4)

METHODS

Isolated fibres and fibre bundles

Single twitch fibres were isolated from the sartorius or the semitendinosus muscle of *Rana temporaria*. After stretching the fibre to 115 % of its slack length the diameter was measured at about seven points with a stereomicroscope at × 200 magnification. The major diameter a was measured first; the fibre was then rotated through 90° and the minor diameter b determined; the cross-sectional area and perimeter were calculated as $\frac{1}{4}\pi ab$ and $\pi\sqrt{(ab)}$, respectively. The quantity $\sqrt{(ab)}$ will be referred to as the diameter (D).

Electrical measurements were also made on bundles of about ten fibres dissected from the semitendinosus muscle, or on fibres from the deep surface of the intact sartorius muscle. In these preparations optical measurements of fibre diameter are not reliable, so diameters were calculated from measurements of the internal resistance per unit length and the mean value of the sarcoplasmic resistivity.

Experimental procedure

Membrane constants were obtained by analysing the low frequency cable properties of the fibre (Hodgkin & Rushton, 1946; Katz, 1948; Fatt & Katz, 1951). Two micro-electrodes were inserted into a fibre; one electrode (filled with 2 M potassium citrate; resistance, about 10 MΩ) was for applying current, and the other (filled with 3 M-KCl; resistance about 10 MΩ) for recording potential. Small rectangular current pulses lasting about 300 msec were passed through the current electrode in inward and outward directions, and the resulting potential changes were recorded at four to seven locations at distances varying between 0·2 and 2·5 mm from the current electrode. The fibres were stretched to 115 % of the slack length in all cases. A constant current condition was ensured by clamping the current with a negative feed-back circuit which was essentially the same as in Moore & Cole (1963).

Slow muscle fibres were not encountered and the fibres reported in this and the subsequent paper (Hodgkin & Nakajima, 1972) showed the following characteristics of twitch fibres: (1) when stimulated by a single shock a propagating twitch was visible under the microscope; (2) inward rectification was present; (3) propagated action potentials were recorded if the fibre was tested electrically at the end of the experiment.

The composition of the Ringer solution was the same as in Table 1 of Hodgkin & Horowicz (1959). The normal Ringer solution corresponded to their solution A: Na^+ 120; Cl^- 121; K^+ 2·5; Ca^{2+} 1·8; HPO_4^{2-} 2·15; $H_2PO_4^-$ 0·85 mg ion/l. The chloride-free sulphate Ringer had the following composition: Na^+ 80·5; K^+ 2·5; Ca^{2+} 8; HPO_4^{2-} 1·08; $H_2PO_4^-$ 0·43; SO_4^{2-} 48 mg ion/l.; sucrose 113 m-mole/l. The hypertonic Ringer was made by adding 350 mM sucrose to normal Ringer solution.

Most of the experiments were conducted at room temperature. When necessary, temperature was changed by controlling the temperature of the water running through a container which enclosed the recording cell.

Sources of errors

(*a*) *Cross-sectional area of isolated fibres.* One source of error arose from the fact that muscle fibres are rarely circular in cross-section. Blinks (1965) compared the average value of actual cross-sectional area with the estimated areas based on (1) the circular, or (2) elliptical assumption (Gordon, Huxley & Julian, 1966). His result showed that on the circular assumption the estimated area was 121 %, and on the elliptical assumption 104 % of the actual area. The present method of estimating the fibre thicknesses a, b is slightly different from that of Gordon *et al.* (1966), and probably overestimates the cross-sectional area by about 10 %. This means that our method may underestimate G_i by 10 %, and C_M or G_M by 5 % in experiments with bundles where the fibre diameter was calculated from the mean value of G_i.

(*b*) *Surface area.* The surface area of the fibre was estimated on the assumption of a cylinder with a diameter $D = \sqrt{(ab)}$. The ratio b/a was on the average 0·8 in our sample of isolated fibres. If the fibre were a column of an elliptical cross-section with diameters a and b, the true surface area would be 1 % larger than our estimate. But this is a conservative estimate of the error since the fibre surface is rather irregular particularly under the electron microscope. Hence, the real values of C_M and G_M may be less than those given in this paper. It will be seen that the errors of G_M and C_M from this source are in the opposite direction to those discussed in the preceding paragraph.

(*c*) *Leakage around micro-electrodes.* When a current electrode is inserted near the potential electrode, the resting potential drops by a few millivolts even when the output impedance of the current source is very high and the feed-back system is well balanced making steady currents insignificant. This drop of resting potential is attributed to a leakage conductance around the electrode. The leakage conductance was from about 0·01 to 0·2 μmho with an average of about 0·1 μmho (10 MΩ, cf. Stefani & Steinbach, 1969).

The resting potential measured by a single electrode would be affected by this leakage. In a large fibre with an input resistance of 100 kΩ this leakage conductance will produce a drop of resting potential of 0·9 mV. In a small fibre of 600 kΩ input resistance, it will cause a 5 mV depolarization.

The leakage conductances around micro-electrodes would also cause an error in estimating the input resistance. If the leakage conductances for both current and potential electrodes were 10 MΩ, the reduction in input resistance would be 11 % in a small fibre and 2 % in a large fibre. However, the magnitude of this error is just about cancelled by an increase of membrane resistance caused by inward rectification. Insertion of two micro-electrodes close together produces depolarization of about 10 mV in the small fibre, and it was estimated that this depolarization increased the input resistance by about 10 %. The overall error of input resistance is probably negligible in the larger fibres and is only one or two percent in the smaller fibres. Similar arguments hold in estimating the error in the length constants.

(*d*) *Non-linearity of resistance.* In muscle the membrane resistance is larger when measured with outward currents than with inward currents, and a large inward current produces the slowly developing hyperpolarization described by Adrian & Freygang (1962). To minimize these complications, the change in potential was kept below about 5 mV. The membrane constants were calculated independently for inward and outward currents, and were averaged at the final stage of calculation.

(*e*) *Defects of theory.* The estimate of the low frequency capacity depends on applying equations appropriate to a simple cable with the membrane element consisting of a resistance and capacity in parallel. From the calculations of Falk & Fatt (1964) it seems that our method of measuring τ_M (p. 109) might underestimate the capacity by a few per cent.

RESULTS

Isolated fibres

(a) Determination of G_1, G_M *and* C_M

The sartorius fibres were treated as leaky cables of infinite length. The space constant λ, and input resistance $\frac{1}{2}\sqrt{(r_m r_1)}$ were obtained from the distribution of potential in the steady state using the relation

$$V_m(x, \infty) = \tfrac{1}{2}I_0\sqrt{(r_m r_1)}e^{-x/\lambda}, \tag{5}$$

where

$$\lambda = \sqrt{(r_m/r_1)}.$$

From Hodgkin & Rushton (1946) the potential change associated with the make of a constant current is

$$V_m(X, T) = \tfrac{1}{4}[I_0\sqrt{(r_m r_1)}]F(X, T) \quad (X \geqslant 0) \tag{6}$$

in which

$$F(X, T) = e^{-X}\operatorname{erfc}\left(\frac{X}{2\sqrt{T}} - \sqrt{T}\right) - e^{X}\operatorname{erfc}\left(\frac{X}{2\sqrt{T}} + \sqrt{T}\right), \tag{7}$$

and $X = x/\lambda$ and $T = t/\tau_m$.

The usual method of measuring the time constant is to make use of the fact that the potential at $x = 0$ should reach 84 % of its final value when $t = \tau_m$. This introduces errors if there is any slow drift of potential (creep) so we determined the time at which $t = 0{\cdot}5\,\tau_m$ for the shortest inter-electrode distance x'. $V_m(X', 0{\cdot}5)/V_m(X', \infty)$ was obtained from a table of $F(X, T)$, and the time to reach this value was then determined.

In the case of semitendinosus fibres, which are about 15 mm long, it was necessary to use the equations for a short cable in determining r_1 and r_m, namely,

$$V_m(x, \infty) = \frac{I_0\sqrt{(r_m r_1)}}{\tanh(l_1/\lambda) + \tanh(l_2/\lambda)}\,\frac{\cosh\left(\frac{l_2 - x}{\lambda}\right)}{\cosh(l_2/\lambda)}, \tag{8}$$

where the lengths l_1 and l_2 are as defined in Fig. 1; l_1 was 3–5 mm and l_2 about 10 mm. Since $l_2 > 3\lambda$, cosh $[(l_2 - x)/\lambda]$ could be approximated by $\frac{1}{2}\exp[(l_2 - x)/\lambda]$. The exponential form was used as a first approximation and this result was corrected later by a factor of 1 % or less. The value of $\tanh(l_1/\lambda) + \tanh(l_2/\lambda)$ was usually 1·95 as against 2 for the infinite cable.

In order to obtain C_M in the semitendinosus fibre we need to know the transient solution in a short cable. This problem can be solved by the method of images, as illustrated in Fig. 1. Let $\chi = x + l_1$ be the distance from the left-hand end of the fibre and let $l_3 = l_1 + l_2$ be the total length of the fibre. Consider an infinite fibre with equal current sources at

$$\chi = 2nl_3 \pm l_1,$$

where $n = -\infty, \ldots, -2, -1, 0, 1, 2, \ldots, \infty$. From symmetry it is clear that there is no longitudinal current at the points midway between sources, for example, $\chi = 0$ and $\chi = l_3$ or more generally at $\chi = nl_3$. Insulating partitions can therefore be placed at any or all of these points without disturbing the current distribution. From this it follows that the voltage transient for a current step in a fibre of finite length is

$$V_m(X, T) = I_0 \frac{\sqrt{(r_m r_1)}}{4} \sum_{n=-\infty}^{\infty} \{F[|X+2n(L_1+L_2)|, T] + F[|X+2L_1+2n(L_1+L_2)|, T]\} \quad (9)$$

in which $F(X, T)$ is defined by eqn. (7), and $L_1 = l_1/\lambda$ and $L_2 = l_2/\lambda$. The following approximation was sufficient for the present case

$$V_m(X, T) \doteqdot \tfrac{1}{4}[I_0\sqrt{(r_m r_1)}]\{F(X, T)+F(X+2L_1, T)+F(-X+2L_2, T)\}. \quad (10)$$

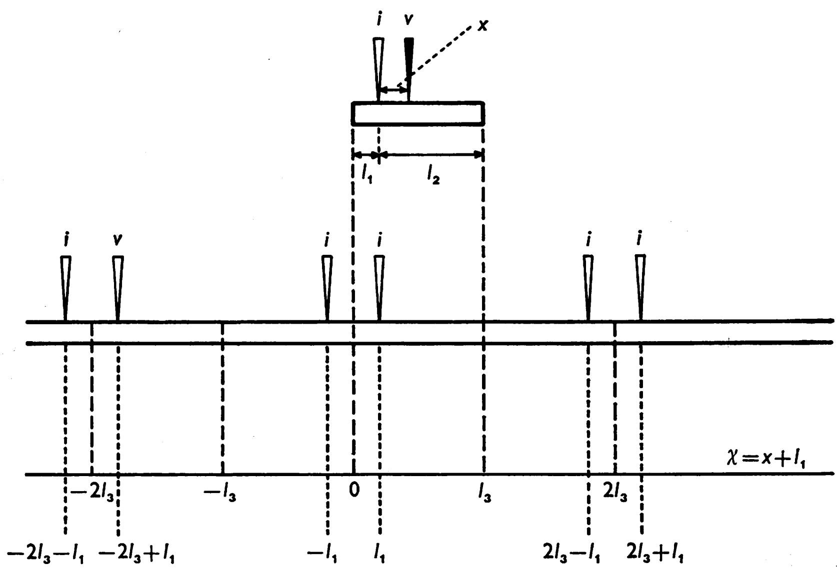

Fig. 1. Above: short cable with sealed ends. Below: infinite cable with current sources to give the same distribution of potential as in the short cable. Note: $\chi = x+l_1$, and $l_3 = l_1+l_2$. i: current injecting electrode. v: potential recording electrode.

The method of obtaining τ_m was essentially the same as in the case of sartorius fibres except that eqn. (10) was used instead of eqn. (6). In experiments using the normal Ringer solution, the value of τ_m based on eqn. (10) differed by only about 5% from the value based on the infinite cable assumption.

(*b*) *Effect of temperature on membrane constants*. The membrane con-

TABLE 1. Effects of temperature change on electrical constants in isolated single fibres

	Fibre	D (μ)	Temp. (° C)	λ (mm)	$\frac{1}{2}\sqrt{(r_m r_i)}$ (kΩ)	τ_m (msec)	Q_{10} of G_i	Q_{10} of G_M	Q_{10} of C_M
Semitendinosus	F-5	162	17·5	2·78	137	32·0			
			2·0	3·57	224	60·8	1·21	1·58	1·03
			17·0	3·06	136	31·3			
	F-7	143	3·9	2·95	246	46·5	1·38	1·65	1·14
			18·8	2·59	134	26·8			
	F-9	137	16·6	2·97	177	34·0			
			2·6	2·98	290	64·2	1·43	1·44	0·93
			17·5	2·96	166	34·9			
	F-10	152	18·3	2·64	119	29·3			
			2·6	2·80	236	63·3	1·48	1·59	0·97
			18·1	2·65	123	29·3			
	F-11	165	18·2	2·47	116	25·6			
			2·4	2·72	224	58·8	1·40	1·63	0·99
			18·8	2·37	115	27·0			
Sartorius	F-26	127	20·0	2·12	155	18·4			
			2·0	2·15	287	33·0	1·36	1·42	1·02
			19·5	2·03	166	18·8			
	F-27	121	19·2	2·28	162	20·5			
			2·0	2·20	270	33·9	1·35	1·33	0·99
			20·0	2·18	161	20·3			
	F-29	106	18·0	2·56	271	27·9			
			2·2	2·55	444	41·4	1·37	1·35	1·05
			20·0	2·57	258	26·8			
	F-30	98	19·2	2·11	236	21·4			
			2·7	2·10	398	33·5	1·35	1·39	1·05
			18·5	1·96	248	21·7			
					Mean ± S.E. of mean		1·37 ± 0·03	1·49 ± 0·04	1·02 ± 0·02

Membrane constants were calculated independently for depolarizing and hyperpolarizing pulses, and were averaged at the final step of calculation.

stants of isolated single fibres were first determined at room temperature, then at about 2–4° C, and finally at about 20° C again. Table 1 summarizes the results obtained with nine isolated fibres. The length constant was not affected or slightly increased by cooling, and the value of $\frac{1}{2}\sqrt{(r_m r_i)}$ was roughly doubled. These effects were reversible. The average values of the temperature coefficient of G_i was 1·37 and of G_M, 1·49. These values have been used to correct for variations of room temperature between experiments, the values in the subsequent sections refer to those at a temperature of 20° C. It was unnecessary to correct C_M, because the Q_{10} of the capacity was close to unity.

The resting potential decreased when the temperature was lowered and recovered almost completely when the original temperature was restored. The average decrease for the fibres in Table 1 was 5·1 mV when the temperature was lowered from 18·5 to 2·5° C.

Del Castillo & Machne (1953) reported that the Q_{10} of G_M was 1·35, which is somewhat smaller than ours. The discrepancy may arise from the fact that, in calculating G_M, they assumed the Q_{10} of G_i to be 1·3 (the value for 0·36% sodium chloride solution). Also they compared G_M on samples of slightly different average diameters. Since G_M depends on fibre diameter, this procedure would have caused some error. Recalculation from their data taking account of these points gave the Q_{10} of G_M as 1·5, in close agreement with the present value.

Tamasige (1950), who used isolated semitendinosus fibres, observed a larger temperature coefficient for G_M and G_i than in the present investigation. Although his value of R_i is close to ours, his value of G_M is only 0·1 mmho/cm² compared with our value of 0·3 mmho/cm². The reason for these discrepancies is not clear.

(c) *Fibre diameter and electrical constants in isolated fibres.* Twenty-one isolated fibres with diameters varying between 38 and 165 μ were measured at room temperature. None of the fibres showed any visible signs of deterioration under the microscope. Table 2, row (1) shows that the mean value of resting potential for the isolated fibres was 92 mV, if corrected for the leakage around the electrode, and this value was the same as the resting potential for fibres from bundles (row 2 of Table 2, also cf. Nastuk & Hodgkin, 1950; Adrian, 1956).

The relationship between fibre diameter and electrical constants is given by Fig. 2. G_i did not vary with diameter (Fig. 2*A*) and its average value in twenty-one fibres at 20° C was $5{\cdot}91 \pm 0{\cdot}13$ mmho/cm (mean ± S.E. of mean, R_i = 169 Ω cm). Using the Q_{10} of 1·37, the value of G_i at 2° C is 3·35 mmho/cm (299 Ω cm).

In contrast, the low frequency capacity per unit area of surface (C_M) increased from about 3 μF/cm² at 40 μ to about 10 μF/cm² at 160 μ (Fig.

TABLE 2. Resting potentials of different samples of fibres

	Mean temperature (° C)	Resting potential, mean (not corrected) (mV)	Resting potential (corrected)* Mean ± S.E. of mean (mV)	Number of fibres
(1) Isolated single fibres	18·7	89	92 ± 0·7	21
(2) Fibres from bundles or whole muscles	20·9	90	92 ± 0·5	86
(3) Small fibres ($40 \leqslant D \leqslant 59\ \mu$)	20·2	86	92 ± 0·7	9
(4) Large fibres ($120 \leqslant D \leqslant 139\ \mu$)	20·6	90	92 ± 1·2	19
(5) Semitendinosus fibres	20·5	89	92 ± 0·5	88
(6) Sartorius fibres	20·6	91	93 ± 0·8	19

* Corrected for the leak around electrode; the leak assumed to be 10 MΩ.

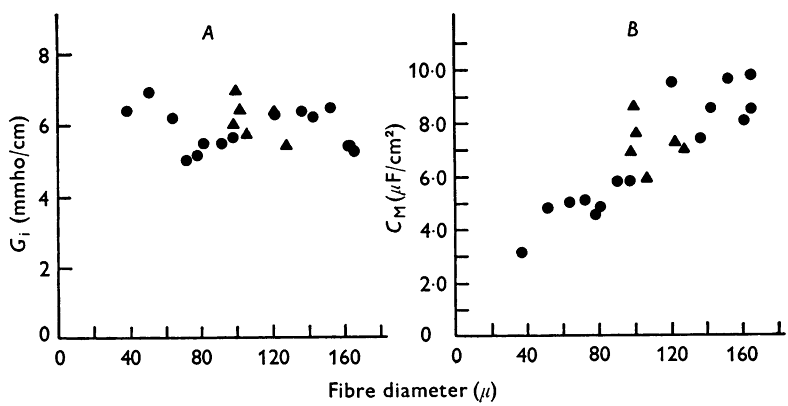

Fig. 2. Internal conductivity (G_i) versus fibre diameter (A), and low frequency membrane capacity (C_M) versus fibre diameter (B) in isolated single fibres. ●, semitendinosus. ▲, sartorius. Experiments were performed at 16·6–21·7° C, and in A the data were corrected to the bath temperature of 20° C.

2B). The values of low frequency conductance (G_M) also seemed to increase with fibre diameter, but the scatter of the values was greater than with C_M. The semitendinosus and sartorius gave almost the same values of electrical constants (circles and triangles of Fig. 2A, B).

(*d*) G_i *in hypertonic solution.* Hypertonic Ringer solutions have often been used to study electrical events in muscle in the absence of mechanical activity (Hodgkin & Horowicz, 1957; Adrian, Chandler & Hodgkin, 1970). It was therefore important to know the sarcoplasmic conductivity of isolated fibres in a hypertonic solution (consisting of Ringer solution plus 350 mM sucrose). In this solution the average value of G_i at 2° C was $2{\cdot}56 \pm 0{\cdot}17$ mmho/cm (mean ± S.E. of mean, $n = 6$, $R_i = 391\ \Omega$ cm), which is somewhat smaller than G_i, in the normal solution at 2° C (3·35 mmho/cm). Since the fibre behaves as an osmometer in this range of osmotic

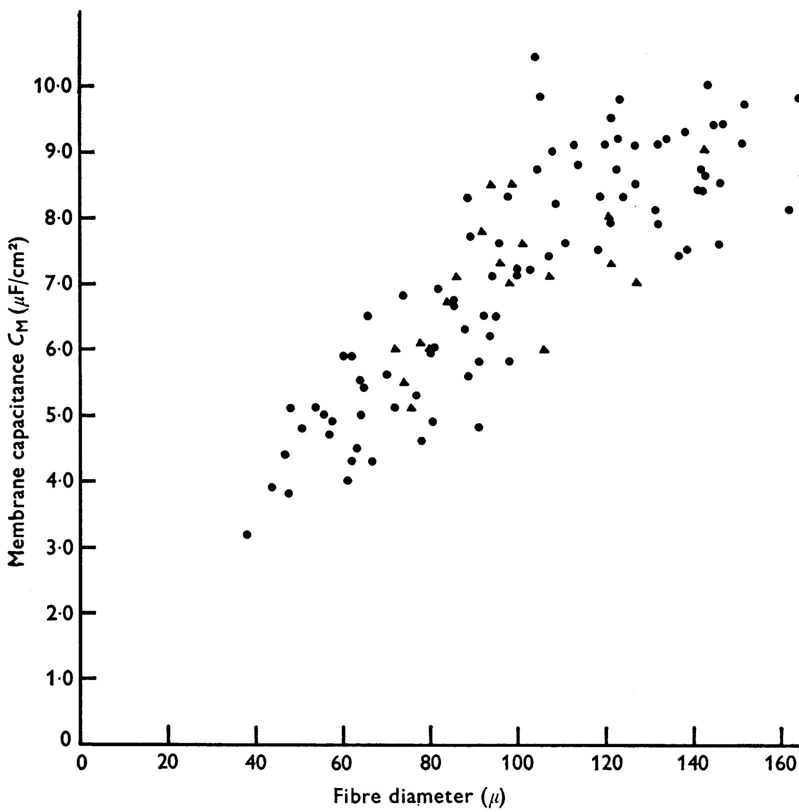

Fig. 3. Variation of low frequency capacity (C_M) with diameter. Most of the points were from fibres *in situ*, but the results in Fig. 2 on isolated fibres have also been included. ●, semitendinosus. ▲, sartorius. Temperature, 16·6–22·5° C.

pressure (Dydyńska & Wilkie, 1963; Reuben, Lopez, Brandt & Grundfest, 1963; Blinks, 1965), G_i ought to increase with osmotic pressure. An increase of myoplasmic viscosity might be responsible for this unexpected reduction of G_i. Freygang, Rapoport & Peachey (1967), who observed a similar phenomenon, proposed the same explanation.

Bundles of fibres

Optical measurements of fibre diameter are not reliable in whole muscle or in bundles of fibres. However, since the sarcoplasmic conductivity is independent of fibre diameter, we can obtain a diameter from eqn. (1) using the standard G_i at the temperature of the experiment. In this way

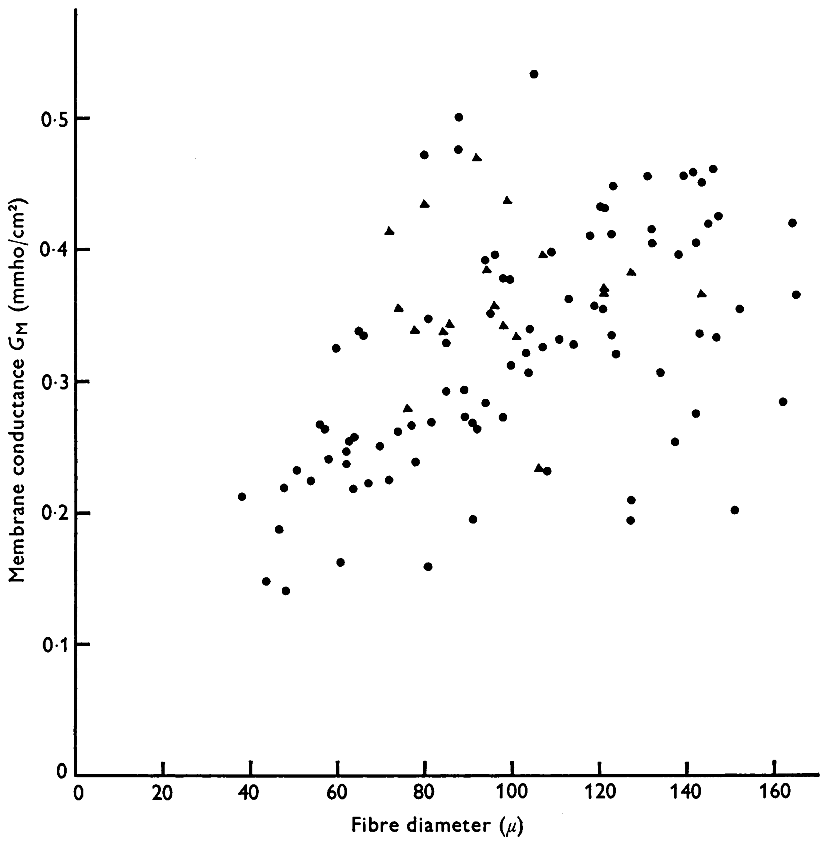

Fig. 4. Variation of low frequency conductance (G_M) with diameter. Most of the points were from fibres *in situ*, but the results on isolated fibres have also been included. ●, semitendinosus. ▲, sartorius. Experiments were performed at 16·6–22·5° C, and the data corrected to 20° C.

the membrane constants of fibres from the whole sartorius and from bundles of semitendinosus fibres were obtained; Figs. 3 and 4 give these results as well as those on isolated fibres.

(*a*) C_M *versus diameter*. In Fig. 3 the low frequency capacity (C_M) is plotted against fibre diameter. The results, which are similar to those in Fig. 2*B*, again show that C_M increases with diameter. They also show that the capacity in the semitendinosus (circles) and sartorius (triangles) are practically the same. The average C_M of the fibre group with diameters between 120 and 139 μ was $8 \cdot 5 \pm 0 \cdot 19$ $\mu F/cm^2$ (mean and S.E. of mean, $n = 19$), whereas that in the range 40–59 μ was $4 \cdot 6 \pm 0 \cdot 17$ $\mu F/cm^2$ ($n = 9$). These results are to be expected if the T-system contributes to the low frequency capacity. In a hypothetical fibre, whose diameter becomes infinitesimal, C_M ought to represent the capacity of the surface membrane only, for in this case the contribution of the T-system should vanish. The electrical model proposed by Adrian, Chandler & Hodgkin (ACH, 1969) predicts that C_M should vary almost linearly with diameter below about 120 μ. Linear extrapolation of C_M to 'zero diameter' for the fibre group below 120 μ in Fig. 3 gave $1 \cdot 15 \pm 0 \cdot 89$ $\mu F/cm^2$ (95% confidence limits), a value which may be regarded as representing the capacity of the surface membrane only. Although this value has a large standard error, it is nevertheless in approximate agreement with the value of 0·9 $\mu F/cm^2$ obtained from more reliable data presented in the next paper (Hodgkin & Nakajima, 1972).

(*b*) G_M *versus diameter*. As shown in Fig. 4, G_M also increases with diameter, but the variation among fibres was greater than with C_M. The greater variation should be regarded as genuine, since C_M values, which have less scatter, were derived from the values of G_M and τ_m. Fig. 4 also shows that G_M of the semitendinosus (circles) and of the sartorius (triangles) are essentially the same. The average value of G_M in the 120–139 μ group was $0 \cdot 365 \pm 0 \cdot 018$ mmho/cm² ($\pm$ S.E. of mean, $n = 19$) whereas that in the 40–59 μ group was $0 \cdot 213 \pm 0 \cdot 015$ mmho/cm² ($n = 9$). Again the ACH model predicts that G_M should vary with diameter in an almost linear manner. Linear extrapolation to zero diameter in the fibre group below 120 μ suggests that the conductance of the surface membrane is $0 \cdot 112 \pm 0 \cdot 071$ mmho/cm² (95% confidence limits). Therefore, in a large fibre the tubules may account for at least half the apparent membrane conductance. But the variability of the membrane conductance made it difficult to assign a definite fraction to their contribution.

(*c*) *Resting potential versus diameter*. Table 2, rows (3) and (4) show that the average value of resting potential for the small fibres was 86 mV compared with 90 mV for the large fibres. This difference probably arose from the fact that small fibres have larger input resistances, so that the leakage conductance around micro-electrodes gave a larger reduction in resting potential. After correcting for this effect, the mean resting potential was found to be the same in both groups of fibres. A similar effect probably accounts for the differences in resting potential between twitch and slow muscle fibres (Stefani & Steinbach, 1969). The resting potentials of the sartorius and semitendinosus fibres were almost the same (rows 5 and 6).

(*d*) *Effects of chloride-free solution.* Electrical constants were measured consecutively in normal and sulphate Ringer; it was assumed that the sarcoplasmic conductivity was the same in both solutions. As shown in Table 3, G_M in the chloride-free solution was 41 % of the value in the normal solution. Thus, the potassium conductance of the membrane seems to be about two-fifths of the total conductance, in rough agreement with the previous estimates (Hodgkin & Horowicz, 1959; Hutter & Noble, 1960). There was a slight increase in the value of C_M and a decrease in diameter in the chloride-free solution. Some of this effect could be attributed to a small loss of intracellular potassium chloride. Another factor is that in chloride-free solutions the slow drift in potential produced by hyperpolarizing currents became pronounced, contributing to an error in the electrical constants. The variability of membrane conductance in the sulphate solution was similar to that in chloride Ringer.

TABLE 3. Changes in G_M, C_M and diameter on substituting SO_4 for chloride ions

	G_M	C_M	Diameter
Ratio of electrical constants in sulphate and in chloride solutions. Mean ± S.E. of mean, $n = 9$	0·41 ± 0·03	1·12 ± 0·06	0·92 ± 0·02

The measurements before and after replacing with SO_4 were made using the same fibre. The average diameter in normal Ringer was 107 μ. Semitendinosus.

TABLE 4. Membrane constants of muscle fibres. Temperature 20° C. G_i = 5·9 mmho/cm

D (μ)	$\frac{1}{2}\sqrt{(r_m r_i)}$ (kΩ)	λ (mm)	τ_m (msec)	G_M (mmho/cm²)	C_M (μF/cm²)
50	810	1·9	22	0·21	4·6
80	320	1·9	19	0·33	6·1
130	145	2·3	23	0·37	8·5

The values were derived from mean values of G_M and C_M of the fibre groups with a diameter range of 20 μ.

DISCUSSION

Although the sartorius and semitendinosus muscle may have different mechanical properties, the electrical constants considered in this paper are essentially the same and will be discussed together. The mean value of R_i, 170 Ω cm at 20°, is smaller than the value of about 250 Ω cm which Bozler & Cole (1935) and Katz (1948) obtained in the sartorius muscle and in extensor longus digitorum IV. As mentioned on p. 108, the cross-sectional area of our fibres might have been over-estimated by about 10%. Allowance for this error would reduce R_i to about 150 ohm cm and would therefore increase the discrepancy. However, neither the uncorrected nor the corrected value is in serious disagreement with the value of 176 Ω cm calculated by Katz (1948) for the adductor muscle of the frog. In that case the measurements of diameter might have been more accurate, since single fibres or bundles of a few fibres were isolated. From studies of the transverse impedance of the sartorius muscle Fatt (1964) obtained the value of 202 ± 53 Ω cm (mean ± S.D.) for R_i at 18·3° C. In view of the completely different theory and method involved in the two sets of measurements it does not seem unreasonable to find a small difference between his result and ours.

In a recent paper Schneider (1970) obtained a value of 102 ± 11 Ω cm (S.E. of mean, $n = 8$) for the resistivity of the sarcoplasm of muscle fibres from *Rana pipiens* immersed in 7·5 mM-potassium Ringer at 25° C. He also states that this value is 10% less than in Ringer containing 2·5 mM-potassium. Correction for differences in temperature and Ringer then gives 131 ± 14 Ω cm, which is not in serious disagreement with 150 Ω cm.

The present experiments provide clear evidence that the low-frequency membrane capacity and conductance of twitch fibres vary with diameter in a manner which is consistent with the idea that the tubular system contributes substantially to the magnitude of these parameters (Falk & Fatt, 1964; Gage & Eisenberg, 1969; Eisenberg & Gage, 1969). It might be argued that large and small fibres have different ionic permeabilities but this would not explain why both C_M and G_M show a similar dependence on diameter. Nor, as will appear from the next paper, would it account for the very satisfactory agreement between the observed relation between C_M and diameter and the curve calculated from the theory of Adrian *et al.* (1969). The situation in crustacea is evidently different from that in frog muscle since Girardier, Reuben, Brandt & Grundfest (1963) and Zachar (1965) found that the membrane conductance per unit surface area of crayfish muscle fibres varied inversely with the fibre diameter.

Since the electrical constants of frog muscle fibres vary with diameter the latter ought to be specified when results are summarized. Table 4 lists values for fibres of 50, 80 and 130 μ. As shown in the next paper the mean diameter of surface fibres from the sartorius is about 85 μ in agreement with Mayeda's (1890) value of 84 μ. The electrical constants of the 80 μ fibres in Table 4 may therefore be regarded as representative of the sartorius muscle.

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