Ionic Conductances of the Surface and Transverse Tubular Membranes of Frog Sartorius Fibers

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ABSTRACT The resting ionic conductances of frog sartorius muscle fibers have been determined in a variety of conditions in order to measure the potassium conductance of the tubular and surface membranes ($g_K{}^t$ and $g_K{}^s$) and the chloride conductance of the tubular and surface membranes ($g_{Cl}{}^t$ and $g_{Cl}{}^s$). In both normal fibers and fibers without tubules, measurements of input resistance and diameter were made at normal pH and at low pH when the chloride conductance was very small. These measurements permitted the separation of the ionic conductances: $g_{Cl}{}^s = 219$ μmhos/cm^2; $g_{Cl}{}^t \doteq 0$ μmhos/cm^2; $g_K{}^s = 28$ μmhos/cm^2; $g_K{}^t = 55$ μmhos/cm^2. Possible sources of systematic error are discussed and a statistical analysis of the effects of random error is presented. The implications of the nonuniformity of membrane properties are discussed along with possible anatomical explanations.

The membrane which encloses the sarcoplasm of frog sartorius fibers consists of the circumferential surface membrane and tubular invaginations of the membrane (located at the Z line in each sarcomere) which constitute the transverse tubular system. It might be expected that the membranes of both systems would have similar properties since they are continuous, but some evidence suggests otherwise. For example the change in membrane potential produced by a sudden change in the extracellular chloride concentration is faster than that produced by a similar change in potassium concentration (Hodgkin and Horowicz, 1960). One explanation for the difference in time course is that the potassium conductance system is less accessible to solutions applied to the outside of a fiber than is the chloride system. If the potassium conductance system were located only in the transverse tubules, while the chloride conductance system was on the surface, one clearly would expect the response to a change in potassium concentration to be slower than the response to a change in chloride concentration.

In the experiments reported here an attempt has been made to determine the distribution of chloride and potassium conductances between the surface and tubular membranes. The membrane conductance has been measured in muscle fibers with and without transverse tubules, with normal or reduced chloride conductance. The four measured conductances allow the four ionic conductances (the conductance of the ions, K^+ and Cl^-, in the two locations, on the surface and in the transverse tubules) to be determined. The results lead to the conclusion that there is very little if any chloride conductance in the transverse tubules, whereas the potassium conductance is shared between the two membrane systems. Furthermore, it was found that the surface membrane possesses the property of anomalous rectification. It was not possible, however, to determine whether the transverse tubules also shared this characteristic. A brief report has been made of some of these results (Eisenberg and Gage, 1968).

METHODS

The glycerol treatment used to remove the tubular system has been described in the previous paper (Gage and Eisenberg, 1969). Tetrodotoxin (10^{-7} g/ml) was added to all solutions. The normal Ringer solution had a pH of 7.2. Solutions with a pH of 5.6 were made by addition of suitable amounts of HCl. Chloride-free solutions were made by substituting glucuronate for chloride. Preparations were flushed with fresh solutions every 20–30 min especially during exposure to solutions of pH 5.6, since this acid pH lies at the limit of the range of the phosphate buffer.

The input resistance of muscle fibers was determined by inserting two microelectrodes close together into the midregion of surface muscle fibers, one to pass current, the other to record potential. Only those fibers with resting potentials of magnitude greater than -70 mv (inside with respect to outside) were used. Fiber diameters and electrode separations were measured using a stereoscopic microscope at high magnification ($\times$ 100).

Current was applied from a constant current generator (Gage and Eisenberg, 1969) which was sufficiently precise that it was not necessary to record current independently: for example, changing the load resistance from 100 megohms to a short circuit (resistance of a small piece of wire) produced no detectable change in output current (the smallest detectable change being about 3%). Thus, "current" records shown here are of the voltage pulse supplied to the input of the constant current generator. The potential recording system used previously (Gage and Eisenberg, 1969) had a rather high input capacitance (about 7 picofarads) and so a different circuit, suggested by Dr. E. A. Johnson, was used (Fig. 1). This circuit has the advantage that the capacitance which limits the frequency response of the over-all circuit is not the input capacitance of the operational amplifier but is rather the capacitance between the input and output of the amplifier. Most of this capacitance is in fact the rather small capacitance across the wall of the microelectrode. In order to minimize this capacitance and thus maximize bandwidth, the level of Ringer solution in the bath was kept low. Measurements were made of the applied current

and of the resulting steady-state membrane potential displacements (recorded at an electrode separation of 30–100 μ). The ratio of these two quantities is defined as the input resistance (R_o). The resistance R_m (and the conductance G_m) of 1 cm^2 of membrane can be calculated from the input resistance, the fiber diameter (d), and an assumed internal resistivity (R_i). It should be noted that R_m and G_m are referred to 1 cm^2 of the outer surface of the muscle fibers. The relationship between these parameters is given by the equation

$$R_m = \frac{1}{G_m} = \frac{\pi^2 d^3 R_o^2}{R_i} \qquad (1)$$

The figure for R_i used in the calculation of G_m in glycerol-treated fibers was 191 ohm-cm (Gage and Eisenberg, 1969); in normal fibers R_i was taken to be 200 ohm-cm (Fatt, 1964).

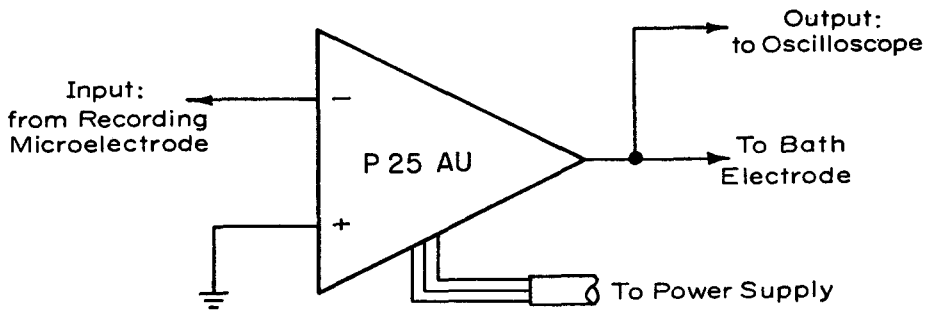

FIGURE 1. The configuration of the voltage-recording amplifier.

The largest source of error in these experiments is in the measurement of fiber diameters. These errors are particularly important since R_m is proportional to d^3. For this reason Falk and Fatt (1964) used an averaging technique which allowed the mean diameter to be used. Means obtained using this technique, as well as linear means, were used in equation (1). As will be seen, the results of the two methods were rather similar.

RESULTS

The main conductance pathways for current across the membrane of normal frog sartorius muscle fibers are shown schematically in Fig. 2 A. At this stage it is convenient to express all conductances in terms of the area of the cylindrical outer surface of the fiber. Later they will be referred to the area of the structures in which they are thought to be located. The resistance in series with the membrane of the transverse tubular system has been ignored in Fig. 2 since it has been shown to be small compared to the other resistances of interest (Falk and Fatt, 1964; Freygang, Rapoport, and Peachey, 1967). In the first analysis no attention is paid to possible sources of error which complicate the calculations but an attempt will be made later to introduce some correction factors.

In normal muscles (with intact tubular systems) the total resting membrane conductance (G_1) equals the sum of the resting chloride conductance on the surface ($g_{Cl}{}^s$), the resting chloride conductance of the tubules ($g_{Cl}{}^t$), the resting potassium conductance of the surface ($g_K{}^s$), and the resting potassium conductance of the tubules ($g_K{}^t$). (Because the sodium conductance is relatively small, it is ignored in these equations.)

$$G_1 = g_{Cl}{}^s + g_{Cl}{}^t + g_K{}^s + g_K{}^t \qquad (2)$$

When the chloride conductance is drastically reduced by lowering pH (Moore, 1966; Hutter and Warner, 1967 *a*, *b*), the membrane conductance

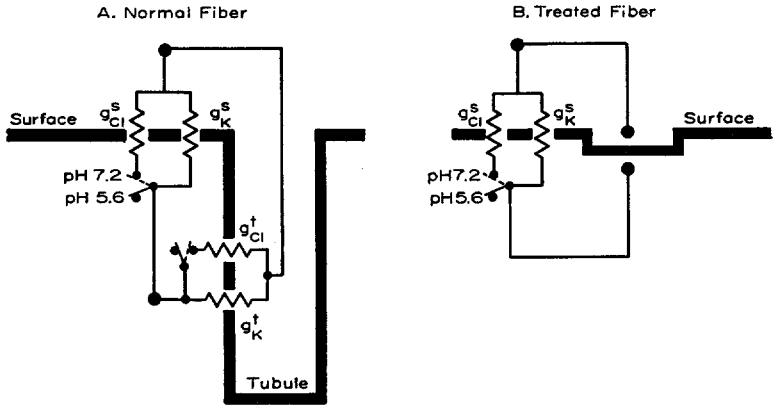

FIGURE 2. A schematic equivalent circuit of the ionic conductances. A. A normal fiber. Since there is little chloride conductance at pH 5.6, the chloride conductance is shown unconnected in that condition. B. A glycerol-treated fiber with virtually no tubular system. Again, since there is little chloride conductance at pH 5.6, the chloride conductance is shown unconnected under that condition.

G_2 is given by the sum of the resting potassium conductance of the surface and that of the tubular membrane (see equation (3) below), since acid solutions, while reducing g_{Cl}, do not affect potassium conductance (Hutter and Warner, 1967 *a*, *b*). Chloride conductance was lowered in this manner, rather than by removing chloride from the solution, because of the instability of resting potentials in the latter case.

$$G_2 = g_K{}^s + g_K{}^t \qquad (3)$$

The components of membrane conductance in glycerol-treated muscle fibers (without transverse tubules) are shown schematically in Fig. 2 B. The membrane conductance (G_3) of glycerol-treated fibers at pH 7.2 consists of the sum of the chloride and potassium conductances of the surface membrane.

TABLE I

Condition	No. of fibers	$\bar{V}_m \pm$ SE	$\bar{d} \pm$ SE	$\bar{R}_o \pm$ SE	$\bar{R}_m$	$\bar{G}_m$	SE
		(*mv*)	(μ)	(*megohm*)	(*ohm-cm²*)	(μ*mho/cm²*)	(μ*mho/cm²*)
1. Normal (tubules intact) pH 7.2	18	−83.4±1.6	52.9±2.0	0.606±0.032 (0.633±0.039)	3395 (3607)	G_1=295 (G_1=277)	46
2. Normal (tubules intact) pH 5.6	42	−87.4±0.9	57.3±1.2	1.06±0.035 (1.11±0.042)	10,922 (11,447)	G_2=92 (G_2=87)	8
3. Glycerol-treated (tubules disrupted) pH 7.2	38	−80.8±1.2	58.1±1.7	0.626±0.031 (0.677±0.034)	3967 (4645)	G_3=252 (G_3=215)	33
4. Glycerol-treated (tubules disrupted) pH 5.6	21	−80.0±1.5	50.6±1.3	1.89±0.16 (2.07±0.15)	24,013 (28,685)	G_4=42 (G_4=35)	6
5. Glycerol-treated (tubules disrupted) Cl-free	9	−84.4±1.7	50.1±1.4	2.17±0.16 (2.25±0.17)	30,596 (32,899)	G_5=33 (G_5=30)	6

$$G_3 = g_{Cl}{}^s + g_K{}^s \tag{4}$$

Finally, the membrane conductance of glycerol-treated fibers at pH 5.6 is equal to the potassium conductance of the surface membrane alone.

$$G_4 = g_K{}^s \tag{5}$$

These equations can readily be solved for the individual ionic conductances:

$$\begin{aligned} g_{Cl}{}^s &= G_3 - G_4 \\ g_{Cl}{}^t &= G_1 + G_4 - G_2 - G_3 \\ g_K{}^s &= G_4 \\ g_K{}^t &= G_2 - G_4 \end{aligned} \tag{6}$$

The conductances G_1 to G_4 were obtained experimentally and are shown in the "G_m" column of Table I. Two figures for G_m are shown in each condition. The first value was obtained by the nonlinear averaging technique (Falk and Fatt, 1964) referred to in Methods. The value in parentheses was derived by using the linear mean of R_o. It can be seen that the linear average is consistently higher than the nonlinear. The standard error of the mean for each value of G_m is given in the column on the right in Table I. These were calculated using the statistical analysis described in the Appendix.

When the first values (i.e. those derived from nonlinear means) for G_1

and G_4 in equations (1) to (4) are used the following results are obtained:

$$\begin{aligned} g_{Cl}^{s} &= 210\ \mu\text{mhos/cm}^2 & g_{Cl}^{t} &= -7\ \mu\text{mhos/cm}^2 \\ g_{K}^{s} &= 42\ \mu\text{mhos/cm}^2 & g_{K}^{t} &= 52\ \mu\text{mhos/cm}^2 \end{aligned} \tag{7}$$

When the values in parentheses (derived from linear means) are used, similar results are obtained:

$$\begin{aligned} g_{Cl}^{s} &= 180\ \mu\text{mhos/cm}^2 & g_{Cl}^{t} &= 10\ \mu\text{mhos/cm}^2 \\ g_{K}^{s} &= 35\ \mu\text{mhos/cm}^2 & g_{K}^{t} &= 52\ \mu\text{mhos/cm}^2 \end{aligned} \tag{8}$$

These results indicate that the chloride conductance is essentially restricted to the surface membrane. (The small values for g_{Cl}^{t} are not significantly different from zero.) The potassium conductance on the other hand is distributed between the surface and tubular membranes. The values for the total chloride and potassium conductances are in good agreement with those found previously for frog skeletal muscle (Hodgkin and Horowicz, 1959), especially if allowance is made for the different relative areas of the tubular and surface membranes in fibers with different diameters.

An attempt was made to obtain more accurate values for the individual conductances with full realization, however, that the results would be limited by the accuracy of our technique which is indicated by the standard errors. Several considerations which were ignored in equations (1 to 4) are included below: (*a*) The possibility that a small number of transverse tubules remain in glycerol-treated fibers. (*b*) The possibility that some chloride conductance is present at pH 5.6. (*c*) The possibility of some "leakage" in glycerol-treated fibers. It can be said at the outset that recalculation using reasonable estimates for these factors does not change the general conclusions already stated. The main effect is seen in the ratio of g_{K}^{t} to g_{K}^{s}.

The equations defining the system become more complex when these factors (the fraction of tubules remaining in glycerol-treated fibers, f; the fraction of the normal chloride conductance left at pH 5.6, p; and the amount of leakage, G_L) are included.

$$G_1 = g_{Cl}^{s} + g_{Cl}^{t} + g_{K}^{s} + g_{K}^{t} \cdots\cdots\cdots \text{(pH 7.2; tubules intact)} \tag{9}$$

$$G_2 = pg_{Cl}^{s} + pg_{Cl}^{t} + g_{K}^{s} + g_{K}^{t} \cdots\cdots\cdots \text{(pH 5.6; tubules intact)} \tag{10}$$

$$G_3 = g_{Cl}^{s} + fg_{Cl}^{t} + g_{K}^{s} + fg_{K}^{t} + G_L \cdots \text{(pH 7.2; tubules disrupted)} \tag{11}$$

$$G_4 = pg_{Cl}^{s} + pfg_{Cl}^{t} + g_{K}^{s} + fg_{K}^{t} + G_L \cdots \text{(pH 5.6; tubules disrupted)} \tag{12}$$

These four simultaneous linear equations can be solved by Cramer's rule and give the following solutions (checked by substitution):

$$g_{Cl}^{s} = \frac{1}{(1-p)(1-f)}[G_3 - G_4 - f(G_1 - G_2)] \quad (13)$$

$$g_{Cl}^{t} = \frac{1}{(1-p)(1-f)}[G_1 + G_4 - G_2 - G_3] \quad (14)$$

$$g_{K}^{s} = \frac{1}{(1-p)(1-f)}[G_4 + pfG_1 - fG_2 - pG_3 - (1-p)G_L] \quad (15)$$

$$g_{K}^{t} = \frac{1}{(1-p)(1-f)}[G_2 - pG_1 + pG_3 - G_4 + (1-p)G_L] \quad (16)$$

It is necessary to estimate values for the three correction parameters, f, p, G_L, in order to use the above solution. In a morphological study of glycerol-treated surface fibers in frog sartorius muscles, the fraction of tubules that remained after glycerol treatment was found to be 0.02 (Eisenberg and Eisenberg, 1968). This value of f was used. The fraction of chloride conductance remaining at pH 5.6 (p) was determined by the following procedure. If there is assumed to be no chloride conductance in a muscle which has been soaked in a chloride-free medium, a comparison of the membrane conductance in the absence of chloride ions with the conductance in a low pH medium enables a value of p to be calculated. Measurements (see Table I) were made of the membrane conductance of glycerol-treated fibers in a chloride-free medium (G_5). G_5 is given by the equation

$$G_5 = g_K^s + fg_K^t + G_L \quad (17)$$

and p can be found by solving equations (12) and (17) simultaneously:

$$\frac{1}{p} = \frac{G_3 - G_4 - f(G_1 - G_2)}{(G_4 - G_5)(1-f)} + 1 \quad (18)$$

(For the sake of simplicity a very small second-order term has been deleted. The error so caused is less than 0.5%.) Substitution of our experimental data into equation (18) gave $p = 0.04$; i.e. 4% of the normal chloride conductance remained in solutions of pH 5.6.

Finally, an estimate can be made of the amount of leakage (G_L). There is little doubt that at least some glycerol-treated fibers are quite leaky since many of the fibers examined at the acid pH had very low resting potentials (and thus were rejected). The presence of leakage is accentuated under these conditions since the membrane resistance is high. The glycerol-treated fibers from which the results shown in Table I were obtained form a highly selected sample consisting of those fibers with resting potentials of magnitude greater than 70 mv. Fibers with such resting potentials are probably not very "leaky." A schematic diagram is shown in Fig. 3 of such a fiber with a leak

conductance G_L, input conductance G_m, and membrane potential V_m. The input conductance without the leak is called G_t and the membrane potential V_m. The value of G_L is obtained from the equation (see Fig. 3)

$$G_L = G_m\left(1 - \frac{V_m'}{V_m}\right) \qquad (18\ a)$$

A value for V_m was obtained from the fibers at the same pH but with intact tubules (Table I). If the entire difference in resting potential between fibers in condition 2 (i.e. normal fibers at pH 5.6) and condition 4 (glycerol-treated fibers at pH 5.6) is ascribed to leakage, a leakage conductance of 3 μmho/cm^2 can be obtained by substitution in equation (18). Another estimate of the leakage conductance (in a different population of fibers) is given by

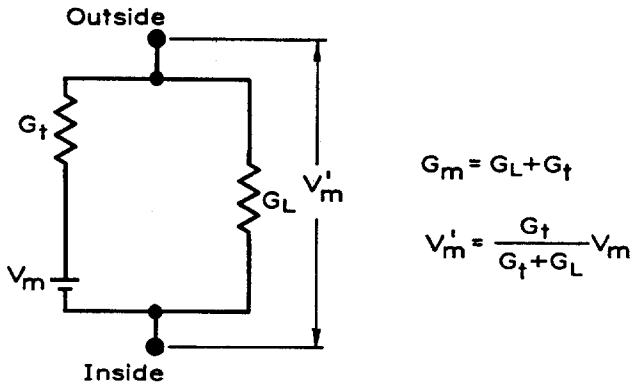

FIGURE 3. The equivalent circuit of the fiber including leakage. The leakage pathway is assumed to be nonspecific; that is, the battery in series with the leakage conductance is assumed to be unimportant. The "true" membrane conductance consists of the conductances to potassium and chloride described in the text. The equations describe the measured membrane potential V_m' and conductance G_m in terms of the true conductance G_t, leakage conductance G_L, and true membrane potential V_m.

a similar comparison of the resting potentials in condition 1 (normal fibers, pH 7.2) and fibers in condition 3 (glycerol-treated fibers, pH 7.2). The value of G_L so calculated is 6 μmhos/cm^2. It should be pointed out that these values are probably overestimates since the difference in membrane potential might be due to factors other than leakage. The value chosen for G_L in the calculations below was 4 μmhos/cm^2.

With the correction parameters described above, the solution (equations (9) through (12)) can be used in an attempt to obtain the best estimates of the individual ionic conductances (which are shown schematically in Fig. 4). These figures were computed from the nonlinear mean values of the membrane conductances

$$g_{Cl}{}^s = 219\ \mu\text{mho/cm}^2 \qquad g_{Cl}{}^t = -7\ \mu\text{mho/cm}^2$$
$$g_K{}^s = 28\ \mu\text{mho/cm}^2 \qquad g_K{}^t = 55\ \mu\text{mho/cm}^2 \tag{19}$$

When the figures in parentheses (the linear means) for membrane conductance (Table I) were used the following values were obtained:

$$g_{Cl}{}^s = 185\ \mu\text{mho/cm}^2 \qquad g_{Cl}{}^t = 10\ \mu\text{mho/cm}^2$$
$$g_K{}^s = 24\ \mu\text{mho/cm}^2 \qquad g_K{}^t = 57\ \mu\text{mho/cm}^2 \tag{20}$$

It can be seen that the values for the separate chloride conductances are relatively insensitive to the corrections described above (compare (7)

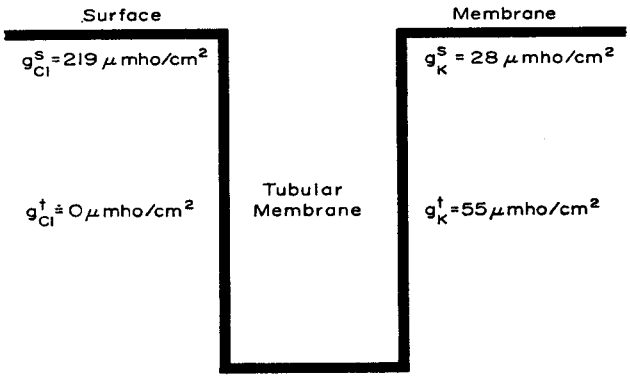

FIGURE 4. A figurative representation of the experimentally determined distribution of chloride and potassium conductance. Note that the figures given are referred to the surface area of a simple smooth cylinder. Tubular membrane area ≐ 4 × surface membrane area (50 μ fiber).

and (19), and (8) and (20)). The total potassium conductance ($g_K{}^s + g_K{}^t$) is also rather insensitive to these corrections. The main change is in the distribution of the potassium conductance; in other words the ratio of potassium conductance in the tubules to that in the surface membrane ($g_K{}^t/g_K{}^s$) is affected significantly when allowance for p, f, and G_L is introduced.

If extreme values for the correction parameters are used, it is possible to change the ratio of the potassium conductances over a wide range. One limit has already been set. When p, f, and G_L are taken as zero, the ratio $g_K{}^t/g_K{}^s$ is 1.2 or 1.5 (depending on the choice of the averaging procedure used in determining the membrane conductances). The ratio is 2.0 or 2.4 when the values for the correction parameters described above are used. If the correction terms are allowed to increase, this ratio can be further increased. Moreover, random errors in the measurements of the conductances could produce significant changes in this ratio. It is necessary therefore to be cautious in giving a figure for the distribution of potassium conductance. In spite of these

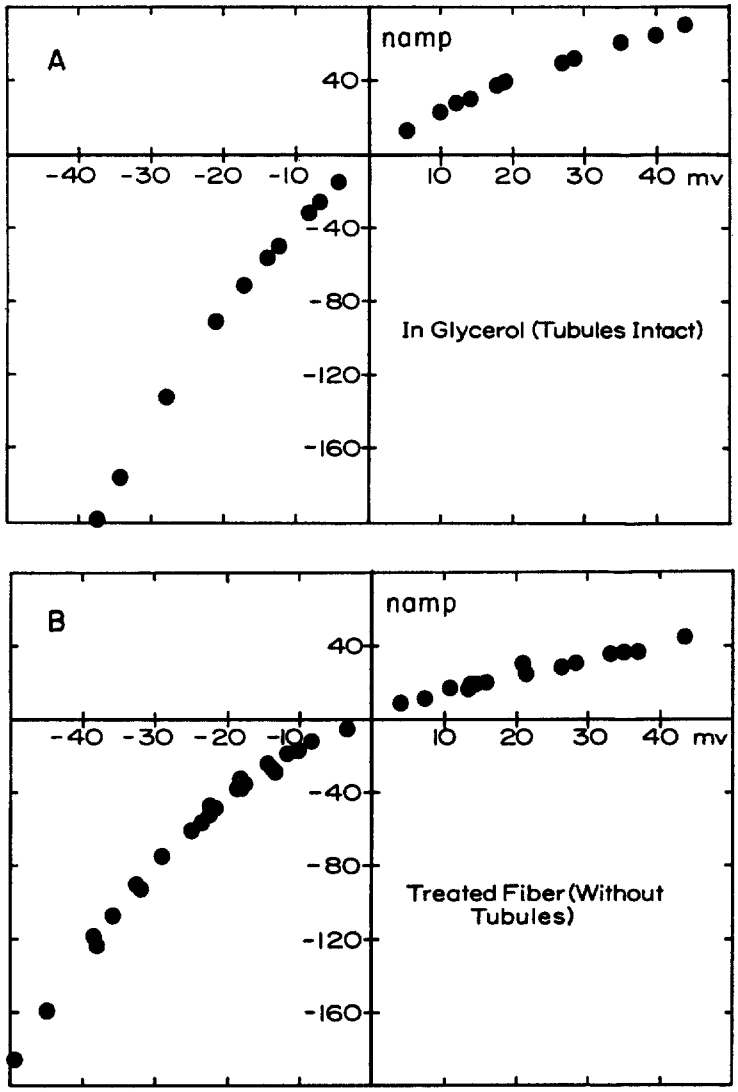

FIGURE 5. The current-voltage relation of a normal fiber (A) in a chloride-free solution containing 100 mM K^+ and 400 mM glycerol (tubular system intact) and (B) after return to a chloride-free Ringer solution containing 100 mM K^+ but no glycerol (tubular system disrupted). Note that the conductance decreases when the direction of current flow is outward.

possible sources of error, it seems safe to conclude that between half and three-quarters of the normal resting potassium conductance resides in the tubules.

Anomalous Rectification

The results presented so far give some information about the location of the resting chloride and potassium conductances. A different nonlinear potassium

conductance system is also present in skeletal muscle and has been termed (Katz, 1949; Hodgkin and Horowicz, 1959; Adrian and Freygang, 1962) anomalous rectification. This phenomenon is particularly evident in solutions containing 100 mM K, presumably because other systems of potassium conductance are inactivated in these solutions. It has been suggested (Hodgkin and Horowicz, 1960) that at least part of this anomalous conductance system resides in the transverse tubular system. It thus seemed worthwhile to look for anomalous rectification in glycerol-treated muscle fibers immersed in 100 mM K solutions in the hope that the location of the system could be defined.

Fig. 5 A shows the current-voltage relation in a normal fiber exposed to a chloride-free solution containing 100 mM K (and 400 mM glycerol) and in

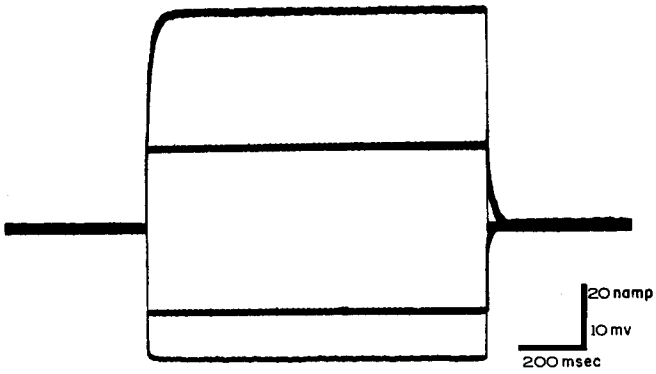

FIGURE 6. The electronic potential produced by current in both inward (downward) and outward (upward) direction. The current records are the inner traces (closer to the base line). Note the larger potential produced by outward current.

a glycerol-treated fiber (Fig. 5 B) without tubules (in 100 mM K, chloride-free Ringer). It can be seen that both curves show the increase in resistance for outward current flow which is characteristic of anomalous rectification. Fig. 6 shows a record from a glycerol-treated fiber in 100 mM K which more strikingly illustrates this rectification. The current pulse in the two directions is equal, yet the potential change is much larger for outward, depolarizing current (upward deflection) than for inward current (downward deflection).

It is difficult to interpret quantitatively these nonlinear current-voltage relationships. Since the potential along the fiber is not constant and the rectification is a function of potential, these curves do not have a simple relation to the current-voltage relation of a uniformly polarized area of membrane. Furthermore, ions besides potassium carry outward current under these conditions (Horowicz, Gage, and Eisenberg, 1968). None the less, it is clear that there is anomalous rectification in the surface membrane.

DISCUSSION

These results indicate that the chloride conductance of muscle fibers is located only in the surface membrane, whereas the potassium conductance is distributed between the tubular and surface membrane. A simple interpretation of these results is that they provide evidence for nonhomogeneity in the conductance properties of the membrane system of skeletal muscle, with different regions of the continuous bounding membrane having different properties. But the results could also be explained by nonhomogeneity in structure. It is possible, for instance, that some ions are not able to enter easily the tubular system or that the presence of structures in series with a specialized region of membrane might influence the over-all conductance properties of the region. This latter possibility has been raised (Gage and Eisenberg, 1969) as a possible explanation for the apparent difference in specific capacitance of the tubular and surface membranes.

The most striking difference between the surface and tubular membranes is the presence of chloride conductance in the former but not in the latter, which seemed to indicate a drastic difference in membrane properties. It has been suggested, however (Fatt, 1964; Rapoport, Freygang and Peachey, 1968), that the transverse tubular system is filled with a material containing fixed negative charge, perhaps analogous to the basement membrane which fills the tubules of cardiac muscle (Sommer and Johnson, 1968). The presence of such fixed charge would tend to reduce the concentration of chloride ions in the tubular system and could thus reduce the over-all conductance to chloride. If chloride is excluded from the lumen of the transverse tubules whereas potassium is free to enter, the findings of Foulks, Pacey, and Perry (1965) concerning the differences between potassium and chloride contractures might be explained.

Application of chloride ions produces contractures in skinned muscle fibers, either by depolarizing the sarcoplasmic reticulum or by depolarizing membranes of "sealed off" transverse tubules (Costantin and Podolsky, 1967). If the tubular membrane itself were impermeable to chloride, application of chloride to the tubular membrane would not change the potential across the tubular membrane and thus would presumably not cause contraction. If the tubular membrane itself were permeable to chloride (the apparent absence of $g_{Cl}{}^t$ being caused by the presence of negative fixed charge in the tubular lumen), chloride could conceivably change the potential across the tubular membrane thus initiating contraction.

The precise distribution of potassium conductance is much more difficult to define because of the greater influence of the correction factors mentioned above. The correction factors for the amount of tubular membrane left in glycerol-treated fibers and for the chloride conductance at pH 5.6 have been

experimentally determined. However, in our analysis the difference in membrane potential between normal and glycerol-treated fibers has been attributed completely to leakage whereas other factors may be involved. For example, if the sodium conductance were confined to the surface membrane, depolarization would be expected when g_K is reduced (as it is in glycerol-treated fibers). Another possibility is that the equilibrium potential for potassium is not normal in glycerol-treated fibers. If the equilibrium potential *is* normal in the somewhat depolarized glycerol-treated fibers, there would be an outward driving force on potassium ions which would tend to reduce g_K (Hodgkin and Horowicz, 1960; Adrian and Freygang, 1962; Horowicz, Gage, and Eisenberg, 1968). If this were so, the values for G_3 and G_4 would be underestimates, and consequently our analysis would tend to overestimate the potassium conductance of the tubules.

If the upper limit of the ratio of the potassium conductances of the tubular and surface membranes is accepted as 2.5, the assumption of homogeneous properties of the tubular and surface membranes would appear to be invalid. Peachey (1965) and Peachey and Schild (1968) estimate that the ratio of the areas of tubular and surface membranes (in a fiber with a diameter of 50 μ) is between 3.5 and 4.5. The potassium conductance per square centimeter of tubular membrane would then be $55 \div 4 \doteq 14$ μmhos/cm^2 in contrast to the surface potassium conductance of 28 μmho/cm^2. Before raising a possible morphological explanation for the apparent ratio of two between the *specific* potassium conductances of the surface and tubular membranes it is perhaps worthwhile mentioning other possible explanations.

1. Experimental error, in either the physiological or morphological results or both. The physiological data give a rather wide range for the values of the ratio of tubular to surface potassium conductance (see Results), and the ratio could be much larger if there were more chloride conductance remaining at pH 5.6 than we measured. On the other hand, the ratio could be smaller if the difference in membrane potential in treated and normal fibers is associated with a change in total potassium conductance.

2. The presence of a considerable degree of folding in the outer membrane. Such folding would reduce the figure for the specific potassium conductance of the outer membrane.

3. The presence of a short effective space constant of the tubular system. If current does not spread far down the tubular system, the amount of tubular area which is functional would be less than that which is morphologically present. This explanation seems unlikely since it cannot simply explain the size of the restricted diffusion space found by Hodgkin and Horowicz (1960) nor the large capacitance of muscle fibers. Furthermore, it seems unlikely on teleological grounds since the function of the transverse tubular system is to permit current to flow into the depths of a muscle

fiber. However, such an explanation would account for the similarity between the ratio of the potassium conductance of the tubular membrane to that of the surface membrane and the ratio of the capacitances of the respective membranes (Gage and Eisenberg, 1969).

4. Part of the tubular system is functionally, but not morphologically closed off. This seems difficult to reconcile with the rapid and complete penetration of the tubular system by a variety of extracellular markers (Huxley, 1964; Page, 1964; Endo, 1966; Hill, 1964; Eisenberg and Eisenberg, 1968).

5. The surface and tubular membranes may indeed have different specific potassium conductances.

Finally, it is interesting to consider a possible morphological explanation for the difference in conductance properties of the two regions of membrane similar to that previously mentioned (Gage and Eisenberg, 1969). The presence of structures adjacent to the tubular membrane might conceivably alter its over-all potassium conductance. It is known that a large part of the tubular membrane is in fact in intimate contact with the sarcoplasmic reticulum (see, for instance, Fig. 4, Gage and Eisenberg, 1969) and this might be expected to confer special properties, perhaps including a reduced specific capacitance and potassium conductance. If only that part of the membrane which is not covered by sarcoplasmic reticulum (about 27%, Peachey, 1965) is permeable to potassium, the specific K permeability of this membrane would be $55 \div (4 \times 0.27) \doteq 51$ μmhos/cm^2, now much greater than the figure of 28 μmhos/cm^2 for the potassium conductance of the surface membrane. If the K permeability system is partitioned between the two types of tubular membrane, with the permeability of the covered membrane being reduced, it is possible to reconcile the two figures (see the similar discussion of the capacitance of the surface and tubular systems (Gage and Eisenberg [1969]).

There may be some functional significance in the ionic conductance properties of the tubular system. It is clear that the total conductance of the tubular membrane is quite low, much lower than that of the surface. This low conductance means that little current can flow across the tubular membrane and thus current can flow further into the fiber. In other words the high specific resistance of the tubular membrane ensures that the effective resting space constant of the tubular system is quite high, at least for slow potential changes. This relatively long space constant may have some importance in the spread of excitation deep into the muscle fibers.

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APPENDIX

The Computation of the Standard Errors of the Mean Membrane Conductances.

The computation of the standard error of the mean ($s/\sqrt{n}$, where s is the standard deviation and n the number of observations) of the membrane resistance, R_m, or conductance ($G_m = 1/R_m$) is not a straightforward problem since neither G_m nor R_m is a measured quantity in our experiments and since there is reason to believe that the measured quantities (input resistance R_o and diameter d) do not vary independently.

It is a simple matter to compute the standard error of the measured quantities, diameter and input resistance. The relationship between membrane resistance and the measured parameters is also known (equation 1). It is not clear, however, how much error in the membrane resistance is produced by a given error in the measured parameters.

Furthermore, if the major source of variation in these experiments is in the measurement of diameter, as seems likely, the observed variation in the input resistance should be caused primarily by the variation in fiber diameter. In other words, variation in diameter should produce variation in input resistance even if the true membrane resistance were the same in every fiber and the errors in the measurement of the input resistance were negligible. It thus is clearly incorrect to treat the variations in diameter and input resistance as if they were independent. Such a treatment would tend to exaggerate the true variation since it would twice allow for the variation caused by errors in the measurement of diameter: first, the variation in the diameter would be used to determine the variation in the membrane conductance; second, the variation in input resistance caused by this variation in diameter would also be used in the calculation of the variation in membrane conductance.

The analysis presented below provides a general method for solving this problem independent of the form of the relationship between the measured parameters. The details of the analysis are presented here since it is possible that the method may be of some general use.

In order to determine the standard error of the function $f(x, y)$ from the errors in x and y, it is first necessary to determine the expected value[1] of $f(x, y)$ from the expected value of x and y (denoted by μ_x and μ_y, respectively). The expected value of any function is given by

$$E\{f(x,y)\} \equiv \int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} f(x,y)g(x,y)\, dy \qquad (21)$$

where $g(x, y)$ is the probability density function which determines the probability that $f(x, y)$ lies in a particular region. That is to say, the probability P that x lies between a and b and that y lies between c and d is given by

[1] Throughout this appendix the language used will be that of the theory of statistics. "Expected value" is the mathematical statement of the intuitive idea of mean value.

$$P(a < x < b; c < y < d) \equiv \int_a^b dx \int_c^d g(x, y)\, dy \tag{22}$$

Since the probability that x and y lie somewhere between $+\infty$ and $-\infty$ is one,

$$\int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} g(x, y)\, dy = 1 \tag{23}$$

The expected value of x (the mean of x) is then found from equation (21) when $f(x, y) = x$.

$$E\{x\} \equiv \mu_x = \int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} xg(x, y)\, dy \tag{24}$$

The expected value of y (the mean of y) is similarly

$$E\{y\} \equiv \mu_y = \int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} yg(x, y)\, dy \tag{25}$$

We now wish to find a convenient expression for the expected value of the function $f(x, y)$ in terms of the expected values of the measured parameters x and y. If the deviations of $f(x, y)$ from the value of $f(\mu_x, \mu_y)$ are not too large, the function $f(x, y)$ can be approximated by the first few terms of the Taylor expansion (see Courant, 1936):

$$f(x, y) \doteq f(\mu_x, \mu_y) + (x - \mu_x)\left[\frac{\partial f}{\partial x}\right]_{\substack{x=\mu_x \\ y=\mu_y}} + (y - \mu_y)\left[\frac{\partial f}{\partial y}\right]_{\substack{x=\mu_x \\ y=\mu_y}} \tag{26}$$

Substitution of this expression into equation (21) and the use of equation (23) give

$$E\{f(x, y)\} - f(\mu_x, \mu_y) = \int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} \left[(x - \mu_x)\frac{\partial f}{\partial x} + (y - \mu_y)\frac{\partial f}{\partial y}\right] g(x, y)\, dy \tag{27}$$

where the derivatives and the function $f(\mu_x, \mu_y)$ are constant during the integrations since they have previously been evaluated at $x = \mu_x, y = \mu_y$.

Substitution of equations (23) and (24) into equation (27) enables this latter expression to be evaluated:

$$\begin{aligned} E\{f(x, y)\} - f(\mu_x, \mu_y) = {} & \left(\frac{\partial f}{\partial x}\right) \int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} xg(x, y)\, dy \\ & - \mu_x \left(\frac{\partial f}{\partial x}\right) \int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} g(x, y)\, dy + \left(\frac{\partial f}{\partial y}\right) \int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} yg(x, y)\, dy \\ & - \mu_y \left(\frac{\partial f}{\partial y}\right) \int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} g(x, y)\, dy \\ = {} & 0 \end{aligned} \tag{28}$$

That is to say, within the limits of the accuracy implicit in equation (26).

$$E\{f(x, y)\} \doteq f(\mu_x, \mu_y) \tag{29}$$

Thus, we have shown that the mean value of the function $f(x, y)$ is approximated, as we might expect, by the function of the mean values $f(\mu_x, \mu_y)$. The accuracy of this approximation can be determined by the inclusion of higher order terms (or of a remainder term) in equation (26) and then by evaluation of the resulting integrals. Determination of the accuracy in general is not possible; determination of the accuracy in any specific case requires considerable further analysis and is not attempted here.

The reader may feel that a considerable amount of analysis was necessary to prove a rather simple point. The analysis presented above, however, is a useful introduction to statistical reasoning and is precisely analogous to that necessary to determine the variance (i.e. the square of the standard deviation) of the function $f(x, y)$ from the variance of x and y. The definition of the variance of $f(x, y)$ is

$$\begin{aligned} \operatorname{Var} f(x, y) &\equiv E\{[f(x, y) - E\{f(x, y)\}]^2\} \\ &\equiv \int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} [f(x, y) - E\{f(x, y)\}]^2 g(x, y)\, dy \end{aligned} \tag{30}$$

which can be approximated as shown in equation (29) by

$$\begin{aligned} \operatorname{Var} f(x, y) &\doteq E\{[f(x, y) - f(\mu_x, \mu_y)]^2\} \\ &\equiv \int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} [f(x, y) - f(\mu_x, \mu_y)]^2 g(x, y)\, dy \end{aligned} \tag{31}$$

Use of the Taylor expansion (26) and the definitions of variance (equation 30) and the definition of covariance (Mood and Graybill, 1963, p. 116) enables us to evaluate the integrals in equation (31):[2]

$$\operatorname{Var} f(x, y) \doteq \left(\frac{\partial f}{\partial x}\right)^2 \operatorname{Var} x + 2\left(\frac{\partial f}{\partial x}\right)\left(\frac{\partial f}{\partial y}\right) \operatorname{Cov} xy + \left(\frac{\partial f}{\partial y}\right)^2 \operatorname{Var} y \tag{32}$$

The details of the integrations are not presented since they require no techniques beyond those used in the evaluation of $f(x, y)$ (see equation [28]).

The meaning of this solution is worth discussing since it is the general solution to the type of problem we have posed and thus may be of some general use. The variance in the derived function is the sum of the variances of the measured parameters each weighted by the appropriate partial derivative. The possible interaction of the measured variables is expressed by the covariance terms. It should be noted that this covariance term will be negative if an increase in one of the measured variables produces an increase in the other, and will be positive if an increase in one of the measured variables produces a decrease in the other. If there is no relationship (in the statistical sense) between the variables to be measured, the covariance term would be zero.

In order to use equation (32) it is necessary to find a method of estimating the

[2] *Note Added in Proof* A similar equation has been previously derived by Wilkinson: *Biochem. J.* 1961. **80**:321.

means, variances, and covariances of the variables from the measured values. Much of statistical theory is devoted to showing that the following estimates are "most efficient" and "unbiased" (Mood and Graybill, 1963).

Function	*Name of best estimate*	*Formula*
Mean value: μ_x	$\bar{x}$	$\bar{x} = \frac{\sum_i x_i}{n}$
Variance: σ_x^2 or Var$x$	s_x^2	$s_x^2 = \frac{\sum_i (x_i - \bar{x})^2}{n - 1}$ (33)
Covariance: σ_{xy} or Covxy	s_{xy}	$s_{xy} = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{n - 1}$

Precisely analogous expressions exist for the mean and variance of y.

The use of these estimates in conjunction with equation (32) allows a solution of the problem. Our data determine the best estimate of the means, variances, and covariances; the theory (i.e. equation (32)) determines the way in which these must be manipulated to determine the variance of the derived function.

The solution of our particular problem is straightforward. The partial derivatives which appear in equation (32) are evaluated by differentiating equation (1); and the estimates of the means, variances, and covariances are determined from the experimental data (by equations (33)). Substitution of the partial derivatives gives the expression:

$$\frac{s_{R_m}}{\bar{R}_m} = \left[4\left(\frac{s_{R_o}}{\bar{R}_o}\right) + 12\left(\frac{s_d R_o}{\bar{d} \cdot \bar{R}_o}\right) + 9\left(\frac{s_d}{\bar{d}}\right)^2\right]^{\frac{1}{2}} \quad (34)$$

where $\bar{R}_m$ denotes the value of R_m determined from the mean values of the membrane input resistance ($\bar{R}_o$) and the mean value of the diameter ($\bar{d}$). The expression has been written in terms of the coefficients of variation (the standard deviations divided by the mean values) since then the equation takes on simple form. The standard errors given in Table I were determined from this equation. It is of interest to note that in all but one case there was indeed a relationship between the input resistance and the diameter and thus the covariance term was significant.

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