*The Equivalent Circuit of Frog Skeletal Muscle Fibers**

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For many years now the transverse tubular system of skeletal muscle has been implicated in the mechanism by which an action potential on the surface membrane initiates contraction in the depths of the muscle fiber. The tubular system has been thought to act as a pathway for current flow, linking the potential change at the surface with potential change across tubular membranes near the axis of the fiber. These potential changes across the tubular membrane presumably act as a trigger, in a manner not known, for the release of calcium from the sarcoplasmic reticulum. This paper discusses the flow of current in the tubular system, and gives a quantitative description of the electrical properties of the tubular system and surface membrane.

One way to study the electrical properties of the tubular system is to measure the various pathways by which current can flow from the sarcoplasm to the extracellular solution; these pathways presumably will include a pathway across the tubular membrane and through the solution filling the lumen of the T system (Fig. 1). The circuit which represents all of these properties is called the equivalent circuit of the muscle fiber. Thus one way to determine the electrical properties of the T system is to determine the equivalent circuit of the muscle

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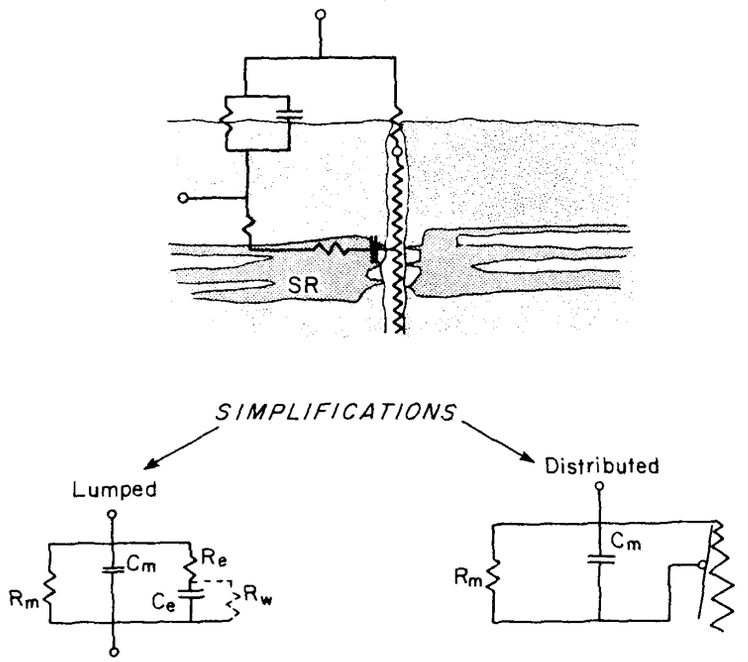

Figure 1. A schematic representation of the equivalent circuit of frog skeletal muscle fibers, as determined by intracellular electrodes. The upper part of the figure is a sketch of the membranous structures thought to be important in the determination of the equivalent circuit. A circuit which represents the properties of each structure is shown as an overlay. It should be pointed out that this circuit is neither complete nor unique but hopefully is sufficient to account for the observed electrical properties. Simplifications of the circuit are shown below; the left hand side showing the simplification which occurs if the predominant series resistance were that of the sarcoplasmic reticulum or mouth of the tubules; the right hand side showing the simplification which occurs if the predominant series resistance were the resistance of the solution filling the lumen of the tubules. All of the circuits represent the properties of a small isopotential length of muscle fiber; the circuit appropriate for a long length of muscle fiber consists of one of these elements distributed along the longitudinal resistance of the sarcoplasm. R_m is the resistance of 1 cm^2 of surface membrane; C_m is the capacitance of 1 cm^2 of surface membrane; R_e is the (lumped) resistance of the sarcoplasmic reticulum and/or mouth of the tubule associated with 1 cm^2 of surface membrane; C_e is the capacitance of the tubular system associated with 1 cm^2 of surface membrane; R_w is the resistance of the membrane of the tubular system associated with 1 cm^2 of surface membrane, it is shown dotted for technical reasons (see text). The peculiar circuit element on the right side of the distributed circuit is meant to represent a capacitance (that of the tubular membrane) distributed along a radial structure.

fiber, and then to interpret one of the branches of the circuit as the properties of the T system. The two pathways for flow of current can be distinguished experimentally if, and only if, they have different electrical properties, in particular if one of them has a resistance in series with its capacitance. Even then it is difficult to detect the presence of two pathways for the flow of current, especially using step functions of applied current, or indeed other non-periodic waveforms of current. In fact, it can be shown both theoretically and experimentally (Lanczos, 1957) that it is most difficult to prove the existence of the two pathways unless sinusoidal excitation is used. Using sinusoidal excitation and rigorous attention to the removal, analysis and correction of artifact caused by stray capacitances, Falk and Fatt (1964) were able to show that, as might be expected, a circuit with at least two components was needed to describe the pathways for current flow from the sarcoplasm to the extracellular solution. Freygang, Rapoport and Peachey (1967) and Schneider (1970) have confirmed this. Our major interest is the detailed configuration of the part of the circuit which represents the T system, and the implications of this configuration for the function of the muscle and for the interpretation of the electrical properties of the muscle fibers.

It is best to proceed by considering what equivalent circuit we might expect from a system with a structure like that of a T system, namely a dense branching array of tubules, using the quantitative anatomical data provided by Peachey (1965). Each tubule itself is a narrow elliptical structure some 250 by 800 Å in cross section. One might expect the core of the tubule to offer significant resistance to the flow of current. Thus, current would not flow unimpeded down the lumen of the tubules but would tend to cross the tubular membrane into the sarcoplasm either directly or by way of the sarcoplasmic reticulum (the partition of current between these paths does not affect the analysis that follows). I am sure this situation is familiar since it is precisely analogous to the flow of current down a cylindrical fiber, the classical problem of cable theory. Because this kind of behavior cannot be described by a finite number of circuit elements, the circuits involved are called distributed circuits; circuits with a finite number of elements are called lumped. It seems natural to expect that the equivalent circuit of the tubular system will be a distributed circuit, different parts of the tubular membrane having different series resistances, each representing the resistance of a length of the solution filling the lumen of the tubule. But upon further reflection, it is easy to see that there may be other circuit elements in series with the resistance of the fluid filling the lumen of the tubules, and it is conceivable that these other resistances could be as large as or larger than the core resistance. In particular, the sarcoplasmic reticulum or a constriction at the mouth of the tubules might contribute significant resistance. If either resistance were much larger than the core resistance of the tubules, all of the membrane of the tubular

system would be in series with much the same resistance, and a lumped circuit, with a finite number of elements would describe the tubular system. In this case current flowing across the tubular membrane deep in the muscle fiber will flow further down a resistive material than current crossing the tubular membrane just under the surface. However, the extra resistance residing in the sarcoplasmic reticulum or mouth of the tubules would swamp this effect and make the tubular membrane quite isopotential. The equivalent circuit emphasizing these effects is shown in Fig. 1. Two possible simplifications of the circuit are apparent, depending on which series resistance, lumped or distributed, predominates. The circuit on the lower lefthand side of the figure is an appropriate representation of a small length of the muscle fiber if the predominant series resistance is that of the sarcoplasmic reticulum or of a constriction at the mouth of the tubules; the resistive leak across the tubular membrane is shown dotted because of technical considerations, see Eisenberg (1967) for a discussion of this point and Eisenberg and Gage (1969) for an attempt to measure this quantity. The circuit on the lower right hand side of the figure is an appropriate simplification if the predominant series resistance is that of the fluid filling the core of the tubules; the peculiar circuit element which comprises the right hand branch of the circuit is meant to represent the radial dependence of the value of the resistance of the luminal solution and is an adaptation of an engineering symbol for a tapered distributed circuit.

It seems likely that the distributed circuit could explain the electrical properties of muscle fibers. In order to test this idea we must make a precise analysis of this distributed circuit; this analysis is quite difficult, essentially because the exponential functions which describe current flow in long cylindrical cells must be replaced by hyperbolic Bessel functions when a disc-like structure such as the T system is considered. The analysis has been done in many ways by now, by Falk and Fatt (1964), later extended by Falk (1968), by Adrian, Chandler and Hodgkin (1969), and by Schneider (1970). In each case the tubular system is treated as a dense meshwork of tubules and the spread of current is calculated using Ohm's law and a differential equation form of the law of conservation of current. Fortunately, all the theories give similar results; unfortunately, the results are very hard to understand intuitively except in the case of sinusoidal excitation. The following treatment is essentially intuitive and physical and is intended to make plausible the discussion that follows. The key results can be proven, however. The basic result of the theory is that the potential across the tubular membrane varies as you go deeper and deeper into the cell. The tubular membrane potential close to the edge of the fiber is close to the potential across the surface membrane, and the potential across tubules deep in the fiber near the center of the cell may be considerably less since current has leaked across the membranes between the surface and the center. In the quantitative discussion of the variation of potential it is very convenient to define a characteristic length of the tubular system, λ_t, analogous to the length constant of cable theory. This characteristic length measures the depth to which current flows in the tubular system; in particular the equation,

$$\frac{u\,(r)}{u\,(a)} = \frac{I_0\,(r/\lambda_t)}{I_0\,(a/\lambda_t)} \tag{1}$$

gives the ratio between the potential across the tubular membrane at a distance a - r from the surface of the cell to the potential across the surface membrane where

- $u\,(r)$ is the potential across the tubular membrane at a distance r from the fiber axis
- a is the radius of the cell, so u(a) is the potential just under the surface membrane
- I_0 is a hyperbolic Bessel function, an elementary but complete discussion of which can be found in Tranter (1969)
- λ_t is the length constant of the tubular system determined at steady state by the ratio of the resistance of the tubular membrane to the resistance of the core of the tubules. See Adrian, Chandler and Hodgkin (1969) for a discussion of the appropriate units for the membrane and luminal resistance. See Eisenberg and Johnson (1970) and Eq. 3 below for a discussion of the generalization of the length constant to the sinusoidal steady-state.

This equation approximates unity if the characteristic length is much larger than the cell radius, that is to say the potential across the tubular membrane is much the same no matter where in the cell one looks. If the characteristic length is less than 0.5 r, the equation is well approximated by a simple exponential relation

$$\frac{u\,(r)}{u\,(a)} \simeq \sqrt{\frac{a}{r}}\; e^{-(a\,-\,r)/\lambda_t} \tag{2}$$

That is to say the decrement of potential in the radial direction when there is decrement is exponential in character, much as it is in the longitudinal direction.

Our task now is to determine whether the lumped or distributed circuit better describes the electrical properties of a muscle fiber. Unfortunately, the impedance data do not decide this question, at least in my opinion. Falk and Fatt concluded that the distributed model fit the impedance data worse than the lumped model; Schneider concluded the opposite. I am not really convinced of either case, particularly because the differences in fit of the two models over the frequency range investigated are small, and could conceivably be due to other effects, for instance leakage of current through the damaged region which surrounds the current microelectrode. If we cannot decide between the distributed and lumped models on the basis of the fit to the impedance data, how can we choose between them? One test that has recently been made (Nakajima & Hodgkin, 1970) is to measure the variation of capacitance with fiber diameter: the distributed model predicts that properties which result from the properties of the entire T system should be proportional to diameter,

whereas properties which result from only a small fraction of the T system or from the surface membrane should be independent of fiber diameter. Nakajima and Hodgkin have recently completed these measurements and they certainly are consistent with the distributed model. It seems to me, however, that they might be consistent with a lumped model and do not provide a decisive test of the two models.

Another way to determine which model best describes muscle electrical properties is to compare the sizes of the various parameters in the model. In particular the two models give different values for the capacitance of the surface membrane: the lumped model gives 2 $\mu F/cm^2$ and the distributed model gives 1 $\mu F/cm^2$. If an independent method of measuring these capacitances were available, we might be able to choose between the models. Of course, one method that seems to be independent is to reason by analogy and say that the capacitance of the surface membrane of muscle fibers should be close to the capacitance of nerve fibers. This is particularly appealing since the capacitance of nerve fibers comes out to be close to the simplest integer, one! This I call the "unitarian hypothesis." Closer examination of the argument shows its weakness, however. The capacitance of a membrane depends on the thickness of the membrane, the area of the membranes across which current can flow and the average dielectric constant of the membrane (which in turn depends on the lipid composition of the membrane, and the fraction of the membrane occupied by material with aqueous-like properties). There is little evidence to indicate that the lipid composition, aqueous fraction or thickness of all membranes is the same, although it would be nice if this were so. Furthermore, it seems likely that there is more area of surface membrane in one centimeter length of muscle fiber than in one centimeter length of a nerve with the same diameter, and thus the specific capacitances should not be equal, even if the membranes were identical. To understand this statement we must make a little detour to discuss the conventions used to describe the capacitance of membranes.

The figure actually measured in these analyses is the capacitance of unit length of the fiber, but one wishes to know the capacitance of one square centimeter of the membrane responsible for this capacitance. That is, one must know the amount of this membrane in one unit length of the fiber. If the fiber is a simple cylinder of membrane like a nerve cell, without infoldings, invaginations, or wrinkles, this figure is easy to compute. Indeed, it is this figure of membrane area which is used in most calculations of the capacitance of "one square centimeter of membrane." If, however, the membrane systems responsible for the capacitance are folded or form a network of tubules there will be much more area present than that of a simple cylinder and the figures for capacitance as conventionally given will be inflated by the ratio of the real area to the hypothetical area. Thus, if the surface membrane of muscle fibers were so wrinkled that there were twice as much area in a given length as in a simple cylinder, the conventional figure for capacitance would be an overestimation of the capacitance of one square centimeter of the surface membrane, an

overestimation by a factor of two in this case. Indeed, there may be this much crinkling of the surface membrane in muscle fibers; the extensibility of muscle fibers, especially compared to nerve suggests this. The electron micrographs which often show extensive vacuolization just below the surface membrane support this idea; and the experiments of Martin (1954) on the variation of conduction velocity with stretch also support this. Thus, the fact that the distributed model gives a figure for the surface membrane capacitance which agrees with the unitarian hypothesis–namely a figure of 1 $\mu F/cm^2$–is not overwhelming evidence for this theory. Indeed, if it turns out that the surface membrane of muscle fibers is quite crinkled (by a factor of 2) the figure given by the lumped model–2 $\mu F/cm^2$–will agree more closely with the unitarian hypothesis than that given by the distributed model!

The impedance measures of Fatt (1964), using extracellular electrodes, provide an independent measurement of the size of the capacitance of the surface membrane. These measurements show a peculiar low frequency capacitance, the interpretation of which is not clear. They also show a capacitance important at high frequencies, which is quite constant over a range of experimental conditions and which it is natural to attribute to the surface membrane. The value of this capacitance is some 2.6 $\mu F/cm^2$, in agreement with the lumped model. The experiments provide further evidence for the lumped model since this capacitance of the surface membrane is constant up to some 100 kc/s, a result not expected from the distributed model.

It is probably worth discussing in detail why the two models make different predictions concerning the high frequency behavior of the tubular system. This discussion is useful in itself but also serves as an introduction to the qualitative analysis of systems, using the idea of a frequency dependent characteristic length. This idea has rather general applicability and has been useful for me and some of my colleagues in developing a feel for what otherwise seemed to be complicated problems. The physics of the situation is rather clear. In the lumped model there is some definite frequency above which the capacitance of the tubular system is not very important in determining the impedance of the whole cell. This occurs because at high frequencies the impedance of the capacitor representing the properties of the surface membrane becomes much smaller than the impedance of the circuit representing the tubular system. This frequency is rather low being around 100 c/s. A different description is necessary for the distributed model. In this model as frequency increases the impedance of the tubular membrane becomes smaller and smaller, thus more current can leak out across the membrane and current flows less and less deeply into the tubular system. An entirely analogous situation arises in the cable theory used to describe the longitudinal spread of current down cylindrical cells. Here as frequency increases the membrane impedance goes down, and current flows out of the cell in a shorter distance. A useful way to describe this phenomenon in cable theory is to define a frequency dependent length constant. The rigorous general definition of this quantity requires some detailed analysis, but the basic

idea is simple. At frequencies where the membrane impedance is set predominantly by the membrane capacitance (and this is usually the case above some 100 c/s) the longitudinal spread of potential is set by the a.c. length constant

$$\lambda^* = \left(\frac{2}{\omega c_m r_i}\right)^{1/2} \tag{3}$$

where

ω = 2π times the frequency of applied current
c_m = capacitance of 1 centimeter length of fiber (F/cm)
r_i = resistance of 1 centimeter length of fiber (ohm/cm)

The equations that precisely describe the longitudinal spread of sinusoidal signals are analogous to those which describe the steady-state spread of potential, if the usual d.c. length constant is replaced by this quantity.

It is natural to try to apply the same procedure to the distributed model of the tubular system and this can indeed be done. The spread of sinusoidal potential down the tubular system is described by equations analogous to the d.c. equations (Eqs. 1 and 2 above) provided the definition of the length constant is generalized just as we have done for cable theory. The advantage of this approach is that it allows us to reason about the frequency dependence of the spread of potential (and crudely about the time dependence as well) in the same way as we think about the d.c. spread. For instance, increasing the frequency or the resistance of the lumen of the tubule, or decreasing the capacitance of the tubular membrane all lower the characteristic length, and thus lower the depth to which current can enter the tubular system.

It is a straightforward task to generalize this discussion to the problem of the frequency dependence of the total capacitance of the tubular system by computing the total charge stored in the capacitance of the tubular system. In the case of the lumped model above some 100 c/s there is no frequency dependence of the total capacitance of a muscle fiber that matters, since there is no significant contribution of the tubular system to the total properties of the cell. In the distributed model this is not the case. As frequency increases, the contribution of the tubular system decreases smoothly, never really vanishing, but becoming unimportant only somewhere near 50,000 c/s. It is for this reason that the models give different predictions about the frequency dependence of the capacitance measured at high frequency, the lumped model fitting more closely to the data. Unfortunately, the only measurements made at these very high frequencies (Fatt, 1964) cannot be interpreted in an unambiguous manner since they fit a model of a circuit with an imperfect capacitator (of phase angle less than 90°), the physical interpretation of which is not clear. Thus, these measurements do not provide decisive support for the lumped model, in my opinion.

It is fortunate that there is still another independent method which ought to measure the capacitance of just the surface membrane. This method consists of measurements of the properties of the glycerol-treated preparation of muscle

fibers, which preparation has little intact tubular system. This preparation was mentioned by Fujino *et al.* (1961) developed by Howell (1969) and independently by Krolenko (1969) and has proven helpful in separating the properties of the tubular system from those of the surface membrane.

My wife and I felt it was most important to determine the extent of disruption of the tubular system in these glycerol-treated preparations, since the presence of a substantial number of tubules would greatly change the interpretation of the properties of the preparations. We decided to use an extracellular marker, horseradish peroxidase, which is known to fill the tubular system with an electron dense reaction product, in order to determine how many tubules were left in glycerol-treated sartorius muscles of *Rana pipiens* (Eisenberg & Eisenberg, 1968). I specify the preparation in such detail because there is reason to believe that the glycerol treatment has different effects on different preparations. Indeed, one might expect that such parameters as the amount and strength of connective tissue, the relative permeability of the membrane to water and glycerol, and the time course and spatial profile of glycerol concentration in the extracellular medium could all be important in determining the extent of tubular disruption.

In any case, the peroxidase method applied to *Rana pipiens* gives highly reliable results, as illustrated in Table 1 (from Eisenberg & Eisenberg, 1968, with permission of the authors). In normal muscle almost all the tubular system is filled with peroxidase. In glycerol-treated preparations (Table 2) there is very little tubular system left intact, and that which is left consists of stumps of tubules extending a few micra from the surface. On the basis of these experiments we concluded that only 2% of the tubular system was intact in glycerol-treated fibers. Since in normal fibers the tubular system has some four times as much area as the surface membrane, only some 8% of the membrane area of these glycerol-treated preparations lies in the remnants of the tubular system.

These results seem quite clear but important conclusions—in fact the interpretation of all the properties of glycerol-treated fibers—depend on the figure for the area of T system present in glycerol-treated fibers. Thus, one should consider all possible sources of error. One phenomenon which could conceivably be significant is that of washout of peroxidase from the tubular system. If the tendency for washout were greatly accentuated in glycerol-treated fibers, either because of a change in the fixation properties of the tubular system or in the time necessary for tubular or extracellular diffusion, the amount of peroxidase remaining in experiments on glycerol-treated fibers might be underestimated. This hypothesis seems unlikely to us. Another source of error would occur if there were structures into which current could flow but peroxidase could not diffuse; for instance if the tubules collapsed, peroxidase might not be able to enter the T system, but there might be enough room for ions to move. It is difficult to eliminate this possibility, but the gross disruption of the fine structure of the T system, and the absence of twitches and various slow potentials, argues against this possibility. Thus, we conclude that as far as

TABLE 1

Normal Muscle

Muscle	Depth of fiber from surface	No. of filled tubules (x)	No. of empty tubules (e)	No. of sites without tubules (n)	Peroxidase reliability $\frac{y}{x+e}$	Fraction of sites at which tubules are found, $\frac{x+e}{x+e+n}$	Fraction of sites at which filled tubules are found, $\frac{x}{x+e+n}$
					%	%	%
A	1	71	4	28	94.7	72.8	68.9
	3	87	1	17	98.9	83.8	82.9
	4	104	0	26	100.0	80.0	80.0
B	1	80	0	11	100.0	87.9	87.9
	3	32	1	7	97.0	82.5	80.0
C	1	191	3	40	98.5	82.9	81.6
	2	349	6	49	98.3	87.9	86.4
	3	162	2	29	98.8	85.0	89.9
	4	222	0	24	100.0	90.2	90.2
Mean, %[a]					98.5	83.7	82.4
Standard Error of Mean, %					0.6	1.7	2.1

[a]Means were computed on an unweighted basis, i.e., each fiber was given equal weight. If the data from each fiber are weighted according to the number of observations made, the means are not changed significantly.

TABLE 2

Treated Muscle Fibers (Disrupted Transverse Tubules)

Muscle	Depth of fiber from surface	Apparent diameter, μ	No. of filled tubules (x')	No. of unfilled sites ($e' + n'$)	Fraction of sites at which filled tubules are found $\frac{x'}{x' + e' + n'}$	Fraction of tubules[a] connected to surface $\frac{x'/(x' + e' + n')}{x/(x + e + n)}$
					%	%
A	1	40	7	263	2.6	3.1
	1	25	18	536	3.3	3.9
	2	15	17	200	7.8	9.5
	2	>25[b]	6	222	2.6	3.2
	2	38	0	128	0	0
	2	20	5	253	1.9	2.3
	3	>30[b]	3	281	1.1	1.3
B	1	47	3	354	0.8	1.0
	1	55	0	392	0	0
	1	>20[b]	7	119	5.6	6.7
	2	50	0	56	0	0
	2	40	23	253	8.3	10.5
	3	25	4	89	4.3	5.2
C	1	>25[b]	0	226	0	0
	2	40	3	311	1.0	1.2
Mean, %[c]					2.6	3.2
Standard Error of Mean, %					0.7	0.9

[a] $x/(x + e + n)$ is taken as 81.1% from combined data.

[b] The apparent diameter is greater than this value, since the edge of the fiber was obscured by a grid bar.

[c] Means were computed on an unweighted basis, i.e., each fiber was given equal weight. If the data from each fiber are weighted according to the number of observations made, the means are not changed significantly.

we can tell the glycerol-treated preparation is a preparation of essentially surface membrane, contaminated by some 8% tubular membrane.

If this conclusion is correct, the electrical properties of glycerol-treated fibers are properties almost exclusively of the outer membrane. Thus, the capacitance of glycerol-treated fibers should represent the capacitance of the surface membrane; hopefully, agreeing quantitatively with either the distributed or lumped model. The capacitance was measured (Gage & Eisenberg, 1969) using steps of current. A capacitance of some 2 $\mu F/cm^2$ was found, close to the values predicted by the lumped model and significantly different from the unitarian value. For some time we felt that this settled the matter in favor of the lumped model, but recently measurements of the high frequency capacitance of glycerol-treated fibers made by Nakajima and Hodgkin (1970) have raised doubts. It is important to discuss the meaning of "high frequency capacitance." To explain this idea, we make a detour into circuit theory. One of the beautiful results of circuit theory is the ease with which the sinusoidal properties of a linear system can be analyzed. Thus, the amount of out-of-phase current can be measured in a system at different frequencies, and from this figure an effective capacitance can be defined, which, of course, will depend on the frequency at which the current is measured. For instance, in the lumped circuit used to describe the T system, the resistance R_e is not very important compared to the impedance of the capacitor C_e at low frequencies; thus one would measure an effective capacitance close to $C_m + C_e$. At high frequencies, on the other hand, the capacitor has a very low impedance and the resistor R_e is important; the effective capacitance is then close to C_m, the capacitance of the surface membrane. In preparations without tubules, only C_m should be present and thus, measurements of the high frequency capacitance in glycerol-treated fibers would be expected to give similar values to measurement of the low frequency capacitance. Impedance measurements on glycerol-treated fibers have not been made yet, but some transient measurements which should give a value close to the high frequency measurement have recently been made by Drs. Nakajima and Hodgkin. By analyzing the time course of the foot of the action potential they have determined a value of 0.9 $\mu F/cm^2$ for the high frequency capacitance. This figure is, of course, significantly different from the 2 $\mu F/cm^2$ that they and we have measured for the low frequency capacitance using rectangular pulses. This result suggests either that there are substantially more tubules left in the glycerol-treated fibers than were measured by the peroxidase experiments or that there are two components of the surface membrane capacitance, one behaving as if it has a resistance in series. The evaluation of these findings requires a full impedance analysis of glycerol-treated fibers, *in my opinion*, particularly in view of the dissimilar findings of Ildefonse *et al.*, (1969) who measured capacitance of glycerol-treated fibers using voltage-clamp techniques. Until such measurements are made I am afraid that we cannot really say which of the two models the glycerol data supports, or indeed whether it shows that further complication is present.

There is another set of experiments which are relevant to the question of which equivalent circuit is the most appropriate for skeletal muscle. These experiments, done by Drs. Vaughan, Howell, and myself (Vaughan, Howell & Eisenberg, 1970) examined the effects of changes in the conductivity of the external solution on the capacitance of normal muscle fibers. It is clear the the distributed model of the tubular system in which the series resistance is ascribed to the solution in the lumen of the tubules could be tested by varying the resistance of this solution. We have tried to do this by replacing some of the sodium chloride in the Ringer solution with sucrose. This procedure should increase the resistance of the solution in the tubular system, thus current should flow less far into the depths of the fiber; in other words, the characteristic length of the tubular system should decrease. We have tried to study this phenomena, measuring the total (low frequency) capacitance of muscle fibers in solutions of low ionic strength by applying step functions of current with one microelectrode and analyzing the voltage recorded with another microelectrode at various points along the cell. If we expect capacitance to reflect the area of membrane across which current flows, we can write the following equation

$$C_e = A\,F\,C_W \tag{4}$$

where

- C_e is the capacitance of the tubular system associated with one square centimeter of surface membrane
- A is the ratio of the area of the tubular system to that of the surface membrane (measured by Peachey, 1965)
- C_W is the capacitance of one square centimeter of tubular membrane
- F is a function which describes the fraction of the area of tubular membrane across which current flows.

As we increase the resistance of the solution in the lumen of the tubules the effective length constant (defined by an equation like Eq. 3) should decrease and the fraction of the area across which current can flow (F in Eq. 4) should decrease; the total capacitance measured should then also decrease. There is a complication however: Rapoport, Peachey and Goldstein (1969) have shown that the tubules swell under these conditions and that there appears to be a large increase in the area (A) of tubular membrane. Furthermore, Freygang, Rapoport and Peachey (1967) have shown that a similar swelling of the tubules produced by hypertonic solutions is accompanied by an increase in the capacitance of muscle fibers. The increase in area in low ionic strength solutions would thus be expected to increase the capacitance, while the increase in luminal resistance should decrease the capacitance. Since the change in area is proportional to the change in ionic strength, but the change in F (proportional to the change in the length constant) depends on the square root of the ionic strength, the increase in area should be more important than the decrease in F. Thus, one would expect the capacitance to increase as ionic strength is reduced, at least as long as the

area of tubular membrane increases linearly: a full theoretical analysis predicts first an increase and then eventually a decrease in the capacitance of the tubular system as ionic strength of the Ringer solution is lowered.

Figure 2 shows our experimental results. Notice the decrease in capacitance to some 2 $\mu F/cm^2$ and the flattening of the curve at that value; also note that similar experiments show no effect on the capacitance of glycerol-treated fibers. It seems as if lowering the conductivity of the external solution decreases the capacitance to the figure of 2 $\mu F/cm^2$. These experiments provide independent support for that figure for the capacitance of the surface membrane and thus for the lumped model. Furthermore, this decrease in capacitance is difficult to reconcile with the analysis mentioned above.

These fibers, bathed in similar solutions of low ionic strength, have another property which fits beautifully with the results described by Costantin (1970): they twitch. This is surprising at first: after all if the capacitance of the tubular system is negligible in these solutions it means that little current can flow into the tubular system. On the other hand, a twitch presumably can occur only if most of the tubular membrane is depolarized (its capacitance discharged). A simple way to explain these findings is to postulate a regenerative electrical system in the tubular membrane which can supply current to depolarize the T

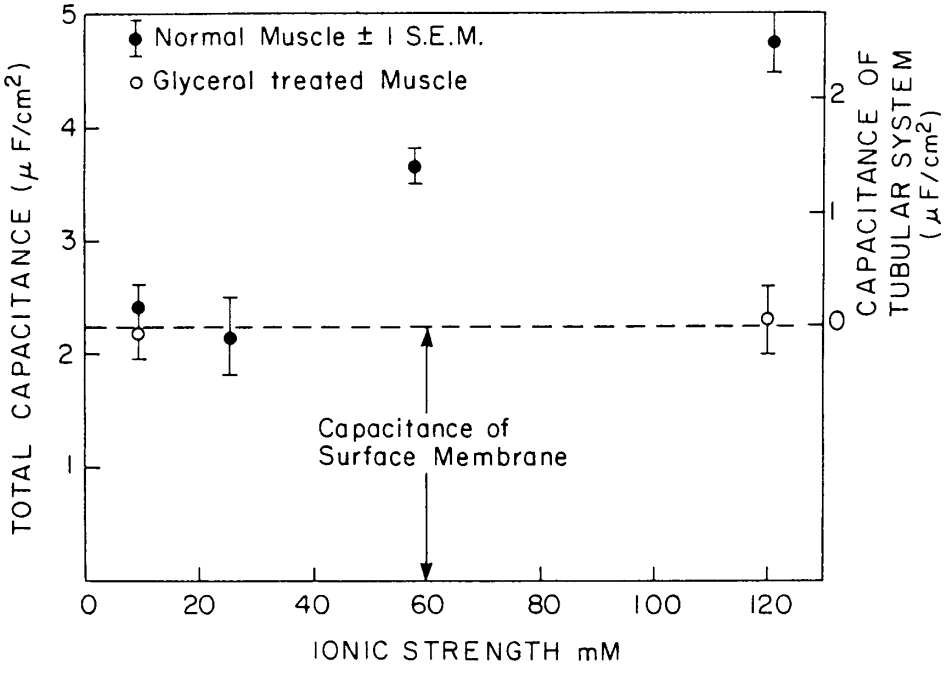

Figure 2. The capacitance, measured using rectangular pulses of current, of frog skeletal muscle fibers, in solutions of varying ionic strength. The solutions were made so as to have the same [K] [Cl] product as normal Ringer. The dotted line indicates the most likely figure for the low frequency capacitance of the surface membrane.

system membranes. The small currents used to measure capacitance presumably do not reach the threshold of this regenerative system, but the larger currents associated with the action potential on the outer membrane probably do.

It is now necessary to summarize this mass of data, and to try to reach a conclusion. Perhaps the best we can say is that taken on balance the lumped model seems to describe the data better than the distributed model, but that the evidence is not overwhelming. More experiments, particularly concerning the impedance of glycerol-treated fibers, and of normal fibers in solutions of low ionic strength, should be useful in resolving some of the present ambiguities.

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