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Determination of Muscle Cable Parameters from a Single Membrane Voltage Response

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Summary. Computer techniques have been developed to achieve a least-squares fit of the Hodgkin and Rushton one time-constant cable equation to the recorded responses of muscle cells to the intracellular injection of square current pulses. In the rat diaphragm the response of the muscle fiber to square current pulses is well fit by a one time-constant model. This makes possible the estimation of the passive electrical properties of muscle sarcolemma using the response of the fiber to a single stimulus. A comparison of the results of this method and the older method of spatial decay in the rat diaphragm shows no significant differences. Average values obtained using the one-point method for estimating membrane resistance and capacitance are 520 $\Omega\,cm^2$ and 4.6 $\mu F/cm^2$, respectively. An estimation of the average specific resistivity of the cytoplasm was made using this method, and it was found to be 213 Ω cm. At levels of injected currents producing large membrane hyperpolarizations where delayed conductance changes make the spatial decay method useless, the one-point method continues to give consistent results since it utilizes only the early portion of the membrane response. Work with the frog sartorius muscle, which is known to display the characteristics of a two time-constant system, shows that this method is capable of estimating the slower components of this system by using only the later portion of the response curve. The immunity of this method to delayed conductance changes and its experimental facility make it useful in estimating the passive electrical properties of muscle fibers, particularly when working with tissues that are small and delicate or that have poor *in vitro* viability.

In work with excitable cells it is frequently necessary to accurately determine the passive electrical properties of the surface membrane at rest. In muscle there are currently two approaches to the measurement of these parameters: the method of steady-state spatial decay and the more recently introduced method of discrete frequency analysis. Both of these methods have inherent limitations in situations where multiple comparative measurements must be made, particularly in regard to the time required for data collection from a single fiber and to the membrane

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damage and subsequent depolarization resulting from the multiple insertions and withdrawals of microelectrodes in each cell examined. These constraints make application of either technique to systems with limited *in vitro* viability and small fiber size difficult.

The passive response of a nerve or muscle membrane to small hyperpolarizing or depolarizing current pulses can usually be described in terms of an elementary electrical analog containing only resistance and capacitance elements and often resembles the behavior of a coaxial electrical cable. In its simplest form, such an analog attributes a single unique time constant to the surface membrane. In 1946 Hodgkin and Rushton derived an equation to describe the time and distance dependence of the response of a nerve axon to the point injection of a square pulse of current across the surface membrane when that membrane was considered to have a single time constant [7]. Later studies in frog muscle suggested that the response of these cylindrical cells was more accurately represented by a model having two discrete radial circuit elements with different time constants [4, 5]. Falk and Fatt in 1963 demonstrated that the two time-constant model and the one time-constant model were equivalent in the later portions of the response to a square current pulse, and others have found that mammalian muscle appears to fit well to a single time-constant model [15]. If either of these conditions holds, it should theoretically be possible to obtain estimates of the passive electrical properties of a muscle cell membrane by measurement of the membrane response at a given distance to a single square current pulse as a function of time and the analysis of this response in terms of the Hodgkin-Rushton equation.

The present paper describes a technique which takes advantage of these considerations and allows the extraction of all passive electrical parameters of a muscle cell from a single membrane voltage response record. This approach is compared in rat muscle with the standard three-point spatial decay method and is shown to yield equal accuracy of measurement while greatly reducing time required for determination in each cell and increasing the number of cells which can be successfully studied. In addition estimates of membrane capacitance are directly obtained which are not available with the earlier method. Applications and limitations of this technique are discussed.

Materials and Methods

Left hemidiaphragms from male adult Wistar rats were used. All measurements were carried out in a mammalian Ringer's solution containing (in mM) 135 NaCl, 4.5 KCl,

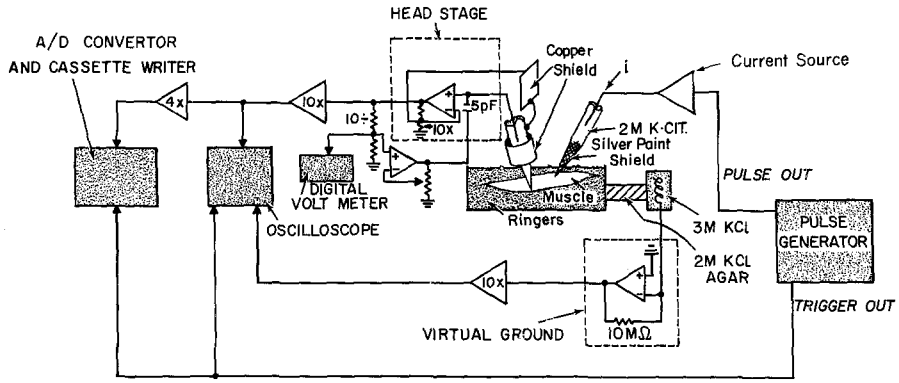

Fig. 1. Diagrammatic representation of electronic equipment. Injected current is measured by the virtual ground system and displayed on the oscilloscope. The fiber responses are simultaneously displayed on the oscilloscope and digitized by the A/D convertor. At the end of the current pulse the 512 digitally recorded words are written on the cassette for later retrieval and data analysis by the PDP-10 computer

1 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 11 Glucose and 12 $NaHCO_3$ which was saturated with 95% O_2 + 5% CO_2 and warmed to 37°. Microelectrodes containing 3 M KCl with resistances between 15 to 40 MΩ were used for recording. Stimulating electrodes were filled with 2 M K-citrate and their resistance ranged from 15 to 30 MΩ. Muscle was maintained at 37 °C by a thermostated water jacket surrounding the chamber and by superfusion with warmed Ringer's at a rate of 5 ml/min. Interelectrode distance was measured with an ocular micrometer fitted to a dissecting microscope.

The electronic components are shown diagramatically in Fig. 1. The current source incorporates positive capacitative feedback capability which allows for trimming of the input current pulse for each stimulating electrode after insertion into individual fibers. Rise times in the current pulse of from 10 to 20 μsec were routinely achievable. Current electrodes were shielded with a conductive colloidal silver paint (G.C. Electronics #22-246) to reduce capacitance artifact. Stimulating currents were measured by a virtual ground system and displayed on the oscilloscope for trimming and recording.

The recording apparatus consisted of a high impedance FET amplifier with adjustable capacitance feedback and a driven guard capable of recording rise times of less than 40 μsec through a 20 MΩ source resistance. The output from this amplifier was simultaneously displayed on an oscilloscope and processed in real time by an analog to digital converter. The sampling rate of the A/D convertor was adjustable in precision steps from 25 to 200 μsec per sample, and this rate served as the time base for the converted data. Data output was temporarily retained in a high-speed shift register during sampling and subsequently transferred to a digital cassette recorder during the brief interval following each test pulse. A single membrane voltage response was recorded as 512 eight bit words each with an accuracy of ±0.05 mV. Data was retrieved for processing directly from the digital cassette by a DEC PDP-10 computer. Data processing routines were written in Fortran IV. The least-squares iterative routines used were based on a technique described by Whitaker and Robinson [16].

Results

Extraction of the passive membrane parameters from the membrane voltage response to a square current pulse is accomplished by finding

those parameters which will give a theoretically predicted response which most closely reproduces the measured response of the membrane. Accurate values for each parameter will be ones which produce a minimum discrepancy between prediction and observation at every time point of the recorded response. In order for this error minimization to be accomplished a phenomenological equation incorporating these parameters must be written which relates membrane response to input current as a function of distance and time, and a means of fitting this equation to the data points must be found. The least-squares technique of curve fitting is the method of choice, but this approach requires that some form of the phenomenological equation be found which is linear.

In a one time-constant membrane model the equation for the response of a muscle fiber as a function of time is analogous to that derived originally by Hodgkin and Rushton [7] for the crustacean nerve:

$$V(x,t)=\frac{R_0}{4}\cdot I\cdot\left(\exp(-x/\lambda)\cdot(1-ERF(v))-\exp(x/\lambda)\cdot(1-ERF(z))\right) \quad (1)$$

where:

$$v=x/(2\cdot\lambda\cdot\sqrt{t/\tau})-\sqrt{t/\tau}$$
$$z=v+2\cdot\sqrt{t/\tau}$$
$$ERF(w)\equiv\frac{2}{\sqrt{\pi}}\int_0^w\exp(-w^2)\,dw$$
$$R_0=\sqrt{r_m\cdot r_i}$$
$$\lambda=\sqrt{r_m/r_i}$$
$$\tau=r_m\cdot c_m.$$

The applicability of this equation to muscle is based in part on the assumption that the muscle fiber can be considered to behave as an infinite cable. That this is indeed the case for the central region of a muscle fiber is well established [5, 6]. This equation cannot be made linear by any of the conventional techniques. However, the method described by Whitaker and Robinson [16], a modification of which is outlined below, is capable of linearizing most differentiable equations [1].

If one assumes that the true values for R_0, λ, and τ are close to some guessed values for these parameters, then Eq. (1) can be approximated by a first-order Taylor Series expansion around the guessed values for the three parameters.

$$V(x,t)=V^*(x,t)+\frac{\partial V^*}{\partial\lambda^*}(\lambda-\lambda^*)+\frac{\partial V^*}{\partial R_0^*}(R_0-R_0^*)+\frac{\partial V^*}{\partial\tau^*}(\tau-\tau^*) \quad (2)$$

where parameters marked with an asterisk indicate guessed values, $V^*(x, t)$ represents the voltage calculated from Eq. (1) using these guessed values, and $V(x, t)$ is the true value of voltage defined by Eq. (1) when the correct values for the membrane parameters are known. Further, the following variables are defined:

$$F_i = V_{\text{observed}}(x, t) - V^*(x, t)$$
$$\Delta\lambda = \lambda - \lambda^*, \quad \Delta R_0 = R_0 - R_0^*, \quad \Delta\tau = \tau - \tau^*$$

then Eq. (2) can be written:

$$F_i = \frac{\partial F_i}{\partial \lambda^*}\Delta\lambda + \frac{\partial F_i}{\partial R_0^*}\Delta R_0 + \frac{\partial F_i}{\partial \tau^*}\Delta\tau. \tag{3}$$

This is clearly a linear function in the new variables $\Delta\lambda$, ΔR_0, and $\Delta\tau$, and there are N of these equations (one for every data point). These can now be solved using the method of least-squares, the equations for which are given in matrix notation below:

$$A = B \cdot C \tag{4}$$

where

$$a_j = \sum_{i=1}^{N} F_i \frac{\partial F_i}{\partial P_j}$$
$$b_{j,k} = \sum_{i=1}^{N} \left(\frac{\partial F_i}{\partial P_j} \cdot \frac{\partial F_i}{\partial P_k}\right)$$
$$c_j = \Delta P_j$$

and

$$P_1 = \lambda, \quad P_2 = R_0, \quad P_3 = \tau$$

then,

$$C = B^{-1} \cdot A. \tag{5}$$

Using this approach, in which the partial errors attributable to each parametric variable are used to provide corrected estimates of these variables for the next iterative solution, an accurate determination of each parameter in the membrane response can usually be arrived at within 5–8 iterations. With typical programming techniques this usually represents less than 8 sec of actual computer operation for the more than 100 data points which are used for each response calculation.

Error Considerations

In the formulation given above for fitting the data to Eq. (1) the diagonal elements of the inverse matrix B^{-1} contain (when convergence

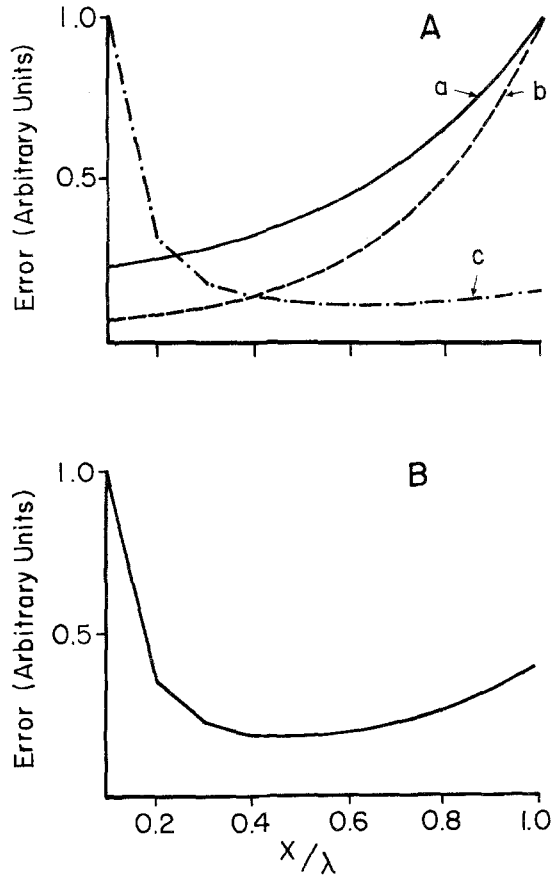

Fig. 2. (*A*) The normalized error to be feared in each of the three cable parameters as a function of interelectrode distance. Curve (*a*) is the error associated with the time constant, (*b*) is that associated with the input resistance and (*c*) is that associated with the length constant. (*B*) The normalized sum of the error in all three parameters as a function of interelectrode distance. In (*A*) each of the curves is given by the expression $Y_j = b_{j,j}^{-1}(x)/b_{j,j}^{-1}(\max)$ where the inverse diagonal elements ($b_{j,j}^{-1}(x)$) are calculated at each value of x over a time period of 0.0 to 3.0 τ at intervals of 0.2 τ, and $b_{j,j}^{-1}(\max)$ is the maximum of the values obtained for each of the parameters. In (*B*) the curve is given by $Y = \sum_{j=1}^{3} b_{j,j}^{-1}(x)/S(\max)$ where $b_{j,j}^{-1}(x)$ is as described above and $S(\max)$ is the maximum value of the sum of the inverse diagonal elements. In all the curves, points were calculated at intervals of x ranging from 0.1 λ to 1.0 λ at steps of 0.1 λ. Note that the minimum occurs between 0.4 and 0.6 times the length constant

is obtained) the proportions of the residual error which can be transformed into the error feared in the estimation of each of the three corresponding parameters. That is:

$$\sigma_{P_j}^2 \propto b_{j,j}^{-1}.$$

Since these elements ($b^{-1}_{j,j}$) are functions of x (the interelectrode distance) one can estimate the value of x for which the effect of random error (signal noise) on each parameter will be a minimum.

Fig. 2*A* shows the relative values for each of the diagonal elements as a function of x calculated for $t=0.0$ to $3.0\,\tau$. Fig. 2*B* shows the sum of the three elements under the same conditions. The minimum value of this function is between 0.4 and 0.6 λ. For the rat diaphragm, where λ is typically 0.5 mm, this corresponds to an interelectrode separation of approximately 200–300 μm. This is fortuitous since this distance is great enough that three-dimensional cable considerations are negligible 12 but is close enough that single fibers can be easily visualized over the distance.

Intracellular Measurements in Rat Diaphragm

The digitized record of a typical membrane response to a square current stimulus recorded intracellularly in a single fiber is shown in Fig. 3*A*. A small amount (100–200 μV) of residual random noise is seen. Fig. 3*B* displays the arithmetic sum of four successive digitized records from the same fiber. This simple addition of a small number of responses to identical stimuli from a given fiber has the effect of enhancing the signal to noise ratio by a factor equal to the square root of the number of responses summed. While the noise level is not a significant computational problem in this case, the improvement in signal to noise ratio between the two curves is easily appreciated.

While Eq. (1) is generally considered to be an adequate model for the nerve axon, it is frequently said to be only an approximation to the response of muscle fibers to step-function currents. This is principally based on the work done in the frog sartorius muscle where the one time-constant model is clearly inadequate for describing the early portion of the response. In this muscle the circuit model for a one time-constant system gives a very poor description of the frequency response of the system for frequencies as low as 10 Hz [5]. For the rat, on the other hand, the one time-constant circuit model has been shown to be adequate to describe the membrane frequency response for frequencies up to at least 450 Hz [15].

In response to step-function currents the differences between the two model types would be expected only in the early portion of the response times (less than the half-rise time) since it has been shown that the two models converge at longer times [5]. Fig. 3*C* and *D* show the recorded summed responses from a rat diaphragm fiber and a frog sartorius muscle

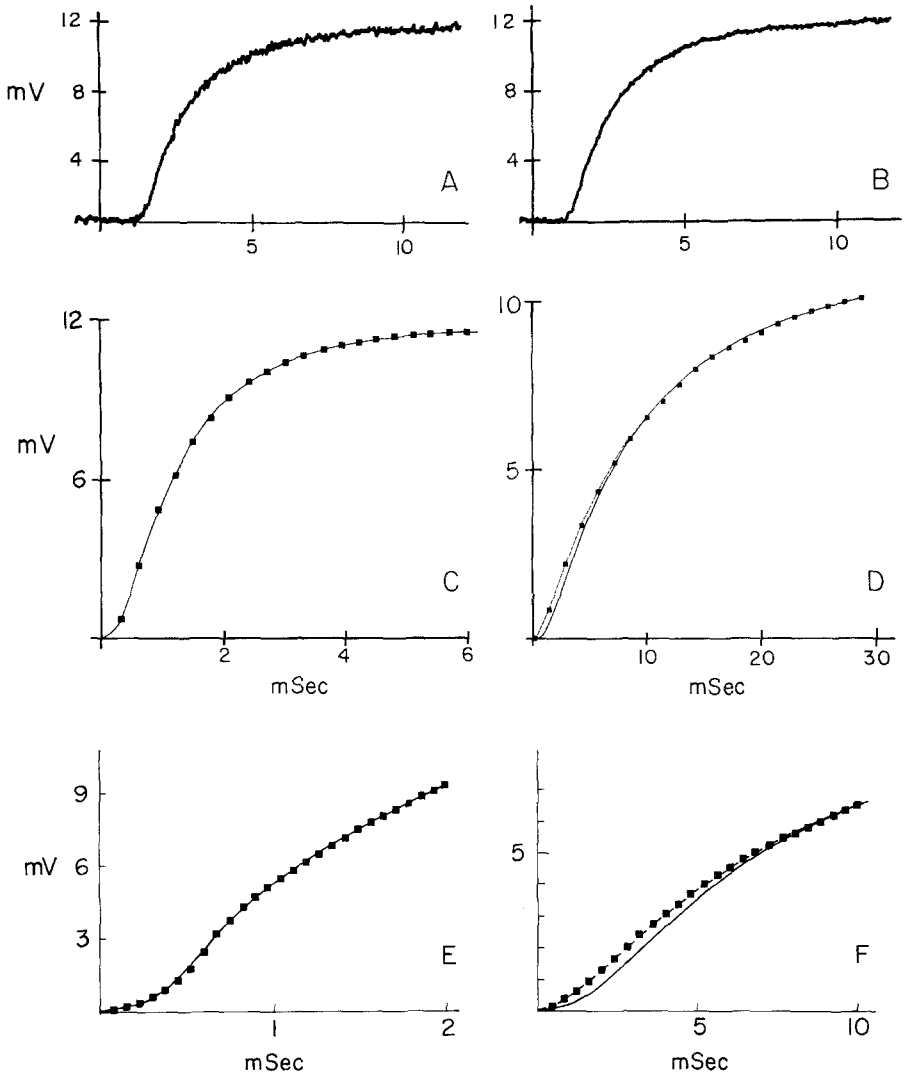

Fig. 3. (*A*) The digitized record of a single response from a single rat diaphragm fiber plotted as a function of time. (*B*) The sum of four responses from the same fiber in (*A*). Note the relative reduction in signal noise. (*C*) The fit of Eq. (1) to the response shown in (*B*) using only the later portion of the response. The interelectrode distance is 248 μm, the current level is 28 nA and the derived membrane parameters are: $R_m = 503\ \Omega cm^2$, $C_m = 4.36\ \mu F/cm^2$ and Radius = 22.4 μm (assumed $R_i = 200\ \Omega cm^2$). (*D*) The recorded response of a frog sartorius muscle fiber and the fit of Eq. (1) using only the later portion of the response. The interelectrode distance is 660 μm, the current is 26 nA and the derived membrane parameters are $R_m = 2683\ \Omega cm^2$, $C_m = 6.45\ \mu F/cm^2$ and Radius = 25.7 μm (assumed $R_i = 169\ \Omega cm^2$). (*E*) and (*F*) the early portions of the responses in (*C*) and (*D*), respectively, with expanded scales. Note that the fit of Eq. (1) is excellent throughout the recorded response in the rat diaphragm fiber (*C* and *E*) while in the frog sartorius fiber (*D* and *F*) Eq. (1) consistently underestimates the early portion of the fiber response indicating the discrepancy between the one and two-time constant systems

fiber (points) and the corresponding predicted response of Eq. (1) (solid lines), the cable parameters for which were derived from the later half of the response curves. In the frog fiber (Fig. 3*D* and *F*) it is clear that the recorded response and the predicted response of Eq. (1) are not the same in the early portion of the curves. Nevertheless, the calculated values for the membrane parameters from this fiber are reasonable estimates of the nontubular membrane resistance and the total membrane capacitance when compared with the results from other workers using more complicated methods [5, 6, 13, 14]. For the rat diaphragm fiber (Fig. 3*C* and *E*) the fit of Eq. (1) to the recorded response is good through the total response time. For the rat diaphragm then, the use of a second time constant to describe the response is not necessary, and the use of the Hodgkin-Rushton equation to derive the cable properties is justified.

Table 1 compares the results from 15 rat diaphragm fibers from which estimates of membrane parameters were made using both the method of spatial decay and the time-varying approach. For the spatial-decay

Table 1. A comparison of the results of the method of spatial decay and the results of the one-point analysis in the measurement of the cable parameters in fibers of the rat diaphragm

Fiber	Spatial decay		One-point		
	R_m	D	R_m	D	C_m
1	354	59	468	70	4.6
2	485	69	478	63	4.5
3	382	67	507	78	5.0
4	304	50	953	80	3.2
5	364	63	502	70	3.7
6	423	72	427	65	5.1
7	282	60	512	73	3.1
8	417	35	562	40	3.1
9	619	64	480	53	4.8
10	521	83	528	78	4.4
11	605	77	546	70	4.3
12	1172	46	1154	50	2.6
13	1310	54	554	30	4.0
14	646	53	421	41	5.3
15	598	74	546	77	4.6
Average	565.9	61.1	576.2	63.1	4.2
SEM	77.2	3.2	52.1	4.1	0.2

R_m = specific membrane resistance (Ωcm^2). D = diameter (μm). C_m = specific membrane capacitance ($\mu F/cm^2$). SEM = standard error of the mean. Assumed value for internal resistivity = 200 Ωcm. For the spatial decay method interelectrode separations were approximately 100, 200, and 400 μm. The responses at 200 μm were recorded and used for the one-point time varying analysis.

method of the steady-state voltage response was recorded at interelectrode distances of approximately 100, 200 and 400 μm. The standard method of spatial decay analysis was used [7].

For the one-point time varying analysis the response at the interelectrode separation near 200 μm was recorded and used for derivation of r_i, r_m and c_m as described above. For either method the cable parameters (the lower case letters) are related to the specific membrane parameters by Eqs. 8, 9, and 10.

$$r_i = R_i/A \tag{8}$$

$$r_m = R_m \cdot P \tag{9}$$

$$c_m = C_m/P \tag{10}$$

where A is the cross sectional area of the fiber, P is the circumference of the fiber, and R_i is the internal resistivity. To evaluate the membrane characteristics, then, one must know either the size of the fiber or the internal resistivity. In this experiment an internal resistivity of 200 Ωcm was used to obtain estimates of the membrane parameters and the diameters from each of the fibers. Experimental data supporting this estimate is given below.

Essentially identical values for average membrane resistance, length constant and fiber diameter were obtained with both methods, although the number of fibers yielding useful data per unit time was far greater with the one-point method, as would be anticipated from the simplicity of the recording technique.

A comparison of measurements of rat muscle membrane and cable parameters found in the literature and the same parameters derived from 124 rat diaphragm fibers using the one-point time varying analysis has been made (Table 2). It can be seen that, although there are some differences in the reported results from other workers and the results from the time varying analysis, the results obtained in this way are well within the range of literature values.

Since the values obtained for the membrane parameters are dependent upon the value assumed for the internal resistivity and since there seems to be little agreement on this value for rat muscle in the literature (estimates range from 125 to 240 Ωcm), experiments were performed to obtain an estimate of the average internal resistivity in the rat diaphragm fibers being used.

Estimates of cable parameters were made on 15 fibers in each of two separate rat diaphragm preparations in an area on each diaphragm which could easily be identified by its relationship to blood vessels and nerves.

Table 2. A comparison of the published results of various methods of measurement of the cable parameters from rat skeletal muscle

hor	Method	Muscle	R_0	λ	τ	D	R_m	C_m	R_i	n
ovick *et al.* [17]	3-point dc	Diaphragm	0.8	0.5	1.5	44	460	3.2	180	124
ade and Barchi [10]	3-point dc	Diaphragm	–	–	–	54	445	–	185	777
del and Senges [11]	3-point dc	Diaphragm	–	–	2.3	49	414	5.5	25	18
tag [2]	3-point dc	Diaphragm	0.8	0.66	2.3	44	722	3.2	182	100
ohara *et al.* [9]	3-point dc	EDL	0.71	0.57	3.1	42	542	5.9	176	16
ohara *et al.* [9]	3-point dc	SOL	1.24	0.46	2.0	30	540	3.8	194	14
nerino and Bryant [3]	3-point dc	EDL	–	–	1.5	47	349	4.2	125	23
stgaard [15]	AC	SOL	0.52	0.67	2.7	62	766	3.6	240	32
s work	1-point time	Diaphragm	0.79	0.54	2.4	45	520	4.6	200	124

R_0 = input resistance (MΩ). λ = length constant (μm). τ = time constant (msec). D = diameter (μm). R_m = specific membrane resistance (Ωcm). C_m = specific membrane capacitance (μF/cm^2). R_i = specific resistivity of cytoplasm (Ωcm). n = number of fibers from which responses were measured.

After the responses were recorded, the specific sections from which measurements were made were isolated, quick frozen in cold isopentane and prepared for transverse cryosectioning. Calibrated photomicrographs were taken of the fixed and mounted sections from which enlarged prints were made. The average cross-sectional area of the surface fibers was then determined with a planimeter. This value and the value for the average longitudinal resistance obtained from the voltage response analyses were then used in Eq. (8) to estimate the internal resistivity.

The average longitudinal resistance for the two preparations was 11.55 MΩ/cm while the average cross-sectional area of the surface fibers was 1844 μm^2 (Table 3). This results in an overall estimate of 213 Ωcm for internal resistivity. It is clear that the apparent average internal resistivity can vary from preparation to preparation and presumably from fiber to

Table 3. Estimation of internal resistivity by comparing longitudinal resistance and cross-sectional area from two preparations

Prep #	r_i (avg)	N_1	A (avg)	N_2	R_i
1	11.63 MΩ/cm	15	1975 μm^2	39	230 Ω · cm
2	11.46 MΩ/cm	15	1749 μm^2	54	200 Ω · cm
Grand avg	11.55 MΩ/cm	30	1844 μm^2	93	213 Ω · cm

r_i = longitudinal resistance. N_1 = number of fibers in electrical measurement. A = cross-sectional area. N_2 = number of fibers in histological measurement. R_i = calculated value for internal resistivity.

fiber. The effects of this variation are somewhat attenuated in the conversion of cable parameters to membrane parameters since the membrane parameters vary as functions of the square-root of the internal resistivity. Because of this fact and in view of the relatively lower values assumed by most other laboratories, a value of 200 Ωcm has been assumed throughout this paper.

A potential source of error in the analysis of passive electrical membrane properties by the steady-state method of spatial decay lies in the nonlinear voltage dependencies of ion conductances in the membrane. In muscle the major resting ion conductance is that attributable to chloride. It has been shown in the frog [8] and more recently in the rat [10] that this conductance exhibits markedly nonlinear behavior as a function of membrane potential, undergoing a time-dependent decrease with hyperpolarization. These changes are detectable for hyperpolarizations as small as 10 mV.

For the dc method of spatial decay this represents a major limitation since it is the late portion of the voltage response which is used to make estimates of the cable parameters. For the one-point time varying analysis described here such non-linear phenomena could be a potential problem since changes in membrane conductance would be expected to alter the shape of the response curve. However, since these changes are both voltage and time dependent and since the time constants (>200 msec) are quite long with respect to duration of the fiber response being analyzed in this technique, one would expect that the time-varying analysis might prove less susceptable to this error.

To investigate this possibility the responses from several rat diaphragm fibers were recorded at injected current levels from 10 nA to approximately 250 nA resulting in graded membrane hyperpolarizations of up to 70 mV. Any nonlinearity or time-varying changes in membrane conductance would be expected to principally affect the estimates of λ and τ obtained from such responses since these parameters determine the shape of the curve. For each fiber studied estimates of both parameters as well as input resistance were made for each level of current injected. The average value of the input resistance was used to calculate the membrane displacement at the site of the current electrode for each fiber and for each current level. Average estimates of the length constant, the membrane resistance, the membrane capacitance and fiber radius were obtained for each fiber and then the percent difference from this average value for each individual estimate of each of these parameters was plotted against the calculated membrane displacement voltage. The results are shown in

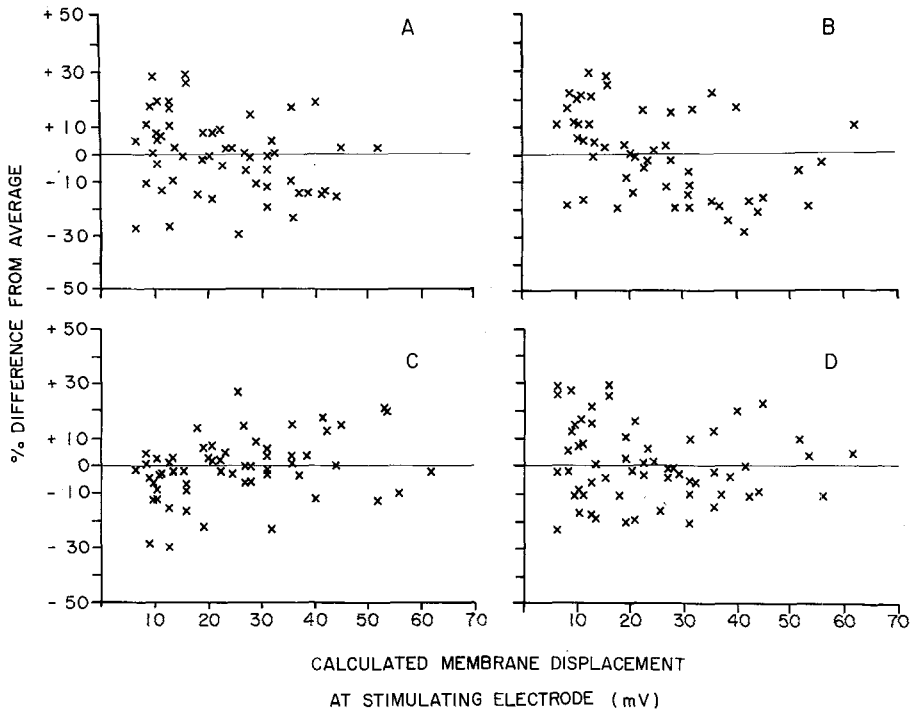

Fig. 4. The percent deviation from average for estimates of membrane and cable parameters made from the same fibers at varying levels of hyperpolarizing current. For each fiber average values were calculated for each parameter and then the percent difference ineach value from this average was plotted against the calculated hyperpolarization at the stimulating electrode. (*A*) The variation in length constant (λ). (*B*) The variation in membrane resistance (R_m). (*C*) The variation in membrane capacitance (C_m). (*D*) The variation in radius. The estimates of all these parameters are essentially constant throughout the range investigated

Fig. 4. While the voltage displacements achieved were clearly adequate to produce the slow time-varying conductance changes in the latter parts of the fiber responses, they affected the early recorded portions little if at all, since the estimates of λ, R_m, C_m and fiber radius are essentially constant throughout the range of displacement voltages.

It is clear from this experiment that the measurements of the membrane parameters based on the one-point system and using only the early portion of the membrane voltage response curve reflect the early ionic conductances, and therefore avoid the problem of voltage dependent ionic conductance changes. This is an important feature since it is the resting or passive properties of the membrane conductances that these methods are designed to measure. For the spatial-decay method the only way to avoid these problems is to use very low input currents which mini-

mize the conductance changes by minimizing the membrane voltage displacement, but this makes measurement difficult and limits accuracy.

Discussion

In 1946 when Hodgkin and Rushton presented their equation describing the response of an axon to a square pulse of current as a function of both time and distance they pointed out that the equation was too computationally complex to be used conveniently as a means of determining passive electrical parameters. They indicated that by allowing the membrane to reach its steady state and measuring this value as a function of distance one could reduce their equation to a simple exponential form which could then be used in the experimental determinations of membrane cable parameters. However, this introduced the requirement of multiple measurements from single fibers with its inherent difficulties. With the more recent widespread availability of high-speed digital computers the problems of computation in the complete formulation are drastically reduced, and it is reasonable to reevaluate the methods used for estimating cable parameters in light of this.

We have shown that the complete Hodgkin-Rushton equation predicting membrane response to a current pulse can be fit to recorded responses using an iterative least-squares technique. For the rat diaphragm this method yields estimates of cable parameters which are comparable to those obtained by experimentally more complex methods. The single-point method has several advantages over methods based on spatial decay since only a single insertion of a recording and a stimulating electrode is required. The time necessary for estimating the fiber parameters in a given cell is reduced at least by a factor of three since a single response and an estimate of interelectrode distance is all that is needed for their definitive evaluation. In practice estimates of fiber cable parameters can be made at rates better than one fiber per minute of experimentation time. In addition, the method is less traumatic to the fibers which are studied since all measurements are made before either electrode is withdrawn, and the problem of damage to the membrane produced by withdrawing and reinserting the electrode is eliminated. It is expected that this method will make the study of smaller and more delicate tissues much more practical.

A problem common to any method of membrane analysis is the transformation of the calculated "cable" parameters (R_0, λ, and τ) derived

from the model response to the membrane parameters (R_m and C_m). Knowledge of either the fiber size or the internal resistivity (from which fiber radius can be calculated) is required in order to make these estimates. Typically a standard value for the internal resistivity is selected which on the average produces reasonable estimates of fiber diameter. In various laboratories this value has been arrived at in different ways, and this had led to a range of assumed values from 125 to 240 Ωcm for rat muscle.

Calculation of the internal resistivity requires determination of both fiber cross-sectional area and longitudinal resistance. Estimates of cross-sectional area based on optical diameter measurements are less reliable than those derived from direct planimetric determinations, but in the latter case sampling error must be considered. Cross-sectional areas determined histologically may not be representative of the fibers sampled electrically due to regional variation in fiber area within the diaphragm. Consequently care must be taken to insure correspondence between the two samples. The technique employed in the present study of careful trimming and measuring only surface fiber cross-sectional areas in the histologic sections minimizes this problem. The value of 213 Ωcm for internal resistivity arrived at in this way indicates that the average internal resistivity is higher than the reported literature average. This conclusion is supported by the work of Westgaard *et al.* [15] who used a die-marking procedure and histologic sectioning to determine a value of 240 Ωcm for the internal resistivity of rat soleus fibers.

The time-varying voltage dependent change in chloride conductance observed with hyperpolarizing current pulses [8, 10] represents a major potential error in cable estimates made using the spatial decay method. This decrease in chloride conductance occurs even with very small hyperpolarizations but has been shown to have a relatively long time constant (>200 msec). For the one-point method of analysis all the information needed for the estimation of the cable parameters (R_0, λ and τ) is contained in the very early portion of the response (the first 10 msec) and errors introduced by these time-dependent conductance changes should not greatly affect the results obtained. The data presented indicate that the one-point system of analysis is indeed immune to these changes for membrane displacements up to at least 70 mV. This value is far above the level at which the spatial-decay method becomes useless because of its dependence upon a steady-state value for the membrane current.

The principal shortcoming of the time-varying method of analysis as it is presented here lies in the inability to obtain estimates on all of the

model circuit parameters for those membranes which are best described by a two time-constant model. The method can be used for determining the slower components of two time-constant models such as the frog sartorius by merely considering that portion of the response which is greater than half of the final response amplitude since this part of the curve is well described by the one time-constant model (5). Indeed, application of this technique has given results which are in good agreement with reported values for the "late" membrane conductances and total capacitance in some preliminary work with frog muscle. However, in order to be able to divide the total capacitance into "early" and "late" components and arrive at a value for the "early" conductance in frog muscle some form of frequency analysis will be required. Currently we are developing a method using Fourier analysis of both input and output signals in order to obtain the necessary frequency dependent response characteristics from a single pulse for full calculation of all parameters in the two time-constant models. This will be the subject of a subsequent publication.

The work presented here with the rat muscle and other preliminary work with mammalian tissues, including the horse and the mouse, indicate that the two time-constant model may not be applicable or at least is not resolvable in the response of these muscles to step-function currents. This conclusion is further supported by the work of Westgaard *et al.* who found that the frequency response of rat muscle was adequately described by a simple one time-constant circuit model for frequencies up to 450 Hz. For these reasons the use of the Hodgkin-Rushton model equation appears to be an accurate and useful technique for rapidly measuring the passive electrical properties of rat muscle membranes.

The experimental simplicity and the rapidity of measurement afforded by this technique also promise to greatly expand the kinds of muscle tissue which can be studied in this manner. The measurement of cable properties in human biopsies or *in situ* on a routine basis seems feasible since the measurements can be done with minimum time and difficulty. Further, the study of small and delicate tissue such as dystrophic mouse muscle or foetal muscle, where electrode damage and *in vitro* viability become major problems for other methods, would be greatly facilitated using this single-point time varying analysis.

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