PREDICTED DELAYS IN THE ACTIVATION OF THE CONTRACTILE SYSTEM

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ABSTRACT The capacitance C'_e, presumed to be located across the walls of the transverse tubules of twitch fibers, was identified in earlier impedance measurements by virtue of having a resistance in series with it. When the voltage V_m across the surface membrane is made to vary, the voltage V_C across C'_e will be delayed with respect to V_m, the extent of the delay depending on the location of the series resistance. Model 1 assumes that the resistivity of the lumen of the tubules is negligible; model 2 assumes that the series resistance arises entirely in the tubular lumen; model 3 assumes that the resistivity of the tubular lumen is small, but not negligible and that the bulk of the resistance arises in a structure directly in series with C'_e and having a similar geometric distribution. If V_m varies sinusoidally, the relative value of $V_{C(\text{max})}$ will fall with increasingly higher powers of the frequency at the center of the fiber if model 2 is applicable, whereas models 1 and 3 predict that $V_{C(\text{max})}$ will fall at high frequency only in proportion to the frequency everywhere in the cross-section of the fiber. Equations have been derived for the voltage change V_C in response to a step change of V_m and during an action potential. On the assumption that contraction is initiated when V_C reaches mechanical threshold, the delay between the activation of myofibrils on the axis of the fiber and at the surface would amount to 2.6 msec in model 2 and 0.25 msec in model 3 for frog fibers of about 100 μm diameter during a twitch.

INTRODUCTION

In a study of the AC impedance of fast muscle fibers with intracellular microelectrodes, Falk and Fatt (1964) obtained evidence for a current path between the outside of the fiber and the myoplasm which consisted of a resistance and a capacitance in series, in addition to two other parallel current paths, one being a capacitance, the other a resistance. The results on frog muscle fibers were analyzed in terms of the circuit shown in Fig. 1, as the simplest model of the inside-outside admittance which could give rise to the observed impedance behavior. The value of capacitance C_m agrees with the value for the membrane capacitance obtained by Cole and Curtis (1936) and by Fatt (1964) using transversely applied AC fields, while the sum of capacitances $(C_m + C_e)$ agrees with the values obtained from the response to an applied current step by Katz (1948) and by Fatt and Katz (1951). The measurements

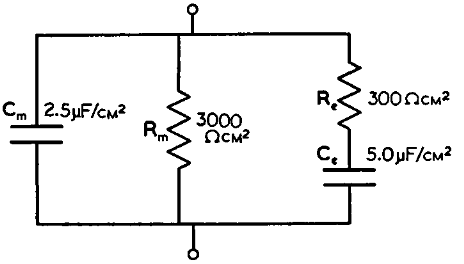

FIGURE 1 Network used by Falk and Fatt (1964) to represent the inside-outside admittance of fibers from the frog sartorius. As a first approximation, each element was considered as lumped at any point along the muscle fiber. The indicated values are close to the average values and are referred to unit area of surface membrane. R_m and C_m were considered to be properties of the surface membrane; R_e and C_e were presumed to reflect the properties of a current path from the myoplasm through the transverse tubular system to the exterior. It was suggested that R_e and C_e might be located in adjacent membranes of the triads.

with transverse electrodes preclude an origin for C_e at the surface of the muscle fiber. Falk and Fatt (1964) suggested that it arises, instead, in the walls of tubules running transversely through the muscle fiber.

Fatt (1964) interpreted measurements on frog muscles made at low frequencies with external transverse electrodes as indicating a current path through channels, presumed to be the sarcotubular system. The resistivity R_x of the tubules could not be reliably estimated in normal Ringer's solution and most measurements were made in low conductivity solutions in which most of the NaCl was replaced by sucrose. It appears that under these conditions there is swelling of the transverse tubular system (Freygang, Goldstein, Hellam, and Peachey, 1964; S. Page, personal communication). It is quite likely that the estimate of the resistivity R_x of the tubule system of 12,000 Ωcm (referred to unit volume of muscle fiber) for muscles bathed by normal Ringer's solution is an underestimate, perhaps by a factor of about 2.5. A revision in the value of R_x to 30,000 Ωcm would require that the channels occupy 0.8 % of fiber volume if they have the conductivity of myoplasm or about 0.3 % if they have the same conductivity as Ringer's solution which would be more in keeping with the value of 0.2–0.5 % based on electron micrographs of the transverse tubules (Page, 1964; Peachey, 1965) or on estimates of a special region into which ions can diffuse fairly freely (Hodgkin and Horowicz, 1960*a*). Even this value of R_x can account only for a small fraction of the total resistance R_e, which appears in series with the capacitance C_e as measured with intracellular electrodes (Falk and Fatt, 1964). The results with transverse electrodes and with internal electrodes are not necessarily in conflict for one might suppose that, in addition to the channel resistance which has a value given by measurements in transverse fields, internal electrodes measure another resistive barrier which is directly in series with capacitance C_e and which has a similar geometry to the structure giving rise to C_e.

The series combination of R_e and C_e may represent properties of a pathway by which the effect of electrical changes occurring across the surface membrane are transmitted to the contractile elements in the interior of the muscle fiber. That this pathway is via the transverse tubular elements of the sarcoplasmic reticulum is made likely by the experiments of Huxley and Taylor (1958) and of Huxley and Straub (1958) who found that local contractures could be obtained by focal depolarization only at the region where the triads of vertebrate muscle were located.

It is now well established that the effective stimulus for eliciting contraction is depolarization of the surface membrane (Kuffler, 1946; Sten-Knudsen, 1954, 1960; Hodgkin and Horowicz, 1960*b*). It is plausible to assume that it is the voltage across the walls of the transverse tubules, i.e. the voltage across the capacitance C_e which is involved in the initiation of contraction. Because there is a resistance in series with the capacitance, there will be a delay before a change of potential, occurring across the surface membrane, can appear across C_e. A suggestion along these same lines was made some years ago by Huxley (1957). At the time, there were no measurements of properties which might be attributed to elements of the internal conducting system. It is the purpose of this paper to show quantitatively what delays might be expected for models of the muscle fiber which differ as to the location of the series combination of capacitance and resistance. The analysis will be based on the assumption that the inward spread of potential change is a passive process. From their studies on local depolarization, Huxley and Taylor concluded that there was no regenerative process concerned in the inward spread of contraction since they observed that the strength of contraction and the extent of inward spread were continuously graded with the strength of stimulus. Although their evidence as to the absence of all-or-none behavior cannot be regarded as decisive, it will be shown that a regenerative process in the transverse tubular system is not required for full activation of the contractile system to follow the occurrence of an action potential at the surface membrane.

THEORY

We shall examine various models of the muscle fiber which differ in the geometric location of the resistance which is in series with the capacitance C_e. For each of these models one wishes to know how the voltage across C_e will vary when V_m, the voltage across the surface membrane, is made to vary. Fig. 2*a* shows a schematic section through the muscle fiber. Except for bulk properties, primed symbols are used for properties considered to arise in the volume of the fiber, unprimed symbols for those considered to arise at the surface. The conversion factor (e.g. C_e of Fig. 1 to C'_e of Fig. 2) is $2/a$, the surface to volume ratio of the fiber assumed to be a circular cylinder of radius a. The surface membrane enveloping the muscle fiber is considered to have the properties C_m and R_m. Penetrating the muscle fiber at various points on its surface is a system of channels, the interior of which has resistivity R_x (referred to unit volume of muscle fiber). It is assumed that the channels carry cur-

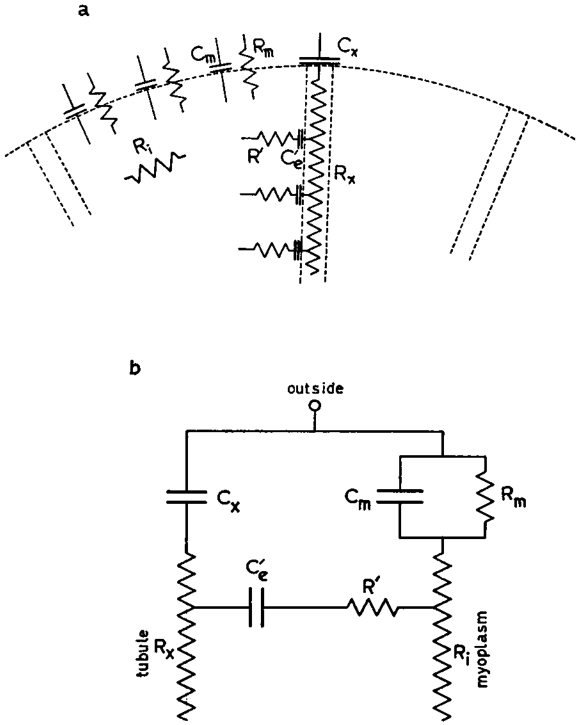

FIGURE 2 Location of the electrical properties of the muscle fiber. (*a*) Schematic section through a muscle fiber, considered to be a circular cylinder. The system of transverse tubules shown as parallel broken lines are assumed to have an internal resistivity R_x and a capacitance C'_e across their walls. The large capacitance C_x, assumed to arise where the tubules reach the surface of the fiber, will be considered infinite for the present analysis. The myoplasm of resistivity R_i is assumed to carry current only in a plane perpendicular to the paper. (*b*) Equivalent circuit showing how electrical coupling between the transverse tubule system and the myoplasm is provided by C'_e and R' in series.

rent only in the transverse plane of the fiber. Evidence for a capacitance C_x at the entrance of the channels was obtained by Fatt (1964) but as it has such a large value, it will be assumed that direct electrical contact is made with the external medium (at reference potential). The electrical connection between the channels and the myoplasm consists of a capacitance C'_e and a resistance R' in series (each referred to unit volume of muscle fiber), distributed along the channel resistance. Within the myoplasm, which has resistivity R_i, it is assumed that the system is isopotential at any given longitudinal position, i.e. potential gradients occur only in the longitudinal direction. Fig. 2*b* shows an equivalent circuit of the muscle fiber containing the elements shown in Fig. 2*a*.

In model 1, the channel resistance R_x will be assumed to be so low that, to a first approximation, voltage gradients in the transverse plane of the muscle fiber can be ignored. All of the measured series resistance R'_e and capacitance C'_e can then be treated as lumped at any point along the muscle fiber. (R'_e is taken to be the sum of R' and an equivalent resistance due to R_x, the distributed nature of which is neglected.)

In model 2, *all* of the measured resistance in series with C'_e is considered to arise from the resistance of channels distributed through the volume of the muscle fiber ($R' = 0$ in Fig. 2). This model has been treated by Falk and Fatt (1964, Appendix D) who gave the steady-state solution. The problem in the transverse plane is that of a tapering cable, the Bessel cable. The analogous problem of heat flow in a circular cylinder is treated by Carslaw and Jaeger (1947, Chap. 7; section 123).

Model 3 assumes the circuit of Fig. 2 and so contains an additional parameter since neither R_x nor R' is to be neglected.

Steady-State Response to AC

Consider the case of a sinusoidally time-varying voltage

$$V_m(t) = V_{m(\max)} \cos\omega t \tag{1}$$

impressed across the surface membrane. The same voltage would appear between the channel system at the surface of the fiber (at $r = a$, r being the radial distance from the center of the fiber) and the myoplasm. We wish to know how the complex voltage $\mathbf{V}_C$ across the capacitance C'_e at any radial distance r from the center of the fiber will vary with angular frequency ω of the applied voltage. The magnitude of $\mathbf{V}_C$ is $V_{C(\max)}$ from which the time-dependent voltage is obtained as

$$V_C(t) = V_{C(\max)} \cos(\omega t + \phi) \tag{2}$$

where ϕ is the phase difference between $\mathbf{V}_C$ and V_m.

In the case of model 1, in which R'_e and C'_e are considered as lumped elements, V_m will be across R'_e, C'_e in series (see Fig. 1) and the voltage across C'_e, $\mathbf{V}_C$ will be uniform in the cross-section of the fiber. The voltage across C'_e will be given by

$$\mathbf{V}_C = V_{m(\max)} \frac{1}{1 + \mathrm{j}\omega C'_e R'_e}. \tag{3}$$

Not only will there be a phase difference between $\mathbf{V}_C$ and V_m, but as the frequency is increased, there will be insufficient time for the charging of C'_e and the relative magnitude of $\mathbf{V}_C$ will diminish. We may write for the magnitude

$$V_{C(\max)} = V_{m(\max)}/[\omega^2(C'_e)^2 (R'_e)^2 + 1]^{1/2}. \tag{4}$$

It will be seen from equation 4 that $V_{C(\max)}$ approximates $V_{m(\max)}$ at low frequencies ($\omega \ll 1/C'_e R'_e$); that $V_{C(\max)} = \frac{1}{2} V_{m(\max)}$ when $\omega = \sqrt{3}/C'_e R'_e$; and that at high frequencies ($\omega \gg 1/C'_e R'_e$), $V_{C(\max)}/V_{m(\max)}$ will fall inversely with the frequency.

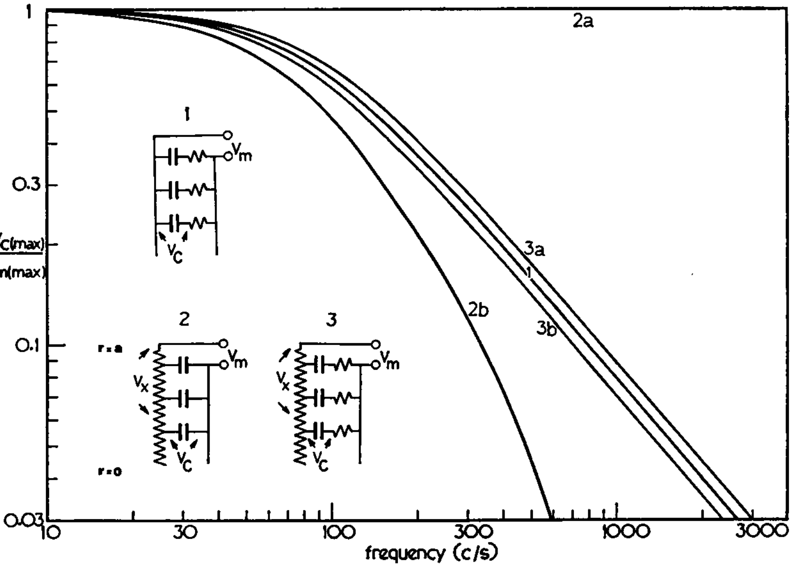

FIGURE 3 Plots of the variation of $V_{C(\max)}/V_{m(\max)}$ with frequency of AC applied across the surface membrane for different models of the muscle fiber. The numbers on the curves in this and in subsequent figures correspond to the models described in the text and the further designations *a* and *b* refer to position in the cross-section of the fiber, *a* being at $r = a$ (at the surface) and *b* at $r = 0$ (along the axis of the fiber). Plotted on double log scales. The insets are diagrammatic representations of the three models considered and show the terminals across which V_m is applied and the positions where V_x and V_C are measured.

$V_{C(\max)}/V_{m(\max)}$ is plotted in Fig. 3, curve 1 against frequency in *c/s* on double-log scales with $C'_eR'_e = 2$ msec, such as might be obtained if C'_e had the value 2400 μF/cm^3 (equivalent to 6 μF/cm^2 of fiber surface, if $2a = 100$ μm) and R'_e had the value 0.82 Ωcm^3 (equivalent to 330 Ωcm^2). The value of 2 msec for $C'_eR'_e$ is slightly larger than the average value of 1.4 msec obtained by Falk and Fatt, but within the range of measured values.

In the case of model 2, in which the series resistance measured with intracellular electrodes is considered to arise from the resistance R_x of channels conducting in the transverse plane of the muscle fiber, the solution (Falk and Fatt, 1964, see equation 45) for the voltage at any position (distance *r* from center of fiber) within the channels will be given with respect to the fiber exterior by

$$V_x = V_{m(\max)}\left(1 - \frac{I_0[(j\omega C'_e R_x)^{1/2}r]}{I_0[(j\omega C'_e R_x)^{1/2}a]}\right) \tag{5}$$

where I_0 represents the modified Bessel function of the first kind and of zero order, its argument given in brackets. (The insets to Fig. 3 show the various models and indicate by means of arrows the respective terminals across which V_m is applied and across which V_x is measured.) The voltage across the capacitance C'_e at any position

r will be

$$\mathbf{V}_C = V_{m(\max)} - \mathbf{V}_x .$$

The absolute value of voltage may be written in terms of tabulated functions as

$$V_{C(\max)} = V_{m(\max)} \left\{ \frac{\mathrm{ber}^2[(\omega C'_e R_x)^{1/2} r] + \mathrm{bei}^2[(\omega C'_e R_x)^{1/2} r]}{\mathrm{ber}^2[(\omega C'_e R_x)^{1/2} a] + \mathrm{bei}^2[(\omega C'_e R_x)^{1/2} a]} \right\}^{1/2} . \tag{6}$$

In this model the voltage across the capacitance at $r = a$ (i.e. where the channels reach the surface of the fiber) will be equal to V_m at all frequencies. At all other positions $(a > r \geqq 0), V_{C(\max)}$ will fall with increasingly high power of the frequency, as the frequency is raised. In order to compare model 2 having distributed resistance R_x, with model 1 having a lumped R'_e, the capacitance C'_e being the same in both models, it is necessary to find values such that an equivalent impedance behavior of the muscle fiber be exhibited, at least at low frequencies. The equivalence is such that $R_x a^2 \simeq 8R'_e$ (see Falk and Fatt, 1964, Appendix D). Equation 6 has been plotted in Fig. 3, curves 2a and 2b with $C'_e R_x a^2 = 16$ msec so that a comparison may be made with $C'_e R'_e = 2$ msec. For a fiber 100 μm in diameter, R_x would have a value of 267,000 Ωcm.

Equation 5 may be generalized by replacing $\mathrm{j}\omega C'_e$ by some other admittance connecting the transverse and longitudinal conducting systems. In the case of model 3 where the electrical connection is made via a resistance R' in series with the capacitance C'_e

$$\mathbf{V}_x = V_{m(\max)} \left(1 - \frac{\mathrm{I}_0\left[\dfrac{(\mathrm{j}\omega\tau_3)^{1/2} hr}{(1 + \mathrm{j}\omega\tau_3)^{1/2} a}\right]}{\mathrm{I}_0\left[\dfrac{(\mathrm{j}\omega\tau_3)^{1/2} h}{(1 + \mathrm{j}\omega\tau_3)^{1/2}}\right]} \right) \tag{7}$$

where $h = (R_x/R')^{1/2} a$ and $\tau_3 = C'_e R'$. $V_{x(\max)}/V_{m(\max)}$ will range between 0 at low frequencies $(\omega \ll 1/\tau_3)$ to a value $1 - \mathrm{I}_0(hr/a)/\mathrm{I}_0(h)$, reached at high frequencies $(\omega \gg 1/\tau_3)$. The voltage across the capacitance at any radial distance will be

$$\mathbf{V}_C = \frac{V_{m(\max)}}{1 + \mathrm{j}\omega\tau_3} \, \frac{\mathrm{I}_0\left[\dfrac{(\mathrm{j}\omega\tau_3)^{1/2} h}{(1 + \mathrm{j}\omega\tau_3)^{1/2}} \cdot \dfrac{r}{a}\right]}{\mathrm{I}_0\left[\dfrac{(\mathrm{j}\omega\tau_3)^{1/2} h}{(1 + \mathrm{j}\omega\tau_3)^{1/2}}\right]} . \tag{8}$$

For this model (see Appendix D of Falk and Fatt, 1964), an impedance behavior of the muscle fiber at low frequencies, observed with intracellular electrodes, corresponding to the behavior of model 1, obtains when

$$R'_e \simeq R' + \tfrac{1}{8} R_x a^2 = R'(1 + \tfrac{1}{8} h^2).$$

The variation of $V_{C(\max)}$ at the surface of the fiber with frequency is shown as curve 3a in Fig. 3. It is identical in shape to curve 1 (for model 1 with lumped elements) since at $r = a$, equation 8 reduces to the same equation as 3 with R'_e replaced by R'. Attenuation of $V_{C(\max)}$ with frequency (shown by curve 3*a*) occurs at frequencies which are higher than is the case for curve 1 by the factor R'_e/R', so that, as seen in Fig. 3, curve 3*a* is simply shifted with respect to curve 1 by a constant amount to the right along the log frequency axis. As in model 1, (but in contrast to model 2), $V_{C(\max)}/V_{m(\max)}$ at any radial distance r for model 3 will fall at high frequencies in proportion to the frequency. At high frequencies, attenuation of $V_{C(\max)}$ at $r < a$ will occur at a lower frequency than at $r = a$, the ratio of the frequencies for any given attenuation being $I_0(hr/a)/I_0(h)$. A plot of $V_{C(\max)}/V_{m(\max)}$ at $r = 0$ as a function of frequency is shown as curve 3*b*, for the case in which $h = 1$ and $C'_eR' = 8/9 \times 2$ msec (also used for curve 3*a*). The value of h is here chosen to correspond to a value of $R_x = 29{,}000\ \Omega$cm (referred to unit volume of muscle fiber and a fiber diameter $2a$ of 100 μ). The values of h and C'_eR' as used in constructing the curves 3*a* and *b* of Fig. 3 would be consistent with impedance measurements on frog fast muscle fibers made with transverse electrodes and with intracellular electrodes.

It will be evident from the foregoing that Fig. 3 would be a prediction of how the threshold voltage required to produce a contracture will vary with frequency when the membrane voltage is made to vary sinusoidally, provided that there were no significant delays other than that required to change V_C to a critical level and provided that a previous half cycle has no influence on the relation between depolarization and activation of the contractile system. In the interior of the muscle fiber, the threshold for contraction would rise very steeply with increasing frequency in the case of model 2, whereas in the case of models 1 and 3, the threshold would rise more gradually and would increase at most only in proportion to the frequency. In carrying out such a test it would be necessary to adopt procedures which would reduce nonlinearities of the electrical characteristics of the muscle fiber and which would ensure that the voltage across the surface membrane will follow the applied sinusoidal voltage (cf. Katz and Lou, 1947).

Response to a Voltage Rising Exponentially with Time

With a steady AC applied to a linear, passive system containing resistances and capacitances, the voltage across each of the elements of the network will remain sinusoidal and of the same frequency as the applied voltage. An analogous situation arises in the case of a voltage rising exponentially with time, $V_m = V_0 \exp(t/T)$, applied across the terminals of the network. The voltage across any of the capacitances will likewise be an exponential of the same time constant T, but will be delayed by a constant amount Δt with respect to the applied voltage. In the case of the lumped series elements C'_e and R'_e (model 1), it can be shown (P. Fatt, personal communication) that the delay in the voltage across C'_e will be

$$\Delta t = T \ln \frac{T + \tau_1}{T} \qquad (9)$$

where $\tau_1 = C'_e R'_e$ (since at any time t, V_m and the voltage across C'_e will be in the ratio $(T + \tau_1)/T$, which is equivalent to a constant delay). It will be seen from equation 9 that when $T \gg \tau_1$, $\Delta t = \tau_1$, which is the upper limit of Δt.

For the case of model 2 with the capacitance across the walls of the channels distributed along the resistance of the channels, it can be shown by use of the superposition theorem and the solution for a step of voltage at the surface of the fiber, given below in equation 13 that the delay between an exponentially rising voltage V_m at the surface of the fiber and the voltage across the capacitance C'_e at any radial position r will be

$$\Delta t = -T \ln \left[2 \sum_{n=1}^{\infty} \frac{T\beta_n}{T\beta_n^2 + \tau_2} \frac{\mathrm{J}_0(\beta_n r/a)}{\mathrm{J}_1(\beta_n)} \right], \qquad r < a; n = 1, 2 \cdots \tag{10}$$

where $\tau_2 = C'_e R_x a^2$ and β_n's are the positive roots of

$$\mathrm{J}_0(\beta_n) = 0 \tag{11}$$

and $\mathrm{J}_0(x)$ and $\mathrm{J}_1(x)$ are Bessel functions of the first kind and of order zero and one, respectively. The series in equation 10 is slowly converging for $\tau_2 \gg T$. An equation of rather similar form to 10 will apply for the case of model 3 but will contain an additional parameter h^2.

In general, for other than sinusoidal or exponentially rising waveforms, the voltage across the capacitance will appear distorted when compared with the applied voltage.

Response to a Step Function of Voltage

We consider next the time dependence of V_C when the voltage V_m is a step function, i.e.

$$V_m = 0, t < 0$$

$$V_m = V_m, \text{ constant} \neq 0, t > 0.$$

In the case of model 1 containing the lumped elements R'_e, C'_e

$$V_C = V_m [1 - \exp(-t/\tau_1)] \tag{12}$$

everywhere in the cross-section of the muscle fiber.

In the case of model 2 in which the series resistance is considered to arise from the resistance of tubules distributed through the muscle fiber, there is an analogous problem of heat flow in a circular cylinder whose surface is kept at constant temperature (Carslaw and Jaeger, 1947, sections 77 and 123). The solution for model 2 is

$$V_C = V_m \left[1 - 2 \sum_{n=1}^{\infty} \exp(-t\beta_n^2/\tau_2) \cdot \frac{\mathrm{J}_0(\beta_n r/a)}{\beta_n \mathrm{J}_1(\beta_n)} \right] \tag{13}$$

where τ_2, β_n, $J_0(x)$, $J_1(x)$ are defined in equations 10 and 11. Roots of equation 11 with corresponding values of $J_1(\beta_n)$ are given in Gray and Mathews (1922) and in Royal Society Mathematical Tables (1960). It may be noted that

$$J_0(0) = 1; \qquad 2\sum_{n=1}^{\infty} \frac{J_0(\beta_n r/a)}{\beta_n J_1(\beta_n)} = 1, \qquad r < a.$$

Equations similar in form to 13 have been considered by Valko, Zachar, and Zacharova (1967) in connection with the diffusion of Ca^{++} in transverse tubules of crayfish muscle fibers. Curve 2*b* in Fig. 4 shows how V_C along the axis of the fiber (at $r = 0$) would vary with time when a step of voltage is applied at the surface so that $V_C = V_m$ at $r = a$ as shown by curve 2*a* of Fig. 4.

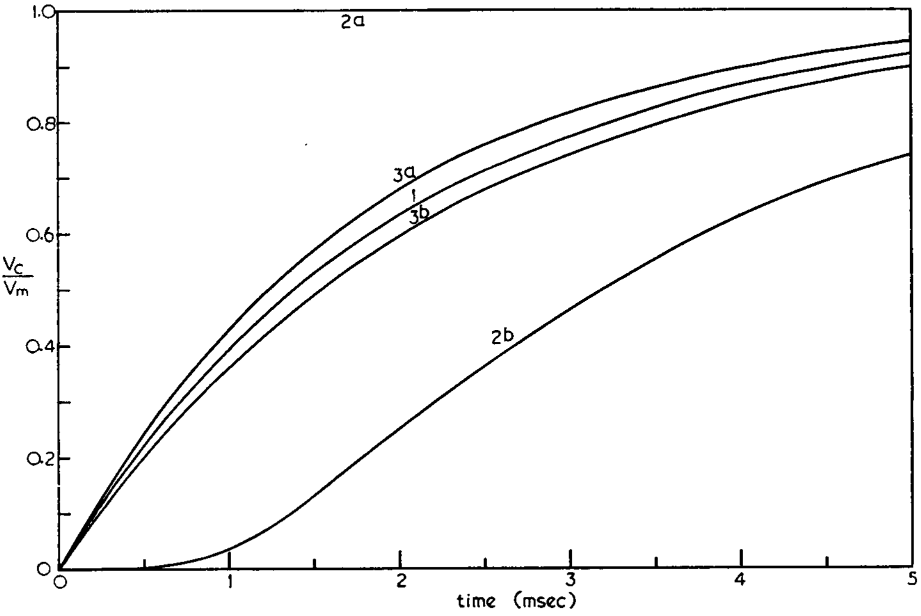

FIGURE 4 Time course of the voltage change V_C across the capacitance C'_e in response to a step change of membrane potential V_m. All the models contain the same amount of capacitance and an equivalent amount of resistance in series. Curve 1 was calculated from equation 12 with $\tau_1 = 2$ msec; curve 2*b* from equation 13 with $r = 0$, $\tau_2 = 16$ msec; curve 3*a* from equation 18 with $\tau_3 = \frac{8}{9} \times 2$ msec; curve 3*b* from equation 17 with same τ_3 as curve 3*a* and with $r = 0$, $h = 1$.

To obtain the solution for model 3 in which the connection between the channels and the myoplasm is via C'_e, R' in series, we use the method of the Laplace transform. The Laplace transform $\bar{V}_x(p)$ of the voltage at any radial distance r along the channels for a step change of V_m is derived from equation 7 as

$$\bar{V}_x(p) = \frac{V_m}{p}\left(1 - \frac{I_0[(p\tau_3)^{1/2}(1 + p\tau_3)^{-1/2}hr/a]}{I_0[(p\tau_3)^{1/2}(1 + p\tau_3)^{-1/2}h]}\right) \tag{14}$$

where $\tau_3 = C'_e R'$. Equation 14 does not contain tabulated transforms but it can be solved for V_x by use of the inversion theorem and contour integration. Evaluation of the residues at the poles of the integrand and application of Cauchy's theorem yields

$$V_x = 2V_m \sum_{n=1}^{\infty} \exp\left[-\frac{\beta_n^2 t}{(h^2 + \beta_n^2)\tau_3}\right] \cdot \frac{h^2 \mathrm{J}_0(\beta_n r/a)}{\beta_n(h^2 + \beta_n^2)\mathrm{J}_1(\beta_n)} \tag{15}$$

where β_n has the same meaning as in equation 11. The voltage across the capacitance will be

$$V_C = \exp(-t/\tau_3) \cdot \frac{1}{\tau_3} \int_0^t (V_m - V_x)\, dt \tag{16}$$

from which we obtain

$$V_C = V_m \left\{1 - 2 \sum_{n=1}^{\infty} \exp\left[\frac{-\beta_n^2 t}{(h^2 + \beta_n^2)\tau_3}\right] \cdot \frac{\mathrm{J}_0(\beta_n r/a)}{\beta_n \mathrm{J}_1(\beta_n)}\right\}, \qquad r < a \tag{17}$$

and

$$V_C = V_m[1 - \exp(-t/\tau_3)], \qquad r = a. \tag{18}$$

Equations 17 and 18 have been plotted in Fig. 4 as curves 3*b* (for $r = 0$) and 3*a* with $h = 1$ and $\tau_3 = 8/9 \times 2$ msec which are the same constants as used in the response to AC shown in Fig. 3, curves 3*a* and *b*.

All three models contain an equivalent amount of resistance and capacitance in series, and can now be compared on a time basis. In the case in which the channel resistance has been neglected (model 1), the voltage across C'_e will rise as a negative exponential function with time constant $C'_e R'_e$ (equation 12 plotted as curve 1 in Fig. 4). With the time constant of 2 msec, the time required for V_C to rise to 40% of its final value is about 1 msec, the time course being the same near the surface and in the muscle interior. On the other hand, in model 2 with all the measured series resistance representing resistance within the lumen of the tubules, a step change of transmembrane potential will appear as a step change of the voltage across C'_e at the surface, while at the center of the fiber the potential will rise with a delay, as seen by the foot on the curve 2*b* in Fig. 4. At the center of the fiber there will be a delay of 2.7 msec before the voltage across C'_e rises to 40% of its final value. If the channels have the low resistance suggested by measurements with transverse electrodes and model 3 is assumed, it can be seen in Fig. 4 that at the surface of the fiber, V_C (curve 3*b*) will be delayed with respect to V_m, to the extent of 0.9 msec when $V_C/V_m =$ 40%, while at the center of the fiber there will be an additional delay of 0.25 msec before V_C reaches the same level. In the distributed models the delay between V_C at the surface and in the interior increases with increasing time.

V_C *During an Action Potential*

We now turn to consider the time course of the voltage across C'_e during the passage of an action potential and the time course of the inward spread of the potential change. We assume that the action potential is across the surface membrane and, as before, that the channel system and associated properties are linear and passive. Since the time course of the voltage change across C'_e for a step change of V_m at the surface is known for different models of the muscle fiber, the response to $V_m(t)$, any arbitrary function of time, can be obtained by the superposition theorem which gives for a system which is quiescent at $t = 0$ (see e.g. Carslaw and Jaeger, 1947, section 10)

$$V_C = \int_0^t V_m(\xi)\,\frac{\partial}{\partial t}\,\mathrm{F}(t - \xi)\,d\xi \qquad (19)$$

where $\mathrm{F}(t)$ is the voltage across C'_e in response to a unit step of voltage at the surface and ξ is the variable of integration replacing t in $V_m(t)$.

In the case of model 1 with lumped elements, application of equation 19 to equation 12 gives

$$V_C = \frac{1}{\tau_1}\int_0^t \exp\,[-(t - \xi)/\tau_1]\cdot V_m(\xi)\,d\xi. \qquad (20)$$

Evaluation of equation 20 is conveniently accomplished by the subtangent method of graphical analysis (Rushton, 1937). The constant τ_1 in the form in which it appears in equation 20 will be referred to as the delay-distortion parameter. Fig. 5 shows a

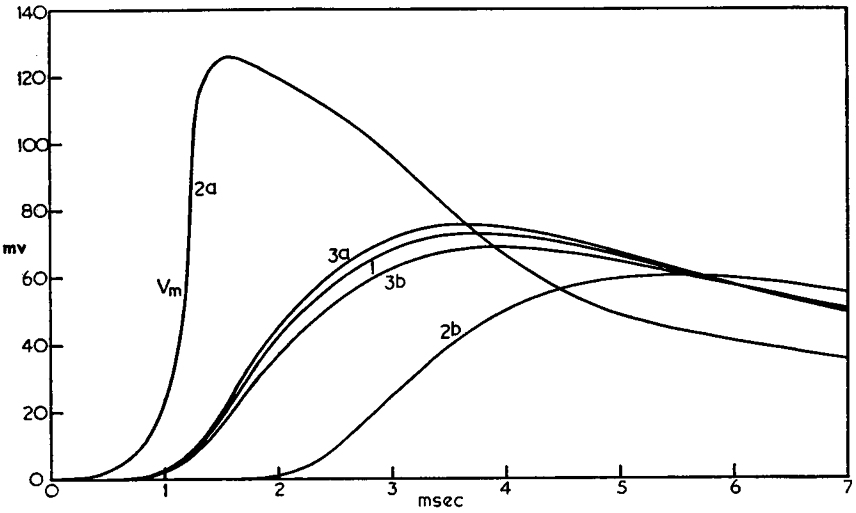

FIGURE 5 Time course of the voltage change V_C across the capacitance C'_e during an action potential labeled V_m. The computed curves are based on the same parameters as used in Figs. 3 and 4. Origin of the time scale is arbitrary. The ordinate represents the displacement of voltage from the resting level.

typical action potential (labeled V_m) recorded from a frog sartorius fiber at 17°C. Curve 1 represents V_C for model 1 with $\tau_1 = 2$ msec.

In model 2, V_C at the surface of the fiber would appear with the same time course as the action potential itself, but there will be a delay in the inward spread. The time course of V_C at any radial distance r from the center of the fiber, from equations 13 and 19, will be given by

$$V_C = 2\sum_{n=1}^{\infty} \frac{J_0(\beta_n r/a)}{\beta_n J_1(\beta_n)} \frac{\beta_n^2}{\tau_2} \int_0^t \exp\,[-(t-\xi)\beta_n^2/\tau_2]\cdot V_m(\xi)\,d\xi, \qquad r < a. \tag{21}$$

It will be noticed that each value of n generates a function which is of similar form to the right-hand side of equation 20, so that for each n the function can be evaluated by subtangent analysis with τ_2/β_n^2 as the delay-distortion parameter and $2J_0(\beta_n r/a)/\beta_n J_1(\beta_n)$ as a weighting factor in the summation over n. Since β_n^2 increases rapidly with increasing n ($\beta_n^2 > 750$ for $n > 8$), if $\tau_2 = 16$ msec, one finds to a close approximation that the summation for $n > 8$ can be written as

$$2\sum_{n=9}^{\infty} \frac{J_0(\beta_n r/a)}{\beta_n J_1(\beta_n)} V_m(t)$$

thus eliminating the need to integrate graphically for large n. Graphical integration for values of $n \leqq 8$ and approximation of the summation for $n > 8$ were used to obtain the time course of V_C at the center of fiber, shown as curve 2*b* in Fig. 5.

To obtain the solution for model 3, use is made of the superposition theorem and equations 17 and 18, resulting in

$$V_C = 2\sum_{n=1}^{\infty} \frac{J_0(\beta_n r/a)}{\beta_n J_1(\beta_n)} \frac{\beta_n^2}{(h^2+\beta_n^2)} \frac{1}{\tau_3} \int_0^t \exp\left[\frac{-(t-\xi)\beta_n^2}{(h^2+\beta_n^2)\tau_3}\right]\cdot V_m(\xi)\,d\xi, \tag{22}$$

$r < a$ when and

$$V_C = \frac{1}{\tau_3}\int_0^t \exp\,[-(t-\xi)/\tau_3]\cdot V_m(\xi)\,d\xi, \qquad r = a. \tag{23}$$

Equation 22 may also be evaluated graphically by subtangent analysis where for each n, the delay-distortion parameter is given by $[(h^2 + \beta_n^2)/\beta_n^2]\tau_3$ and the weighting factor is $2\,J_0(\beta_n r/a)/\beta_n J_1(\beta_n)$. The time course of V_C at $r = a$ and at $r = 0$ during an action potential is shown in Fig. 5, curves 3*a* and 3*b* for this model with $\tau_3 = \frac{8}{9} \times 2$ msec and $h^2 = 1$.

Since it is probable that the channel system is involved in the initiation of contraction, the time required for V_C to be displaced by 45 mv (depolarization) from its resting level is of particular interest, for as shown by Hodgkin and Horowicz (1960*b*), when this threshold depolarization of the surface membrane is exceeded, tension increases steeply as a function of depolarization, reaching 90% of its maximal value with a further displacement of about 15 mv. We see from Fig. 5 that at the threshold for contraction, V_C for model 1 is delayed with respect to the action potential by about 0.9 msec and the delay increases to about 1.3 msec before V_C reaches 60 mv. The interval over which V_C exceeds threshold for contraction is appreciably greater than that of the action potential so that it would be possible for the active state of the contractile material to last longer than the suprathreshold duration of the action potential (see Falk, 1961; Sandow, Taylor, and Preiser, 1965).

In the case of model 2, Fig. 5 (curve *2b*) shows that V_C at the center of the fiber will be considerably delayed with respect to V_C at the surface, the delay amounting to 2.6 msec for V_C to change by 45 mv (the threshold depolarization for contraction) and increasing to 4 msec before reaching 60 mv (the depolarization at which near-maximal contraction could be initiated). The peak value of V_C at the center of the fiber is 61 mv, lower for this model than for the others considered.

In model 3 in which the resistivity of the channels is considered low, but not negligible, the contraction threshold would be reached near the surface of the fiber (curve *3a* of Fig. 5) in 0.85 msec from the time the action potential changed by 45 mv, while a further 0.25 msec would be required before a potential change of 45 mv spread inward to the center of the fiber (curve *3b*). The time required for V_C near the surface to reach 60 mv is 1.1 msec while at the center it amounts to about 1.5 msec (measured from the time at which the action potential has risen to 60 mv). In this model also, the duration of the potential change which would be suprathreshold for contraction exceeds that of the action potential.

Effect of a Resistive Leak across the Tubule Walls

The models considered thus far have ignored any leakage resistance, denoted R'_{ce}, across the walls of the tubules (which would be in parallel with C'_e, see Fig. 2), the existence of which would cause a voltage gradient in the transverse plane of the fiber with a DC voltage V_m applied across the surface memvrane. The magnitude of the effect can be obtained by replacing $j\omega C'_e$ in equation 5 by the DC conductance, $1/(R'_{ce} + R')$ connecting the channels with the myoplasm. The ratio of the voltage V across the walls of the tubules at any r to V_m will be

$$V/V_m = \frac{I_0[R_x^{1/2}(R'_{ce} + R')^{-1/2}r]}{I_0[R_x^{1/2}(R'_{ce} + R')^{-1/2}a]}. \tag{24}$$

From the data of Adrian and Freygang (1962) and from consideration of an equivalent circuit of the muscle fiber, Falk and Fatt (1964) estimated the value of R'_{ce}, if referred to unit area of muscle fiber surface, to be about 8000 Ωcm^2 for a fiber in Ringer's solution at its normal resting potential. For a fiber of 100 μm diameter, this value of R'_{ce} would be equivalent to 20 Ωcm^3 if referred to unit volume of fiber.

In model 2, if R_x has the value 267,000 Ωcm, then along the axis of the fiber V/V_m would be $1/I_0$ (0.57) $= 0.923$. If R_x has the value of 29,000 Ωcm and $R' = 0.73$

Ωcm^3 as used in computing curves for model 3, then V/V_m on the axis of the fiber would be $1/I_0\,(0.19) = 0.993$. The value of R'_{ce} is sufficiently large that its effect can be neglected for model 3, but its effect, although small, may not be entirely negligible for model 2 with large R_x.

It is possible that R'_{ce} is a nonlinear element responsible for "anomalous rectification" described originally by Katz (1949). The nonlinearity is such that with an outward applied current (which is the required direction for producing contraction), R'_{ce} increases. An increase of R'_{ce} would, of course, diminish any decrement in the transverse plane of the fiber.

DISCUSSION

The studies of Hill (1949) indicate that the active state of muscle is fully turned on during a twitch. The calculations in this paper, based on measured impedance parameters of muscle, show that the passive spread of potential V_C across the capacitance of the walls of the sarcotubular system would be sufficient to result in near-maximal activation of the contractile system. However, there would have to be only a short delay in the further stage of coupling to the contractile system, or else, if there were a significant delay in activating the contractile system, there would have to be an equally long delay in turning it off when V_C falls. Early mechanical changes such as latency relaxation (Rauh, 1922; Sandow, 1944) and an increased resistance to stretch (Hill, 1951) suggest that the delays in the process leading to contraction are brief. It seems quite possible that a substantial fraction of the time of 1.5 msec from the stimulus to the onset of latency relaxation at 20°C (Sandow, 1952) reflects the delay in changing V_C to its threshold value.

At present there is little information on which to base a close comparison of the predicted delays for various models of the muscle with experimental observations. Using high-speed cinematography, Gonzalez-Serratos (1966, 1967) has looked for the delay between the activation of myofibrils near the surface and those at the center of isolated frog muscle fibers during a twitch initiated by a propagated action potential. He made use of the fact that when a muscle fiber is compressed along its longitudinal axis, the myofibrils become wavy (wavelength of about 50 μm), the waves disappearing during active shortening of the muscle fiber. Measurements were made on large fibers, about 180 μm in diameter. Gonzalez-Serratos estimated a delay of about 1 msec between the activation of myofibrils at the surface and those on the axis of the fiber at about 20°C. Owing to technical difficulties, he was unable to time the mechanical changes in relation to the time of arrival of the action potential.

A delay of only 1 msec between surface and axis of large diameter fibers is significantly less than the theoretical minimum delay for the voltage across C'_e to change to its threshold value in model 2 in which all of the resistance in series with C'_e was considered to have its origin in the lumen of the transverse tubules. A delay of 2.6

msec before the voltage across C'_e at the center of the fiber would change by 45 mv (taken to correspond to the threshold for contraction) during an action potential was calculated on the basis of parameters for fibers of 100 μm diameter. The parameter which determines the delay for model 2 is $\tau_2 = C'_e R_x a^2$ and if one makes the reasonable asumption that C'_e (referred to unit volume) and the relative volume occupied by the transverse tubules are independent of fiber diameter, then τ_2 will vary as the square of the fiber diameter. If Gonzalez-Serratos' measurements correctly reflect the delay, they can be taken as evidence against model 2.

It should also be mentioned that there was no evident large dependence of $C'_e R'_e$ on fiber diameter in the original analysis of Falk and Fatt (in which it was assumed that C'_e and R'_e arose in structures of similar geometry) as would have been the case if the resistance arose in fact, in the lumen of the tubules. In the case of model 3 on the other hand, in which it is assumed that part of the resistance arises in the tubular lumen, whose resistivity R_x corresponds with measurements with transverse electrodes, and a part in a structure with a geometry similar to C'_e, the product $C'_e R'_e$ would be expected to vary by less than 50% for fibers of diameter between 50 and 200 μm.

A delay of 0.25 msec in the change by 45 mv of the voltage across the capacitance along the axis of the fiber as compared with the surface during an action potential was calculated for model 3, based on a fiber diameter of 100 μm. The effect of an increase in fiber diameter on the delays during an action potential has not been calculated. An estimate can be obtained from the response to a step change of V_m, since it will be noticed from a comparison of Figs. 4 and 5 that the delay between axis and surface for V_C rising to 40% of its final value, in the case of V_m (a step function) is about the same as for a change of V_C by 45 mv in the case of an action potential. The delay between axis and surface for $V_C/V_m = 0.4$ with the parameter $h^2 = \frac{1}{8} R_x a^2/R'$ having the value 3, so as to correspond to a 173 μm fiber, amounts to 0.77 msec. This value is only slightly less than Gonzalez-Serratos' estimate for fibers of comparable diameter during a twitch.

Gonzalez-Serratos also studied the change in amplitude of the myofibrillar waves during a twitch at different temperatures and observed that the delay between central and surface myofibrils increased by a factor of about 2 for a decrease of temperature of 10°C. Assuming that the threshold voltage required for contraction does not change with temperature, one would expect this result with either model 2 or model 3. In model 2, the delay in the central part of the fiber would increase at low temperatures because R_x would be expected to increase to the extent of 1.3-fold per 10°C fall in temperature (if the tubules have the same viscosity as water, it being assumed that the conducting material is ionic), and because the action potential would have a slower time course, its maximum rate of rise decreasing by a factor of 2 for every 10°C decrease in temperature. Likewise in model 3, the effect of a slowing of the action potential would be to increase the delay between V_C at the axis and the surface of the fiber on an absolute time scale. There would be an additional factor increasing the delay which would depend on the physical structure which accounts for R', the resistance directly in series with C'_e. The parameters which determine the delay in model 3 are $\tau_3 = C'_eR'$ and R_xa^2/R'. If R' is independent of temperature, then the ratio R_x/R' will increase by a factor of 1.3 with lowering of temperature by 10°C, so that the delay between center and surface will be augmented. If R' has its origin in a membrane somewhat leaky to ions, then R' would be expected to increase by the factor 1.3 for each 10°C fall in temperature in the same way as the resistance of the surface membrane (del Castillo and Machne, 1953), R_x/R' remaining constant with temperature. Falk and Fatt (1964) suggested that R' might represent a property of the membrane of the lateral elements of the triads, lying in close apposition to the walls of the transverse tubules.

From the above considerations, it will be seen that the effect of temperature on the delay between axis and surface of a fiber does not enable one to discriminate between various alternatives. A doubling of the delay between the initiation of contraction at the center and at the surface for a 10°C decrease in temperature would, in any case, not be inconsistent with a passive inward spread of potential.

Since the action potential is prolonged by a decrease in temperature, the maximum rate of fall of the action potential having a Q_{10} of about 2.5 (Falk, 1961), the time during which V_C would be above threshold would increase substantially at low temperature. The prolongation of the active state at low temperature (Ritchie, 1954) might be accounted for in this way. Under other conditions, as well, an increased duration of the active state can be correlated with an increased duration of the action potential or with lowering of mechanical threshold (Hodgkin and Horowicz, 1960*c*; Falk, 1961; Sandow et al., 1965).

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