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THE KINETICS OF MECHANICAL ACTIVATION IN FROG MUSCLE

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SUMMARY

1. The kinetics of mechanical activation were examined in muscle fibres of the frog's sartorius muscle, using a voltage clamp to control membrane potential, tetrodotoxin to eliminate electrical activity and microscopic observations to determine the mechanical threshold.

2. The strength–duration curve was determined over a range of membrane potentials varying between -52 mV (rheobase) and $+90$ mV. At 4° C the critical duration was about 11 msec at -30 mV, 4 msec at 0 mV and 2 msec at $+40$ mV.

3. For pulses where $V > -10$ mV the threshold criterion at 4° C was that the 'area above -30 mV' must exceed about 120 mV msec.

4. The effect of a brief subthreshold pulse declines with a time constant of about 3 msec at -100 mV and about 8 msec at -85 mV at 4° C.

5. Although the strength–duration curve is well fitted by assuming a first-order mechanism in which the rate of release of activator increases with membrane potential, other experiments show that the over-all mechanism is probably second order in time.

6. A short pulse must be at least 50 % threshold if it is to give a visible contraction when added to a long pulse which is just below rheobase.

7. Delayed rectification was conspicuous with medium or long pulses which were just below the mechanical threshold, but short pulses could give contraction without turning on any appreciable potassium conductance.

8. The Appendix extends Falk's (1968) treatment of the charging of the tubular system under a voltage clamp.

INTRODUCTION

Although there is an extensive literature on the electrical excitability of muscle, relatively little is known about the criteria which an electrical stimulus must satisfy if it is to give a contraction in the absence of a pro-

pagated action potential. Nor is there any information about the way in which two subthreshold pulses affect the mechanism which activates muscular contraction. The aim of this paper is to fill in some of these gaps using a voltage clamp to control membrane potential, tetrodotoxin to eliminate electrical activity, and microscopic observation to determine whether or not the muscle fibre contracts. Although the method is indirect the results were surprisingly repeatable and gave some information about the nature of the activating mechanism. An obvious weakness is the reliance on a visual end-point, but the results obtained by this simple method are in fair agreement with those of Adrian, Costantin & Peachey (1969) who used polarized light, much higher magnification and cinephotography to determine which parts of the muscle contract.

Several observers have noticed that the critical membrane potential at which contraction first occurs is close to that at which delayed rectification begins. It has also been found that agents like calcium or nitrate which alter the mechanical threshold have a very similar effect on the 'threshold' of delayed rectification (Costantin, 1968; Kao & Stanfield, 1968). In order to decide whether there is any direct relation between the two phenomena, the kinetics of mechanical activation were compared with those of the system controlling delayed rectification. The results showed that there was a clear cut difference between the strength–duration curves of the two variables, and that brief pulses, which were just threshold for contraction, gave less delayed rectification than longer, threshold pulses.

Experiments on electrical activation raise the question of the rate at which electrical effects might spread in the tubular system. The Appendix deals with this problem, which has been considered previously in a slightly different context by Falk & Fatt (1964) and Falk (1968).

METHODS

Two, or occasionally three micro-electrodes were inserted into a muscle fibre at the pelvic end of the frog's sartorius muscle. Contractions were observed with a binocular microscope at a magnification of 100.

In most cases two micro-electrodes were used, one for applying current and the other for recording voltage. They were separated by about 50 μ with the voltage electrode nearer the end of the fibre and at a distance of 100–200 μ from it. The voltage electrode was filled with 3 M-KCl and the current electrode with 2 M-K citrate. The feed-back system was essentially similar to that described by Costantin (1968) or Adrian, Chandler & Hodgkin (1966). The feed-back amplifier had an open-loop gain of 4000–10,000 and limited at ± 30 V output. In experiments in which delayed currents were measured, three electrodes were employed and membrane currents were obtained from the voltage difference between the middle and end electrode (Adrian *et al.* 1966). In the present experiments the control voltage was taken from the middle electrode rather than from the end electrode, since it is important in studying contraction that the point controlled should be as near the current electrode as possible. A diagram showing the electrode arrangement is given in Fig. 8.

Tetrodotxin at a concentration of 10^{-6} g/ml. was present in all solutions. The chloride Ringer fluid was solution A in Table 1 of Hodgkin & Horowicz (1959); the sulphate solution was based on solution H of the same Table, but with 2·5 mM-K and 187·5 mM-Na instead of 190 mM-K. All the experiments were carried out at 2–6° C.

RESULTS

A. *The strength–duration curve*

Figure 1 shows the wave form of three pulses whose amplitude was sufficient to give a just perceptible contraction. The short, strong pulse in the top record was not rectangular because the feed-back amplifier

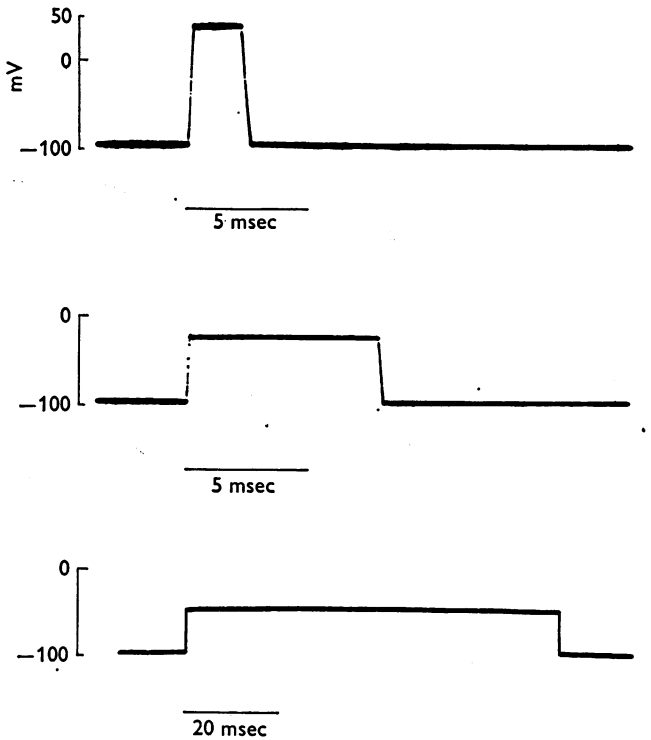

Fig. 1. Records of three pulses which gave a just perceptible contraction. The abscissa is time and the ordinate is the internal potential in mV. Fibre 1·3 (Table 1). Temperature 5·5° C.

limited at ± 30 V and could not deliver more than 6 μA through an electrode of resistance 5 MΩ. Since few electrodes could pass a current as high as 6 μA, it was not practicable to increase the swing of the amplifier. The duration of all pulses was measured at half amplitude.

Figure 2 illustrates a typical strength–duration curve at a temperature

of 3° C. The vertical lines through some of the points for brief pulses were drawn between the voltages which did, or did not, give a visible contraction. With longer pulses the threshold was measured to within 2 mV or less. In determining the curve it was important to avoid applying pulses which exceeded the threshold by more than a few millivolts. The observer knew when the voltage pulse was applied but was not told its magnitude. As can be seen, the points fell on a smooth curve, and four measurements at

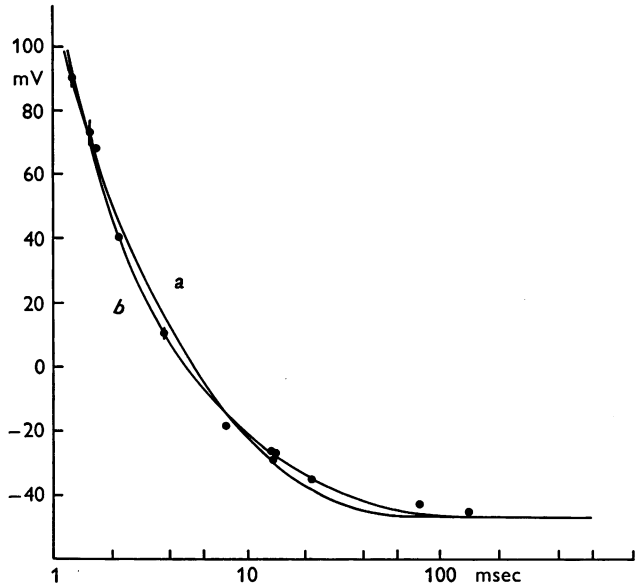

Fig. 2. Strength–duration curve of contractile mechanism at 2·6° C. Fibre 2·1. The curves were drawn from eqn. (5) with the α's and β's given by eqn. (8) curve *a*, or eqn. (9) curve *b*. For curve *a*, RT/F was taken as 23·8 mV. The rheobase was taken as −47·6 mV in both curves, hence $y_c = 0{\cdot}135\bar{y}$ in *a* and $y_c = 0{\cdot}210\bar{y}$ in *b*.

a duration of 13–14 msec, which were made at the beginning, end and near the middle of the run, gave very similar thresholds. Table 1 summarizes the results obtained in several experiments of this type.

B. *Quantitative description of strength duration curve*

In discussing the kinetics of activation one cannot get far without some kind of quantitative hypothesis. The model described here works well for the strength–duration curve, but, as will appear presently, it is undoubtedly an over-simplification.

Suppose that depolarization releases an activator which might be calcium, or something which determines calcium concentration, from a

store in which there is a large reserve. Then assuming first-order kinetics the concentration of activator might be given by

$$\frac{dy}{dt} = \alpha\bar{y} - \beta y, \tag{1}$$

where y is the concentration of activator, $\bar{y}$ is the fixed concentration in the store, t is time, α is the rate constant for release of activator and β is the rate constant for return of activator to the reservoir. If α and β change instantaneously with membrane potential and are not functions of time, the solution of eqn. (1) is

$$y = y_0 e^{-\beta t} + \frac{\alpha}{\beta}\bar{y}(1 - e^{-\beta t}), \tag{2}$$

TABLE 1. The strength–duration relation of the contractile mechanism

Fibre reference	Diameter (μ)	Temperature (° C)	Resting potential (mV)	Rheobase (mV)	$t(-30)$	$t(0)$	$t(+40)$	$t(+90)$
					(msec)			
1·1	75	4·8	−84	−52	9·2	3·7	1·8	—
1·2	70	5·1	−98	−53	8·8	3·5	1·6	—
1·3	80	5·5	−97	−51	10·5	4·0	2·1	—
2·1	75	2·6	−88	−46	15	4·8	2·3	1·3
2·3	45	4·5	−92	−54	—	2·1	—	—
3·1	110	3·2	−94	−53	—	5·6	—	—
3·2	100	4·8	−97	−54	—	3·4	—	—

The last four columns give the critical durations at voltages of −30, 0, +40 and +90 mV; these values were determined from curves drawn through the experimental points. Fibres from the same muscle have the same first digit in the reference number. Fibre diameters may be in error.

where y_0 is the concentration at the holding potential. On the assumption that contraction occurs when y reaches a critical concentration y_c, the strength–duration curve should be given by

$$t' = \frac{1}{\beta} \ln \frac{\alpha/\beta - y_0/\bar{y}}{\alpha/\beta - y_c/\bar{y}}, \tag{3}$$

or

$$t' = \frac{1}{\beta} \ln \frac{\alpha/\beta - \alpha_0/\beta_0}{\alpha/\beta - \alpha_R/\beta_R}, \tag{4}$$

where α_0, β_0 are the values of α, β at the holding potential and α_R, β_R are the values at the rheobase; t' is the duration of the rectangular pulse.

If the resting concentration of activator, y_0, is negligible eqn. (3) reduces to

$$t' = -\frac{1}{\beta} \ln \left(1 - \frac{\beta y_c}{\alpha\bar{y}}\right). \tag{5}$$

In order to obtain a strength–duration curve of qualitatively the right

form it is necessary that α/β should increase with V. At the left-hand end of the curve, where $\alpha/\beta \gg y_c/\bar{y}$, eqn. (5) simplifies to

$$t' \doteqdot y_c/\alpha\bar{y}. \tag{6}$$

For $V > -10$ mV the points in Fig. 2 are roughly fitted by assuming that α increases linearly with V according to the eqn.

$$\frac{\alpha\bar{y}}{y_c} = A(V+30\text{ mV}) \tag{7}$$

where $A = 6{\cdot}7\text{ mV}^{-1}\text{ sec}^{-1}$.

TABLE 2. Values of the constant A

Fibre reference	Diameter (μ)	Temperature (° C)	Internal potential, V (mV)	A ($\text{mV}^{-1}\text{ sec}^{-1}$)
1·1	75	4·8	+50	7·7
			− 1·5	9·6
1·2	70	5·1	+20	9·5
			− 2·5	9·8
1·3	80	5·5	+38	7·1
			+ 8	7·5
			+ 1	8·8
2·1	75	2·6	+90	7·1
			+73	7·1
			+68	6·8
			+41	6·7
			+10	6·8
			−18·5	(11·3)
2·3	45	4·5	− 6	17
3·1	110	3·2	+19	5·7
3·2	100	4·8	+ 7	9·4
Mean		4·1		8·5

The constant A is defined as the reciprocal of the area above -30 mV, i.e.

$$\frac{1}{A} = \int_{t_1}^{t_2} (V+30)\,dt,$$

where V is the internal potential and t_1 and t_2 are the times at which $V = -30$ mV on the upstroke and downstroke of the pulse.

This is illustrated by Table 2 in which values of A are given at different membrane potentials. Since the pulses were not perfectly rectangular, A was obtained as the reciprocal of the area in mV sec of that part of the voltage time record which exceeded -30 mV. At 4° C the mean value of A was $8{\cdot}5\text{ mV}^{-1}\text{ sec}^{-1}$ and its reciprocal was 118 mV msec. This result will be used later (p. 222) to estimate the effectiveness of the action potential as a stimulus to the contractile system.

Except in the region where eqn. (6) applies it is not possible to deduce much about the dependence of α and β on V from the strength–duration relation. Curve a in Fig. 2 was drawn by assuming that α and β vary with

membrane potential in the same way as the flow of a monovalent ion on the constant field assumption, that is

$$\alpha = \beta e^{\phi} = \frac{\phi \times 0{\cdot}028 \text{ msec}^{-1}}{1 - e^{-\phi}} \tag{8}$$

where $\phi = VF/RT$. The critical concentration y_c was chosen to give approximate agreement between the theoretical and experimental values of the rheobasic potential ϕ_R, using the formula $y_c/\bar{y} = e^{\phi_R}$. Although it is remarkable that such a simple formula should fit so well, the agreement provides no evidence for the validity of the underlying hypothesis. Curve b which is an equally good fit was calculated from

$$\alpha = \beta e^{\psi} = \frac{\psi \times 0{\cdot}0133 \text{ msec}^{-1}}{1 - e^{-\psi}} \tag{9}$$

where $\psi = \frac{1}{10}(V + 32)$. In spite of the apparent similarity of the two strength–duration curves, the physical implications of the two equations are entirely different. According to eqn. (8) the equilibrium concentration of y changes e-fold in 24 mV, whereas eqn. (9) implies an e-fold change in 10 mV. It was also found that a reasonable fit could be obtained by regarding β as constant and making α the only voltage-dependent quantity. The conclusion from these attempts at a quantitative description is that the strength–duration curve is not a useful starting point for a formal theory of activation, although it may be helpful in deciding between theories developed on other grounds. The most interesting point which emerged from the analysis is that the threshold criterion for brief pulses at 4° C is that the area above −30 mV should exceed 120 mV msec.

C. *Temporal summation of two subthreshold pulses*

The object of these experiments was to estimate the rate constant β which determines how long the state of increased mechanical excitability persists after a brief subthreshold stimulus. Figure 3 illustrates the pulse structure. The first pulse (S_1) had a duration of 2·52 msec and the second pulse (S_2) lasted 1·70 msec, the amplitude of both being 99 mV. When S_1 was applied alone, the critical duration which gave a just perceptible contraction was estimated as 2·70 msec at the beginning of the run and 2·68 msec at the end. It was found that S_1 and S_2 gave a slight contraction when separated by 3·04 msec but not when separated by 3·40 msec. During each pulse the on rate $\alpha\bar{y}$ should greatly exceed the off rate βy, so the activation should increase linearly; between the two pulses $\beta y \gg \alpha\bar{y}$, so the activator

should decay exponentially. Hence the value of β at the holding potential can be calculated from

$$\alpha t_1 e^{-\beta' t_D} + \alpha t_2 = \alpha t_c, \tag{10}$$

or

$$\beta' = \frac{1}{t_D} \ln \frac{t_1}{t_c - t_2}, \tag{11}$$

where t_1 is the duration of S_1; t_2 is the duration of S_2; t_c is the critical duration at which S_1 alone (or S_2 alone) just gives a contraction; t_D is the critical interval between S_1 and S_2 at which contraction occurs. β' is

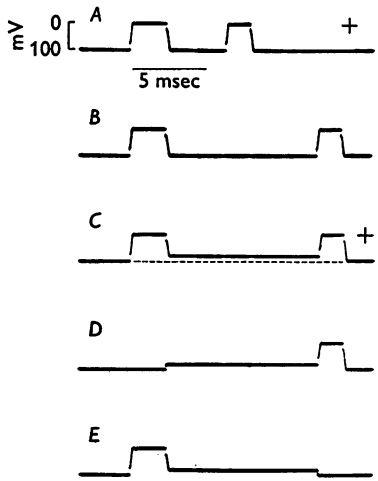

Fig. 3. Temporal summation of two subthreshold pulses. + indicates a contraction. Fibre 2·2 (Table 3); temperature 3·9° C.

the value of β at the holding potential and α (which cancels) is the on rate constant during the pulse. In the example given, t_c = 2·69 msec, t_1 = 2·52 msec, t_2 = 1·70 msec and t_D = 3·04 msec; hence β = 0·31 msec^{-1}. With short, brief pulses the visual end-point was poor and estimates of β sometimes differed by a factor of two in one experiment. It was therefore impracticable to test the assumption that mechanical excitability decays exponentially, or to see whether the rate constant of the decay is independent of the strength of the first stimulus (cf. Katz, 1937). However, we did obtain evidence about the effect of membrane potential on the rate at which the activator decayed. The method was to vary the potential during the interval between the two pulses with a third pulse of amplitude S_D. In Fig. 3*B*, S_1 and S_2 were separated by 8·2 msec and S_D = 0; this combination gave no contraction. However, if S_D was increased to 14 mV there was a clear contraction (record *C*). Since neither $S_D + S_2$ (record *D*) nor $S_1 + S_D$ (record *E*) gave a contraction, the positive result in

C is an indication that the mechanical excitability decayed more slowly when the fibre was depolarized. Estimates of β obtained by this method are given in Table 3. The data for fibre 2·2, which are considered to be the most reliable, are consistent with

$$\beta = 0{\cdot}15 \text{ msec}^{-1} \exp -\frac{V+90}{15}. \tag{12}$$

According to eqn. (8), which fits the strength–duration curve, β should be 0·11 msec^{-1} at $V = -90$ mV. This is of the right order of magnitude, but the data in Table 3 suggest that β varies more rapidly with membrane potential than one would expect from the equations used to fit the strength–duration curve. The strength–duration and summation experiments could be reconciled by using more complicated expressions for either α or β, but the data were not good enough to justify further analysis. In the small type section on p. 219 it is shown that the results in Table 3 can be explained without assuming a direct dependence of β on membrane potential.

D. *Effect of varying V after a short pulse*

According to the hypothesis outlined on p. 211 the activator concentration obeys a first-order equation and should reach a maximum at the end of a rectangular pulse. If this is correct the three pulses shown in A, B and C of Fig. 4 should be equally effective. On the other hand, if activation is a second order process the effectiveness of a pulse should be increased when followed by a depolarization, as in A, and decreased when followed by a hyperpolarization as in C. The result was that an after-depolarization slightly increased the effectiveness of the pulse and that an after-hyperpolarization slightly decreased it. To make the three pulses just threshold the duration or the magnitude of S_1 had to be altered, as shown in Table 4. The result is roughly consistent with that expected if the activation process were first order and the potential which altered α and β lagged behind the membrane potential with an exponential delay of the order of 1 msec. This assumption seems plausible, since Falk & Fatt (1964) represent the electrical contribution of the tubular system to the impedance of a sartorius fibre by an R–C element with a time constant of about 1·4 msec. The data in Table 4 might also be explained by keeping α and β as instantaneous functions of the membrane potential and introducing a time lag of 1–2 msec between the variable y and activation.

E. *Addition of long and short pulses*

This section is concerned with a curious effect which we came across while investigating the interaction between long and short stimuli. In Fig. 5, S_1 is a long rectangular pulse with an amplitude just below rheo-

base; when increased by 1·5 mV it gave a clear contraction. According to the hypothesis outlined on p. 211 the activator concentration y at the end of S_1 should be close to y_c; from eqn. (8) if S_1 is 1 mV below rheobase then $y = 0{\cdot}96\, y_c$. This can be tested by adding a strong brief pulse S_2 at the end of S_1. In the experiment of Fig. 5 the amplitude of S_2 was 100 mV and

TABLE 3. Values of the rate constant β determined from the summation interval at different internal potentials

Fibre reference	Temperature (° C)	Resting potential (mV)	V_D (mV)	β (msec^{-1})
1·1	4·8	−84	−84	0·10
2·2	3·9	−99	−99	0·26
			−134	> 0·42
			−99	0·29
			−99	0·31
			−85	0·11
			−99	0·24
			−85	0·13
			−79	0·07
			−99	0·31
2·3	4·5	−92	−92	0·24
			−92	0·56
			−77	0·22

β was calculated from the summation interval t_D by eqn. (11).
V_D is the potential during the summation interval.

TABLE 4. Effect of varying the potential $V(S_2)$ after short a pulse (Fig. 4 and text)

	Fibre reference	Resting potential mV	$V(S_2)$ mV	$t_c(S_1)$ msec
A	2·3	−92	−136	2·6
			−92	2·5
			−69·5	2·3
B	1·1	−84	−130	3·9
			−84	3·3
			−53·4	2·2

$t_c(S_1)$ is the critical duration of the first pulse. In A, the potential during the first pulse, $V(S_1)$, was −6 mV and $t_c(S_1)$ was measured directly as described in the text. In B, $V(S_1)$ was varied and t_c at $V(S_1) = -5$ mV was calculated from the strength–duration curve. Rheobase A = −54 mV; rheobase, B = −49 mV. Temperature 4–5° C.

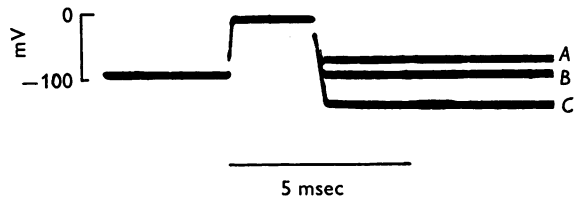

Fig. 4. Pulse structure discussed in section D of text.
Records from fibre 2·3 Table 4.

its duration (1·74 msec) was 0·47 times that required to give a contraction; on a simple basis it should raise y to 0·47 y_c when applied alone. When combined with S_1 as in record C one might expect the effects to add linearly, and, since

$$0{\cdot}96y_c + 0{\cdot}47y_c = 1{\cdot}43y_c$$

there should be a contraction. This did not happen unless S_2 was greater than 60–70 % threshold. When S_2 was less than 60 % threshold it appeared to have no effect at all, and in repeated trials the threshold for S_1 alone was found to be indistinguishable (to within about 1 mV) from that using $S_1 + S_2$. A simple explanation might be that the mechanical excitability

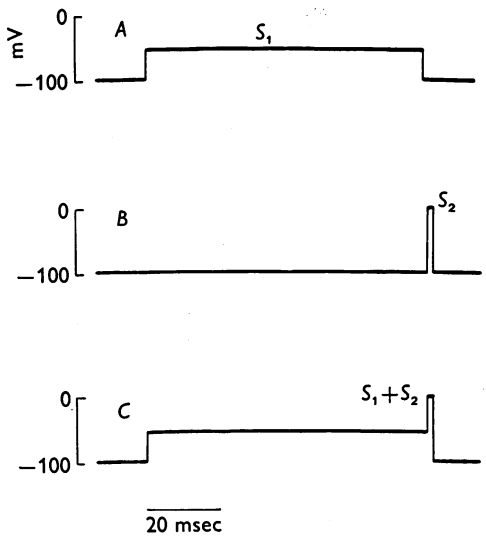

Fig. 5. Addition of long and short pulses (section E of text). Record A shows a long pulse S_1 which was at most 1·5 mV below threshold. Record B shows a short pulse S_2 whose duration was 47 % threshold. In record C the two pulses were combined but there was no contraction.

reached a peak early in S_1 and had declined to a low level by the time S_2 was applied. However, this possibility was eliminated by shortening S_1 and showing that at no time would $S_1 + S_2$ cause a contraction if $S_1 <$ threshold and $S_2 <$ 0·5 threshold. Figure 6A is an attempt to quantify the effect. Here the critical duration of S_2, which provides an indication of the mechanical threshold, is plotted against the membrane potential during S_1. The graph shows that the long pulse did lower the mechanical threshold but that the greatest reduction which could be obtained was about 50 %. A similar result was obtained in another experiment (Fig. 6B) but the maximum reduction with S_1 just below rheobase amounted to less than 20 %.

A result similar to that in Fig. 6 is expected in a regenerative system and was observed many years ago by Rushton (1932) in his studies of electric excitation. It is surprising to find it as a feature of mechanical activation, because except under rather special conditions (Sugi & Ochi, 1967) local contractions are graded and there is no evidence of anything like a triggered response (e.g. Huxley & Taylor, 1958; Hodgkin & Horowicz, 1960*b*).

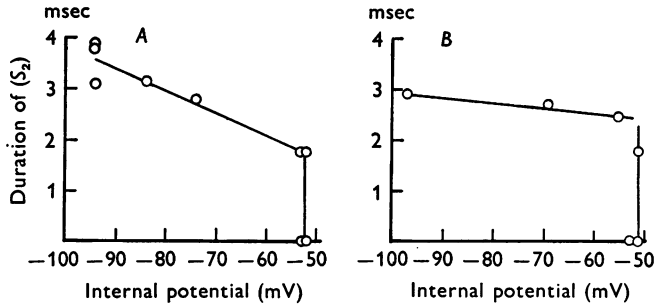

Fig. 6. Addition of long and short pulses in two experiments. The abscissa gives the membrane potential during the first, long pulse S_1. The ordinate is the duration of the second pulse S_2. The amplitude of S_1 and the duration of S_2 were adjusted so that the combination was just threshold. The duration of S_1 was 1·2 sec in *A* and 195 msec in *B*. Resting potential = holding potential = −94 mV in *A* and −97 mV in *B*. For further explanations see text.

Figure 7 illustrates a possible explanation. The abscissa is the concentration of activator and the ordinate $\dot{y}$ gives the rate of destruction of activator (or the rate at which it is returned to a store). It is assumed that something like substrate inhibition occurs and that the rate of destruction declines if y exceeds a concentration y'. The horizontal interrupted lines show the rate of release of activator at different membrane potentials. In the steady state, destruction and release of y must be equal, so the intersection of a horizontal line with the curve gives the steady level of y for any particular voltage. Since the line for $V = -50$ mV coincides with the peak of the curve, a voltage just less than −50 mV gives a steady concentration of y'. If the depolarization is slightly greater no steady state is possible and the activator then builds up until it reaches the critical concentration y_c. If $y'/y_c = 0{\cdot}4$, the greatest concentration which can be built up by a steady depolarization is only 40 % of that required to initiate contraction. This means that a short pulse must exceed 60 % threshold if it is to be effective when added to a subrheobasic depolarization. The explanation is formally similar to that used for certain types of hyperpolarizing response.

The experiments which have just been described suggest that the effects of a just subthreshold pulse might decay more slowly than those of a weak pulse. as described for nerve excitation by Katz (1937). If correct, this raises an objection to the conclusion that the rate constant β depends directly on membrane potential. The argument for dependence was that in the experiment of Fig. 3, $S_1+S_D+S_2$ caused a contraction, but no contraction was seen with S_1+S_2, S_1+S_D, or S_D+S_2. However, from section D, we know that S_1+S_D is more effective than S_1 alone, and if there is a large Katz effect one could explain the effectiveness of the combination $S_1+S_D+S_2$ without invoking a direct dependence of β on V.

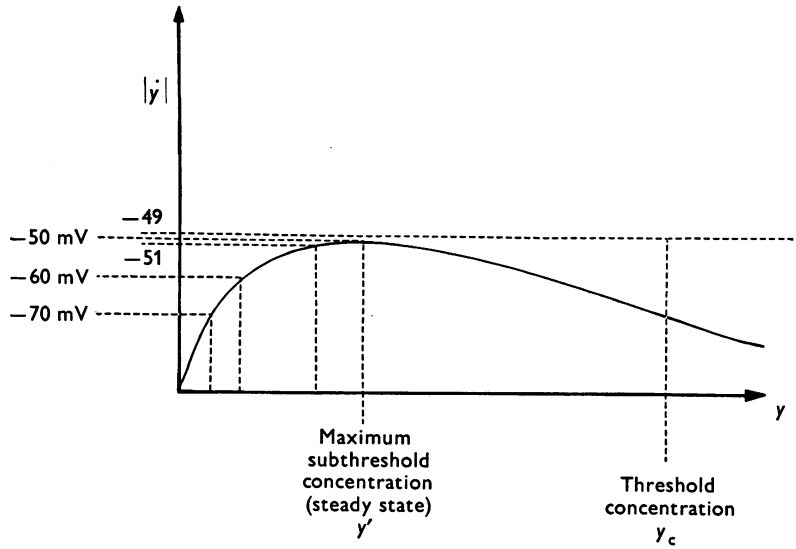

Fig. 7. Diagram illustrating possible explanation of Fig. 6 (see text). The abscissa (y) is the concentration of a hypothetical activator. The ordinate for the curve is the rate of destruction of activator and for the horizontal interrupted lines is the rate of release of activator.

F. *Comparison between the kinetics of mechanical activation and delayed rectification*

The aim of these experiments was to see whether pulses of different duration, with an amplitude just sufficient to give a contraction, all give the same amount of delayed rectification. The membrane current was measured by the three-electrode method with control from V_{2l}, the voltage on the middle-electrode (Fig. 8). The membrane current was obtained from the difference $(V_{2l}-V_l)$, to which it is proportional if the resistance per unit length of the fibre is constant. A sulphate Ringer fluid was used in order to reduce chloride current and make delayed rectification more conspicuous. With this solution, the rheobase for contraction was at about -37 mV instead of -50 mV as in the experiments with chloride Ringer fluid.

Figure 8 shows voltage and current traces for threshold pulses of different duration and amplitude. Delayed rectification can be seen in records

B and *C*, but is either absent or masked by capacity current in record *A*. The family of curves in Fig. 9 have been corrected for capacity current by subtracting a scaled correction from each trace. The correcting differences were obtained by averaging the capacitative transients observed with anodal and small cathodal voltages. The results show that the delayed current at threshold was greatest with the 21 msec pulses and declined to

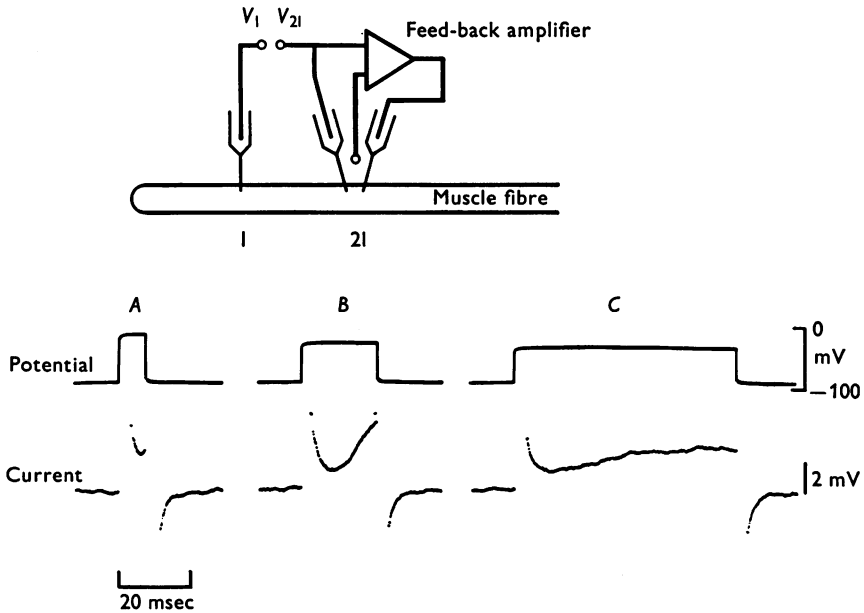

Fig. 8. Top. Arrangement of electrodes. The spacing of the electrodes from the end of the fibre was 250 μ:250 μ:100 μ.

Middle: voltage V_{2l} recorded from middle electrode.

Bottom: membrane current measured as $(V_{2l} - V_l)$.

Note that the delayed current in *B* was larger than in *A* or *C*. All three voltages were adjusted to give a just perceptible contraction. Temperature 4·4° C. Holding potential −90 mV. Resting potential −84 mV. Sulphate Ringer fluid containing 2·5 mM-K. On the current records the factor for obtaining membrane current per unit length is 160 (nA/cm) per mV. Fibre diameter, 75 μ.

about $\frac{1}{5}$ in the 7 msec pulse; the difference becomes slightly larger if conductance rather than current is used as a criterion of delayed rectification. It is also evident that both delayed current and conductance were greater in the 12 msec pulse than in the 62 msec pulse.

Figure 10 compares the strength–duration curve for contraction (A) with that for delayed rectification (B), using the time to reach a small fixed conductance as the criterion in curve B. The voltage V_{2l} was used for the contraction threshold and V_l for delayed rectification. The two curves clearly have a different shape, suggesting that the underlying mechanisms

have different temporal characteristics. A possible objection to the conclusion is that with short pulses the observed contractions might be confined to the immediate vicinity of the current electrode. Local contractions near the current electrode were observed by Adrian *et al.* (1969) and are to be expected theoretically. However, such local contractions would be hard to see at the magnification used in our experiments and we think that our visual threshold probably corresponded to some more substantial movement.

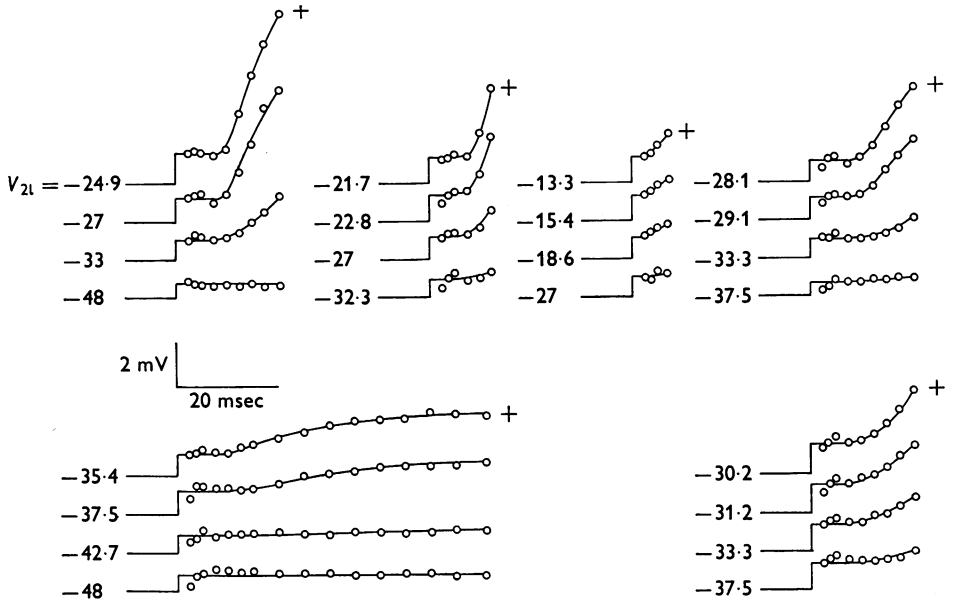

Fig. 9. Families of curves showing current as a function of time at different membrane potentials with pulses of different duration. In each family, the top curve, which is marked with a +, gave a contraction, the others did not. The curves were obtained from records like those in Fig. 8 but were corrected for capacity current. For further details see Fig. 8.

An interesting point about Fig. 10 is that the threshold potential at 20 msec changed progressively during the experiment from −26 mV to −36 mV. However, there was no alteration in the delayed rectification threshold and the points from different families all fall on the same curve. This is another indication that contraction does not occur when the conductance increases by a fixed amount.

DISCUSSION

The experiments in section A show that at 4° C a pulse to +40 mV must last about 2 msec in order to activate the contractile mechanism. This raises the question of the effectiveness of the action potential as a stimulus

for contraction. A direct way of approaching the problem is described by Adrian *et al.* (1969) but it is interesting to make a rough calculation from the present results. For this purpose we assume that the approximation in eqn. (7) can be extended down to $V = -30$ mV, that $\alpha = 0$ for $V < -30$ mV and, further, that $\alpha\bar{y} \gg \beta y$ in eqn. (1). The maximum value of y/y_c is then given by

$$\frac{y}{y_c} = AB,$$

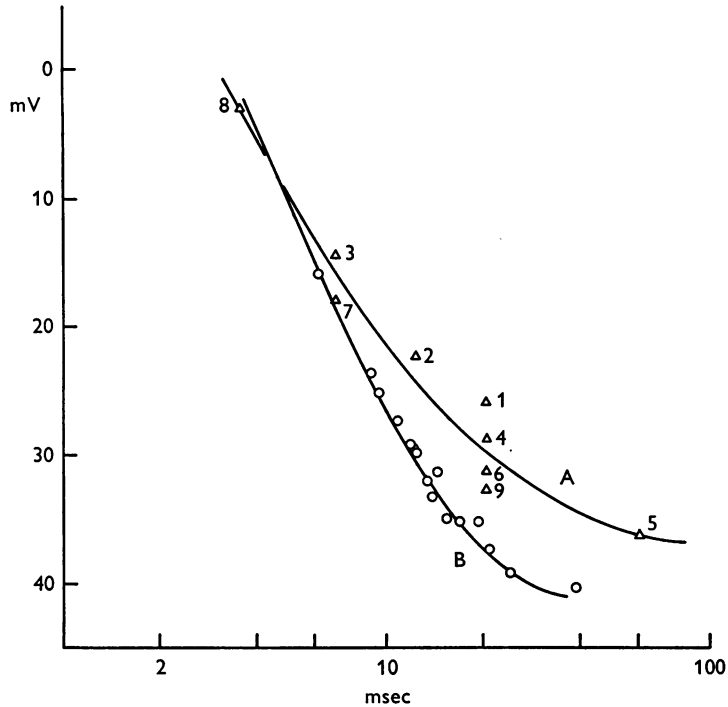

Fig. 10. Strength–duration curves for A, contraction, and B, delayed rectification, calculated from Fig. 9. The criterion for delayed rectification was that the 'potassium conductance' g_K should increase to a small arbitrary extent, roughly equal to the leakage conductance in Fig. 9. g_K was taken as proportional to $(V_{2l} - V_l)/(V_l - V_K)$ with $V_K = -80$ mV. Numbers against the triangles (curve A) indicate the order of the experiment.

where $A = 8{\cdot}5$ mV^{-1} sec^{-1} at 4° C, and B is the area in mV sec of that part of the action potential which exceeds -30 mV. From the data of Nastuk & Hodgkin (1950) we estimate B as about 0·25 mV sec at 4° C, hence $y/y_c = 2{\cdot}1$ and the action potential is well above the mechanical threshold.

It would be interesting to repeat these calculations for 20° C but unfortunately the temperature coefficient of the constant A has not been measured. C. Y. Kao & P. R. Stanfield (personal communication) and

Adrian *et al.* (1969) give strength–duration curves at room temperature but in neither case are there values for internal potentials more positive than about -10 mV. On the assumption that temperature did not alter the shape of the curve, a Q_{10} of about 2 for A is obtained from a comparison of Kao & Stanfield's data with our own. Since the spike duration increases 2·5–3-fold between 7 and 17° C (Nastuk & Hodgkin, 1950) it would seem that a spike is likely to be slightly less effective at room temperature than at 4° C, but still well above threshold.

Section F indicates that with large depolarizations the rise in potassium conductance lags slightly behind the activation of contraction. If this relation held over a wide range of conditions it would provide a mechanism which would automatically cut off the supply of activator, by repolarizing the membrane, soon after the threshold for contraction had been reached. This might be advantageous in keeping the twitch brief and its activation economical.

Of the remaining results, the only one which needs comment is the finding that a depolarization which is very close to rheobase cannot, apparently, reduce the threshold for a short pulse to zero. This was explained by postulating something like regeneration in the activation mechanism. It now seems possible that the exceedingly steep relation between tension and membrane potential observed by Hodgkin & Horowicz (1960*b*) in the region of -50 mV might be explained in a similar way, though it would be necessary to introduce other factors, such as variability throughout the fibre, in order to account for the complete curve relating tension to membrane potential.

APPENDIX

Distribution of potential in transverse tubular system

Part of the large capacity of muscle is thought to reside in the network of transverse tubules (Falk & Fatt, 1964). In considering the electrical activation of contraction we need to know how quickly and completely the tubular system would charge if the potential difference between sarcoplasm and external fluid is made to undergo a sudden displacement. Falk & Fatt (1964) have dealt with the a.c. properties of the tubular network, and Falk (1968) gave solutions for a voltage clamp on certain assumptions, but did not deal fully with the case considered here.

We assume that the tubules are open at the surface and form a regular network of the kind illustrated in Fig. 11. The diameter of the tubules is taken to be small compared with the mesh, and the mesh itself to be small compared with the diameter of the muscle. The lumen of the tubules has a specific conductivity G_L (mho cm^{-1}) and their membrane a capacitance

and conductance per unit area of C_W and G_W respectively. From these basic constants it is necessary to calculate the three practical constants which appear in the equation for current spread in the tubular system. These are $\bar{C}_W$ and $\bar{G}_W$, the capacitance and conductance of the tubular membrane per unit volume of muscle fibre, and $\bar{G}_L$, the effective radial conductivity of the lumen in mho cm^{-1}. If ρ is the fraction of the total

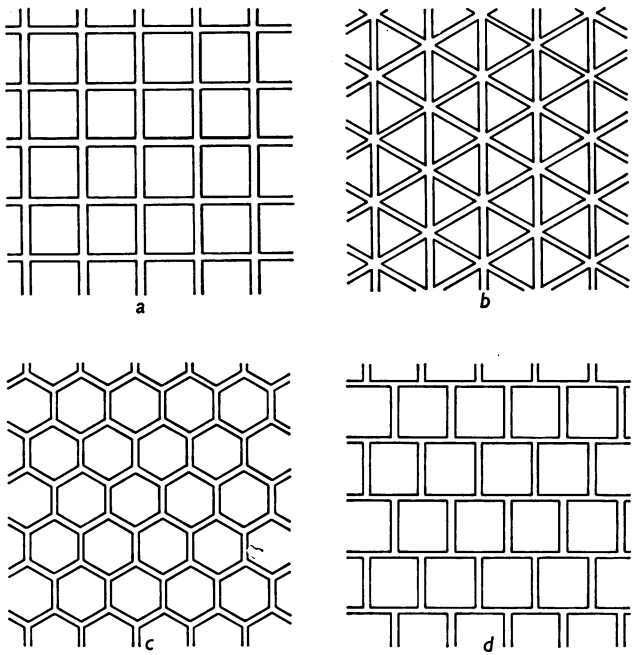

Fig. 11. Hypothetical arrangement of tubular networks considered in text; *a*, square, *b*, trigonal, *c*, hexagonal, *d*, staggered squares.

muscle volume occupied by tubules and ζ is the volume to surface ratio of the tubules then

$$\bar{C}_W = C_W\rho/\zeta, \tag{1}$$

$$\bar{G}_W = G_W\rho/\zeta, \tag{2}$$

$$\bar{G}_L = G_L\rho\sigma, \tag{3}$$

where σ is $\frac{1}{2}$ for the networks *a*, *b* and *c* in Fig. 11; network *d* is anisotropic with $\sigma = \frac{1}{2\cdot 5}$ for current flowing parallel to the vertical of the T junctions, and $\sigma = \frac{1}{2}$ for the direction at right angles.

It is easy to see that σ should be $\frac{1}{2}$ in certain simple situations. Thus, if the field is parallel to one set of tubules in network *a* this set makes a contribution of $\frac{1}{2}G_L\rho$ and the other set

contributes nothing. In network *b*, if one set is parallel to the field it contributes $\frac{1}{3}G_L\rho$, and the other two sets each contribute

$$\frac{G_L\rho}{3}\cos^2\frac{\pi}{3} = \frac{G_L\rho}{12};$$

if one set is at right angles it contributes zero and the other two each contribute

$$\frac{G_L\rho}{3}\cos^2\frac{\pi}{6} = \frac{G_L\rho}{4}.$$

A more general proof can be given in the following way. Consider two planes normal to the applied field. For network *a*, if half the tubes make an angle ϕ with the field, then the other half must be at $\phi-\frac{1}{2}\pi$. The number of tubes which intersect the two planes is proportional to $\cos\phi$ and $\cos(\phi-\frac{1}{2}\pi)$ respectively, whereas the length of the tubes is proportional to $\sec\phi$ and $\sec(\phi-\frac{1}{2}\pi)$. Hence the contributions to conductivity are proportional to $\cos^2\phi$ and $\cos^2(\phi-\frac{1}{2}\pi)$ giving

$$\sigma = \tfrac{1}{2}[\cos^2\phi+\cos^2(\phi-\tfrac{1}{2}\pi)] = \tfrac{1}{2} \tag{4}$$

for all values of ϕ.

Applying the same argument to the trigonal network *b*, we find

$$\sigma = \tfrac{1}{3}[\cos^2\phi+\cos^2(\phi-\tfrac{1}{3}\pi)+\cos^2(\phi+\tfrac{1}{3}\pi)] = \tfrac{1}{2} \tag{5}$$

for all ϕ.

Since the trigonal network may be split into three identical hexagonal networks it follows that the $\sigma = \frac{1}{2}$ relation also applies to the hexagonal network.

Since the inside of the fibre is considered to be equipotential the outward radial current per unit length is

$$i_r = 2\pi r\bar{G}_L\frac{\partial u}{\partial r}, \tag{6}$$

where u is the displacement of the potential difference across the tubular membrane from its resting values, given in the sense internal potential minus potential of lumen of tubule; r is the distance in the radial direction. The outward current through the tubular membrane is given by

$$i_W = \frac{\partial i_r}{\partial r} = 2\pi r\left(\bar{G}_W u+\bar{C}_W\frac{\partial u}{\partial t}\right), \tag{7}$$

so

$$\frac{\bar{G}_L}{r}\frac{\partial}{\partial r}\left(r\frac{\partial u}{\partial r}\right) = \bar{G}_W u+\bar{C}_W\frac{\partial u}{\partial t}, \tag{8}$$

or

$$\kappa\frac{\partial^2 u}{\partial r^2}+\frac{\kappa}{r}\frac{\partial u}{\partial r} = \frac{u}{\tau_W}+\frac{\partial u}{\partial t}, \tag{9}$$

where

$$\kappa = \bar{G}_L/\bar{C}_W \quad \text{and} \quad \tau_W = \bar{C}_W/\bar{G}_W.$$

The propagation constant κ has the same dimensions as a diffusion coefficient but is numerically much greater. The values adopted on p. 228 lead to a κ of 5×10^{-3} cm^2/sec which is about 700 times greater than the diffusion coefficient of an ion like calcium.

The steady-state solution of eqn. (9) is

$$\frac{u}{u_a} = \frac{\mathrm{I}_0(r/\lambda_\mathrm{T})}{\mathrm{I}_0(a/\lambda_\mathrm{T})}, \tag{10}$$

where I_0 is the hyperbolic Bessel function of zero order,

$$\lambda_\mathrm{T} = \sqrt{(\kappa\tau_\mathrm{W})} = \sqrt{\frac{\bar{G}_\mathrm{L}}{\bar{G}_\mathrm{W}}},$$

a is the fibre radius and u_a is the potential at $r = a$. The tubular current density at the surface of the fibre is

$$I_a = \bar{G}_\mathrm{L}\left(\frac{\partial u}{\partial r}\right)_a, \tag{11}$$

so

$$\frac{I_a}{u_a} = \frac{\bar{G}_\mathrm{L}\mathrm{I}_1(a/\lambda_\mathrm{T})}{\lambda_\mathrm{T}\mathrm{I}_0(a/\lambda_\mathrm{T})}, \tag{12}$$

where I_1 is the hyperbolic Bessel function of first order. This reduces to

$$\frac{I_a}{u_a} = \frac{a\bar{G}_\mathrm{W}}{2}, \tag{13}$$

when

$$a/\lambda_\mathrm{T} = 0.$$

The total resistance of the tubular system, referred to unit area of fibre surface, is given by the approximation

$$R_\mathrm{T} = \frac{u_a}{I_a} \doteqdot \frac{2}{a\bar{G}_\mathrm{W}} + \frac{a}{4\bar{G}_\mathrm{L}} \tag{14}$$

which holds with 0·5 % accuracy for $a/\lambda_\mathrm{T} < 1$. With the values chosen on p. 228 (for which $a/\lambda_\mathrm{T} = 0{\cdot}4$) the first term on the right-hand side, which represents the tubular membrane resistance is 3333 Ω cm² and the second, which is the effective resistance of the tubular fluid, is 67 Ω cm². With $a/\lambda_\mathrm{T} = 0{\cdot}4$, the ratio u_0/u_a is 0·96 so the displacement of tubular membrane potential is nearly constant over the radius of the fibre.

For a voltage clamp, the boundary conditions are $u = 0$ initially, and $u = u_a$ at $r = a$ for $t > 0$, where u_a is constant. It is convenient to rewrite eqn. (9) in the following dimensionless form

$$\frac{\partial^2 u}{\partial R^2} + \frac{1}{R}\frac{\partial u}{\partial R} = \nu^2 u + \frac{\partial u}{\partial T}, \tag{15}$$

where

$$R = r/a, \quad T = \kappa t/a^2 \quad \text{and} \quad \nu = a/\lambda_\mathrm{T} = a(\kappa\tau_\mathrm{W})^{-\frac{1}{2}}.$$

The solution of eqn. (15) which satisfies the boundary conditions can be found by Dankwerts's method (Crank, 1956) and is

$$\frac{u}{u_a} = 1 - 2\sum_{n=1}^{\infty} \frac{\nu^2 + \alpha_n^2 \exp -[(\nu^2 + \alpha_n^2)T]}{\nu^2 + \alpha_n^2} \frac{\mathrm{J}_0(\alpha_n R)}{\alpha_n \mathrm{J}_1(\alpha_n)}, \tag{16}$$

where J_0 and J_1 are Bessel functions of the first kind and $\alpha_1, \alpha_2, \ldots \alpha_n$ are the positive roots of $J_0(\alpha) = 0$. When $\nu = 0$ this reduces to eqn. (13) of Falk (1968). An alternative form of eqn. (16) is

$$\frac{u}{u_a} = \frac{I_0(\nu R)}{I_0(\nu)} - 2 \sum_{n=1}^{\infty} \frac{\alpha_n \exp - [(\nu^2 + \alpha_n^2) T]}{\nu^2 + \alpha_n^2} \frac{J_0(\alpha_n R)}{J_1(\alpha_n)}. \tag{17}$$

The current density in the tubular region at the surface consists of two components of which the d.c. part is given by eqn. (12) and the transient part by

$$I_a' = \frac{2 u_a \bar{G}_L}{a} \sum_{n=1}^{\infty} \frac{\alpha_n^2 \exp - [(\nu^2 + \alpha_n^2)\, T]}{\nu^2 + \alpha_n^2}. \tag{18}$$

During the latter part of the charging process I_a' decreases exponentially with a final time constant τ_f given by

$$\tau_f = \frac{\tau_W \tau_1}{\tau_W + \tau_1}, \tag{19}$$

where $$\tau_W = C_W / G_W$$

and $$\tau_1 = a^2 / \alpha_1^2 \kappa. \tag{20}$$

For a voltage clamp the effective capacity of the tubular system, referred to unit area of fibre surface, is

$$C_T = \frac{1}{u_a} \int_0^{\infty} I_a' \mathrm{d}t. \tag{21}$$

On inserting I_a' from (18) we obtain

$$C_T = \frac{a \bar{C}_W}{2} \sum_{n=1}^{\infty} \frac{4 \alpha_n^2}{(\nu^2 + \alpha_n^2)^2}. \tag{22}$$

When the membrane resistance is infinite $\nu = 0$ and since

$$\sum_{n=1}^{\infty} 4 \alpha_n^{-2} = 1$$

we obtain

$$C_T = \frac{a \bar{C}_W}{2} \tag{23}$$

as is expected, since charge is distributed uniformly over the whole of the tubular capacity. In the example quoted below, where $\nu = 0{\cdot}4$, the summation term is 0·96, and for $\nu = 1$ it is 0·80, so that for $a/\lambda_T < 1$ there is little error in using eqn. (23) to estimate the effective tubular capacity.

In order to gain some idea of the electrical properties of the tubular system the following values will be adopted for the basic constants:

a, fibre radius, 4×10^{-3} cm.

ρ, fraction of fibre volume occupied by tubules, 3×10^{-3}.

ζ, volume to surface ratio of tubules, 10^{-6} cm.

C_W, capacity per unit area of tubular wall, 1 μF/cm^2.

G_W, conductance per unit area of tubular wall, $0{\cdot}5 \times 10^{-4}$ mho/cm^2.

G_L, conductivity of lumen, 10^{-2} mho/cm.

σ, network factor, $\frac{1}{2}$.

The value of ζ and ρ are taken from Peachey (1965); G_L is assumed to be slightly less than the conductivity of Ringer fluid at 20° C. C_W and G_W were chosen to give an apparent membrane capacity and resistance similar to those observed by Fatt & Katz (1951), i.e. 7 μF/cm^2 and 3000 Ω cm^2, on the assumption that tubular and surface membranes are identical. If most of the chloride conductance is in the surface (Hodgkin & Horowicz, 1960*a*; Eisenberg & Gage, 1967), the estimate of G_W might be three times too large.

The practical constants and parameters calculated from these units are:

$\bar{C}_W$, capacity of transverse tubular system (TTS) per unit volume, 3×10^3 μF/cm^3.

$\bar{G}_W$, conductance of membranes of TTS per unit volume, 0·15 mho/cm^3.

$\bar{G}_L$, conductance of lumen of TTS per unit volume, $1{\cdot}5 \times 10^{-5}$ mho/cm.

τ_W, time constant of tubular wall, 20 msec; ($\tau_W = C_W/G_W$).

λ_T, space constant of TTS, 100 μ; ($\lambda_T^2 = \bar{G}_L/\bar{G}_W$).

κ, propagation constant of TTS, 5×10^{-3} cm^2/sec; ($\kappa = \bar{G}_L/\bar{C}_W = \lambda_T^2/\tau_W$).

ν, 0·4; ($\nu = a/\lambda_T$).

a^2/κ, unit of time, 3·2 msec; ($T = \kappa t/a^2$).

Quantities which may be compared with experimental measurements are:

C_T, input capacity of TTS per unit area of fibre surface, 5·76 μF/cm^2.

C'_M, capacity of surface membrane excluding TTS, 1 μF/cm^2.

$C'_M + C_T$, apparent capacity per unit area of fibre surface 6·76 μF/cm^2.

τ_f, final time constant of charging under voltage clamp, 0·54 msec.

R_T, input resistance of TTS, referred to unit area of fibre surface 3400 Ω cm^2.

R'_M, resistance of surface membrane excluding TTS, 20,000 Ω cm^2.

$\dfrac{R_T R'_M}{R_T + R'_M}$, apparent membrane resistance, 2906 Ω cm^2.

Falk & Fatt (1964) give values which should be roughly equivalent to $C_T = 4$ μF/cm^2 and $\tau_f = 1{\cdot}3$ msec. However, there was some scatter in their results and it is not clear whether their values apply to a fibre with a radius of 40 μ. A method of estimating λ_T and κ from the spread of

mechanical activation is described by Adrian *et al.* (1969) in isolated fibres from the frog's semitendinosus muscle. These results suggest that κ may be about 8×10^{-3} cm^2/sec, but that λ_T is 60 μ, which is somewhat smaller than our estimate of 100 μ. On the other hand, if most of the chloride conductance is in the surface, as suggested by Einsenberg & Gage (1967), the estimated λ_T would be about 170 μ.

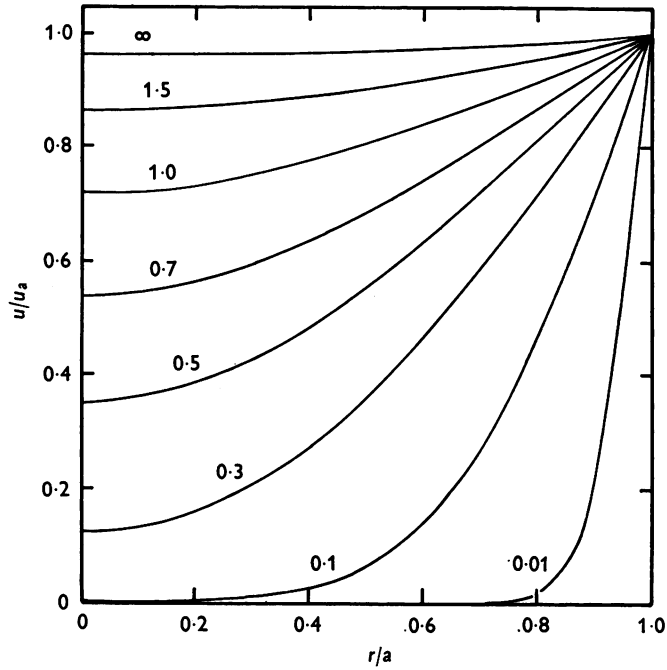

Fig. 12. Potential distribution in tubule at different times after sudden displacement of potential at surface of fibre. The abscissa is r/a where r is radial distance and a is the radius of the fibre. The ordinate is u/u_a where u is the potential difference at r, and u_a is the potential difference at $r = a$. The numbers against the curves are time in msec after the step for a fibre with $a^2/\kappa = 3{\cdot}2$ msec. To obtain value of T for each curve the numbers given should be multiplied by 0·3125.

Figure 12 illustrates the distribution of tubular potential at various times after the beginning of a voltage step at the surface, calculated from eqn. (16). It shows that the potential in the centre of a fibre of radius 40 μ reaches half its final value in about 0·65 msec; at a distance of 30 μ from the axis the half time is about 0·1 msec.

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