How much mechanical activity follows a membrane depolarisation of known amplitude and duration? For the steady state case we have the result of Hodgkin (1958), significant in view of what follows, that contracture tension varies for low levels approximately as the sixth power of the depolarisation. To answer the more difficult question involving time, one might think that something like "voltage clamp" technique is necessary. I shew that this is not so.

Brown and Sichel (1936) using single sartorius fibres found a graded tension response to increasing transverse electric field stimulation when the membrane was rendered non-propagating by partial depolarisation. One can now improve on their methods in several directions. First of all one can use the result of Nastuk and Hodgkin (1950), that regenerative membrane currents are carried by sodium, to obtain an electrically passive membrane independent of resting potential. Second one can use latency relaxation (Sandow, 1947; Abbott and Ritchie, 1951) as the earliest and an independent measure of activity. Finally by study of the threshold strength-duration relations in the propagating case one can determine the equivalent circuit of the membrane and its intra and extracellular environment, and thus determine the actual time course of membrane potential under a given externally applied field.

METHODS

Principal muscle used was frog sartorius (Rana pipiens) although other muscles, R. Capitis Collique and R. Penis of the turtle (Pseudemys elegans and Chrysemys picta), were used the main results were not qualitatively different except in the case of voltage dependence for R. Penis which showed a threshold behaviour rather than sixth power law. Sartorius had the advantage that latency relaxation was the largest, this being for normal Ringer solution in the ratios 50:5:1 respectively.

Muscle chamber and transducer set-up is shewn in fig. 1. Electrodes were of platinum foil, 9.0 × 0.9 cms., sealed at the bottom end to the wall of the test tube (14 mms. I. D.). Two transducers were used. For the highest sensitivity a gramophone piezo pick-up gave 18 mV/mgm.; in place of the needle was a glass ("melting point") tube with a hook formed on the end, a fine set-screw stop took up loads exceeding 5 gms. For calibration and loads up to 50 gms. an ionisation transducer (K. S. Lion, 1956) was mounted alongside the crystal with a similar glass hook. Sartorius was mounted with flat surface normal to

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Fig. 1. Experiment arrangement. $T_h$, $T_s$, ionisation and crystal transducers. $E$, electrodes. $TH$, thermos bottle temperature bath. $M$, muscle. $G$, glass supporting rod. $R$, rack and pinion adjustment.

direction of electric field, and adjusted to 20 percent greater than rest length to give maximum latency relaxation. Coupling of either transducer to the muscle was by a length of strong smooth twine knotted with the tendon and looped at the end. Anything more rigid than this gave, when latency relaxation was being measured, trouble from sound pick up. Further to eliminate the latter the whole set-up was mounted on a 125 lb. block of cast iron suspended on 8, 18" lengths of 1/2" pressure tubing, which then had a highly damped period of about one second. To obtain the highest resolution (0.1 mgm.) it was necessary to observe complete silence.

Since the impedance of the electrode system was about 20 ohms, square pulses from a generator were fed to it through a toroidal matching transformer. For levels above 5 volt/cm. however instead two rapid acting relays were used, back to back, driven by two pulse generators with a variable delay between them; source of power here being an accumulator. All pulses were monitored either, in the case of latency relaxation, on the tension record itself or otherwise on a separate oscillograph.

C.R.O. records were photographed to give positive transparencies which were then copied in an enlarger on to graph paper for measurement. Sweeps were calibrated with 1 msec. time mark generator.

For propagated threshold strength-duration measurements, curare, at $3 \times 10^{-4}$ gm/cc., was used to produce neuromuscular block; this is essential since the nerve threshold is somewhat lower than that of muscle membrane. For latency relaxation observations however it is not necessary since the nerve response is clearly distinguished by its delay.

Normal ringer had the composition—

<table>
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<tr>
<th></th>
<th>Na</th>
<th>K</th>
<th>Ca</th>
<th>Cl</th>
<th>HCO₃⁻</th>
<th>H₂PO₄⁻</th>
<th>pH</th>
</tr>
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<tr>
<td>mM</td>
<td>125</td>
<td>2.5</td>
<td>1.0</td>
<td>125.5</td>
<td>2.5</td>
<td>0.5</td>
<td>7.2</td>
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Choline salts were prepared by neutralisation of choline bicarbonate by corresponding acid. Except where otherwise stated temperature was 10°C, which gave the maximum latency relaxation in sodium ringer.
RESULTS

Charging time of the membrane

The threshold strength \((E)\)-duration \((\tau)\) relation for transverse field stimulation in normal (sodium) ringer is given in fig. 2; it corresponds to a two time-constant formula of the form

\[
\frac{E_0}{E} = \phi(\tau/\tau_m) = 1 - Ae^{-\tau/\tau_m} - (1 - A)e^{-\tau/\tau_m^*}
\]

(1)

found by Blair (1937) for (effectively) longitudinal field stimulation of muscle. If we assume that the membrane fires at a fixed potential, independent of the rate of rise within the limits indicated here, then (1) with \(E_0 = \epsilon \Delta V_m\) and \(\tau = t\), also describes the rise \(\Delta V_m\) of membrane potential to a step-function applied field \(E\). In support of this we note that the time constants in (1) are independent of temperature as they should be if determined solely by membrane capacity and extracellular resistances; whereas the kinetics of membrane conductances are well known to have \(Q_{10}\)'s of 3 or so. Figure 2 also exhibits the convenient fact \(\Delta A = \frac{1}{2} \Delta \tau_m\), true for all the muscles studied; an unexplained feature however is that the constant \(A\) has \(Q_{10} = 2\).

![Fig. 2. Threshold strength-duration for sartorius muscle in transverse field \(E\) volts cm\(^{-1}\) of duration \(\tau\) msecs. The absolute threshold (rheobase) \(E_0 = 2\) volts cm\(^{-1}\). The time constant \(\tau_m = \frac{1}{2} \tau_s = 2.2\) msecs., see eq. (1) of text.](image)

Latency relaxation in sodium ringer

This is shewn in fig. 3; as previously reported (Goodall, 1958) it increases with applied field and reaches values some ten times greater than that found by Sandow (1947) and Abbott and Ritchie (1951). To see how much of this is due to synchronisation of stimulus I make use of the analytical approximation
Fig. 3. Latency relaxation in normal ringer at 10°C. Numbers refer to stimulation strength as multiple of absolute threshold. Duration 1.3 m secs.

$$f(z) = z^6 - z^3$$

$$f(z, z') = \int_0^z \frac{f(z + z') - f(z)}{z'} \, dz$$

(2)

to describe the time course of tension in the latent period. The effect of an uniform spread of starting times, occupying a fraction $z'$ of the latent period ($z = 1$) and described by the function $f(z, z')$, is shewn in fig. 4. Comparing this with fig. 3 it is seen that this is not a sufficient cause for the effect of higher fields, for example the locus of the minima in experimental curves is convex to the time axis, the opposite of that computed.

Fig. 4. Effect of uniform spread $z'$ of starting time on the form and amplitude of latency relaxation calculated from eq. (2) of text. Points $x$, experimental values from curve 4 of fig. 3.
Stimulation in choline ringer

The conclusion from the above is that we are seeing a response from action potentials driven to greater than free values by the applied field. To test this we can remove the regenerative membrane current, without any other change (i.e., resting potential), by substituting choline for sodium in the ringer. There is then no threshold and for all amplitudes the membrane potential should be given by (1) as indicated above. When this is done (fig. 5) it is found that the amplitude $y_R$ of latency relaxation tends to the sixth power of the applied field for low levels and constant square pulse width. Since this is the same as found by Hodgkin (1958) for tension response from uniform steady state depolarisations ($AV_m$), the corresponding twitch tension $y_T$ was measured and found to follow the same behaviour (fig. 6), with $y_R \approx -0.017 y_T$. From here on then, $y_R$, $y_T$ were used interchangeably as measure of activation.

![Diagram](image-url)

**FIG. 5.** Latency relaxation in choline ringer; numbers refer to stimulation strength in volts cm$^{-1}$, pulse duration 1.3 msecs.

Induction time of excitation coupling

When the pulse width ($\tau$) in the above experiments is varied at constant field one obtains the result shewn in fig. 7, namely after an apparent induction period the response is linear with duration. To see whether the delay is accounted for by membrane charging time we can combine the result (1) with
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FIG. 6. Log-log plot to show approach to sixth power law at low levels for response $y$ against applied field strength $E$. Curves $R$, $T$, latency relaxation and twitch: ordinate scale mgms. and 100 mgms. respectively. Solid lines, sixth power relation.

FIG. 7. Response in choline ringer as function of pulse duration $\tau_E$ temperature as parameter. Field strength, $E=12$ volts cm$^{-1}$. Ordinates of curve for $0^\circ$ have been multiplied by three.
the sixth power law (fig. 6) and integrating obtain

\[ g(x) = \int_0^\tau \phi(x)^6 \, dx + \int_x^\infty (1 - \phi(x))^6 \, dx \]

for the response resulting from the application of a pulse of duration \( \tau = \tau_m \); this function is plotted in fig. 8 for the two extreme values \( A = 0, 1/2 \). The intercept of the linear asymptote on the time axis I call the apparent induction time \( \tau_c \). It is seen immediately that the experimental value is far smaller than the calculated and furthermore its rather large temperature dependence is not accounted for by that of the constant \( A \) in (1); at higher temperatures it appears to reach a constant limiting value \( \tau_c = 0.4 \) m sec.

![Figure 8. Effect of pulse duration on response as calculated from eq. (3). for the two extreme values of the constant A.](image)

Figure 9 is a record of twitches with increasing pulse duration; it shews that the duration of the active state, as judged by time to peak of tension, is prolonged.

**Effect of resting potential and ions**

From the above it is seen that excitation coupling can be described quantitatively by the slope and intercept of the linear portion as in fig. 7. One can then begin to study factors which affect these as in fig. 10, where instead of slope I have recorded amplitude for some \( \tau > \tau_c \). There is a characteristic effect of resting potential on the amplitude which is seen by varying the potassium
and calcium independently and using the results of Jenerick (1953) and Jenerick and Gerard (1953) for its dependence on these ions. The surprising result here is that excitation coupling is quite indifferent to the absence of calcium (at least to the level found in normal reagents), I found this to be the case even after three hours and three changes of solution. As far as the induction time $\tau_c$ is concerned calcium has no effect while potassium increases it as shewn. Finally one can show that the effect of anions is primarily a direct one on excitation coupling; for the 30 percent increase in latency relaxation amplitude in choline nitrate ringer is the same as that found by Kahn and Sandow (1955) in sodium nitrate ringer. Perhaps more significantly the resting potential for maximum response is shifted upwards.

Localisation of excitation coupling

That the effect of an undirectional pulse is localised (to one side of each fibre) is shewn by fig. 11, where the latency relaxation response is shewn to one and two pulses. When the latter are of opposite sign their effect is purely additive in time; but when they are of the same sign the effect of the second pulse is to increase the amplitude of the response without prolonging the latent period. One saw in fig. 5 that increasing excitation by depolarisation amplitude

![Diagram](image-url)
FIG. 10. Effect of ions on excitation coupling. A, Exchange of chloride for nitrate, $V_R$ is resting potential changed by potassium concentration. B, Three levels of calcium, abscissa potassium concentration. C, variation of apparent induction time with potassium concentration (or equivalent resting potential).

FIG. 11. Two pulse experiment: $2^+$, $2^-$ two pulses of same and opposite signs. 1, single pulse; 2–1, difference.
decreases latent period, when this is done by prolonging the pulse the two effects practically cancel.

DISCUSSION

The results obtained here are consistent with and can be interpreted in terms of those of Huxley and Taylor (1959) in respect to local activation. Thus the discrepancy in the time constants noted above could be due to the sensitive spots, found by these authors, having a lower capacity per unit area than the part of the membrane having the regenerative sodium conduction. Similarly the anion shift could be due to change of the anion electrochemical gradient at these spots, since Adrian (1956) found no change of resting potential. However the form of the temperature dependence of induction time indicates that at lower temperature it is being determined by a chemical reaction of some kind.

The indifference to external calcium is a result of some interest; it should not be confused however with the effect of calcium depletion under prolonged calcium-free conditions (Goodall, 1957) which is to slow up the initial rate of contraction. First attempts to investigate this by the above methods met with the difficulty that excitation coupling is slowly lost in choline ringer and this is most pronounced in R. Capitis Collique where the calcium depletion effect can otherwise be most easily shown.

SUMMARY

(1) Threshold strength-duration for transverse field stimulation of muscle in normal ringer is given by a relation with two time constants which are independent of temperature.

(2) Increase of latency relaxation with transverse field strength of stimulation in normal ringer (Goodall, 1958) is not due to synchronisation of stimulus.

(3) In choline ringer the amplitude of both latency relaxation and twitch height varies for low levels of stimulation as the sixth power of the field for pulses of constant duration.

(4) This amplitude depends linearly on duration after an apparent induction time.

(5) The induction time is temperature dependent and smaller than would be expected from the charging time of the membrane derived from (1).

(6) The amplitude for constant stimulation depends on resting potential, having a maximum somewhat below the normal resting potential.

(7) On replacing chloride by nitrate the amplitude is increased and this maximum shifted to a higher potential.

(8) Induction time increases with potassium concentration and is independent of calcium.

(9) Two pulse experiments show that activity following an undirectional pulse is localised to one side of the fibres.
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REFERENCES