The electrical properties of the muscle fibre membrane

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An analysis is made of the electric properties of frog muscle using the 'rectangular pulse' technique of Hodgkin & Rushton (1946). The experiments were made on isolated fibres and small bundles of the M. adductor magnus and on the M. extensor longus dig. IV. Sub-threshold currents of about 0·1 sec. duration were applied and wave form and attenuation of the extrapolar potential changes determined.

In the vicinity of the cathode the relation between current and voltage across the fibre membrane is non-linear, and there is evidence of a local electric response with currents of more than 30 % threshold strength. At the anode, however, the membrane behaves as a conductor of approximately constant resistance (§ A).

At an average temperature of 22° C, the following mean values were obtained:

(a) Fibre diameter: 75μ in the bundles of M. adductor magnus, and 45μ in M. extensor longus dig. IV.

(b) Characteristic length of the muscle fibres: 0·65 and 1·1 mm. respectively.

(c) Membrane time constant: 9 and 18·5 msec.

(d) Specific resistance of the myoplasm: about 230 Ωcm.

(e) Transverse resistance of the fibre membrane: 1500 and 4300 Ωcm.^{2}.

(f) Membrane capacity: about 5 μF/cm.^{2}.

The numerical differences between isolated fibres and whole muscle arose chiefly from a different value of the membrane resistance, the significance of which is discussed (§§ B 1 and D).

The value of the membrane capacity of muscle is about five times higher than that reported for various other cell membranes and confirmed here for isolated crustacean nerve fibres. The large membrane capacity must be an important factor in determining the slow electrical time scale of muscle.

The relations between the electric constants of the resting muscle fibre and some of its physiological properties (time factor of excitation, propagation velocity, rate of decline of the end-plate potential) are discussed (§ E).

The location of the membrane, or interface, at which electromotive changes occur is discussed, and a number of reasons are given which indicate that the site of the electrotonic potential changes must be at the surface of the muscle fibres.

INTRODUCTION

In a recent paper Hodgkin & Rushton (1946) have described a method by which the electric constants of a non-medullated nerve fibre could be determined. This method has been applied to frog muscle with the object of measuring the resistance and capacity of the fibre membranes. An absolute measurement of these quantities is of interest for two reasons. First, frog muscles are used extensively to study the rates at which ions exchange between cell and surrounding fluid (Boyle & Conway 1941; Steinbach 1944; Krogh 1946), and one may expect to find a quantitative relation between the permeability to ions and the electric conductance of the cell membrane (see Cole 1940). Secondly, the resistance and capacity of the membrane are of great physiological importance in all excitable tissues in that they influence the rates at which potential changes develop and spread along the fibre surface. For instance, the time factor of electric excitation, the velocity of the action poten-

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tial wave and the time course and spatial spread of the end-plate potential must all depend upon the magnitude of membrane resistance and capacity.

Muscle fibres are known to be relatively slow in their electric reactions: the impulse velocity is 10 to 20 times less than in the motor nerve (Adrian & Owen 1921; Schaefer 1936), and the time factor of excitation is about 20 times larger (Lucas 1907/8; Rushton 1930; Schaefer, Schölmerich & Haass 1938; Blair 1941). Even if we compare muscle with thinly medullated nerve, we find that the propagation velocity in a frog muscle fibre of $75\,\mu$ diameter is less than one-half that in a $30\,\mu$ crustacean axon. It will be shown that the slower electrical time scale of skeletal muscle fibres can be attributed to their larger membrane capacity.

The experiments were made on isolated fibres or fibre bundles as well as on whole muscles. It will be necessary to describe in detail the results obtained with both types of preparations, for each had its particular advantages and limitations.

METHOD

(1) *List of symbols*

r_i = internal resistance of the fibres per unit length (Ω/cm.).

r_o = resistance of the outside fluid per unit length (Ω/cm.).

r_m = transverse resistance × unit length of the fibre membrane (Ω cm.).

R_i = specific resistance of fibre interior (myoplasm) (Ω cm.).

R_o = specific resistance of outside fluid (Ringer solution) (Ω cm.).

R_m = transverse resistivity of membrane (Ω cm.^{2}).

C_m = membrane capacity (μF/cm.^{2}).

λ = characteristic length of fibre = $\sqrt{[r_m/(r_i+r_o)]}$ (cm.).

τ_m = $R_m C_m$ = time constant of membrane (μsec.).

m = $r_i r_o/(r_i+r_o)$ = parallel resistance of inside and outside resistance per unit length (Ω/cm.).

V_A = electrotonic potential at the polarizing electrode (V).

I = current flowing through the polarizing electrode (A).

y = V_A/I = 'electrotonic resistance' (Ω).

x = extrapolar distance (cm.).

Vol.$_t$ = total volume of muscle (c.c.).

Vol.$_i$ = internal volume of fibres (c.c.).

Vol.$_{ext.}$ = outside fluid volume (c.c.).

ρ = radius of fibres (cm.).

l = length of fibres (cm.).

N = number of muscle fibres in parallel.

S = membrane area per unit length (cm.).

(2) *Apparatus*

The method of measuring the electric characteristics of muscle fibres is identical with that described by Hodgkin & Rushton (1946) for lobster nerve. Unless special mention is made, their procedure and nomenclature was adopted in the present work. The technique consists essentially in the application of a subthreshold 'rect-

angular' current to the fibres, and in an analysis of the potential changes produced in the extrapolar region. The circuit shown in figure 1 (a) differs from that of Hodgkin & Rushton (1946) only in the use of a mechanical contact breaker and of a balanced earth point in the polarizing circuit. In this figure the electrodes and earth point have been arranged so as to record extrapolar potential changes. To complete the measurements, the polarizing current was monitored across R_6, then leads E_2 and E_4 were reversed to determine the value of m. Each procedure required, of course, resetting of the earth point G.
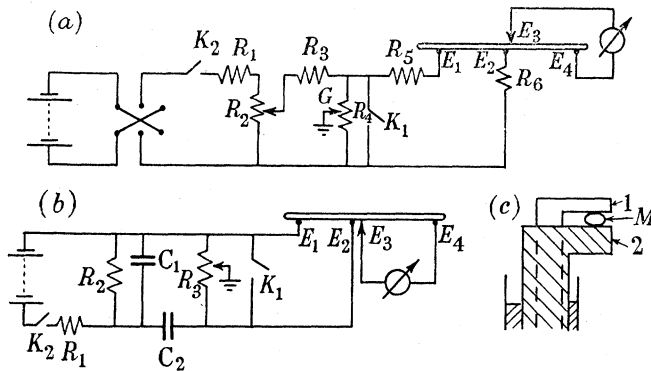

FIGURE 1. (a) Measurement of extrapolar potentials. Electrodes as arranged for isolated fibres. K_1, K_2, keys of Lucas rotating contact breaker; R_1, 15,000 Ω; R_2, and R_4, 5000 Ω; potentiometers; R_3, 60,000 Ω; R_5, 0·5 to 5 megohms; R_6, monitor resistance of 10,000 to 100,000 Ω; E_3, sliding electrode; E_3, E_4, input leads to cathode follower grids. (b) Brief double condenser pulses. Electrodes as arranged for M. extensor, dig. IV. K_1, manual short-circuit; K_2, key of contact breaker; R_1, 20,000 Ω; R_2, 2000 Ω; R_3, 5000 Ω; C_1, 0·1 μF, C_2, 0·05 μF. Monitor resistance not shown. (c) Electrode connector of E_3 used for M. extensor dig. IV. 1, bridge of filter paper; M, muscle; 2, sharpened wooden connector.

In some experiments, a brief subthreshold current pulse was applied to the muscle, using a double condenser discharge as shown in figure 1 (b). The time course of the discharge was adjusted so that it should imitate the brief action of the nerve impulse in a curarized preparation (Eccles, Katz & Kuffler 1941).

The muscle was connected via two cathode followers to a D.C. amplifier of the type described by Hodgkin & Huxley (1945). The two channels of the amplifier were balanced to within 1:200, and this, together with the use of a balanced polarizing current, eliminated most unwanted artefacts. In some experiments the contact breaker was coupled by a small condenser to the output of the cathode follower in order to mark the beginning and end of the rectangular current with a brief spike. The grid current in the input circuit was less than 10^{-9} A, and usually about 10^{-10} A. The recording speed in these experiments was relatively low, for the time constant τ_m of the membrane was of the order of 10 to 20 msec. For this reason the delay introduced by stray capacities of the apparatus was negligible. When single fibres were used, resistances of a few megohms were encountered in both polarizing and recording circuits, but even under these adverse conditions the lag of the whole apparatus (including all connexions from contact breaker to cathode ray tube) could be represented by an exponential curve with a time constant of less than 70 μsec. and could thus be disregarded. The time base was calibrated with 50 cycle A.C.

(3) *Preparation and procedure*

Small bundles containing 1 to 4 muscle fibres were isolated from the M. adductor magnus, or M. adductor longus, of English *Rana temporaria*. The muscle was left attached to the bones, or tendons, at either end. The dissection was carried out in a Ringer bath to which usually some curarine was added. This does not affect the excitable properties of the muscle membrane (Lucas 1907/8; Schaefer *et al.* 1938), but it reduces the risk of injuring the muscle fibres at an advanced stage of the dissection when the last nerve branches are severed. In some experiments, the solution was replaced by ordinary Ringer before the fibres were mounted on electrodes.

During dissection, the muscle was placed on a glass slide as described by Ramsay & Street (1941), and its attachments were tied to rubber bands. When a clean stretch of 10 to 15 mm. had been obtained, the preparation was gripped at both ends by screw-controlled forceps and raised into a layer of light liquid paraffin. Electrodes similar to those described by Hodgkin & Rushton (1946) were then applied and their distances measured with an eyepiece scale. The sliding lead E_3 was placed opposite the polarizing electrode E_2, and the polarizing circuit was balanced by temporarily connecting E_4 to earth and adjusting G for zero deflexion. E_4 was then reconnected to its input grid and a preliminary series with varying current intensities of alternating directions was made. After threshold had been determined, a suitable current strength, usually near threshold, but with anode at E_2, was selected and the required measurements were made. At the end of the experiment, the preparation was transferred to a Ringer bath, and the fibre diameters were measured with a 48-fold magnification.

A few comparative experiments were made on isolated nerve fibres of *Carcinus maenas*, using the same apparatus but replacing Ringer by sea-water electrodes.

It was found on several occasions that isolated muscle fibres survived well in a Ringer bath and gave vigorous responses after 24 hr. soaking, yet after being transferred to paraffin oil and mounted on electrodes, would fail to conduct within 1 or 2 hr. It is not clear whether this was the result of the immersion in paraffin oil or of the handling during the experiment, but it raised doubts as to the physiological condition of the fibre membranes. Comparative experiments were therefore made on whole muscles which should give one greater assurance concerning their physiological state. After preliminary work on the sartorius, the M. extensor longus dig. IV was used. This muscle is of cylindrical shape, about 15 mm. long, often less than 0·5 mm. thick, and consists of approximately 50 parallel fibres (variations between 15 and 70 fibres were observed, the former case apparently being an anomaly). In transverse sections of the muscle two or three spindles were frequently seen, and it is possible that these structures introduced some complication. But as they occupy only a few per cent of the total cross-sectional area, they are not likely to affect the extrapolar potential seriously. At times, two or three fibres of the muscle were found to diverge and join another tendon, and it was then difficult to avoid injuring them. These fibres were either carefully removed from the rest of the muscle, or the preparation was discarded. The muscle was mounted in a moist paraffin-wax

chamber. The electrodes were calomel half-cells connected to the muscle by pools of Ringer and sharpened strips of wood which had been soaked in Ringer's solution. One of the wooden connectors was clamped to an ebonite rod which could be driven, parallel to the muscle, by a coarse micrometer. The dimensions of the muscle were measured with a binocular microscope and eyepiece scale.

(4) Sources of error

The analysis of the results is based on the theory of a uniform cable of linear dimensions with distributed capacity and leakage resistance (Hodgkin & Rushton 1946; Lorente de Nó 1947). In practice the conditions of the experiment conformed only approximately to the theoretical assumptions, and it is necessary to consider the errors which may have arisen in this way.

Thickness of muscle. The theory demands that the current flow should be strictly parallel in the internal and external conductors, and that there should be no transverse potential differences (p.d.'s) except between inner and outer surface of the membrane. This can be attained only if the length constant λ is very large compared with the thickness d of the tissue (Hodgkin & Rushton 1946). In an average experiment, λ/d was about 4 to 5. To check whether this was a tolerable ratio, two empirical tests were employed.

(a) If there were an appreciable transverse potential gradient in the external or internal medium, it would cause a part of the extrapolar potential to be established instantly. This would form a discrete component of the recorded potential change, added to the electrotonic membrane potential which develops gradually and is progressively slowed as lead E_3 is moved away from the polarizing electrode. Furthermore, the first component should reverse sign when E_3 is placed on the opposite side of the muscle, while the membrane component would not reverse. Finally, the membrane potential should be abolished by chloroform, while potential gradients in the fluid conductors would remain. In isolated fibres and the extensor dig. IV, only the 'membrane' component was observed, which indicates that these preparations were sufficiently thin. With the sartorius muscle, however, both components showed up markedly (§ C).

(b) The effective thickness of the tissue can be reduced to one half by applying the polarizing current to both sides of the muscle instead of only one. This was done by means of a bridge of filter paper which touched the upper surface of the muscle as well as the wooden electrode (figure 1 (c)). If the spread of the extrapolar potential is altered significantly by this procedure, then the ratio λ/d is evidently not large enough. The tests made with this method will be described in more detail below: they confirmed that the M. extensor longus dig. IV was a satisfactory preparation for the present purpose, while the sartorius was not. In the experiments described in §D, the double-sided contact of electrode E_3 was used throughout.

Width of and distance between electrodes. In the ideal case, electrodes E_2 and E_3 should have infinitesimal width (δ), and their distance D from electrodes E_1 and E_4 should be infinite. In practice, the ratio λ/δ was about 5 to 10, and the ratio D/λ not less than 4 to 5. These imperfections are not likely to vitiate the measurements

of length and time constants (λ and τ_m) by more than a few per cent, and no attempt was made to correct for them.

When the extensor dig. IV was used, the arrangement of electrodes E_2 and E_3 (figure 1 (b)) inevitably compromised the measurement of the potential change at the polarizing electrode itself: for when E_2 and E_3 made contact, polarizing current flowed through the common part of the electrode and produced a small p.d. in the recording circuit. Furthermore, the electrotonic potential recorded at this point suffered an appreciable decrement, for the combined width of E_2 and E_3, and of the droplet of fluid forming at the point of contact, amounted to about 0·4 mm. This affects the measurement of the constants y and r_i/r_o (cf. Hodgkin & Rushton 1946), but an approximate correction can be made by extrapolating the observed relation between $\log V_1$ and x to a distance of 0·4 mm. $\times \sqrt{[r_i/(r_i+r_o)]}$, which was approximately 0·3 mm.

Distribution of fibres. The theoretical treatment deals with a single uniform cable, but it can equally be applied to a bundle of identical cables arranged in parallel with conducting interspaces. In most experiments we have an aggregate of fibres of very different diameters. In the extensor dig. IV, for example, variations in fibre size of 3·5 : 1 (25 to 90μ) are common, and extreme variations of 10 : 1 (10 to 100μ) have been observed. The electric constants derived from such a non-uniform assembly must be treated with caution. They may be regarded as the properties of an 'average' fibre, but not much confidence could be placed on this statement, if it was not supported by measurements on isolated fibres.

Measurement of fibre size. The most serious technical error arose from inaccuracies in determining the fibre diameters. These inaccuracies were due to variations of diameters along the muscle, to elliptical rather than circular cross-sections of fibres and to uncertainty regarding the thickness of adventitious tissue. In extreme cases the error may have been as high as ± 20 %, and one may regard ± 10 % as an average estimate. Fortunately, some of the relevant constants, e.g. λ and τ_m, are independent of this measurement, but others, such as the absolute values of R_m and C_m, are affected by it. The value of R_i is proportional to the square of the diameter, and is therefore the least reliable quantity which has been measured.

In the case of the M. extensor dig. IV the method used for determining surface area and internal volume of the fibres needs further description. The dimensions of the muscle were measured with a low-power microscope, and the fibres were counted after fixing and sectioning. The two required quantities are (i) the volume per unit length, Vol.$_i$/1, and (ii) the membrane surface S per unit length, of all the muscle fibres. From the electrical measurements the longitudinal resistance of the outside fluid was known. Using this result and the specific resistance of Ringer solution, the outside fluid volume was calculated and was subtracted from the optically determined total volume to find the internal fibre space. This procedure takes no account of poorly conducting material on the outside of the fibres such as connective tissue fibrils, hence the estimate of the internal volume is somewhat too large.

The total membrane surface could be calculated easily if all fibres had the same diameter: it would then simply follow from $S = 2\sqrt{(\pi N \text{Vol.}_i/1)}$, where S is total membrane area per unit length, Vol.$_i$/1 total internal volume per unit length, and N the number of fibres. Because of the large scatter of fibre sizes, this formula must

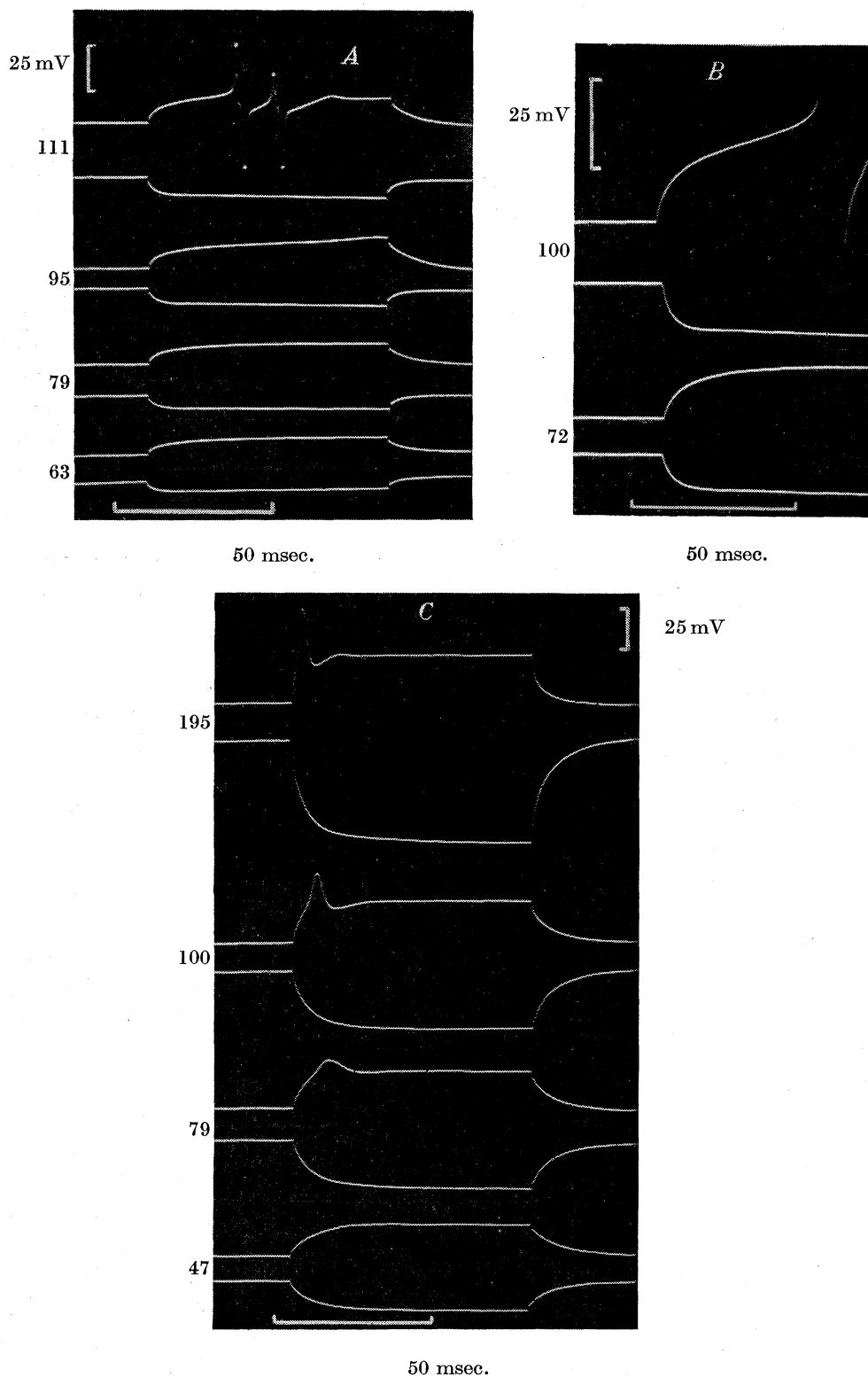FIGURE 2. Potential changes at the polarizing electrode. Cathodic potentials shown as upward deflexions. Strength of current indicated in relative units. Calibration: 25 mV and 50 msec. *A*, single muscle fibre. Peaks of diphasic spikes are marked by dots. *B*, four muscle fibres. Spike potential (not shown) 85 mV. *C*, anomalous response of a muscle fibre, after a block had developed near the recording electrode.

give too high a value. The order of magnitude of this overestimate was determined in one muscle by measuring the diameters of its 57 fibres. It was found that the true value of S was about 10 % less than calculated from the above equation. As the scatter of fibre diameters appeared to be much the same in all preparations, a reduction factor of 0·9 for the calculated value of S was employed throughout.

In the case of *Carcinus* axons, the outside diameter of the fibre was measured while it was mounted on electrodes in paraffin oil. The internal volume was obtained as for the M. extensor dig. IV, by subtracting the electrically determined outside fluid space. This results in an overestimate of the axon diameter, but no further correction was attempted.

The errors already discussed are of a technical nature: in addition there were a number of small inaccuracies arising from genuine divergences between the behaviour of nerve or muscle and the simple cable theory. These will be discussed under the appropriate headings below.

(5) *Control experiments on dead tissue*

To ensure that the electrotonic potentials were correctly attributed to the living cell membranes, the muscles were treated with chloroform. This was applied to isolated fibres by gently stroking their surface with a fine glass rod which had been in contact with a chloroform-saturated Ringer solution. Extensor muscles were exposed to chloroform vapour which was introduced into the moist chamber by means of a pad of filter paper. Within a short time the extrapolar potential changes disappeared, except for a small rectangular wave recorded at the polarizing electrode which did not exceed a few per cent of the original potential (see also Hodgkin & Rushton 1946). The action of chloroform was as complete in whole muscle as in isolated fibres, the only difference being the rapidity of its onset. The effect on isolated fibres was immediate, while that on the extensor muscle was delayed for several minutes.

RESULTS

A. *The relation between current intensity and membrane potential*

Before we can attempt to measure the membrane conductance of muscle, we must find out whether it obeys Ohm's law within at least a limited range of current intensities. That this can only be approximately so, is indicated by the fact that the A.C. impedance of muscle varies continuously with the polarizing current strength (Dubuisson 1935; Katz 1942), and that subliminal currents produce a local response at the cathode (Kuffler 1942). Nevertheless, direct measurements show that the relation between electrotonic potential and applied current is not far from linear with cathodic currents of less than one-third threshold and with anodic currents of considerably greater intensity (figure 4). It is true that a very slight, continuous, upward curvature of the voltage:current relation was sometimes observed (see also Schaefer *et al.* 1938), but as a first approximation the membrane characteristic appears to follow Ohm's law in the range indicated in figure 4.

Near threshold, the relation between catelectrotonic potential and current strength becomes distinctly non-linear. This effect was observed in isolated fibres

as well as in whole muscle (figure 4). There was a significant difference between the time courses of anodic and cathodic potentials, the latter continuing to rise for a longer time. At threshold, propagated spikes arose after a period of gradual inflexion upwards (figures 2 A, B and 3 A) and all transitions between the plateau and the inflexion of figure 2 B could be obtained by fine adjustment of the current strength. The whole family of potential-time curves is so similar to the local responses in *Carcinus* nerve described by Hodgkin & Rushton (1946) and Hodgkin (1947) that no further comment is required.
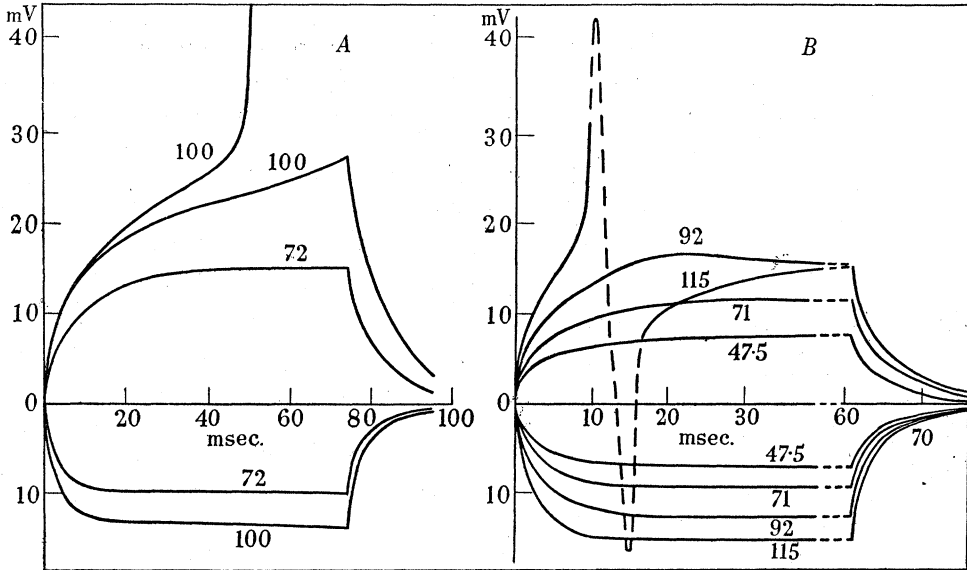

FIGURE 3. Superimposed records of potential changes at the polarizing electrode. Cathodic potentials shown as upward deflexions. Relative current strength shown in figure. *A* and *B*, two experiments on bundles of four fibres.

In several preparations, a different type of local response was seen. It consisted of an initial hump, rather than a prolonged 'creep' of the catelectrotonic potential. In one case, illustrated in figure 2 C, the hump increased in size and became oscillatory as the current intensity was raised. After the local responses had died out, the electrotonic potential settled to a lower level than at the anode. A behaviour of this kind was observed in fibres of low excitability, and it sometimes developed in the course of an experiment. Although it cannot be regarded as characteristic of normal muscle, a very similar type of reaction has previously been described in the giant axons of cephalopods (Arvanitaki 1939; Cole & Curtis 1941) where it appears to be the usual event.

In small bundles of fibres the inflexion of the local response occurred at about 20 mV (varying in 7 experiments between 16 and 32 mV). The size of the spike varied between 35 and 85 mV. Conduction velocity was determined from the two peaks of the diphasic response and, in 5 experiments at 22° C, was 1·0 to 1·45 m./sec., with a mean of 1·27 m./sec. This is considerably less than the velocity in whole

sartorius muscle (1·6 m./sec. at 20° C, see Eccles *et al.* 1941). There are probably two reasons for this difference: first, one would suspect the isolated fibres to have a reduced 'safety margin' and therefore to conduct at an abnormally low rate (see Method). Secondly, the resistance on the outside of the fibres was very high (see § B 4), and this must cause a considerable drop in velocity (cf. Hodgkin 1939; Katz 1947 *b*). For example, a 60μ fibre which conducted at a speed of 1 m./sec., had a ratio of outside : inside resistance of 1·02: the velocity of this fibre *in situ* would have been $1·0 \times \sqrt{[(r_i + r_o)/r_i]} = 1·42$ m./sec.
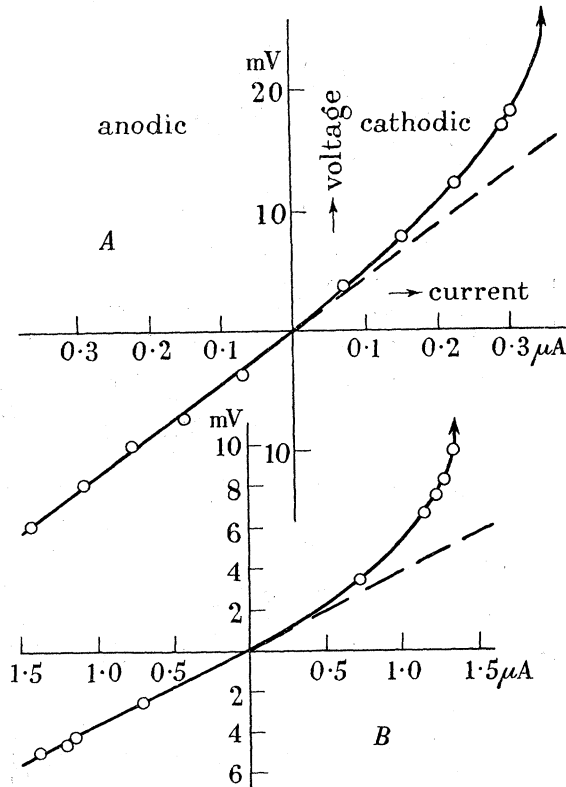
FIGURE 4. 'Voltage/current' relation at the polarizing electrode. *A*, from the experiment of figure 3 *B*. *B*, from a M. extensor dig. IV. Ordinates: maximum amplitude of local potential change in mV. Abscissae: current strength in μA. Threshold of propagating spikes indicated by arrow. *Note.* In experiment B the deflexions were steady, while in A, with currents near threshold, the amplitude of the cathodic potential began to decline before the break of the current (cf. figure 3 *B*).

B. *Electric constants of isolated muscle fibres*

It follows from these observations that the muscle membrane gives a local electric response in the region of the cathode which may persist for 100 msec. or more, and this causes the apparent membrane resistance to depart significantly from Ohm's law. To minimize this complication the analysis was restricted to the potential changes at the anode.

FIGURE 5. Anodic potential changes. *A*, M. adductor magnus, four fibres (diameters 70, 70, 45, 45μ). *B*, M. adductor magnus, two fibres (diameters 130 and 50μ). *C*, *Carcinus* ne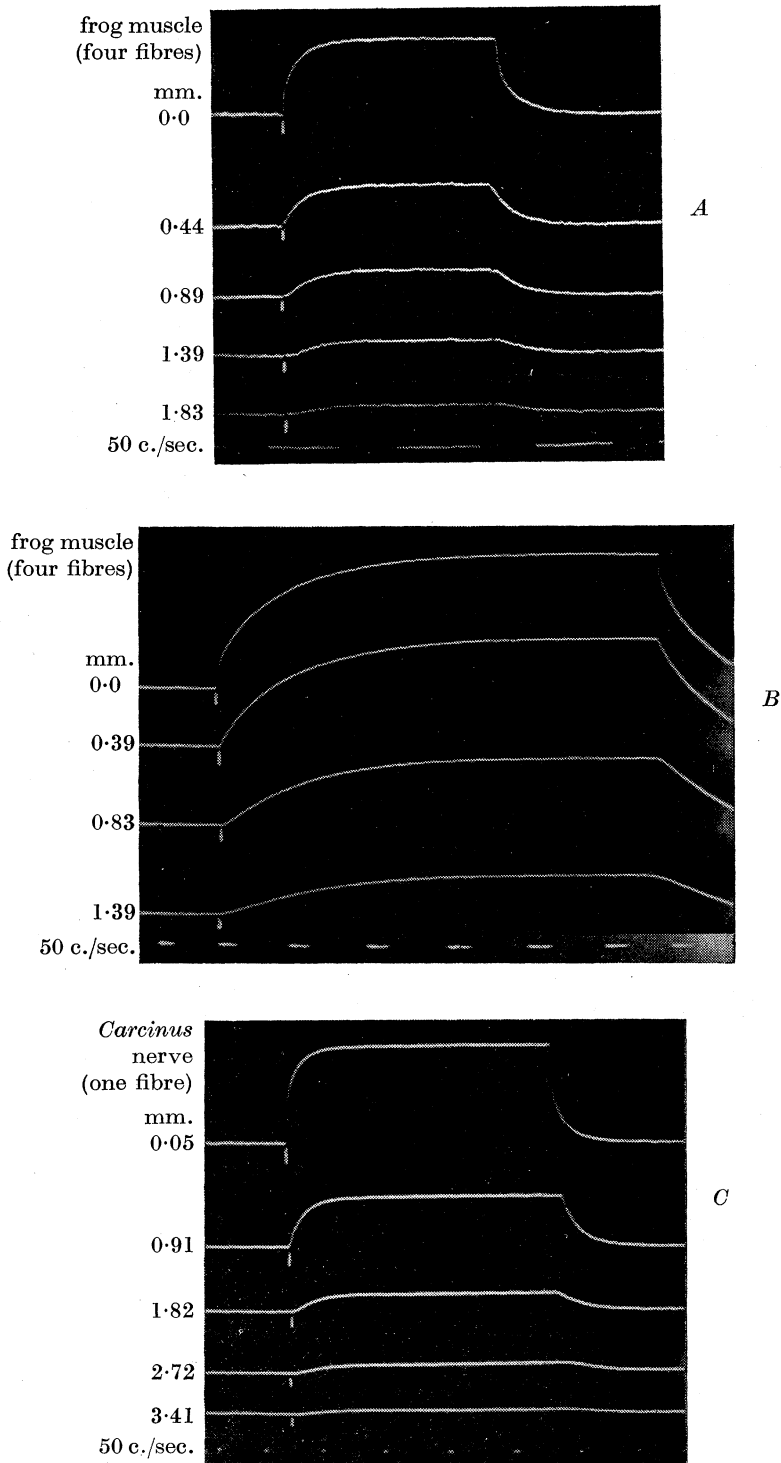rve. Single fibre of 43μ outside diameter. Extrapolar distances shown in mm. Instant of current make is indicated by a dash. Time marks at 20 msec. interval.

(1) *Extrapolar decrement and the value of* λ

In figures 5 *A* and *B*, the extrapolar potentials are shown in two preparations containing four and two muscle fibres respectively. There are considerable differences in the spatial spread and time course of the anelectrotonic potentials. In figure 5 *B*, the potential spreads *farther* and rises and falls more *slowly* than in figure 5 *A*. This indicates that in the former case the fibres had a substantially higher membrane resistance, while their membrane capacity may not have been very different from that in figure 5 *A*.

For comparison, the potential changes in a nerve fibre of *Carcinus maenas* are illustrated in figure 5 *C*. It is clear at once that the extrapolar potential spreads *farther* and rises and falls more *quickly* in the 30μ nerve axon than in the larger muscle fibres. This suggests that the membrane resistance of frog muscle is rather less, and its membrane capacity considerably larger, than in crab nerve.
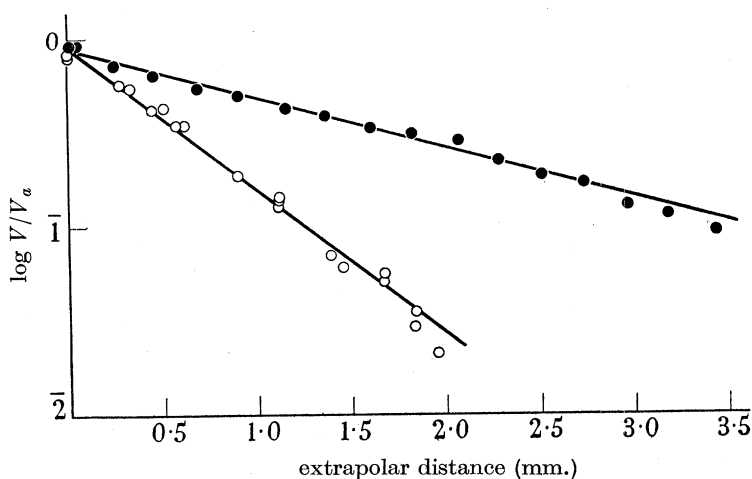
FIGURE 6. Extrapolar decrement of the steady potential change. Ordinates: $\log_{10}$ of voltage. Abscissae: extrapolar distance in mm. ○, four fibres of M. adductor magnus. Four series of observations. ●, *Carcinus* axon. Two series of observations.

To find the magnitude of these membrane constants, the next step is to determine the characteristic length λ and the time factor τ_m of the fibres. In figure 6, the final value of the extrapolar potential is plotted logarithmically against distance. There is no serious divergence from a linear relationship which shows that the decrement is approximately exponential as predicted by theory. The value of λ is 0·58 and 1·15 mm. in the experiments of figures 5 *A* and *B*, and 1·64 mm. in the *Carcinus* axon of figure 5 *C*.

The results of 9 experiments on small groups of muscle fibres are shown in table 1. The mean value of λ was 0·65 mm. and it varied between 0·47 and 1·15 mm.

The figures of table 1 are unselected results from muscle fibres capable of conducting impulses. The physiological conditions, however, of these fibres varied considerably. Some survived a prolonged experiment well, while others failed to conduct within 1 or 2 hr. after mounting in liquid paraffin (see Method), and the membranes of these fibres may have been abnormally leaky. In calculating mean

TABLE 1. ISOLATED MUSCLE BUNDLES

'weight'	number of fibres	fibre diameter (μ)	temperature $(^{\circ}C)$	λ (mm.)	τ_m (msec.)	R_i $(\Omega\,cm.)$	R_m $(\Omega\,cm.^2)$	C_m $(\mu F/cm.^2)$	percentage external fluid space
2	3	100, 100, 30	21·5	0·48	4·6	280	1030	4·5	11
1*	2	70, 70	28	0·47	6·5	131	650	10	15·5
4	4	70, 70, 45, 45	23	0·58	5·9	150	1010	6	22
3	4	90, 90, 90, 45	21	0·59	7	182	1370	5	12
1	3	120, 80, 80	21	0·91	13	266	2000	6·5	21
1	2	130, 50	22	1·15	27	175	4500	6	10·5
4	4	95, 75, 45, 30	23	0·66	7	151	1500	5	19
2	1	65	21	0·78	15	152	2100	7·3	17·5
1	1	60	22·5	0·7	6·5	220	1510	4·3	26·5
weighted mean	(1–4)	75	22·5	0·65	9	176	1500	6	17

* This preparation had been subjected to prolonged accidental stimulation.

values, the experiments have been 'weighted' as shown in column 1 of table 1, taking into account (i) consistency of threshold, (ii) clean appearance of fibre surface, and (iii) number and consistency of individual observations.

There were no significant differences between experiments on single fibres and on mixed bundles of 2 to 4 fibres. The values of λ observed in the two single-fibre experiments were close to the mean value of all the other experiments. In view of the marked disparity of fibre sizes in small muscle bundles, one might have expected some divergence from the exponential decrement, but no serious deviation was found (figures 6 and 9). There appear to be two explanations for this result: (a) The contribution which the small fibres make to the extrapolar potential may not be large enough to produce a significant departure from the exponential decay. (b) As Hodgkin & Rushton (1946) have pointed out, the membrane conductance per sq.cm. might be less in a fibre of smaller diameter. Such a relation is indicated by measurements on a variety of non-medullated axons (table 7), and it is easy to see that if muscle cells of all sizes are to come into ionic equilibrium with their environment in approximately the same time, the conductance of the resting cell membrane must vary in proportion to the volume/surface ratio. If a relation of this kind holds, the scatter of λ in fibres of different size would be greatly reduced.

(2) *The time constant τ_m*

The time constant of the membrane was determined by two methods: (i) by plotting the half-times of rise and fall of the extrapolar potential against distance; and (ii) by analyzing the potential change at the polarizing electrode (figure 7). The first procedure depends upon an empirical relation found by Hodgkin & Rushton (1946), viz. that the half-value of the electrotonic potential propagates with constant velocity which is equal to $2\lambda/\tau_m$. It is a fairly accurate method for it takes account of a series of measurements and is not subject to the errors introduced by the finite width of the electrodes.

Because of these errors the second method is likely to give an overestimate of τ_m. This seems to be borne out by the results, for the mean value of τ_m obtained by this

method was 25 % greater than the mean of the 'half-time' measurements. The figures in table 1 represent the average results obtained by both methods.

In some experiments the anelectrotonic potential showed a slight prolonged creep which is seen, for instance, in figure 3 *A*. A similar effect was seen occasionally in whole muscle (cf. also Schaefer *et al.* 1938). It was clearly due to some secondary change of the membrane potential which is not explained by the simple cable theory, but the effect was small and did not appreciably alter the result of the analysis.
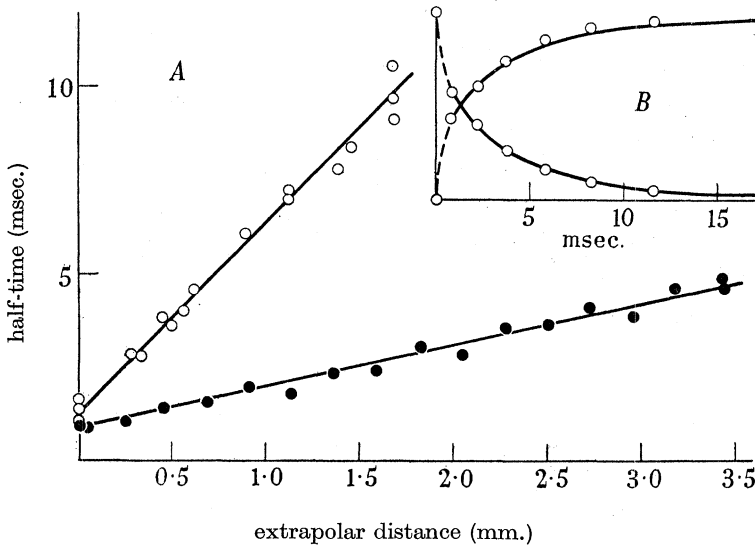

FIGURE 7. Determination of τ_m. *A*, 'propagation velocity' of half-value of the extrapolar potential. Ordinates: time required for rise, or fall, to one half of the steady deflexion. Abscissae: extrapolar distance. Same experiments as in figure 6. Each point represents the mean of two determinations made on rise and fall of the record. ○, four fibres of M. adductor magnus. Four series of observations. ●, *Carcinus* axon. Two series of observations. *B*, potential change at the polarizing electrode. Same muscle as in A. The two curves are tracings of rise and fall. The circles are calculated for $X = 0$ and $\tau_m = 5\cdot8$ msec. Method *A* gives $\tau_m = 5\cdot9$ msec.

The mean value of τ_m in all 9 experiments was 9 msec., but there were variations between 4·6 and 27 msec. It will be noted in table 1 that there was a correlation between the values of λ and τ_m in the individual experiments, and it appears that the variations of both quantities arose from an inconstancy of the membrane resistance (see also § B 4).

(3) *The longitudinal resistance of muscle*

The current encounters two kinds of resistance in the muscle: (*a*) the parallel resistance r of the interior of the fibres and the surrounding medium ($r = ml$, where l is the length of muscle and $m = r_i r_o/(r_i + r_o)$); and (*b*) a resistance y due to the back e.m.f. of the membrane at the polarizing electrodes ($y = V_A/I$, where V_A is the steady electrotonic potential at the electrode and I the current passing through it). Both values, m and y, are required for the further evaluation of the resistance and capacity of the membrane.

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To find y, the current I was measured by recording the potential drop across a monitor resistance R_6 (figure 1 (a)). The value of m was determined by reversing electrodes E_2 and E_4, recording the voltage gradient at various points of the equatorial region, and measuring the current as before.

In the measurement of m, some inaccuracy arises from small irregularities along the fibre surface, such as the electrode contacts and fine droplets of saline. Their effect is to give rise to a small electrotonic potential which is added to the expected rectangular wave form (see also Hodgkin & Rushton 1946). Another frequent complication consisted of a small but definite overshoot of the potential change at the make and break of the current. This could not be explained by surface irregularities or by the imperfections of electrode width and interelectrode distance, all of which produce a 'creep' and not an 'overshoot', nor could it be traced to an artefact. The effect was not observed if a *Carcinus* axon or a silk thread was used, and it was not due to inconstancy of the applied current which was monitored across R_6. Whatever their causes, the net result of all these complications was to introduce some uncertainty, for it was difficult to decide whether the initial or final deflexion of the record should be taken. Fortunately, the error never exceeded 10 %.

If we multiply the value of m by the cross-sectional area of the preparation we obtain the specific resistance of muscle tissue. This quantity may be compared with values previously determined by different methods. Hartree & Hill (1921) measured the longitudinal resistance of chloroform-treated frog's sartorii with alternating current (50 c./sec., 1 mA) and obtained a specific resistance of $159\,\Omega$ cm. at 20° C. Bozler & Cole (1935) found that the transverse high-frequency resistivity of a frog's sartorius at $16\cdot5^\circ$ C was about $185\,\Omega$ cm. In table 2, the results of 9 experiments on small bundles of fibres, and of 14 experiments on extensor muscles are shown, the mean values, at 22° C, being 156 and $157\,\Omega$ cm. respectively. There is a large scatter, especially in the isolated fibres, but this is almost certainly due to the large error in finding the cross-sectional area of the preparation (see Method).

TABLE 2. SPECIFIC RESISTANCE OF MUSCLE

A. Isolated fibres and small bundles

temperature (° C)	21·5	28	23	21	21		
specific resistance (Ω cm.)	228	116	128	160	184		
							mean
temperature (° C)	22	23	21	22·5			22·5
specific resistance (Ω cm.)	164	135	135	155			156

B. Extensor dig. IV

temperature (° C)	20	20	23	22	23·5	25	26·5	
specific resistance (Ω cm.)	189	151	182	153	158	151	143	
								mean
temperature (° C)	25	25	22·5	17	18·5	20		22
specific resistance (Ω cm.)	140	129	163	163	169	157		157

As Hartree & Hill (1921) pointed out, the resistivity of frog muscle is approximately equal to that of a 0·36 % NaCl solution. It is clear that with all three methods, only the parallel resistances of external and fibre medium were involved, for the membranes offer no appreciable impedance to high frequency A.C. (Bozler & Cole

1935), nor to any current after treatment with chloroform (see Method, cf. also Guttman 1939), nor are they involved to any appreciable extent with measurements made in the equatorial region (cf. Hodgkin & Rushton 1946). Thus, the high specific resistance of the whole tissue shows that the conductivity of the myoplasm is low, probably rather less than one-half that of the Ringer solution outside.

(4) *The electric constants of muscle fibres*

Having determined the values of λ, τ_m, y and m, and knowing the volume and surface of the fibres, we are now in a position to calculate the relevant constants. For this the following equations are used (see Hodgkin & Rushton 1946).

$$(a) \qquad r_i/r_o = m\lambda/2y,$$

$$(b) \qquad \text{Vol.}_{\text{ext.}}/\text{cm.} = R_o/m(1 + r_o/r_i),$$

$$(c) \qquad R_i = \Sigma(\pi\rho^2)\, m(1 + r_i/r_o),$$

$$(d) \qquad R_m = Sm\lambda^2(2 + r_i/r_o + r_o/r_i),$$

$$(e) \qquad C_m = \tau_m/R_m.$$

(a) The value of r_i/r_o was not very accurate as it was affected by random errors of three measurements. It is, however, a useful quantity for it gives an indication of the amount of external shunting and enables one to calculate the magnitude of the membrane action potential, which is equal to the observed spike multiplied by $(1 + r_i/r_o)$. The recorded action potentials in single fibres and in synchronous volleys of small bundles varied between 35 and 85 mV. The calculated action potentials across the fibre membrane were 70 to 120 mV. The upper value is close to the figures given by Hodgkin & Huxley (1945), Hodgkin & Rushton (1946) and Hodgkin (1947) for various non-medullated nerve fibres.

Some of the small fibre bundles had been carefully freed from loose connective tissue, and this seemed to be reflected in a low value of r_i/r_o. In the 9 experiments on isolated bundles, r_i/r_o varied between 0·25 and 0·96, with a mean of 0·47. In 13 experiments on the M. extensor dig. IV, r_i/r_o was on the average 1·08 and varied between 0·47 and 1·75. An early experiment on the extensor muscle gave the exceptional value of 3·2, in this case no attempt had been made to drain off excess fluid.

(b) The outside fluid volume is shown in tables 1 and 5. In isolated fibres the external fluid space amounted to 17 % (10·5 to 26·5 %), in the foot muscles to 27 % (17 to 45 %) of the total volume. These figures are higher than the 13 % which have been obtained by chemical methods on the frog's sartorius (Boyle, Conway, Kane & O'Reilly 1941). The difference is probably due to the fact that the sartorii had been blotted with filter paper, a procedure which could not be adopted on the present preparations.

(c) The specific resistance R_i of the myoplasm is obtained from the previous data and the cross-sectional area of the fibres. The individual values are probably accurate only within 40 %. The results are shown in tables 1 and 5. In isolated fibres the unweighted mean value at 22·5° C was 188 Ω cm., in the foot muscles it was 255 Ω cm. at 22° C. The average of all 23 experiments at 22° C was approximately 230 Ω cm., i.e. about 2·9 times the resistivity of Ringer solution. By a different method, Bozler

& Cole (1935) arrived at $260\,\Omega$ cm. at $16\cdot5^\circ$ C and concluded that the conductivity of muscle fibres is one-third of that of Ringer solution. According to Boyle & Conway (1941), 23 % of the fibre volume is taken up by non-solvent space, hence the specific resistance of the 'fibre water' would be about $180\,\Omega$ cm. at 22° C.

(d) The value of the membrane resistance depends upon several measurements, though mainly those of λ, m and S. There is little doubt that the observed variations of R_m are too large to be explained by random errors, and they probably reflect the variable physiological state of the fibre membrane (see also Hodgkin & Rushton 1946; Lorente de Nó 1947).

The average value of R_m in small bundles was $1500\,\Omega$ cm.^{2}, and the extreme range 650 to $4500\,\Omega$ cm.^{2}.

(e) The mean value of C_m in these experiments was $6\,\mu\mathrm{F/cm.}^2$, varying between $4\cdot3$ and $10\,\mu\mathrm{F/cm.}^2$. It will be seen below that the membrane capacity of the whole muscle did not differ substantially from this value, but that its membrane resistance was considerably higher.

(5) *Experiments on single nerve fibres of* Carcinus maenas

The specific membrane capacity of muscle fibres is much higher than the value of 1 to $1\cdot5\,\mu\mathrm{F/cm.}^2$ which has been found for many other cell membranes (Cole 1940; Hodgkin & Rushton 1946). This suggests that the muscle membrane has a higher dielectric constant or is thinner than the other membranes which have been investigated. Before accepting this conclusion, it was considered advisable to make control experiments on nerve axons of *C. maenas* (see Method).

An example of the results has been shown in figures 5 to 7 which illustrate the fact that the length constant λ is greater and the time constant τ_m smaller in *Carcinus* nerve than in frog's muscle. A summary of 3 experiments is shown in table 3 which agrees substantially with the values obtained by Hodgkin (1947). The value of R_i is rather lower than Hodgkin's figure, but this difference cannot be pressed in view of the multiple errors which affect the measurement of this constant. The membrane capacity was $0\cdot72$ to $1\cdot75\,\mu\mathrm{F/cm.}^2$, with a mean of $1\cdot17\,\mu\mathrm{F/cm.}^2$. In Hodgkin's 8 experiments, C_m was $0\cdot7$ to $2\cdot03\,\mu\mathrm{F/cm.}^2$ with a mean of $1\cdot11\,\mu\mathrm{F/cm.}^2$. Thus the membrane capacity of *Carcinus* axons obtained in the present experiments agrees satisfactorily with that of other observers, and this lends more weight to the statement that there is a genuine difference in the case of muscle.

TABLE 3. *CARCINUS* NERVE FIBRES

temperature ($^\circ$ C)	outside diameter of preparation (μ)	λ (mm.)	τ_m (msec.)	R_i (Ω cm.)	R_m (Ω cm.^{2})	C_m ($\mu\mathrm{F/cm.}^2$)
17	42	$1\cdot6$	$2\cdot1$	62	2940	$0\cdot72$
$16\cdot5$	32	$1\cdot81$	$5\cdot5$	36	3150	$1\cdot75$
16	43	$1\cdot64$	$3\cdot9$	66	3700	$1\cdot05$

C. *Experiments on the whole sartorius muscle*

As the physiological state of the isolated fibres was probably not normal, the experiments were repeated on whole muscle. The limitations which are imposed

by the thickness of the tissue and by the presence of a non-uniform population of fibres have been discussed above (Method). Preliminary experiments were made on sartorius muscles, with the following results.

Effect of thickness of muscle. The extrapolar potential of the sartorius shows two distinct components, a rectangular wave whose sign reverses when the recording electrode is placed on the opposite side of the muscle, and a proper electrotonic potential. The nature of the two components has already been discussed (see also Biedermann 1896–8). The rectangular wave-form arises from the transverse resistance of the muscle volume and, as pointed out by Schaefer *et al.* (1938), it can be balanced by feeding a suitable portion of the applied voltage into the recording circuit. Unfortunately this procedure does not alter the fact that the current flow in this relatively thick sheet of muscle is not parallel, and that therefore a quantitative analysis of the results becomes impracticable. The layer of muscle fibres which makes intimate contact with the polarizing electrode is subjected to a steeper longitudinal potential gradient than the more remote layers, hence the apparent value of λ varies with the position of the fibres which have been selected for measurement. This was confirmed by exploring the surface of the muscle with a capillary electrode. There was a very sharp electrotonic decrement near the polarizing electrode, but a much more diffuse spread of the potential on the opposite side of the muscle.

If the polarizing electrode is applied to both sides of the muscle, these complications are reduced. In this way the thickness of the tissue is effectively halved, and the situation conforms more nearly to the theoretical case. In table 4, the results of an experiment are summarized. As expected, the apparent value of λ became greater when the polarizing electrode was applied to both sides of the muscle, but there is no certainty that the true value of λ had been reached. The quantities of R_m and C_m in table 4 were computed on the assumption of uniform fibre size (450 fibres of 73μ diameter). The results of series B agreed fairly well with those of tables 1 and 5, but not much confidence could be placed in them, and these experiments were discontinued.

TABLE 4. APPARENT CONSTANTS OF SARTORIUS MUSCLE

	λ (mm.)	τ_m (msec.)	R_i (Ω cm.)	R_m (Ω cm.^{2})	C_m (μF/cm.^{2})
A, E_2 applied to one side	0·55	10·3	218	1180	8·7
B, E_2 applied to both sides	0·8	10·8	248	2080	5·2

Effect of external perimysium. The outer surface of the sartorius muscle is covered by a strong sheath of connective tissue, several microns thick, while the inner surface of the muscle is freely exposed. It was of interest, therefore, to measure the extrapolar potential when electrodes $E_{1\text{-}4}$ were applied to alternate sides of the muscle. If the external perimysium were responsible for an appreciable part of the electrotonic potential, then this potential should become much greater if polarizing and recording electrodes are placed in contact with the perimysium, and most of the current is forced to penetrate it, than if the electrodes are on the opposite side of the muscle.

No significant difference, however, was observed, and it appears, therefore, that the external perimysium provides no serious hindrance to the movement of sodium and chloride ions.

D. *Experiments on the M. extensor longus dig. IV*

The same test as used in the experiment of table 4 showed that the M. extensor longus dig. IV was satisfactory for the present experiments (see Method). In an experiment in which the polarizing anode was applied (i) to one side, (ii) to two opposite sides, and (iii) to the four sides of the muscle, no significant difference in the spread of the extrapolar potential was observed, the values of λ being 1·56, 1·61 and 1·44 mm. and the values of τ_m 29·6, 32·2 and 31·5 msec. respectively. Furthermore, the extrapolar potential had no 'rectangular' component except when there was a direct fluid contact between electrodes E_2 and E_3 (figure 1 (*b*)). To minimize any residual errors which might arise from divergent current flow, the anode was always applied to both sides of the muscle.
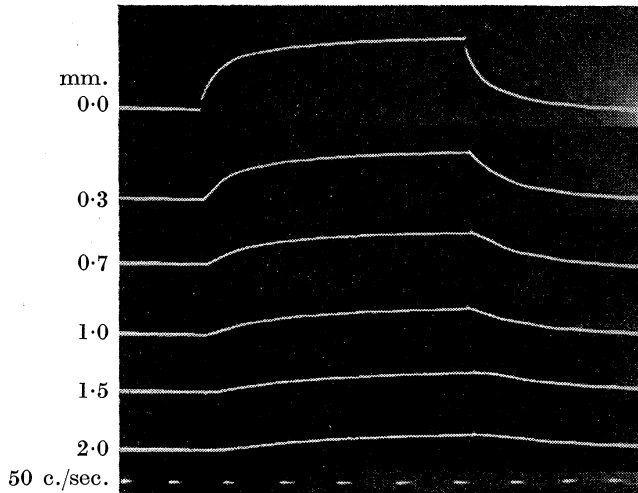

FIGURE 8. Anodic potential changes in M. extensor longus dig. IV. Distance between electrodes E_2 and E_3 shown in figure. Instant of make and break marked by a faint spike. At 'zero distance' a small instantaneous deflexion arose from the common resistance of polarizing and recording electrode (see Method).

The methods of measurement differed from those described above in only one respect, namely, that an extrapolation had to be made when calculating the value of y (see Method). This was necessary because of the appreciable width of the electrode contacts. For the same reason, the value of τ_m was determined by relying solely upon 'half-time' measurements at various distances (§ B (2)), and not upon an analysis of the potential at the contact of E_2 and E_3. These limitations, however, are not likely to diminish the accuracy of R_m and C_m.

An example of the extrapolar potential changes is shown in figure 8. Results of a similar experiment are plotted in figure 9, which indicates that the wave form and

attenuation of the electrotonic potential in these muscles agrees satisfactorily with the cable equations.

The results of 15 experiments are summarized in table 5, to which only a few comments are required.
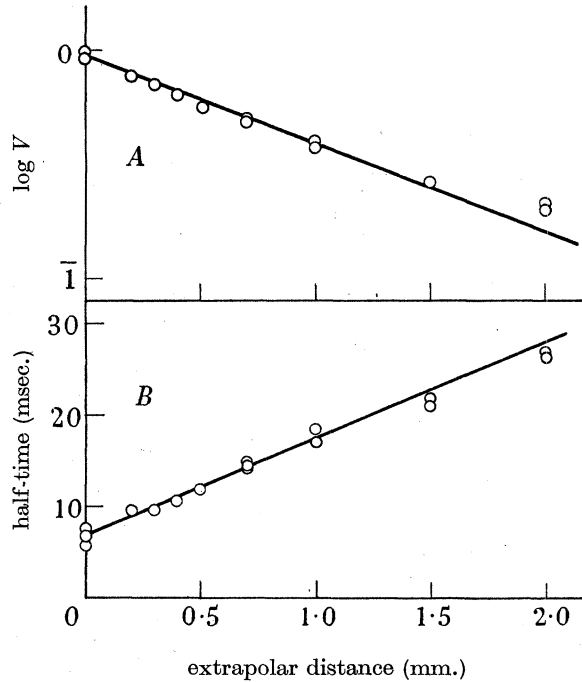

FIGURE 9. Measurement of λ and τ_m in M. extensor dig. IV. *A*, spatial attenuation, plotted as in figure 6. *B*, 'propagation velocity' of half-value, plotted as in figure 7. In each case, two series of observations, made with current pulses of 90 and 220 msec. duration respectively.

TABLE 5. M. EXTENSOR LONGUS DIG. IV

temperature (° C)	λ (mm.)	τ_m (msec.)	R_i (Ω cm.)	R_m (Ω cm.²)	C_m (μF/cm.²)	percentage external fluid space
20	0·97	12	320	2600	4·6	26
20	1·5	30·5	330	9500	3·2	45
22	0·98	15·5	226	3900	4·1	28
23	1·06	11·9	355	4100	2·9	29
23·5	0·88	12·5	234	3000	4·2	21
24	1·45	21·7	—	—	—	—
25	0·75	7·3	222	1500	4·9	25
26·5	1·2	22·5	218	5300	4·3	28
25	0·96	15	218	2900	5·2	27
25	1·34	38	329	6400	5·9	22
25	1·53	20	214	5000	4·0	38
22·5	1·20	25·5	290	6200	4·1	31
17	0·93	11·5	210	3200	3·6	23
18·5	1·04	17	206	4200	4·1	17
20	0·86	13	210	2200	5·8	24
mean 22	1·1	18·5	255	4300	4·4	27

The average diameter of the fibres is considerably smaller than in the thigh muscles, 45μ as compared with 75μ. A similar result was obtained by Mayeda (1890) who gave the following values:

                  frog's sartorius: mean diameter 84μ, range 19 to 152μ;
                  extensor longus dig. I: mean diameter 46μ, range 11 to 80μ.

This difference between foot and thigh muscles must be borne in mind when comparing their electric constants, for a correlation between fibre diameter and membrane conductance has been suggested by Hodgkin & Rushton (1946).

The values of λ, τ_m and R_m are, on the average, substantially greater in the extensor muscle than in the isolated bundles of thigh muscles (see table 1). This may reflect a genuine difference between the two types of muscles and would agree with the suggested relation between fibre size and permeability. But it is probable that a major part of the difference in R_m, and in the other constants which depend upon R_m, is to be explained by the better physiological condition of the undivided muscle.

There does not appear to be a marked difference in the values of C_m in tables 1 and 5, and the mean values become more nearly equal if account is taken of the different methods in deriving them. To make the comparison between isolated fibres and whole muscle more precise, the mean value of C_m in table 1 should be replaced by one which is based entirely on 'half-time' measurements. We obtain then for the isolated bundles a mean value of C_m of $5\cdot3\ \mu\mathrm{F}/\mathrm{cm}.^2$ with a standard error of the mean of $0\cdot55\ \mu\mathrm{F}/\mathrm{cm}.^2$ (9 experiments), and for the extensor dig. IV a mean of $4\cdot4\ \mu\mathrm{F}/\mathrm{cm}.^2$, with a standard error of $0\cdot23\ \mu\mathrm{F}/\mathrm{cm}.^2$ (14 experiments).

E.   *The relation of the membrane constants to physiological properties of muscle*

The large capacity of the membrane is probably an important factor in determining the long time-scale of the electric reactions of muscle. This will be considered more fully on three examples: (i) the time factor of excitation of muscle (Lucas's (1907/8) and Rushton's (1930) α-constant); (ii) the conduction rate; and (iii) the end-plate potential in curarized muscle.

(1) *The time factor of electric excitation*

It was first shown by Keith Lucas (1907/8) that the strength-duration curve of a sartorius muscle has two or three components of which only the slowest is due to the muscle fibres. This conclusion was temporarily disputed, but it is now clearly established that the 'chronaxie' of muscle at 20° C is of the order of 10 msec., provided the electrodes have been arranged so as to ensure an approximately parallel current flow and that the points of current entry and exit are at least a few mm. apart (Rushton 1930; Schaefer *et al.* 1938; Ramsay & Street 1941; Blair 1941). Schaefer *et al.* (1938) have recorded electrotonic potentials from a sartorius muscle and concluded that the half-time in the vicinity of the polarizing electrode was approximately equal to the chronaxie. These authors assumed that the development of the electrotonic potential as well as that of the excitation process were governed by a single exponential time factor. This view can only be regarded as a rough approximation, but the general conclusions reached by Schaefer *et al.* (1938) and the order of magnitude of their quantities are substantially correct.

The threshold of electric stimulation is not governed solely by the 'passive' attainment of a certain catelectrotonic potential (see Rushton 1937; Katz 1939; Hodgkin & Rushton 1946). It is evident from figure 3 A that the applied current must be maintained during the period of inflexion until the local regenerative process at the cathode has reached sufficient intensity to propagate itself. Hence, the time factor which is derived from a strength-duration curve does not characterize a simple electrotonic charging process, but a sequence of events of which only the first stage is determined by the resistance and capacity of the resting membrane. The later stages depend upon the character of the local response and involve active changes of the membrane constants. Nevertheless, a large value of R_m and C_m of the resting, or anodically polarized, membrane entails a slow initial development of the excitatory disturbance and must be reflected in a large time factor of excitation.

(2) *The propagation velocity of muscle*

The conduction velocity in a sartorius muscle, at $20°$ C, is approximately 1·6 m./sec. (Eccles *et al.* 1941). In isolated fibres of 70 to 80μ diameter the conduction rate was about 20 % less, even after allowing for the high external resistance. This difference, however, may be attributed to a state of lower excitability (§A). In the M. extensor dig. IV the propagation velocity was measured after curarization, and values close to that of the sartorius muscle were found. At $20°$ C, the foot and front of the muscle spike, i.e. presumably the largest fibres, travel at a speed of 2·2 m./sec. The spike summit gave a value of 1·6 to 1·8 m./sec. Occasionally, a slow fibre could be stimulated separately from the rest. The lowest speed which could thus be observed was 1·0 m./sec. at $20°$ C.

These values are lower than those of crustacean nerve axons of the same or smaller diameter (cf. Hodgkin 1939; Prosser 1946). A comparison of conduction rates in different fibres is difficult, because the conduction of impulses depends upon many variable factors, and usually only one or two of these variables are known. Moreover, an entirely adequate theory of propagation has not yet been developed. The local circuit theories of Blair (1934), Rashevsky (1936), Rushton (1937) and Offner, Weinberg & Young (1940) lead to an equation of the type $v = S'L/\alpha$, where v is conduction velocity, L length constant, α time constant of the membrane and S' a 'safety factor' which depends upon the size of the action potential and the excitability of the membrane. These theories are based on the assumption of a sharp boundary between resting and active region, and according to Offner *et al.* (1940), the values of L and α of the *active* region should be used.

If we consider propagation *in situ*, or in a large volume of tissue or saline, we can transform the above equation into

$$v = \frac{S'}{\sqrt{(2R'_m)}} \times \frac{\sqrt{\text{radius}}}{C_m\sqrt{R_i}},$$

where R'_m is the active membrane resistance (Ω cm.^{2}) and C_m the membrane capacity, changes of which during activity are neglected (see Cole & Curtis 1939). Thus conduction velocity depends upon five quantities of which three, namely, the radius of the fibres, the membrane capacity and the specific internal resistance, have been

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measured in some invertebrate nerve and in muscle fibres, while the 'safety factor' is not accurately known, and the active membrane resistance has only been worked out for the squid giant axon. It is clear, however, that according to all these theories the membrane capacity plays an important part in determining conduction speed. In table 6, the values of $C_m^{-1} \times \sqrt{(\text{radius}/R_i)}$ have been listed for squid nerve, crab nerve and frog muscle and compared with the conduction velocities in a large volume of saline. There is a reasonable correlation between the three physical factors and the conduction velocity, though the matter cannot be pursued until the other factors have been clarified.

TABLE 6. CONDUCTION VELOCITIES *IN SITU* AT $20°$ C

	radius (μ)	C_m (μF/cm.^{2})	R_i (Ω cm.)	$\dfrac{\sqrt{\text{radius}}}{C_m \sqrt{R_i}}$ (rel. units)	conduction velocity (m./sec.)	data taken from
squid nerve	250	1·1	30	26	25	Curtis & Cole (1938), Hodgkin (1939), Cole & Hodgkin (1939)
crab nerve	15	1·1	60	4·5	4·5	Hodgkin (1939, 1947), this paper
frog muscle	30	4·8	230	0·75	1·6	this paper

(3) *The membrane constants and the end-plate potential in curarized muscle*

When neuro-muscular transmission is blocked by curarine, a nerve impulse produces a subthreshold depolarization of the muscle, the end-plate potential (e.p.p.), which spreads a few mm. along the fibre surface (Eccles *et al.* 1941). This e.p.p. decays much more slowly than the muscle spike, and two alternative explanations have been put forward for this behaviour. According to Coppée (1943), the e.p.p. arises from a prolonged activity at the nerve endings and is, in fact, a negative after-potential of the motor end-plate. According to Eccles *et al.* (1941), however, the period of activity during neuro-muscular transmission is very brief, of the order of 4 msec., and the prolonged decline of the e.p.p. is due to the slow dissipation of charge along and across the resting fibre membrane.

If it is true that the depolarizing action of the transmitter is turned 'on' and 'off' within about 4 msec., then it should be possible to reproduce the e.p.p. by applying an equally brief subthreshold current pulse to the muscle. As the most accurate information on the spread of the e.p.p. was obtained on whole muscle, this experiment was made on the M. extensor dig. IV, using the circuit diagram of figure 1 (b). Tracings of the extrapolar potential are shown in figure 10; they may be compared with the e.p.p. at a junctional focus of the sartorius (taken from Eccles *et al.* 1941, figure 5). The resemblance of the two types of potential is close and becomes even closer if account is taken of differences in temperature and the fact that the rate of decline of the e.p.p. has a Q_{10} of 1·3 (Eccles *et al.* 1941). The potential changes differ appreciably only in the rate of attenuation over the first mm.: the e.p.p. spreads further, probably because the individual nerve endings are scattered over a narrow zone of muscle (Eccles *et al.* 1941, p. 367). There is, however, little doubt that a very

brief influence, such as the subthreshold current pulse shown in figure 10, leaves behind a potential change which decays slowly and is almost indistinguishable from an e.p.p.

In his study of isolated junctions of the M. adductor longus, Kuffler (1942) recorded e.p.p.'s which are quicker than those obtained in whole muscle. This difference may be attributed to two causes: (i) in whole muscle the e.p.p. is recorded
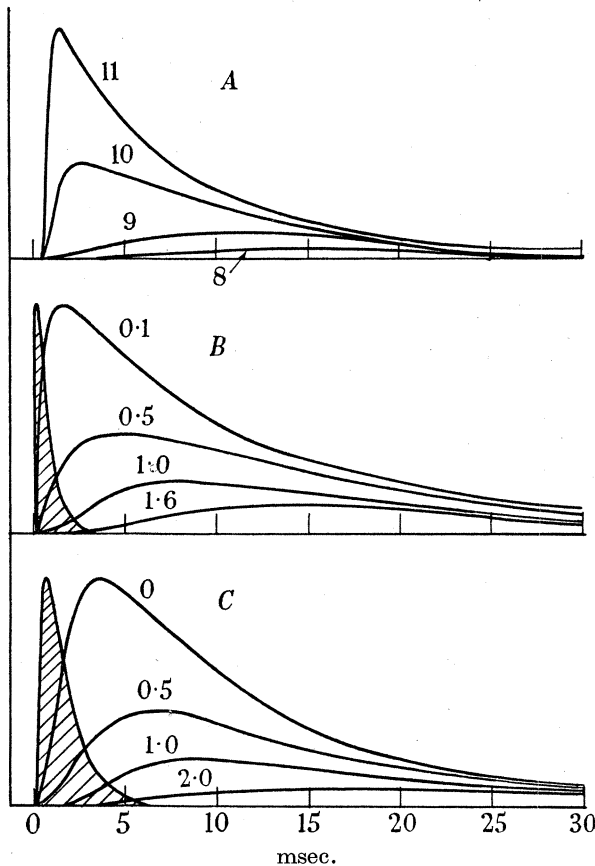

FIGURE 10. End-plate potential of curarized frog muscle (A) and its reconstruction by a brief current pulse (B and C). A, from Eccles *et al.* (1941, figure 5), showing e.p.p. in frog's sartorius at 27·5° C. Distances from pelvic end shown in mm. B and C, M. extensor dig. IV. Extrapolar potentials produced by a brief current pulse which is shown by the shaded area. Distances between leads E_2 and E_3 in mm. B, 22·5° C., $\lambda = 1·04$ mm., $\tau_m = 17$ msec. C, a longer current pulse. 18·5° C., $\lambda = 1·2$ mm., $\tau_m = 25$ msec. The curve at 'zero distance' has been corrected for a small current escape (cf. figure 8).

at a small distance from the majority of the junctions, and (ii) the isolated fibre had presumably a fairly low membrane resistance. If the mean value of τ_m ($= 9$ msec.) obtained on isolated fibres is valid for Kuffler's preparations, we can pursue the analysis of the e.p.p. a step further. In figure 11 the e.p.p. recorded from the junction of a curarized muscle fibre (in paraffin oil) has been reproduced (Kuffler (1942), figure 8). By a simple numerical analysis suggested to me by Professor A. V. Hill and using Hodgkin & Rushton's (1946) equation 4·1 we can now determine the

current pulse which would produce an electrotonic potential, at the electrode, identical with the e.p.p. The result of this analysis (figure 11 A) is a brief pulse which rises and falls within 4 msec. This agrees very well with Kuffler's (1942) conclusion, viz. that the active phase of the transmitter cycle is completed within 4 msec. A similar, but more approximate, analysis has been made for the e.p.p. in a curarized M. extensor dig. IV (figure 11 B). In this case, the 'transmitter pulse' derived in figure 11 A has been adopted and the e.p.p. reconstructed by reversing the above analysis.
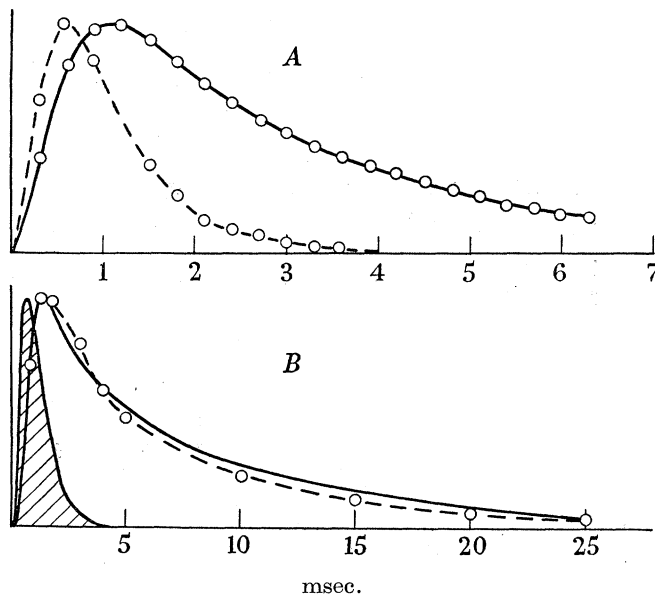

FIGURE 11. A, full line: from Kuffler (1942, figure 8), showing e.p.p. at an isolated junction of M. adductor longus. Broken line: analyzed subthreshold current which is equivalent in its action to the neuro-muscular transmitter. Analysis based on $x = 0$, $\tau_m = 9$ msec. Circles show results of the numerical analysis. B, full line: e.p.p. in a curarized M. extensor dig. IV; $\lambda = 0.86$ mm., $\tau_m = 13$ msec. Shaded area: equivalent current pulse, as in A. Circles and broken line: electrotonic potential produced by this pulse at $x = 0.1\lambda$.

Thus, the results of the cable analysis offer strong supporting evidence for the thesis that the active phase of myoneural transmission is brief, and that the e.p.p. is prolonged merely because the muscle membrane can hold electric charge for many milliseconds. There are, however, limitations to the accuracy of the present analysis, for the depolarization by an electric current does not completely imitate that by a chemical transmitter (see Katz 1942), nor can we reproduce the exact situation of the synapse by an externally applied electrode.

The suggestion of a brief action and prolonged passive decay applies to curarine-treated muscle, but not necessarily to other cases, e.g. not to eserinized muscle in which the active period is known to be lengthened (Eccles et al. 1942). Coppée's (1943) evidence was derived largely from experiments with various other drugs, under whose influence the active phase of the transmitter cycle may well have been prolonged.

DISCUSSION

The experiments described in this paper confirm that the electrical properties and reactions of the muscle membrane are very similar, apart from quantitative differences, to those of a non-medullated nerve axon. In view of the different structure and function of the two types of cells, the analogous behaviour of their surface membranes has frequently been questioned. For example, Dubuisson (1942) in a recent review has discussed the possibility that the distribution of ions in muscle fibres is essentially different from that in nerve, and that the bulk of the muscle potassium might be enclosed in the anisotropic disks of the fibrils rather than being dissolved in the cell water. Although such arguments are speculative, one must seriously consider whether the muscle fibre may not be subdivided into smaller 'cable elements', and whether potential changes at the boundaries between myofibrils and sarcoplasm might not conceivably be responsible for the electrotonic potential of the muscle fibre.

It is worth while, therefore, to summarize the evidence which shows that the surface membrane of the muscle fibre is the most probable seat of the observed potential changes.

The structures at which the electrotonic potential (or the e.p.p.) and the action potential of muscle arise, appear to be identical, for not only does the spike develop from the catelectrotonic potential, but the latter is temporarily abolished and gradually rebuilt after the spike has passed (Kuffler 1942; see also § A above). The two voltages do not sum, as they would if they arose at two separate types of membrane in series with one another.

Hence, the argument concerning the site of the membrane applies to the spike as well as to the electrotonic potential. The fact that the spike is an all-or-none reaction which involves the muscle fibre as one unit makes it difficult to believe that the action potential can be built up by numerous smaller units inside the cell. More direct evidence has been obtained recently by Graham & Gerard (1946) who recorded large resting and action potentials when a microelectrode was inserted into a muscle fibre. With this technique the potential across the surface membrane of the fibre must have been recorded, for it is inconceivable that a recording electrode could have penetrated a myofibril without destroying it.

The ratio r_i/r_o in the present experiments is relevant to this problem. In clean muscle bundles r_i/r_o was as low as 0·25, with a mean of 0·47. Assuming that the electrotonic potential arose at the *surface* membrane, r_o and r_i are the resistances, respectively, of the interstitial fluid and the fibre contents. But on the hypothesis that the electrotonic potential arose at the interfaces of myofibrils and sarcoplasm, r_i would be the resistance of the myofibrils and r_o the parallel resistance of sarcoplasm and surrounding Ringer space. According to Dubuisson (1942, p. 451) the volume proportions of Ringer space, sarcoplasm, and myofibrils are 14·5 : 28·5 : 57. If we use these data, the specific resistance of Ringer solution (83 Ω cm. at 22° C) the average resistivity of muscle (157 Ω cm.) and the mean value of r_i/r_o (0·47), we can test the validity of both hypotheses. On the first assumption it can be shown that the specific resistance of the outside fluid would be 71 Ω cm., which is not far from the

correct value of $83\,\Omega$ cm. On the second hypothesis, the external space includes the sarcoplasm, and we can calculate what the specific resistance of 'myofibrils' and 'sarcoplasm' should be to satisfy this hypothesis. The results are 132 and $983\,\Omega$ cm. for fibrils and sarcoplasm respectively. That is to say, the fibrils would have to be better conductors than the cell sap if we are to record appreciable potential changes from them. According to Schmitt, Bear, Hall & Jakus (1947) the myofibrils are made up of numerous protein filaments which presumably contribute little to the conduction of current. It is, therefore, more probable that these structures, far from being 'core-conductors', are effectively short-circuited by the potassium-rich fibre water (Boyle & Conway 1941), and that their only contribution to the electrical measurements is that of an insulator which takes up a fraction of the cell volume and thereby increases the value of R_i.

One may reasonably conclude that R_m and C_m represent properties of the surface membrane of the muscle fibres. In table 7 the known membrane constants of several excitable tissues have been listed; they include frog's muscle as well as three types of non-medullated nerve. When surveying the values of R_m one gains the impression that there is a relation between fibre size and membrane conductivity, as pointed out by Hodgkin & Rushton (1946). In comparing frog tissue with that of marine invertebrates, account must be taken of the different salinity of external and internal medium. The conductance of a sq.cm. of membrane is roughly the same in a muscle fibre as in an equally large crustacean nerve fibre, in spite of the fact that the salt solutions which surround the muscle membrane have a 4 times lower conductivity. This would seem to suggest that the surface membrane of muscle is a less effective ion barrier than that of a nerve axon of the same diameter. The membrane capacity, however, is much larger in muscle than in the three non-medullated nerves. It is possible that both differences arise from a single fundamental property: for example, if the muscle membrane were thinner, or more thoroughly impregnated with ions, than the nerve membranes, the differences of R_m and C_m would be explained.

TABLE 7. ELECTRICAL CONSTANTS OF NERVE AND
MUSCLE FIBRES (TEMP. APPROX. 20° C)

fibre type	diameter (μ)	λ (mm.)	τ_m (msec.)	R_m (Ω cm.²)	C_m (μF/cm.²)	R_i (Ω cm.)	R_o (Ω cm.)	data from
squid nerve	500	2·5	0·7	700	1	30	22	Curtis & Cole (1938); Cole & Hodgkin (1939)
lobster nerve	75	1·5	2	2000	1	60	22	Hodgkin & Rushton (1946)
crab nerve	30	2	5	5000	1	60	22	Hodgkin (1947), and this paper
frog muscle (bundles of add. magnus)	75	0·7	9	1500	6	200	87	this paper
frog muscle (extensor dig. IV)	45	1·1	18	4000	4·5	260	87	this paper

The conductance R_m^{-1} has been regarded as an index of the total ion density and permeability of the membrane (Cole 1940). This is a reasonable concept, for it seems to imply no other assumption than that the resistance to the transport of ions across the membrane should be the same whether the moving force arises from a concentration gradient or from an electric potential difference. The analogy would fail, however, unless the value of R_m can be measured under conditions in which the membrane behaves truly as a passive conductor. To approach this condition, 'inward' currents were used and the analysis was restricted to the anodic region in which an approximately linear relation between current and voltage was found. Nevertheless, such linearity is not final proof of the 'passive' behaviour of the fibre membrane. It is conceivable—and a model has recently been described which illustrates this point (Katz 1947a)—that any current, anodic or cathodic, alters the ion permeability of the membrane and leads to a regenerative potential change across it. Thus, until there are direct means, for example, by the use of tracer ions, of testing the relation between electric conductance and ion permeability of the membrane, the theoretical significance of the value of R_m will remain uncertain.

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