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AN ANALYSIS OF THE END-PLATE POTENTIAL RECORDED WITH AN INTRA-CELLULAR ELECTRODE

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According to present knowledge, the process of neuromuscular transmission can be described by the following scheme: nerve impulse → acetylcholine → end-plate potential → muscle impulse → contraction. The evidence for this chain has been summarized by Eccles (1948), Hunt & Kuffler (1950) and Rosenblueth (1950), whose reviews may be consulted for further references. While there is little doubt that acetylcholine is released by the nerve impulse and depolarizes the end-plate, the mechanism of these two actions is at present unknown and requires further investigation. The most immediate electrical sign of neuromuscular transmission is the end-plate potential, a local depolarization of the muscle fibre which is presumably due to the direct action of the neuromuscular transmitter. By measuring this electrical change under suitable conditions, some light can be thrown on the preceding steps of the transmission process. The object of this paper is to investigate the properties of the end-plate potential even more closely than has previously been attempted, making use of the method of intra-cellular recording which has been developed by Graham & Gerard (1946), Ling & Gerard (1949) and Nastuk & Hodgkin (1950). This method offers several advantages: resting and action potentials at individual junctions can be recorded in whole muscles, without microdissection or even removing the muscle from a Ringer bath, and the measurements do not suffer from uncertainties which are usually associated with the shunting effect of the interstitial fluid.

The immediate concern of the present work is to determine the electric charge which passes through the end-plate membrane during the transmission of one impulse and to throw some light on the mechanism by which the transfer of ions across the end-plate is brought about.

METHODS

The method of intracellular recording was similar to that described by Ling & Gerard (1949) and Nastuk & Hodgkin (1950) except that the same muscle fibre, and often the same spot of the fibre, was used for a series of measurements and thus subjected to repeated insertions and withdrawals

of the microelectrode. This introduced an extra risk, for after a number of insertions local damage eventually resulted causing resting and action potentials to decline. It was, therefore, not always possible to complete a set of measurements and, in the non-curarized preparation, active movement of the muscle greatly increased the hazards of the experiment. But in spite of these inherent difficulties the method of repeated local insertions was satisfactory in many cases and gave consistent results which we could not have obtained by other means. For example, a quantitative study of the end-plate potential (e.p.p.) requires that the electric response in the same fibre should be measured at various distances from the junction. Furthermore, the size of the e.p.p. varies in individual muscle fibres much more than their resting potentials or spikes, and it was therefore desirable to compare measurements on the same junction when examining the effect of ions or drugs on the transmission process. Finally, it was only by successive insertion of the electrode at different points along a muscle fibre and so finding the position of maximum e.p.p. (cf. Fatt & Katz, 1950*a*, *b*), that we could be certain of having located an end-plate accurately. (The term 'end-plate' is used here to describe the post-synaptic area of a muscle fibre which is in contact with the motor nerve endings, ignoring the fact that in frog muscle the shape of the junction resembles a 'bush' rather than a 'plate'.)

Preparation. The nerve-sartorius preparation of the frog (*Rana temporaria*) was used and mounted in the chamber shown in Fig. 1. The chamber was moulded from paraffin wax set in a Petri dish and was so arranged that it could be completely drained of fluid from a depression at one side of the

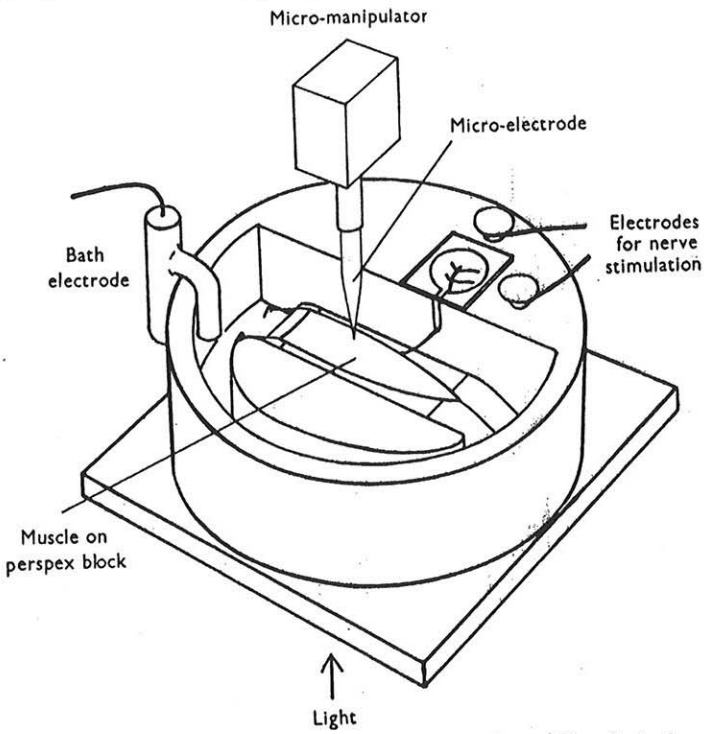

Fig. 1. Nerve-muscle chamber with stimulating and recording electrodes.

central trough. The muscle lay flat, deep surface uppermost, on a transparent Perspex block forming the floor of the central part of the chamber. It was held in this position by threads tied at each end which were looped under silver wire hooks embedded in the paraffin wax. The electrodes for nerve stimulation were situated in a separate moist compartment which was reached by the nerve

via a narrow groove. The Petri dish was fixed to a glass plate which was itself carried by a mechanical stage on another large plate. Illumination was provided by light passing up through both glass plates and through the Perspex block. The preparation was viewed with a binocular dissecting microscope of magnification × 39. An eyepiece micrometer served for measurement of short distances along the muscle fibres, while coarser movements were obtained with the mechanical stage and read on the attached vernier.

The depth of fluid in the bath above the muscle was about 3 mm. It was kept at this low value in order to minimize the capacity to earth across the glass wall of the microelectrode.

The bath electrode was in the form of an agar-Ringer solution bridge connecting to a chlorided silver spiral. This led to earth via small series resistances through which steady calibration voltages and square pulses could be applied.

The microelectrode was held by a short piece of rubber tubing which led through an agar-Ringer solution bridge to a chlorided silver ribbon. The microelectrode assembly was carried on a de Fonbrune micromanipulator, the controls for which were placed outside the shielding metal box containing the preparation.

Microelectrode and amplifier. Capillary microelectrodes of external tip diameter less than 0·5 μ. and filled with 3 M-KCl were used. The wire connecting the micro-electrode to the amplifier was shielded, and the shield connected to the cathode of the first valve (RCA 954). A balanced d.c. amplifier with input cathode followers of low grid current and reduced grid-to-earth capacity was

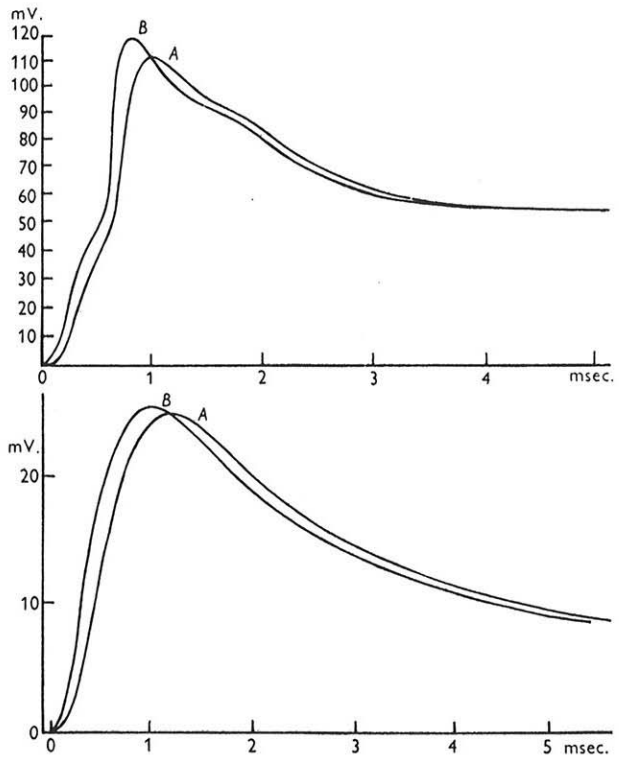

Fig. 2. Effect of amplifier distortion. Curves *A* are tracings of oscillograph records, curves *B* are corrected for high-frequency attenuation. Upper part: end-plate response during normal transmission (the muscle was treated with prostigmine, hence the large residual potential change). Lower part: curarized preparation, showing a pure e.p.p.

used, similar to those described by Nastuk & Hodgkin (1950) and Huxley & Stämfli (1949). The amplifier was calibrated by applying voltages in steps of 10 and 1 mV., and this procedure was also used to balance and measure resting potentials. In addition, a square pulse generator was used to test the time constant of the recording apparatus. This time constant varied with the resistance of the microelectrode which was apt to increase in the process of penetrating a muscle fibre. In some experiments a shielded junction box was inserted between microelectrode and amplifier. The box contained a micro-switch and 20 MΩ. shunt which could be placed across the input when a square pulse signal was applied. In this way it was possible to measure the resistance of the electrode when it was in the recording position. In other experiments a check was kept on the temporal distortion of a square pulse. The voltage wave-form at the amplifier input differed from the square pulse applied between bath and earth, in that it showed two distinct components, an instantaneously rising fraction which can be attributed to the initial displacement of charge at the glass wall of the immersed microelectrode and an exponentially rising portion which gives an indication of the time constant of the recording system. This time constant was usually between 50 and 200 μsec., and in some cases caused appreciable distortion in certain details of the electric response. In Fig. 2 a tracing is shown, together with a correction obtained by 'subtangent analysis' (cf. Rushton, 1937). The difference is rather more pronounced in this than in other experiments. On the whole, it was felt preferable to present the results without such correction, but it should be remembered that most time measurements given below are a little too large, exceeding the true value by about 0·1 msec.

Experimental procedure. In measuring membrane potentials the reference level was the potential of the bath on the surface of the fibres. As the tip of the microelectrode was moved from the bath into the interior of the fibre, the potential of the tip suddenly dropped by about 90 mV., and this drop was measured by compensating the deflexion of the cathode ray with a calibrated voltage input. The electric response, spike or end-plate potential, to a single stimulus was then recorded, the electrode withdrawn, and the return of its potential to the original level was checked, the whole process taking usually about 15 sec. During successive insertions, apart from random variations of a few per cent, there was usually a slight progressive decline of the membrane potential, and as a rule the experiment on an individual fibre was discontinued when the resting potential fell below 80 mV. In curarized muscles, it was sometimes possible to make more than twenty successive measurements on the same fibre before excessive injury occurred, and even in normal twitching muscle twelve to fifteen successive electrode insertions could, on some occasions, be carried out without serious injury. We presume that in these eases a fortunate combination of circumstances allowed the muscle to withdraw from the impaled electrode at the beginning of the twitch without damaging either the electrode or itself. On some occasions, an unusual sign of injury was observed which appeared to be due to damage of fine nerve branches rather than muscle fibres: in these cases, the resting potential of the muscle was undiminished, but its end-plate response suddenly failed, and it was sometimes observed that nearby end-plates in adjacent muscle fibres had also failed, indicating that some damage had been inflicted to the common nerve axon. The important fact was that the continued observations of resting potential and electric response in any given fibre provided by themselves an adequate check of the state of the preparation.

Localization of end-plates. Fig. 5 shows a series of records obtained by recording at different points inside a curarized muscle fibre. As the micro-electrode approaches the end-plate the first sign is always a small and slow end-plate potential. With the electrode closer, there is a characteristic change in the amplitude and especially in the time course of the e.p.p. and it is possible, with some experience, to estimate the residual distance of the end-plate from observations of the shape of the response. In this way, the focal point can be approached quickly, with two or three insertions, and its position is then found more accurately by moving the electrode in 100 or 200 μ. steps. In Figs. 6 and 7 the changes in amplitude and time of rise with distance are shown in two experiments. It was unusual to find fibres which could be followed over long distances along the surface of the sartorius muscle: the outlines of individual fibres often become obscured by adjacent fibres and by nerve branches, blood vessels and connective tissue which tend to run across the surface, especially near the end-plate foci. If a part of the

fibre is not perfectly clear in its outlines, there is a risk that the electrode tip might slip unnoticed into an overlapping neighbour, and we presume that the dotted curve in Fig. 6 arose from such an accident. In the experiment of Fig. 7 no such difficulty was experienced, and there was satisfactory agreement at every observed point between two series of measurements. In the great majority of the experiments, it was not necessary to follow individual fibres for any great length, but it was essential to be certain of the positions of individual end-plates throughout the experiment. For this purpose, the muscle was curarized at the beginning of the experiment, and a suitable number of points of maximum e.p.p. (i.e. 'end-plates') were located in the manner already described. By carefully mapping the microscopic field, noting all outstanding landmarks, it was possible to identify the fibre, and return to the same spot within 50 μ. The reliability of this procedure could be judged only from results; but the electric response at an end-plate differs so much from that of the immediate surroundings that no ambiguities arose, and it was clear that the proportion of failures in identifying previously located end-plates was minute.

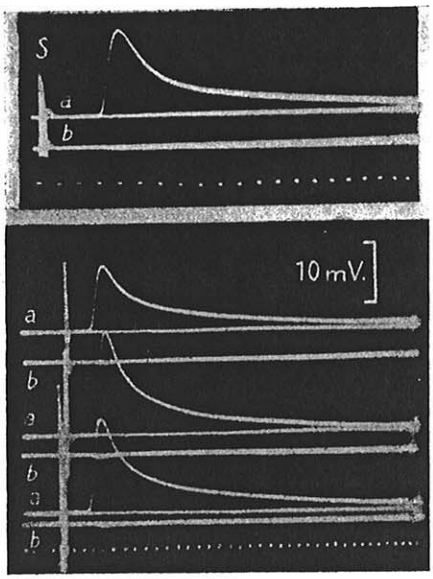

Fig. 3. End-plate potential in curarized muscle. Examples of focal recording, with the microelectrode (*a*) inside the fibre, (*b*) on the surface of the fibre. *S*: stimulus artifact. Time marks: msec.

Extra-cellular potential changes. The error introduced into measurements of end-plate and action potentials by the existence of a small potential difference outside the fibre had to be considered. The magnitude of the external electric field is proportional to the radial current density at the surface of the fibre and to the specific resistance of the external solution, and it leads to a slight reduction and distortion of the observed membrane potential change. The size of the external potential change was checked in a number of experiments. After recording an end-plate or action potential with the electrode inside the fibre, another record was obtained when the electrode tip had just been withdrawn from the fibre. Examples are shown in Fig. 3. The external potential change varied a good deal: values between less than 1% and 5% of the internal action potential were obtained. Even in low-sodium solutions (4/5 of sodium chloride replaced by osmotically equivalent sucrose), where the effect of external potentials would be greatest, the amplitude of the external potential did not exceed 8% of the internal one, so that only a small correction was

required when measurements made in solutions of different conductivities had to be compared. In most experiments of the present paper the conductivity of the bath remained constant (about 90 Ω. cm. at 20° C.), and no correction for external potentials was applied.

Direct stimulation. In some experiments an electric current was sent through the fibre in order to stimulate it directly or to determine its electrotonic 'cable constants' (cf. Hodgkin & Rushton, 1946; Katz, 1948). For this purpose another microelectrode was attached to a second micromanipulator which consisted of a combination of adjustable Palmer blocks and a vertical micrometer drive. The arrangement of stimulating and recording electrodes is shown in the diagram of Fig. 4. The procedure was to insert the recording electrode first, and then to introduce the stimulating microelectrode into the same fibre, either very nearby (at a distance of 20–50 μ.) or 1–2 mm. away. Repetitive subthreshold current pulses were used to indicate the moment when the stimulating electrode penetrated the required fibre, as this coincided with the sudden appearance of an electrotonic potential on the screen of the cathode-ray tube. There was usually also a drop of the resting potential by a few millivolts (cf. Nastuk & Hodgkin, 1950). In some of these experiments, a double-beam tube was used, the membrane potential being recorded by the first, and the current monitored by the second channel (see Fig. 4).

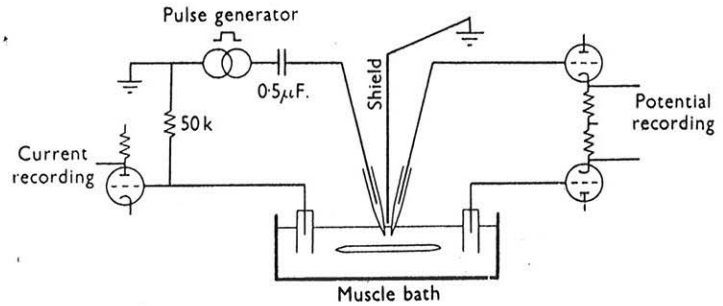

Fig. 4. Arrangement for measuring current and potential across the fibre membrane with two internal electrodes. Note: shield to reduce artifact.

The current passing through electrode and muscle membrane was usually a few tenths of a microampere. This required that several volts be applied to the electrode and gave rise to a high voltage gradient across the wall of the microelectrode tip. Not every electrode was suitable for these experiments, and with some there was evidence of dielectric breakdown occurring at the electrode tip: as the impressed voltage was increased, the current through the electrode would then suddenly rise and by-pass the muscle fibre, failing to produce or maintain a potential change across the membrane.

Temperature. Room temperature was recorded during the experiments, but checks made with a thermocouple indicated that the termperature of the preparation was about 1-1·5° C. lower, evidently because of evaporation occurring from the surface of the shallow portion of the chamber (Fig. 1). To avoid osmotic disturbance of the muscle, the bath was changed at intervals of less than 1 hr.

Solutions. In many experiments, a modified Ringer's solution was used with the composition: 113 mM. sodium, 2·0 mM. potassium, 3·6 mM. calcium, 1 mM. phosphate, 121 mM. chloride. The phosphate buffer maintained the pH at 6·8. This differed from normal Ringer mainly by its higher calcium content (3·6 mM. instead of 1·8 mM.). The advantage of this solution was that it raised the threshold of the muscle fibre, relative to the e.p.p., by about 25% and therefore caused the e.p.p., both in curarized and normal preparations, to become somewhat more conspicuous. In other experiments, normal Ringer was used, either buffered by the addition of 1·0 mM. phosphate or unbuffered. Other solutions containing D-tubocurarine chloride (Burroughs Wellcome), or prostigmine bromide (Roche) were made up as described in the experimental section.

RESULTS

A. *End-plate potential in curarized muscle*

The electric response of the end-plate becomes relatively simple when neuromuscular transmission is blocked by curarine (Eccles, Katz & Kuffler, 1941; Kuffler, 1942*a*). The effect of this drug is to reduce the amplitude of the e.p.p. below the threshold of the muscle fibre, so that no impulse arises and a local subthreshold potential change remains. Its general characteristics have previously been worked out on the whole muscle (Eccles *et al.* 1941). By

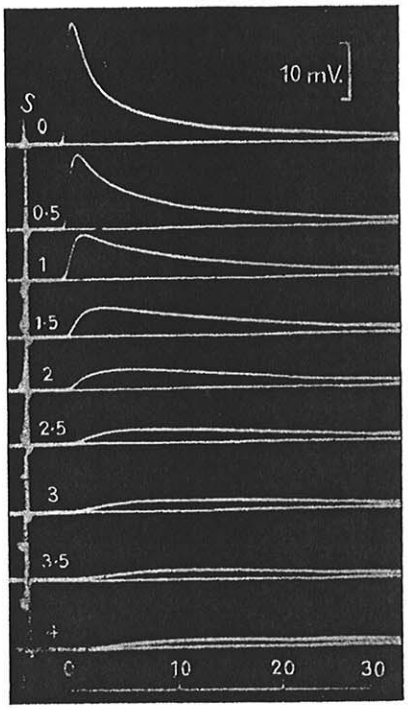

Fig. 5. End-plate potential in a single curarized muscle fibre. The position of the micro-electrode was altered in successive $\frac{1}{2}$ mm. steps. The numbers give the distance from the end-plate focus, in mm. $\times 0{\cdot}97$. *S*: stimulus artifact. Time in msec.

placing a microelectrode into a curarized muscle fibre the situation becomes further simplified, for the observed response is now confined to that of a single end-plate. The method which was used in approaching the end-plate region of an individual fibre has been described above, and the results of an experiment are illustrated in Fig. 5. The e.p.p. consists of a single monophasic wave, which is rapidly attenuated as it spreads along the fibre. At the centre of the junctional region, the e.p.p. in a completely curarized muscle attains an amplitude of as much as 20–30 mV., but there are large variations of size in

different fibres of the same muscle, the observed range of e.p.p. amplitudes with a given dose of curarine being greater than 10-fold. As the e.p.p. represents a graded subthreshold response, there is of course no reason to expect constancy of its amplitude, but it is remarkable that under given experimental conditions a 10- or even 20-fold variation should be found in the size of the end-plate response of different fibres. One may suspect that there are corresponding variations in the size of the junctional contact areas, or in the quantities of acetylcholine ejected from individual nerve endings.

When the e.p.p. is recorded at a 'focal' point, as in Fig. 3, it is found to rise suddenly, reaching its peak in little more than 1 msec. and declining to one-half in another 2 msec. The characteristic features of the 'focal' e.p.p. are listed in Table 1, which again shows that there is large variation in amplitude, but relatively little variation in time course.

TABLE 1. End-plate potential in curarized muscle

(These figures were obtained from ninety-four end-plates with focal recording. Most of these were selected end-plates giving e.p.p.'s of at least 10 mV. in a completely blocked muscle. Figures marked with asterisk*: these are uncorrected figures. To allow for the sytematic errors mentioned in 'Methods', subtract about 1° C. from temperature readings, and 0·1 msec. from time measurements, in this and all subsequent results. Temperature, 20° C. (16–24° C.)*. Calcium concentration, in most cases 3·6 mM. Curarine concentration, $3–5 \times 10^{-6}$ D-tubocurarine chloride (Burroughs Wellcome).)

Resting potential (mV.)	E.p.p. amplitude (mV.)	Time from onset to peak (msec.) (mean and S.E. of mean)	Time from onset to half-decline (msec.)
90 (75–107)	2·5–29 (usually 10–20)	1·3±0·02 (1·0–1·6)*	3·9±0·08 (2·4–6·0)*

A more complicated picture is sometimes obtained when the electrode is inside a fibre which has a small e.p.p., but next to one with a very large e.p.p. In this case the record becomes seriously distorted by the external field due to the adjacent 'sink', and a combination is recorded of (i) a small true change of membrane potential and (ii) an external p.d. due to the neighbouring fibre. The two changes are of opposite electric sign, and may give rise to a diphasic potential change, starting with a brief downward deflexion (the micro-electrode becoming at first more negative). The diphasic response was seen only under these special conditions, and it should be realized that the initial phase is *not* a 'membrane potential', for it can be seen when the microelectrode is in the bath on the surface of the adjacent fibre. A similar explanation applies to the small diphasic or polyphasic disturbances which were observed in non-curarized muscle (cf. Nastuk & Hodgkin, 1950), and which are due to the external fields of impulses travelling in adjacent fibres.

Effect of curarine concentration on end-plate potential and resting potential

With an increased dose of curarine, the e.p.p. becomes further reduced in size without any other obvious changes. Conversely, it will be seen that the removal of curarine is associated with a large increase in the rate of rise of the e.p.p., but its peak amplitude then becomes obscured by the intervention of the muscle spike. The effect of curarine on the size of the e.p.p. is summarized in Table 2. It is noteworthy that the resting potential of the end-plate membrane

is not significantly altered by curarine, the mean values being $90{\cdot}5 \pm 0{\cdot}5$ mV. (S.E. of mean, 176 measurements) for the non-curarized end-plates and $90 \pm 0{\cdot}6$ mV. (94) for curarized end-plates. The differences of resting potential

TABLE 2. Effect of curarine on size of end-plate potential

A. Completely blocked muscle. Comparing the peak amplitudes at eight end-plates, with 5×10^{-6} and $2{\cdot}5 \times 10^{-5}$ D-tubocurarine chloride. Temperature, 21° C.

	5×10^{-6} curarine (mV.)		$2{\cdot}5 \times 10^{-5}$ (mV.)		
Fibre	Resting potential	E.p.p.	Resting potential	E.p.p.	E.p.p. reduction
I	93	7·8	90	0·6	0·077
II	93	9·8	92	0·95	0·097
III	86	7·0	90	0·7	0·1
IV	80	6·5	86	0·95	0·146
V	83	6·9	91	0·55	0·08
VI	85	6·6	85	0·8	0·121
VII	83	17·4	81	2·5	0·144
VIII	86	20·7	90	2·7	0·13
Mean	86	10·3	88	1·22	0·112

B. Comparing normal and curarized muscle (4×10^{-6} D-tubocurarine chloride). The e.p.p. was measured at a fixed point of its rising phase, 0·44 msec. after its onset.)

	4×10^{-6} curarine (mV.)		Normal (mV.)		
Fibre	Resting potential	E.p.p. at 0·44 msec.	Resting potential	E.p.p. at 0·44 msec.	E.p.p. reduction
I	95	4·5	96	24·6	0·18
II	89	4·7	94	22·7	0·21
III	90	12·1	97	36·4	0·33
IV	82	8·8	98	34·8	0·25
V	96	6·4	100	32	0·2
VI	89	9·9	97	40	0·25
VII	86	5·4	86	28·4	0·19
VIII	88	12·2	84	36	0·34
IX	86	6·1	84	19·7	0·31
Mean	89	7·8	93	30·5	0·25

in the paired observations of Table 2 are also not significant. In Table 2 A, the mean difference is $-2 \pm 1{\cdot}4$ mV., the more deeply curarized end-plates having a slightly larger resting potential, while in Table 2 B, the mean difference is $+4 \pm 2$ mV.

An analysis of the spatial spread and decay of the end-plate potential

In two experiments, illustrated in Figs. 6 and 7, a fairly complete series of records was obtained at various distances from the focal point. In Fig. 8 several tracings are superimposed which show the characteristic decline and temporal spreading of the wave-form as the electrode is moved outward from the end-plate. It has been shown by several authors (Eccles *et al.* 1941; Kuffler, 1942*b*; Katz, 1948) that the e.p.p. arises from a rapid initial displacement of electric charge by the neuromuscular transmitter: this active phase appears to subside within a few milliseconds, and thereafter the time course of

the e.p.p. is determined by the rate at which the charge spreads along, and leaks across, the muscle membrane. There are certain consequences of this hypothesis which can be subjected to a quantitative test.

The distribution of charge in a resting muscle fibre is described by the classical cable theory (see Hodgkin & Rushton, 1946). According to this theory, the total charge which the transmitter has placed on, or displaced from, the

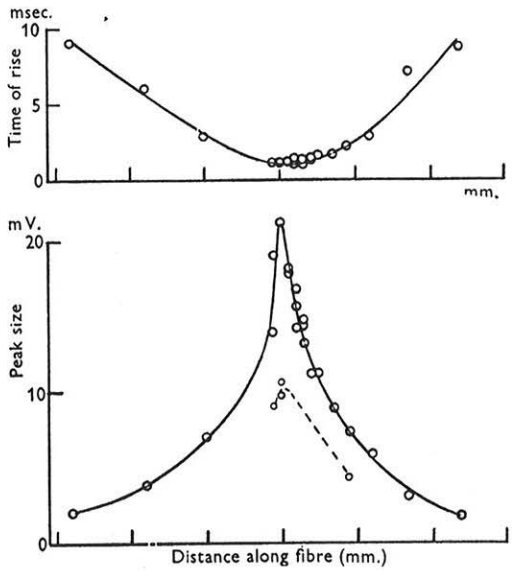

Fig. 6

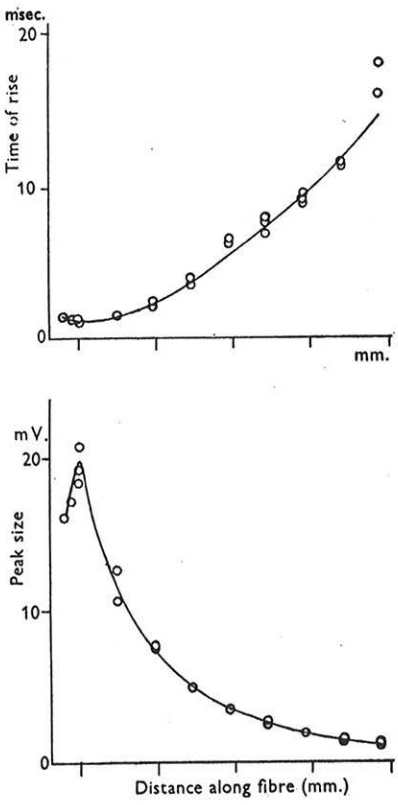

Fig. 7

Fig. 6. Spread of e.p.p. in a single curarized fibre. Lower part: peak amplitude of e.p.p. is plotted against distance along the fibre (small circles: see text, p. 324). Upper part: time of rise of the e.p.p. is plotted against distance.

Fig. 7. Spread of e.p.p. in another fibre. Same co-ordinates as in preceding figure.

muscle fibre should decay exponentially after the transmitter has subsided. The time constant of the decay of end-plate charge should be the same as the time constant of the muscle membrane as determined by other methods (cf. Katz, 1948). In order to measure the total displacement of charge, the spread of the e.p.p. along the fibre was plotted at various moments (Fig. 9). The area

under each curve gives a relative measure of the charge on the fibre surface (or rather of the 'deficit of charge', the fibre surface having been depolarized). The area can be found accurately at short times, up to about 10 msec.; at longer times, an extrapolation is required for distances greater than 4 mm., but this introduces only a slight inaccuracy in the final points. The logarithm of the area is plotted against time in Fig. 10: the end-plate charge is seen to reach a maximum at about 1·5–2 msec. and from then on to decay exponentially with a time constant of 20·6 msec. In another experiment, the maximum was

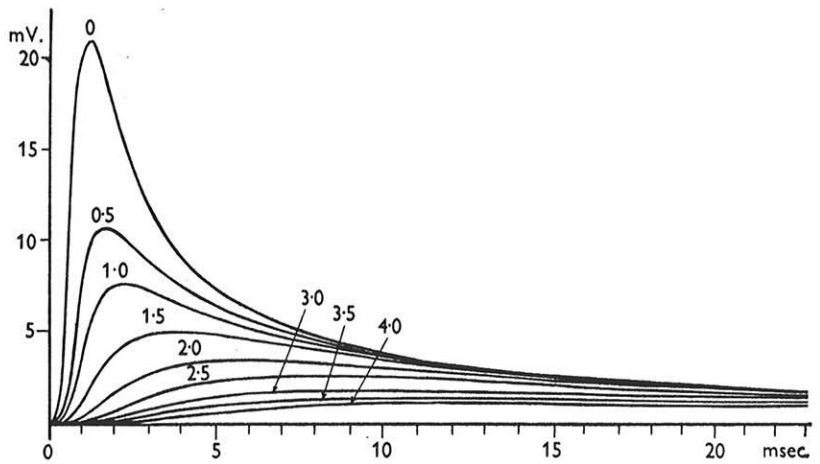

Fig. 8. Tracings of e.p.p.'s at different distances from end-plate focus. In superimposing the records, the stimulus artifact (see Fig. 5) was taken as the common point. The numbers give the distances, in mm. × 0·97, from the end-plate centre.

attained in 2–3 msec., and the charge then subsided with a time constant of 27·4 msec. These time constants are within the range of values previously found for frog muscle (see also Tables 4 and 5), and this confirms the view that, beyond the first 2 or 3 msec., the e.p.p. in curarized muscle is no longer actively maintained, and that its further time course is determined simply by the resistances and capacity of the muscle fibre.

It was pointed out to us by Mr A. L. Hodgkin that the theoretical equations describing the spread of charge along the fibre become greatly simplified if the charge has been applied *instantaneously at a point* of the fibre (see Appendix I). During the e.p.p., charge is placed on (or displaced from) points of the fibre which are usually spread out 50 to 100 μ. on either side of the centre of the nerve endings, and the displacement is nearly complete within 2 msec. These distances and times are small compared with the length and time constant of the fibre and tentatively their finite size may be disregarded, especially when analysing measurements at one or more millimetres from the end-plate focus. According to the simplified theory outlined in Appendix I, the duration T of the rising

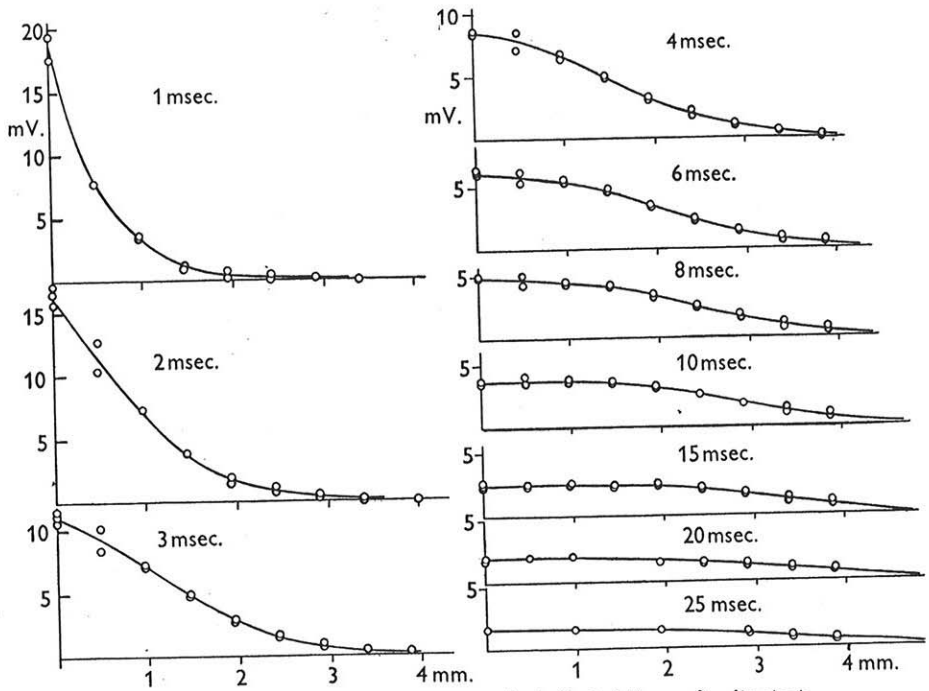

Fig. 9. Spatial distribution of e.p.p. at the indicated times after its start.

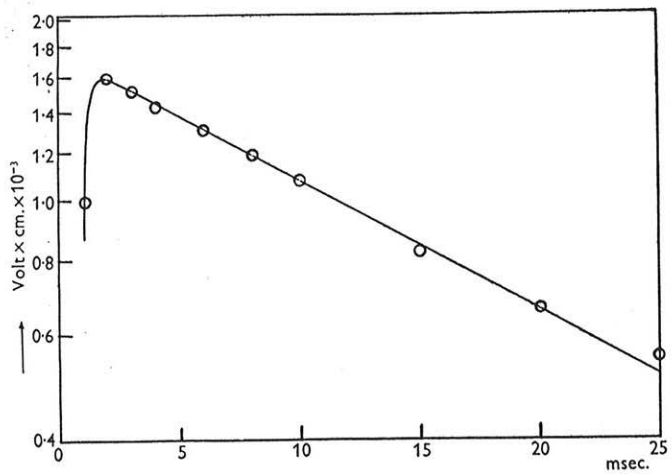

Fig. 10. The exponential decay of the end-plate charge. Ordinates: area ($\int V dx$) of the potential—space curves of Fig. 9, in mV. × cm., on a logarithmic scale. Abscissae: time in msec. Note: to convert ordinate readings into coulombs, multiply by $2c_m$, where c_m is membrane capacity per unit length of fibre, and the factor 2 is required because the e.p.p. spreads in both directions along the fibre.

phase of the e.p.p. at any given point, should then be related to the distance x of this point from the focus by the following equation

$$\frac{x^2}{\lambda^2}=\frac{4T^2}{{\tau_m}^2}+\frac{2T}{\tau_m}, \tag{1}$$

where τ_m is the time constant of the membrane and λ the length constant of the muscle fibre. These constants are related to the resistances and capacity of the fibre as follows:

$$\tau_m=R_m\,C_m,$$

where R_m is the transverse resistance and C_m the capacity of 1 cm.^{2} of membrane, and, when the fibre is immersed in a saline bath,

$$\lambda^2=\frac{R_m}{R_i}\times\frac{\rho}{2},$$

where R_i is the specific internal resistance and ρ the radius of the fibre (for further details and nomenclature, see Hodgkin & Rushton, 1946, and Katz, 1948).

Having found τ_m, we can now use the curves of Fig. 8 and plot values of $4T^2/{\tau_m}^2+2T/\tau_m$ against x^2 (Fig. 11). According to equation (1) this should give a straight line with slope $1/\lambda^2$. The observed relation is approximately linear,

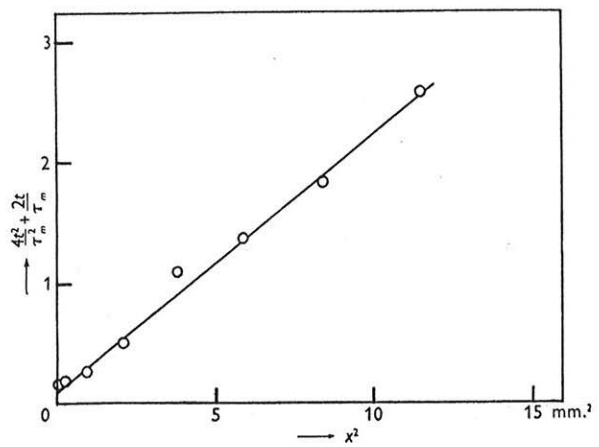

Fig. 11. Analysis of the e.p.p. in curarized muscle (see text and Appendix I). The theoretical relation is linear (slope $=1/\lambda^2$) and passes through the origin. The divergence from theory at small values of t and x is due to the fact that the rise of the e.p.p. is not instantaneous.

and λ is found to be 2·15 mm. In another experiment, the value of λ determined in the same way was 2·4 mm. This may be compared with the values of λ on p. 335, which were obtained by the 'square-pulse analysis' of the electrotonic potential: the mean value in seven fibres is 2·4 mm. (varying between 2·2 and 2·6 mm.).

To find R_m and C_m, we must make an assumption about the size and internal conductivity of the muscle fibres. The average fibre diameter in a sartorius muscle is about 75–80 μ. (Mayeda, 1890; Katz, 1948), but the fibres on the deep surface of the muscle are larger than average (Hill, 1949). Moreover, in searching for a distinct fibre which can be followed along the muscle, it is probable that one of the largest superficial fibres was chosen. We were inclined to take 100 μ. for fibre diameter and about 250 Ω.cm. for internal resistivity (cf. Bozler & Cole, 1935; Katz, 1948). The more direct experiments described on p. 335 indicate, however, that the fibre diameter was more nearly 140 μ., and this value was therefore adopted. The values calculated for R_m and C_m are 3300–4100 Ω.cm.^{2} and 6–7 μF./cm.^{2}, respectively (see Table 3). As shown in Table 5, these results

TABLE 3. Membrane constants derived from end-plate potential

Fibre	τ_m (msec.)	λ (mm.)	R_m (Ω.cm.^{2})	C_m (μF./cm.^{2})
I	20·6	2·15	3300	6
II	27·4	2·4	4100	7

are within the range of values obtained by more direct methods, and we regard the quantitative agreement as a further confirmation of our premises, namely that the e.p.p. is produced by a brief impulse of transmitter activity.

While this conclusion applies to curarized muscle, it does not hold under all conditions, and certainly not when the preparation has been treated with a cholinesterase inhibitor (see p. 337). Even in the curarized preparation, there was some variation in the time course of the e.p.p. at different end-plates (cf. Table 1) which may have arisen from variable persistence of the transmitter/end-plate reaction. Some variation, however, in the time course and spread of the e.p.p. around its focus must be expected because the spatial distribution of nerve endings varies considerably in individual muscle fibres. As Kühne (1887) has shown, the motor nerve terminals in frog muscle spread along the fibre over a distance which may vary between 30 and as much as 500 μ. The exact shape of the e.p.p. recorded at the centre of this region and the sharpness of its spatial peak must depend upon the spread of the nerve-muscle junction. If this covers a length of a few hundred microns, it will give rise to a relatively blunt peak of the e.p.p. The same effect arises, even with sharply localized junctions, if the nerve endings happen to lie on the buried side of the muscle fibre; the microelectrode cannot then be brought very close, and fine longitudinal adjustment makes little difference. In the course of locating large numbers of end-plates, considerable variations in the sharpness of localization were observed. In some cases, the position of the electrode was more critical even than shown in Fig. 6, while in other cases, a shift of 200 μ., in either direction from the centre, produced little diminution in e.p.p. size. One may surmise that this was associated with an extensive spread of the nerve endings, or their being located on the opposite side of the fibre.

Direct measurement of the membrane constants

It was desirable to determine the resistance and capacity of the muscle fibres, under similar experimental conditions, but in a more direct way than used in the preceding section. For this purpose, the rectangular pulse technique was employed as described by Hodgkin & Rushton (1946) and Katz (1948), except that intracellular electrodes were used to pass current through the membrane, and to record the resulting change of potential across it (see Methods, Fig. 4). The current was an inward directed pulse through the membrane, of about 70 msec. duration and 0·2 μA. intensity, which caused the membrane potential to increase by about 40 mV. The current was delivered by a rectangular pulse generator, but its shape and intensity depended upon the resistance of the microelectrode which was liable to change during the current flow. This showed itself usually in a gradual reduction of current strength, from its initial peak to a more steady level which was reached after some 10–20 msec. The current pulse was examined on a double-beam oscilloscope, and it was ascertained that a period of sufficiently steady current flow, and steady membrane potential, preceded the break of the pulse. Under these conditions, the level of the membrane potential reached at the end of the pulse, and the transient potential changes following its break, could be used to determine the relevant fibre constants.

Applying the cable theory of Hodgkin & Rushton (1946) to the present case, we find that, for a distance x between the two internal electrodes, the steady potential change V recorded at one electrode is related to the steady current I flowing through the other electrode by the following equation:

$$V = \frac{I}{2}\sqrt{(r_m r_i)}\,\exp\,[-x/\sqrt{(r_m/r_i)}], \qquad (2)$$

where r_m and r_i are, respectively, the transverse resistance of the membrane times unit length and the longitudinal resistance of the fibre per unit length. The term $\frac{1}{2}\sqrt{(r_m r_i)}$ is the effective resistance between inside and outside, measured at a point far from the tendon, while $\sqrt{(r_m/r_i)}$ is the length constant λ.

Thus the values of r_m and r_i can be found from measurements with two different electrode separations. The time constant of the membrane can be determined from the time course of decay of the membrane potential, for instance by measuring the time of decline to 15% with zero separation, or by comparing half-times at different distances (cf. Hodgkin & Rushton, 1946).

The electrodes were placed into the same muscle fibre about 10 mm. from the pelvic end. The 'polarizing' electrode was left there, while the recording electrode was moved from a position only 20–30 μ. away to a distance of 1·6 mm. and finally back to the original or an intermediate point. The resting

potential was measured at the recording electrode, and the current was monitored in every case. The results are shown in Table 4. The values of λ, τ_m, r_m and r_i were obtained directly, while those of d, R_m and C_m are based on an assumption regarding the internal conductivity of the fibre. Its specific

TABLE 4. Membrane constants derived from 'square pulse analysis'

(Temperature 19° C. R_i is assumed to be 250 Ω.cm.)

Fibre	Resting potential (mV.)	λ (mm.)	τ_m (msec.)	$\frac{1}{2}\sqrt{(r_m r_i)}$ (Ω.)	"d" (μ.)	R_m (Ω.cm.²)	C_m (μF./cm.²)
I	89	2·3	31	210000	132	4000	8
II	91	2·3	37·5	215000	131	4100	9
III	85	2·4	29	135000	168	3400	8
IV	79	2·6	33	215000	139	4900	7
V	90	2·2	33	232000	123	3900	8
VI	89	2·2	34	193000	135	3600	9
VII	82	2·5	44	230000	132	4800	9
Mean	86	2·4	34·5	204000	137	4100	8

resistance R_i was taken as 250 Ω.cm., in accordance with earlier measurements of Bozler & Cole (1935) and Katz (1948). The fibre diameter was then calculated from

$$d = \sqrt{\left(\frac{4}{\pi} \times \frac{R_i}{r_i}\right)}, \tag{3}$$

the mean value of d being 137 μ. This seems rather large, but it is within the known range of fibre diameters of frog's muscle (Mayeda, 1890), and it is likely that during the present experiments the largest fibres have been selected.

The values of R_m and C_m obtained in this series are listed in Table 5, together

TABLE 5. Summary of different measurements of R_m and C_m in muscle

Method and reference	Preparation	R_m (Ω.cm.²)	C_m (μF./cm.²)
External electrodes (Katz, 1948)	Small bundles and isolated fibres (75 μ.)	1500	6
	Toe muscle (45 μ.)	4000	4·5
Internal electrodes, e.p.p.	Sartorius	3700	6·5
Internal electrodes, 'square pulse'	Sartorius	4100	8

with other measurements on frog muscle. The most notable feature in this table is the large value of the membrane capacity (4·5 – 8 μF./cm.²) which exceeds that of several non-medullated nerve axons by a factor of 5.

The displacement of electric charge at an end-plate by the neuromuscular transmitter

With the use of these figures we can calculate the quantity of electric charge which is removed from the surface of a curarized muscle fibre, during the local action of a nerve impulse. This quantity is of special interest because it gives us an indication of the depolarizing power of the neuromuscular transmitter, and of the minimum number of ions which flow through the active end-plate surface. Presumably, when acetylcholine is released from the nerve endings it reacts with the end-plate so as to form a local 'sink' into which the surrounding muscle membrane discharges. But whatever the mechanism of this action, the discharge of the muscle fibre during the e.p.p. must be brought about by a transfer of ions across the end-plate membrane, and the number of ions which are transported across the end-plate surface must be large enough to provide for the observed displacement of charge.

It might be argued that, even during a subthreshold e.p.p., some regenerative reaction occurs in the surrounding muscle membrane which reinforces the local transfer of ions, quite apart from the primary action at the end-plate itself. But if such a regenerative process were at all important, it would have a noticeable effect on the time course of the e.p.p. and on the membrane constants derived from it. For example, the resistance and time constant so determined should have a larger value than when measured with the usual method of anodic polarization (p. 335). No such difference was observed, and we feel justified in assuming that the muscle membrane is discharged passively into the 'sink' at the motor end-plate.

It was shown in Fig. 10 that during the first 2 msec. of the e.p.p. the displacement of charge from the muscle membrane reaches a maximum and then declines exponentially. The maximum charge amounts to $3{\cdot}2 \times 10^{-3}$ V.cm. multiplied by the capacity per unit length of fibre. During normal impulse transmission considerably more charge is transferred across the end-plate: the results of Table 2 indicate a three- to five-fold amplification, following the withdrawal of curarine.

With a membrane capacity of 6 μF./cm.^{2} and a fibre diameter of 135 μ., the capacity of 1 cm. of fibre is $2{\cdot}45 \times 10^{-7}$ F., and the transfer of charge during the subthreshold e.p.p. is 8×10^{-10} coulombs. This corresponds to a net transport of at least 8×10^{-15} mol. of univalent cations inward, or anions outward, across the end-plate membrane. In the absence of curarine, the figure increases to $2\text{–}4 \times 10^{-14}$ mol. This is the *minimum* quantity of ions which the transmitter causes to flow across a single end-plate during one impulse. It is a surprisingly large amount, considering the small size of the end-plate area: it is equivalent, for instance, to the transfer of sodium across 0·8 mm.^{2} of non-medullated axon membrane during a single nerve impulse (Keynes & Lewis, 1950).

The number of ions which contribute to the production of the e.p.p. must, in fact, be larger than this estimate, as it represents only the net transfer of charge, i.e. the excess of cations over anions moving in one direction. Presumably the movement of ions is brought about by a reaction between acetyl-

choline and its receptors in the end-plate: it may involve a direct entry of acetylcholine ions into the muscle fibre or a permeability change leading to increased flux of other ions across the surface (cf. Fatt, 1950). It would be an important step in the study of this problem if the quantity of acetylcholine released by a nerve impulse at a single end-plate could be compared with the quantity of ions required for the production of an e.p.p.

The local discharge of the fibre surface is opposed, relatively slowly, by the flux across the membrane of potassium, chloride and other 'diffusible' ions which are responsible for the gradual return to the resting level. In the curarized muscle, the initial displacement of charge is much more rapid than subsequent leakage across the membrane, and there is very little overlap between the two phases. A very different situation arises when the action of the transmitter is prolonged, by the use of a cholinesterase inhibitor.

The effect of prostigmine on the end-plate potential

The action of several anti-cholinesterases has been studied carefully by Eccles & MacFarlane (1949), who found that there is invariably a marked lengthening of the active phase during which the e.p.p. is built up. In Fig. 12

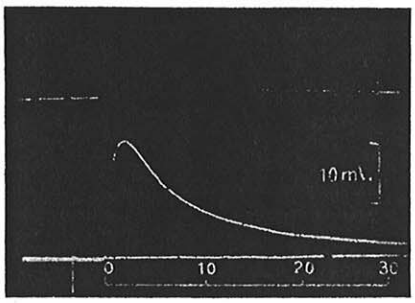

Fig. 12. Effect of prostigmine, in curarized muscle. The lower record was obtained from the same end-plate, after addition of prostigmine bromide (concentration 10^{-6}). Time, msec.

an intracellular record of this effect is shown. In a curarized muscle fibre, (3×10^{-6} D-tubocurarine chloride), an e.p.p. was observed rising to a peak of 7 mV. in 1·1 msec. and falling to one-half in another 2·1 msec. After an addition of 10^{-6} prostigmine bromide, the response at the same end-plate built up to a more rounded peak of 19 mV. in 2·1 msec., and then fell to one-half in another 5·2 msec. The effect is very similar to that previously described on whole muscle, with a moderate dose of eserine (Eccles, Katz & Kuffler, 1942) and other cholinesterase inhibitors (Eccles & MacFarlane, 1949).

It was shown by Eccles *et al.* (1942) that eserine produces a much more dramatic lengthening of the e.p.p. in the uncurarized muscle, though recording

becomes then more complicated because of the presence of muscle spikes. We have confirmed their observation under somewhat different conditions. Nerve-muscle transmission can be blocked by lowering the external sodium concentration to one-fifth (cf. Fatt & Katz, 1950*b*) leaving an e.p.p. of similar shape, though usually of somewhat slower rise and fall than in the curarized muscle (see Tables 6 and 1). If prostigmine is added to the solution, a striking

TABLE 6. End-plate potential in sodium-deficient solution

(Mean values of twenty-five experiments at 20° C. Na concentration reduced to one-fifth by substitution of isotonic sucrose.)

Resting potential (mV.)	E.p.p. peak amplitude (mV.)	Time from onset to peak (msec.)	Time from onset to half-decline (msec.)
83	·9–28	2·1 (1·1–2·6)	5·9 (3·2–7·8)

change occurs, shown in Fig. 13. The e.p.p. is lengthened enormously, much more than in the experiment of Fig. 12. Instead of passing through a sharp peak, the e.p.p. rises to a plateau which is maintained for some 30–40 msec., and then declines to one-half in 0·1 sec., as compared with 6 msec. (see Fig. 14).

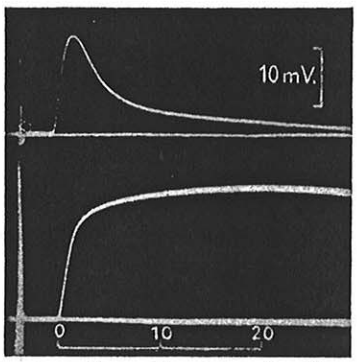

Fig. 13. Effect of prostigmine in a 'low-sodium' muscle. Upper record: E.p.p. in sodium-deficient muscle (4/5 of Na replaced by sucrose). Lower record: after addition of prostigmine bromide (10^{-6}). Time, msec.

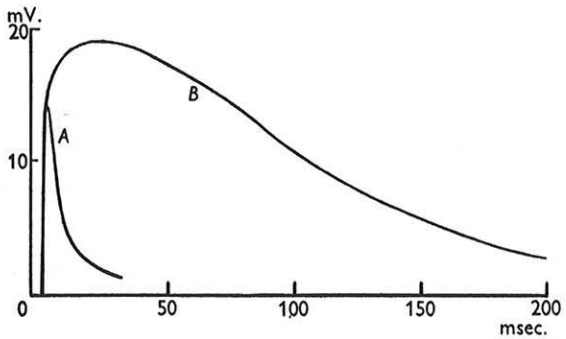

Fig. 14. Superimposed tracings of e.p.p.'s. *A*: low-sodium muscle. *B*: like *A*, but after adding prostigmine bromide (10^{-6}).

There is strong evidence (see Brown, Dale & Feldberg, 1936; Eccles *et al.* 1942; Eccles & MacFarlane, 1949) that this effect is due to the protection of acetylcholine against rapid hydrolysis, and that therefore the amount of acetylcholine, initially released by the nerve impulse, continues to build up the e.p.p. and to maintain it against the simultaneous spread and leakage of charge along and across the surface membrane. The quantity of ions which passes through the end-plate in the prostigmine muscle must greatly exceed the figure given above for the curarized muscle. An estimate of the excess can be obtained by comparing the 'areas', i.e. the time-integrals, of the e.p.p.'s in the two cases: this area is about 50 times larger for the prostigmine-e.p.p. of Fig. 13, than for a 'curarine'-e.p.p. of the same initial rate of rise.

This is an important point in connexion with the alternative modes of acetylcholine action which have been suggested (Katz, 1942; Fatt, 1950). If, for instance, acetylcholine were to depolarize the end-plate by direct penetration, the quantity of ions released by a single impulse must provide not only the electric charge which is placed on the muscle fibre during the ordinary e.p.p. but the much larger quantity which is needed to maintain the e.p.p. in eserine- or prostigmine-treated muscle.

It is of interest to trace the time course of the transmitter/end-plate reaction and its changes under the influence of prostigmine. This can be done approximately by an analysis of the e.p.p. which has been previously described (Katz, 1948, p. 529). The analysis depends upon a knowledge of the time constant of the membrane, and on the assumptions (i) that the time constant is not appreciably affected by prostigmine, and (ii) that the transmitter reaction can be treated as the equivalent of an applied current pulse. There is good evidence that the first assumption holds true (Eccles *et al.* 1942) but the second is oversimplified (cf. Section B below), though not likely to lead to serious error in the present comparison. In Fig. 15 the result of such an analysis is shown for (*a*) the e.p.p. of curarized muscle, (*b*) of curarine-prostigmine-treated muscle and (*c*) of Na-deficient and prostigmine-treated muscle. The curves show, strictly speaking, the time course of three current pulses which, with a membrane time constant of 25 msec., would alter the membrane potential in a manner identical with the three observed types of e.p.p. It will be noted that, even in the presence of a cholinesterase-inhibitor, there appears to be an initial impulsive phase of transmitter action, which is followed by a long period of low-level activity. Similar phenomena have been described and discussed in detail by Eccles *et al.* (1942) and Eccles & MacFarlane (1949).

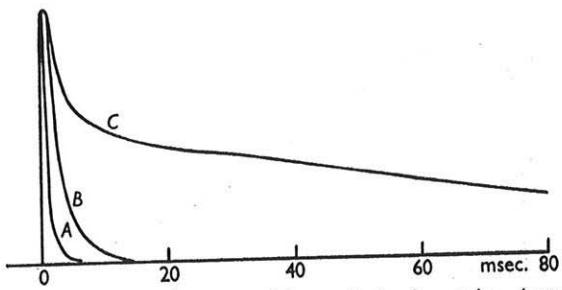

Fig. 15. 'Transmitter action' curves, obtained by analysis of e.p.p.'s. *A*: muscle treated with curarine; *B*: curarine + prostigmine; *C*: low-sodium + prostigmine. The ordinates have been scaled to the same maximum.

B. *The electric response of the normal end-plate membrane*

In the normal muscle fibre, the e.p.p. rises at a much greater rate and leads to a propagating spike and contraction. The electric response at the end-plate differs from a conducted action potential in a characteristic manner (Figs. 16–19, 21). A large e.p.p. invariably precedes the spike and forms a 'step' during the rising phase of the record. After the peak a discrete 'hump' is seen in most cases, indicating a continued action of the transmitter during the falling phase of the potential.

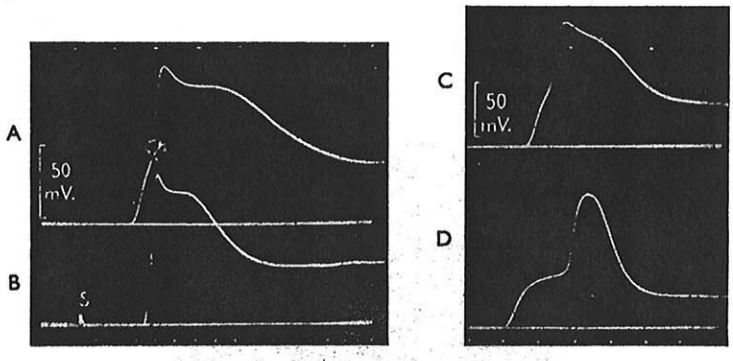

Fig. 16. End-plate responses in normal preparation. Four end-plates, showing step, spike and hump (except in D where the safety margin is low and a delayed spike without hump is seen). *S*, stimulus artifact. Time marks, msec.

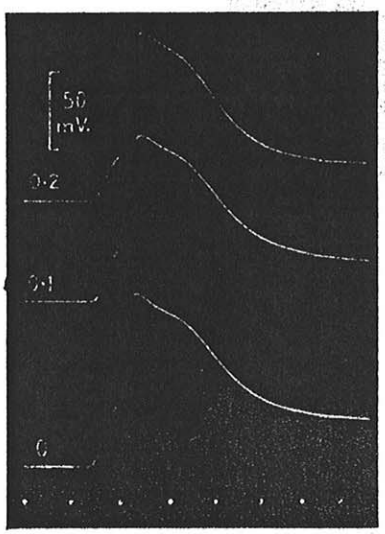

Fig. 17

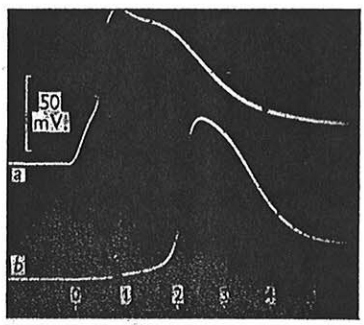

Fig. 18

Fig. 17. Three records from the same muscle fibre, at distances of 0, 0·1 and 0·2 mm. from the end-plate focus. Time marks, msec.

Fig. 18. Records from the same fibre (*a*) at the end-plate and (*b*) 2·5 mm. away. Time, msec.

The usual procedure was, first, to locate a number of end-plates in a fully curarized muscle and then remove the drug by 30 min. washing in Ringer's solution (see Methods). The same results were obtained in a few cases in which the end-plates were found, by trial recordings, in normal untreated muscle (e.g. Fig. 18). This method naturally involved a considerable wastage of fibres, and was only used as a check to ascertain that the preliminary curarine-treatment had no irreversible effects.

Measurement of 'step' and 'hump'

Before trying to analyse the components of the end-plate response, it is of interest to describe and measure its characteristics. The diagram of Fig. 20 shows the points which were chosen as a convenient measure of 'start', 'step', 'peak' and 'hump'.

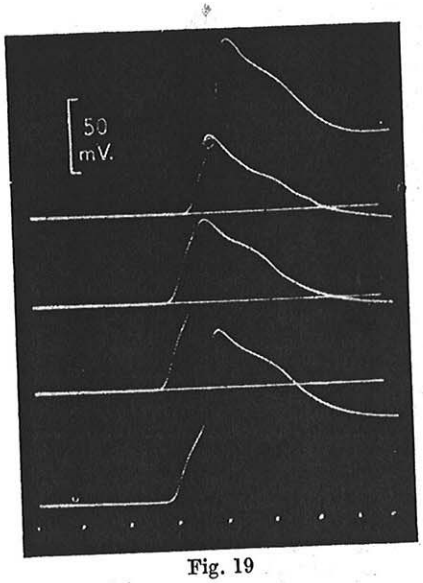

Fig. 19

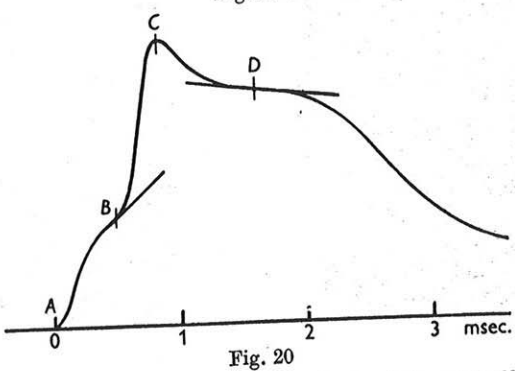

Fig. 20

Fig. 19. Examples of end-plate responses in prostigmine-treated muscle fibres. Note the larger residual potential change. Time marks, msec.

Fig. 20. Diagram showing 'characteristic points' of the end-plate response.

The 'start' (point *A*) was taken as the point of just perceptible deflexion (about 0·3 mV., with low amplification). At the end-plate, the response rises sharply above the baseline, and this measurement was accurate within less than 0·1 msec. The 'peak' (point *C*) provided no difficulty, but the positions of 'step' (*B*) and 'hump' (*D*) are subject to some uncertainty. The height of the step was measured near the point of inflexion, at a level at which the spike could be seen to take off. The hump was measured at the mid-point of the flat shoulder on the falling phase. The separation between peak and hump was not always distinct (cf. Fig. 16) and the position of the hump, therefore, not always well defined. We estimate the accuracy of our measurements as being within 2–3 mV. and 0·1 msec. for the step (*B*), and 5–8 mV. and 0·2–0·3 msec. for the hump (*D*).

TABLE 7. Electric response at the normal end-plate

(Times are measured from the onset of the potential change. Active-membrane potential: p.d. across active membrane = action potential minus resting potential. Errors are the standard errors of the mean.)

	Temperature (° C.)	Calcium concn. (mM.)	Resting potential (mV.)	Action potential peak		Active-membrane potential (mV.)	End-plate 'step'		End-plate 'hump'	
				(mV.)	Time (msec.)		(mV.)	Time, (msec.)	(mV.)	Time (msec.)
	20 (16–23·5)	3·6 (1·8–9)	91±0·43	113±0·77	1·1 (0·54–2·4)	22±0·67	41±0·6 (25–54)	0·6 (0·31–1·65)	97 (80–117)	1·8 (1·3–2·7)
Number of experiments	—	—	(135)	(134)	(134)	(134)	(135)	(135)	(104)	(104)

Results from 135 end-plates are summarized in Table 7. Most experiments were made with a solution containing 3·6 mM. calcium, i.e. twice the amount normally in Ringer. In Table 7 are included the results of twenty-five experiments in which ordinary Ringer (1·8 mM. calcium) had been used, and thirty experiments in which prostigmine bromide in a concentration of 10^{-6} had been added. These various solutions affected the measurements only in one respect, namely that the height of the initial end-plate step was less with 1·8 mM. calcium (33 mV.) than with 3·6 mM. calcium (41 mV.). The statistical significance of this difference is further shown in Table 8, in which nine 'paired' measurements on the same end-plates are summarized. Prostigmine has an important effect on the membrane potential after the spike (Fig. 19; cf. Eccles *et al.* 1942), but made no appreciable difference to the present results.

TABLE 8. Effect of calcium on end-plate step

	Calcium concn. (mM.)	Resting potential (mV.)	Step height (mV.)	No. of exps.	Step ratio and S.E. of mean
Total measurements	1·8	91	33 (26–44)	25	—
	3·6	91	41 (25–50)	71	—
Paired measurements	1·8	92	32	9	1·25± 0·046
	3·6	92	39	9	

As with the curarine experiments (Table 1) a high degree of variability was again encountered in the size of the e.p.p. which differed at individual junctions much more than the resting or action potential of the membrane. In the present measurements, this variability showed itself, not in the level of the e.p.p. at

which the muscle impulse takes off—this was relatively constant—but in the *time* needed for the e.p.p. to rise to this threshold level. The variations in the entire muscle must have been greater than is apparent from Table 7, for most of the present results have been obtained from end-plates which had been selected during the preliminary curarine treatment because their e.p.p.'s were found to be large and easy to locate. The differences in the rate of rise of the e.p.p. must mean that even under normal conditions there are large variations in the safety margin of transmission at individual junctions. Such variation has been known for a long time: it was demonstrated by Adrian & Lucas (1912) and by Bremer (1927) who showed that during fatigue or partial curarization a variable number of fibres can be made to respond by varying the interval between two nerve impulses. Another example will be shown on p. 358 below in the variable susceptibility to anodic block at different end-plates.

We did not include in Table 7 the results from a small number of fibres in which the e.p.p. failed to reach the impulse threshold. A delayed spike was then usually recorded coming from a remote junction in the same fibre (cf. Katz & Kuffler, 1941, also Fig. 28*C* below). The local failure was presumably due to some abnormal condition of the muscle, but it was found side by side with end-plates at which transmission did not seem to be impaired and served further to illustrate the high degree of variability in junctional transmission.

The results of Table 7 show a wide dispersion in two other respects: (i) in the latency of the spike peak, and (ii) in the presence of a discrete hump which was clearly discernible in only some of the records. Both variations result from the variable size of the initial e.p.p.: the time to the peak includes the variable duration of the initial step, and a discrete hump could be seen only when the spike took off sufficiently early during the e.p.p. so that a residual transmitter effect, 2 msec. after the start, was not obscured by the spike peak.

In Table 2B, the responses of the same end-plates are compared (i) in fully curarized muscle and (ii) after withdrawal of curarine. The e.p.p. height was measured at a fixed interval, 0·44 msec. after the start. The results indicate that the e.p.p. in the fully curarized muscle was reduced to about one-quarter (with variations between 0·18 and 0·34). In another muscle, the same dose of curarine reduced the e.p.p.'s to about one-eighth. In general, a dose of curarine seemed to depress small e.p.p.'s more than large e.p.p.'s, and the dispersion in e.p.p. sizes, therefore, appeared to be greater in curarized than in normal muscle.

Comparison of the electric response at and off the end-plate

In Fig. 21, an experiment is illustrated in which the action potential was recorded at various distances along the same fibre. As the microelectrode moved away from the end-plate, the complex response (step-peak-hump, Fig. 21, 4–6) changed into a simple conducted spike which travelled in both directions, at a uniform velocity of about 1·4 m./sec. The initial step declined in the manner already shown for the rising phase of the curarized e.p.p., and its sharp ascent was replaced by the gradually increasing 'foot' of the conducted potential wave. The 'hump' was noticeably reduced, a few hundred microns away from

the end-plate (e.g. Fig. 17), and vanished as the electrode was moved farther. The shape of the conducted spike varies somewhat from fibre to fibre, and its peak has often an 'angular' appearance as in Fig. 24 (*M*) below, but there is

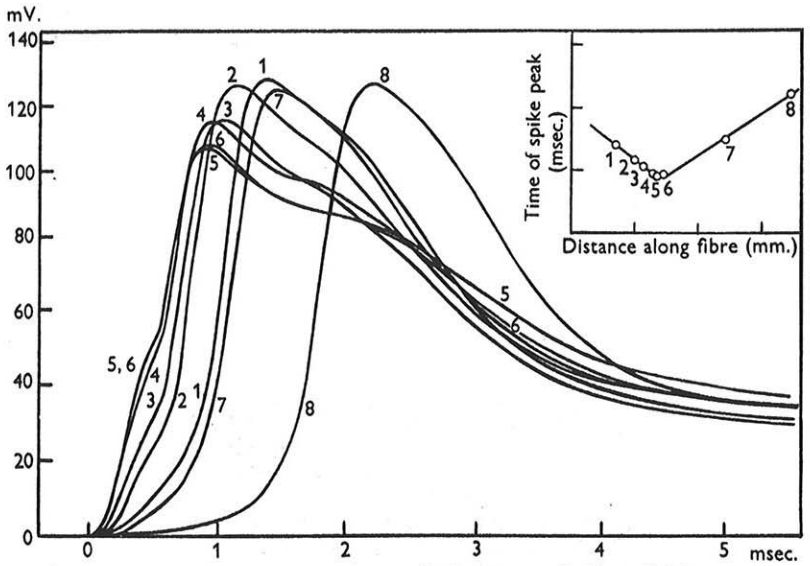

Fig. 21. The transition of electric activity from end-plate to muscle fibre. Calcium concentration, 9 mM. Temp. 17° C. The microelectrode was moved along the fibre, and records were obtained at the following positions (distance from position 1): (1) 0 mm.; (2) 0·3 mm.; (3) 0·45 mm.; (4) 0·6 mm.; (5) 0·65 mm.; (6) 0·75 mm.; (7) 1·75 mm.; (8) 2·75 mm. The resting potential was between 88 and 92 mV. during these records. Note the gradual changes in the shape of the action potential and spike latency. Inset: the time of the spike summit is plotted against distance, showing a propagation velocity of about 1·4 msec. in both directions from positions (5) and (6).

no doubt that the hump-like protrusion is a distinct feature of the end-plate response.

Fig. 21 indicates that the amplitude of the action potential increases by some 10–20 mV. as it is conducted away from the end-plate. In Table 9, the mean values of a large number of measurements, at and off the end-plate, have been listed. The resting potentials do not differ appreciably in the two situations,

TABLE 9. Active-membrane potential *on* and *off* the end-plate

(Mean values and S.E. of means.)

	I Resting potential (mV.)	II Action potential (mV.)	Active membrane potential (II − I)	No. of exps.
End-plate	91±0·43	113±0·77	22±0·67	134
Off the end-plate	88±0·6	123±1	35±1·1	52

Reduction of active-membrane potential at the end-plate: 13±1·3 mV.

but the amplitude of the spike is considerably higher in the nerve-free portion than at the end-plate. The 'active-membrane potential', i.e. the level of the reversed p.d. during the peak, is $35 \pm 1{\cdot}1$ mV. (S.E. of mean of 52 experiments) off the end-plate and $22 \pm 0{\cdot}67$ mV. (134) at the end-plate. Thus a difference of over 10 mV. remains to be accounted for. In individual experiments the value of this difference was subject to considerable variation, but this arose to a large extent from variations in the size of the e.p.p. The peak of the action potential at the end-plate was significantly depressed only when it originated at an early moment and was followed by a discrete hump. Apparently the diminution of the active-membrane potential depended upon the persistence of intense transmitter activity at the time of the peak, and no such reduction occurred when the spike arose too late for this interaction to take place.

In order to obtain more conclusive information on the nature of the end-plate response, the initiation of a spike by the neuromuscular transmitter was compared with that by artificial electric stimulation.

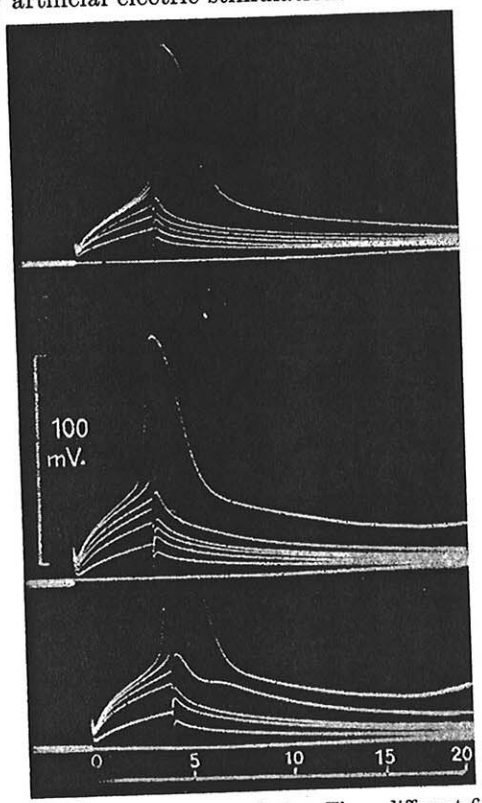

Fig. 22. Membrane potential during direct stimulation. Three different fibres, in which potential changes were recorded, near the cathode, with several subthreshold and one superthreshold current pulse. Note inflexion and local response with subthreshold current pulses. Time, msec.

The end-plate step

Two microelectrodes were placed into the same muscle fibre, less than 50 μ. apart, and one was used as a stimulating electrode by passing an outward current pulse through the membrane, while the change of the membrane potential was recorded by the other electrode. With a sufficient current strength, the membrane potential falls from its resting level, of 85–90 mV., to a point at which a spike is generated. The process is shown in Fig. 22, the time course of the curves being very similar to those previously obtained with external electrodes (Hodgkin & Rushton, 1946; Katz, 1948). The important point is that the height of the step which precedes the action potential is substantially the same as the height of the end-plate step during neuromuscular transmission. The step was measured in the same way as indicated in Fig. 20, and the results are shown in Table 10. In twenty experiments, using 3·6 mM-$CaCl_2$, the mean

TABLE 10. Comparison of initial 'step' with direct and indirect stimulation

	Direct stimulation			End-plate potential		
Calcium concn. (mM.)	Resting potential (mV.)	Step height (mV.)	No. of exps.	Resting potential (mV.)	Step height (mV.)	No. of exps.
3·6	86	39 (31–50)	20	91	41 (25–50)	71
1·8	85	36 (30–41)	7	91	33 (26–44)	25

height of the step was $39 \pm 1{\cdot}2$ mV. as compared with $41 \pm 0{\cdot}6$ mV. for the e.p.p., while in seven experiments with 1·8 mM-$CaCl_2$, it was 36 mV. (compared with 33 mV. for the e.p.p.). The result was the same whether the stimulus was applied at the nerve-free end of the fibre, or at the end-plate position. The level at which the spike originated seemed to be independent of the time taken to reach it: this time depended upon the current strength, and in the different records varied between 0·3 and 8 msec.

The measurement of the step height is related to the excitation threshold of the muscle fibre, that is to the critical level at which the membrane potential becomes unstable. This level can be found by using a short threshold shock (see Hodgkin, Huxley & Katz, 1949), or by breaking the current at the critical point at which the membrane potential is left 'in the balance', neither rising nor falling for a short time after the break. In practice, the current pulse was increased in small steps, and the largest potential change which just failed to flare up into a spike was taken as an indication of 'threshold' (Fig. 22). Using this method, the threshold depolarization was found to be several millivolts higher than the step, measured in the conventional way adopted above. Measurements of 'step height' and 'threshold level' are shown in Table 11, the means of eight experiments being 38 and 44 mV. respectively. These values are considerably larger than the figure of 15 mV. recently reported for the giant axon of the squid (Hodgkin *et al.* 1949), but the experimental conditions differ

in two important respects: (*a*) the threshold of the squid axon was measured by uniform stimulation of a long length of fibre instead of *at one point*, and (*b*) the resting potential of the isolated squid axon is about 30 mV. less than that of

TABLE 11. 'Step' height and threshold level

(Calcium concentration 3·6 mM. in all fibres except VIII where it was 1·8 mM.)

Fibre	I Resting potential, (mV.)	II Step height, (mV.)	III Threshold level (mV.)	Difference (III – II), (mV.)
I	87	39	44	5
II	85	35	37	2
III	82	36	42	6
IV	78	31	36	5
V	81	38	47·5	9·5
VI	91	42·5	50·5	8
VII	90	46	50	4
VIII	88	38	43·5	5·5
Mean	85	38	44	6

frog muscle. If we were to define 'threshold' as a critical *membrane potential*, rather than a critical *depolarization*, the difference between the two sets of measurements would almost vanish, the 'threshold' being at about 45 mV. in either case.

It is safe to conclude from the present experiments that the height of the end-plate step is determined by the threshold of the surrounding muscle membrane, and that the threshold of this region does not differ by more than a few per cent from the threshold of other parts of the muscle fibre.

The end-plate spike

The analogy between an applied current and the neuromuscular transmitter helps us to account for the height of the initial step, but it fails to account for the further course of the end-plate response, for its reduced amplitude and the appearance of a hump on its declining phase.

It might be suggested that the size of the spike would, for some reason, be smaller at the point where it originates than after it has been conducted over a distance, and that this would account for the discrepancy of the active-membrane potentials in Table 9. It was important, therefore, to compare the active-membrane potentials for a locally initiated and a conducted spike. Two successive records were taken from the same point of a muscle fibre, in a nerve-free part: first observing the conducted spike which was elicited some distance away, either via the nerve or by a direct stimulus through another internal electrode. The second microelectrode was then inserted close to the recording point (less than 50 μ. distant) and another, direct, stimulus was applied. An example is shown in Fig. 23 where the peaks of the two action potentials are seen to differ by only 2 mV. The twitch during the first response usually pro-

duced some local damage associated with about 10% drop of the resting potential, but evidence will be presented, on p. 354 below, that this did not cause an immediate noticeable change in the active-membrane potentials, whose measurements therefore remained valid.

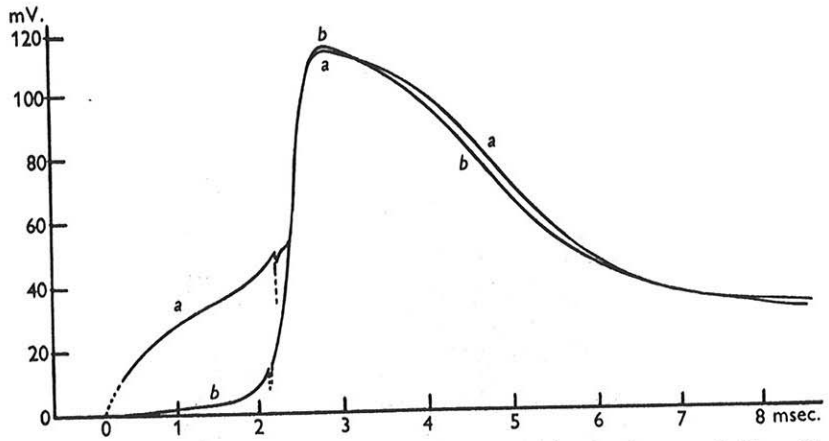

Fig. 23. Comparison of conducted and 'locally originating' spikes in a muscle fibre. The stimulating electrode was (*a*) 40 μ., and (*b*) 2 mm. from the recording electrode.

The effect of local mechanical damage can be appreciated quantitatively in the following manner. In the unstimulated fibre, the resistance across the membrane was on the average 200,000 Ω. (Table 4) and the potential difference 90 mV. Neglecting any initial leakage (cf. Nastuk & Hodgkin, 1950) a 9 mV. (10%) reduction of the resting potential implies that a small leak has sprung around the microelectrode, amounting to a shunt of 1·8 MΩ. (cf. Appendix II). During the spike, the effect of this shunt becomes much less important because the resistance across the membrane has fallen to about 20,000 Ω. (see p. 356 below); the reduction of the active-membrane potential (35 mV.) caused by a leak of 1·8 MΩ. is less than 0·5 mV.

In six experiments in which conducted and locally initiated spikes were compared, the active-membrane potential of the former was on the average 1·5 mV. less than that of the latter, an insignificant difference (S.E. of mean ± 1·3 mV.) and of opposite sign to that required.

The second possibility which had to be examined was that the features of the end-plate response might be imitated by a direct stimulus, if the applied current pulse were maintained throughout the period of electrical membrane activity. When this was done, a number of interesting changes were produced which will be described in the following section, but they bore no resemblance to the end-plate response. On the contrary, under the influence of a maintained outward current, the action potential continued to build up to a higher peak, and there was no indication of a hump during the decline. This effect of the applied current was seen invariably, whether the current was passed through the end-plate or through other regions of the fibre surface.

It might further be suggested that the characteristic features of the end-plate spike depend upon special properties of the muscle fibre at the junction, quite irrespective of release and local action of the transmitter. To decide this question, the action potential must be recorded at the end-plate when it is set up (*a*) by a nerve impulse (*N*) and (*b*) by a direct stimulus (*M*). Two successive records were obtained from the same end-plate with alternative stimulation of *N* and *M*. The sequence of the shocks was varied in different experiments. Usually, however, the direct stimulus was applied first, as it caused only one

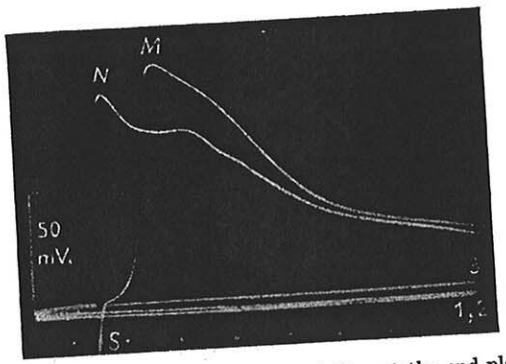

Fig. 24. Response to nerve (*N*) and direct (*M*) stimulation at the end-plate region of a muscle fibre. The direct current pulse was applied about 1·5 mm. away. *S*: stimulus artifact at the end of the direct pulse; 1 and 2: baselines (the lower corresponding to *N* the upper to *M*) showing a small drift of resting potential between the two stimuli, 3: electrotonic potential due to a subthreshold pulse. (The unusual notch in the *N* response following the hump, was due to an external spike potential of adjacent fibres.) Time marks, msec.

fibre to contract. An example is shown in Fig. 24, and for better comparison the two records have been superimposed, by displacing one along the time axis, in the tracings of Fig. 25. It is clear that the muscle spike (*N*) arising from an e.p.p. fails to reach the level which it attains (*M*) in the absence of the e.p.p. The results of fifteen similar experiments are summarized in Table 12. The

TABLE 12. Comparison of *M* and *N* spikes at the same end-plates

	M			*N*			Difference (*M* − *N*) of active-membrane potential (mV.)
Fibre	Resting potential (mV.)	Action potential (mV.)	Active-membrane potential (mV.)	Resting potential (mV.)	Action potential (mV.)	Active-membrane potential (mV.)	
			A. Three selected experiments				
I	88	119	31	85	101	16	15
II	90	121	31	85	97	12	19
III	90	115	25	93	103	10	15
Mean	89	118	29	87	100	13	16
			B. Fifteen experiments				
Mean	87	111	24	80	91	11	13±1·3

first part of this table contains the measurements on three fibres, in which little or no local damage occurred, and the resting potential did not change appreciably between the two records. The mean values of all measurements are shown in the second part of Table 12; they include several experiments in which a substantial

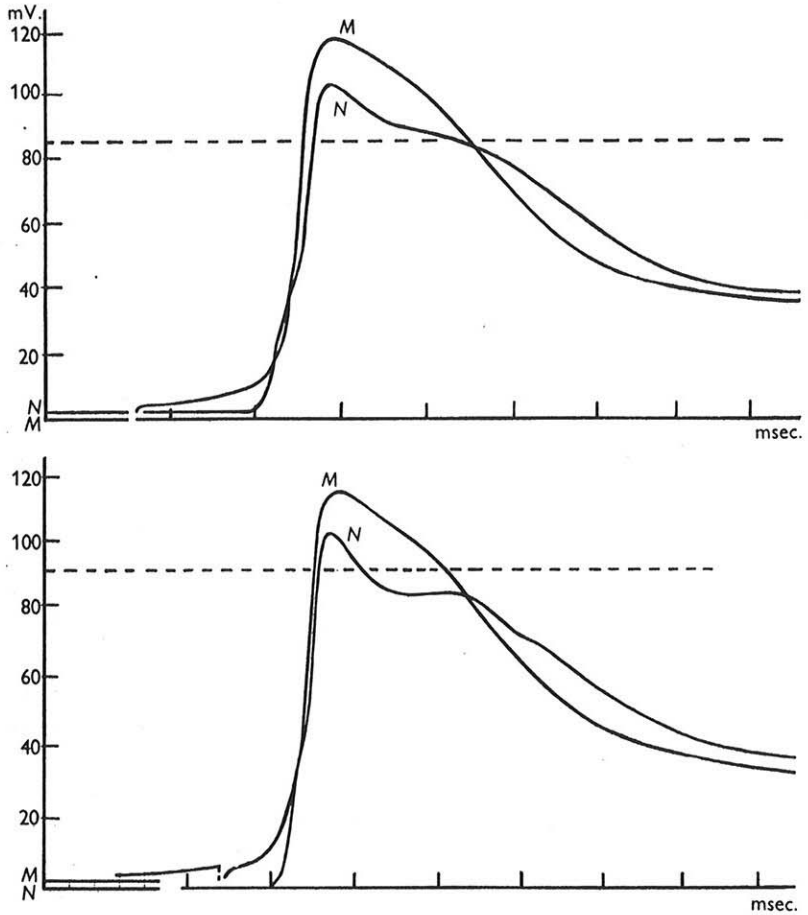

Fig. 25. Tracings of *N* and *M* responses at the end-plate region. Examples from two end-plates. Broken line: zero p.d. across membrane. A small drift of the resting potential between successive stimuli is indicated by the displacement of *M* and *N* baselines.

drop of the resting potential occurred after the first stimulus, but as pointed out above (see p. 354 for further details), the measurement of the active-membrane potential remains valid. Whether we take average values or selected experiments in Table 12, the result is equally conclusive. It shows that the reduction of the active-membrane potential must be attributed to the effect of the neuromuscular transmitter. This reduction amounts to more than 10 mV., and

if we compare Table 12 with the previous results of Table 9, it appears that the differences in active-membrane potentials recorded *on* and *off* the end-plates are thus entirely accounted for.

This observation throws some light on the mode of action of the neuromuscular transmitter. The reaction between acetylcholine and the end-plate not only fails to reach the level which is attained by the membrane action potential, but it drags the action potential down to a lower level. A simple explanation of this fact would be provided if the transmitter/end-plate reaction were to 'short-circuit' the active muscle membrane. The spike arises from a sudden and specific increase of sodium permeability, casusing the membrane potential to approach the equilibrium level of a 'sodium electrode' (Hodgkin & Katz, 1949; Nastuk & Hodgkin, 1950; Hodgkin, 1951). The e.p.p. does not appear to be produced in this way, and we assume that during the action of acetylcholine the end-plate undergoes a much more drastic change of its surface properties and becomes permeable to ions generally. We assume, in other words, that the end-plate membrane suffers a transient insulation breakdown of the kind postulated by Bernstein and Höber, in contrast with the active change of the surrounding muscle membrane which is now known to depend upon a selective permeability to sodium. This 'short-circuit' hypothesis has a number of interesting consequences, some of which have been worked out in Appendix II and subjected to experimental test in the following sections.

In the first place, the hypothesis implies that under the most favourable conditions—in the absence of blocking agents and with the maximum quantity of acetylcholine being released—the e.p.p. could approach, but not exceed, simple depolarization. In order to depolarize, i.e. to provide an effective short-circuit for the resting potential, the leakage resistance across the end-plate has to be considerably less than the 200,000 Ω. across the resting muscle membrane (see Appendix II). To reduce the active-membrane potential from 35 to 22 mV., the leakage across the end-plate has to be of the same order as the reduced resistance across the active fibre surface. It will therefore be of great interest to find the value of this active-membrane resistance.

During normal impulse transmission, the required leakage of the end-plate 'sink' may also be estimated from the known rate at which the resting muscle membrane is discharged through this sink (normally 40–50 mV. in about 0·5 msec.) and the two independent estimates should be compared.

In Appendix II, the depolarizing effect of a shunt resistance suddenly placed across the muscle membrane has been calculated and the results indicate that the leakage of the active end-plate is of the order of 20,000–30,000 Ω. during normal transmission. In the following section, experiments are described which indicate that the resistance across the active fibre surface is of the same order of magnitude.

Finally, it follows from the 'short-circuit' hypothesis that the size of the e.p.p.

should be directly proportional to the resting potential. For example, if we were to raise the membrane potential to twice its normal resting level, by 'anodizing' the end-plate region, the same short-circuiting effect of the transmitter should then produce twice as large an e.p.p., and this relation should hold under all conditions, during neuromuscular block as well as in normal impulse transmission.

Resistance changes of the active fibre membrane

Measurements with alternating current have shown that the membrane impedance of nerve and muscle fibres undergoes a rapid diminution during the passage of an impulse (Cole & Curtis, 1939; Katz, 1942). Cole & Curtis concluded that the 'high-frequency conductance' (see Cole, 1949) of the axon membrane increases about 40-fold during the spike. The exact time course of this change was difficult to determine, but it is certain that the membrane conductance reaches its peak very rapidly during the rising phase of the action potential.

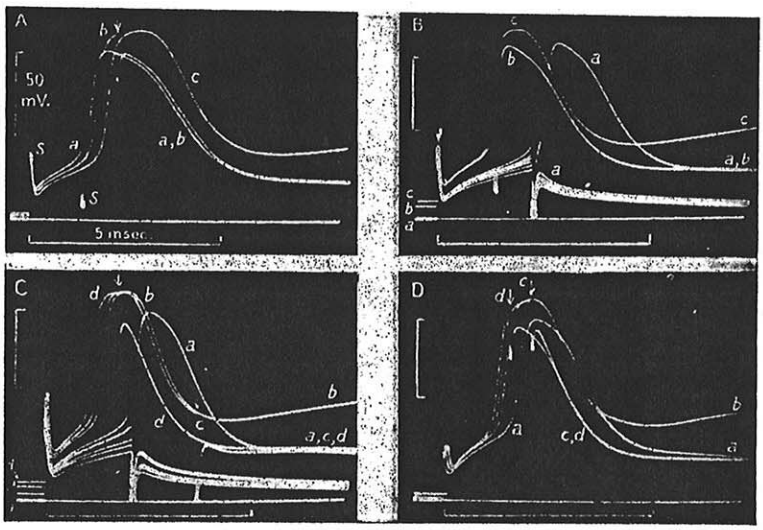

Fig. 26. The effect of an extrinsic current during the muscle spike. Examples from four muscle fibres. A: three responses, with the current (*a*) being broken at the beginning of the spike, (*b*) near its peak (marked by arrow), (*c*) being continued throughout. Note the rapid transition at the arrow, from 'current-on' to 'current-off' curve. B: (*a*) several subthreshold and one superthreshold pulse; (*b*) and (*c*), two responses with current *off* and *on*, respectively. C: showing several subthreshold and four superthreshold stimuli. With the latter, the current was broken either at the beginning (*a*), or during the spike indicated by arrows (*c*), (*d*), or maintained throughout (*b*). In B and C, note drift of resting potential (initial baseline displacement), but little or no change in active-membrane potential. D: four spikes, with the currrent alternatively *off*, *on*, or broken at moments marked by arrows. Note rapid change at the arrows, from current-*on* to current-*off* type of response. S: stimulus artifacts at make and break of current pulse.

It is possible to obtain an estimate of the changes of membrane resistance by passing a constant current through the fibre membrane and measuring the p.d. which the current adds (henceforth called the 'extrinsic p.d.') during and

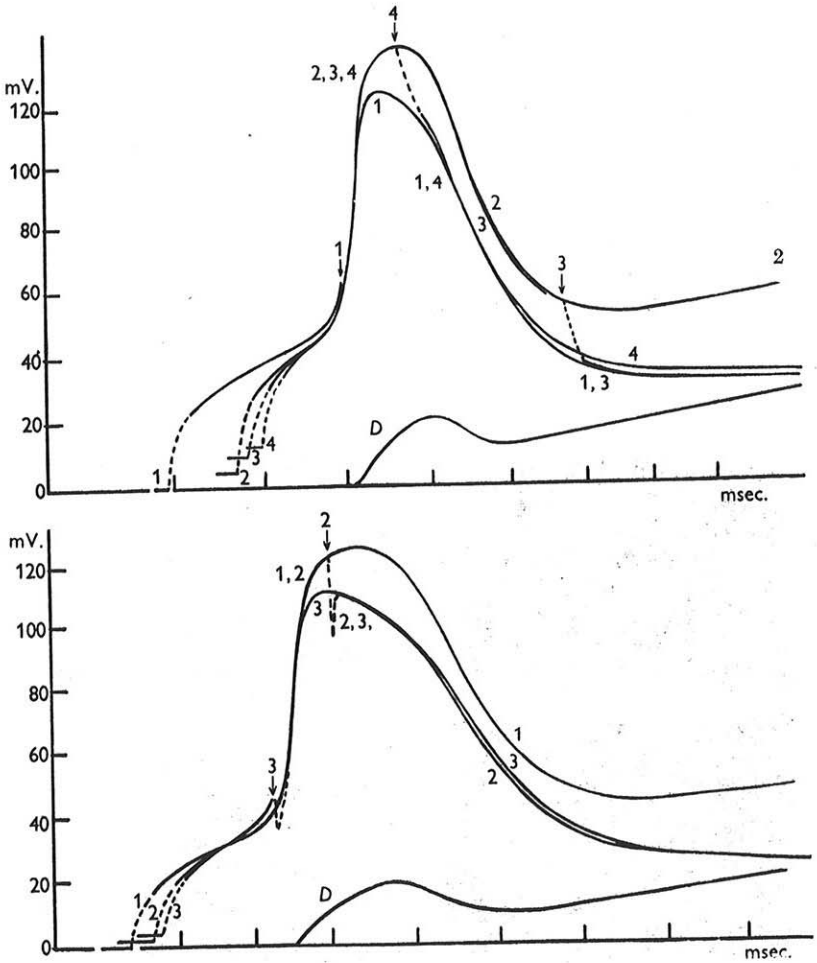

Fig. 27. Effect of extrinsic current during spike. Superimposed tracings. Examples of *on* and *off* curves from two muscle fibres. The 'extrinsic potential' built up by the current pulse during the spike is shown by curve *D* (which is obtained by subtracting the mean *off* from the *on* curve). Note: although the resting potential fell progressively, as seen by the displacement of initial baselines, the active-membrane potentials showed little change.

after the spike. The procedure was to stimulate with a second microelectrode and to break the applied current at various moments after threshold had been reached (Fig. 26). The experiment depended upon a comparison of successive spike records, with the current on or off, and it was necessary in the first instance to decide whether successive records, obtained usually with progres-

sively falling resting potential, are strictly comparable. It has already been mentioned that a 10 or 20% drop of the resting potential, due to mechanical injury, does not necessarily invalidate the measurements of the active-membrane potential. The justification for this is shown in Fig. 27. In this figure, successive records have been superimposed, by shifting the individual spikes horizontally, until the ascending phases met, but *without* shifting them vertically. Although the resting potentials (initial baselines) differed by several millivolts from one record to the next, the action potentials remained almost exactly superimposable. If the current was maintained beyond the initial subthreshold period, there was:

(i) No noticeable change in the maximum rate of rise of the spike.

(ii) A distinct addition to, and broadening of, the peak.

(iii) An increase in the maximum rate of fall of the spike.

(iv) A later gradual redevelopment of the extrinsic potential difference.

If the current was broken at any moment during this sequence, the membrane potential returned from the 'current on' to the 'current off' curve within a fraction of a millisecond. It would appear from the results in Fig. 27 that we are justified in using the 'off'-curve as a baseline, from which the extra p.d. due to the maintained outward current can be measured. The only region in which reliable measurements could not be made was the steep ascending phase. A slight lateral displacement of the superimposed records would make a considerable difference here. It is unlikely that the applied current produces a large extra p.d. during this phase: (*a*) because the upstrokes of the superimposed curves, as in Fig. 27, cannot be displaced from each other by more than 30 μsec. without noticeably mismatching the later parts of the curves; and (*b*) because the membrane conductance is known to reach its peak during the rising phase, and hence only a small extrinsic p.d. could be expected. It was, indeed, somewhat surprising to find that such a conspicuous potential change is produced by an applied current at the peak of the spike. Previous measurements (Kuffler, 1942*b*) indicated that no such addition occurs and that, on the contrary, the whole of the electrotonic potential collapses during the spike peak. But the discrepancy between Kuffler's and the present results is explained by the fact that we have recorded the action potential within 20–40 μ. of the cathode, while in the previous work an electrode of about 2 mm. width was used which would reduce the observed p.d. effectively to zero. It will be noted from equation 2 (see p. 334) that immediately at the cathode the p.d. produced by an applied current is proportional to $\sqrt{(r_m r_i)}$ so that, even when the membrane resistance has dropped to 1% of its resting value, one-tenth of the previous electrotonic potential should still be observed. But the length constant $\sqrt{(r_m/r_i)}$ is then also reduced to one-tenth so that, 1 or 2 mm. away, the extrinsic p.d. will be effectively abolished.

The time course of the extrinsic p.d. is indicated in Fig. 27 (*D*). As has been pointed out, the initial part of this curve (*D*) coinciding with the steep ascending phase of the spike is uncertain and depends upon the exact point at which the records have been superimposed. There is, however, no such uncertainty about the later course of the extrinsic p.d., measured during and after the peak of the spike. It has a characteristic shape with a maximum 0·5–1·0 msec. after the peak of the spike, and a minimum about 1 msec. later. This was observed in all fifteen experiments of this type, and it suggests that during the spike the muscle membrane undergoes two separate phases of increased ion permeability, one associated with the rise, the other accompanying the fall of the action potential. It will be noted that a relatively large extrinsic p.d. is built up during the slow initial phase of decline (the 'angle'), but it drops to a minimum later when the action potential falls more rapidly. This result seems to be analogous to recent observations by Weidmann (1951) on mammalian heart muscle and has an interesting bearing on the ionic theory of the impulse developed by Hodgkin & Huxley (1950; see also Hodgkin *et al.* 1949). According to this theory, two separate permeability changes occur during the spike: the rising phase is associated with a momentary increase of permeability to sodium ions, but this is a transient change which becomes rapidly exhausted or inactivated. It is followed after a brief delay by a phase of high potassium permeability which leads to a rapid return of the membrane potential to its original level. The present results provide evidence for two separate changes of membrane conductance, a transient increase during the ascending phase, and a second increase during the fall. The two conductance changes may well be associated with the two separate phases of sodium and potassium transfer envisaged by the theory of Hodgkin & Huxley.

In nine experiments, the strength of the outward current was measured simultaneously with the extrinsic p.d. From the ratio of the two values, an approximate estimate of the membrane resistance can be obtained. The estimate depends upon the assumption that the time constant $r_m c_m$ of the active membrane is brief compared with the time course of the extrinsic p.d. and the associated resistance change. Although this is over-simplified, it is approxi-

TABLE 13. Resistance across fibre membrane during the falling phase of the spike
(Measured at the 'dip', cf. Fig. 27, *D*.)

Summary of the values of $\frac{1}{2}\sqrt{(r_m r_i)}$ in nine experiments:

9000 23,000 14,000 24,000 24,000 21,000 42,000 22,000 12,000 Ω.

Mean 21,000 Ω.

mately true for the relatively slow changes after the peak of the spike, when the time constant of the membrane appears to be of the order of 0·3 msec. (compared with 30 msec. in the resting muscle). In Table 13 the ratio of extrinsic p.d./outward current is given, at the time of the 'dip', in nine experiments. The

mean value is about 20,000 Ω., varying between 9,000 and 42,000 Ω. These values represent the transverse resistance $\frac{1}{2}\sqrt{(r_m r_i)}$ of the active muscle fibre, measured during the falling phase of the spike: they are about one-tenth of the resting value (Table 4) which indicates that the membrane resistance r_m, at that moment, is only about 1% of the resting values, 40 Ω.cm.^{2} instead of 4000 Ω.cm.^{2}. During the rising phase of the spike, the resistance is presumably even lower. At the time of the spike summit, the extrinsic p.d. is of about the same size as during the 'dip', and we may tentatively regard the value of 20,000 Ω. as representing the active-membrane resistance during the peak of the spike as well as later during its falling phase.

The time constant of the active membrane is, therefore, also of the order of 1% of the resting value, about 0·3 msec. compared with 30 msec. This is borne out by the fact that the added extrinsic p.d. disappears within a fraction of a msec. when the current is broken during the spike (Fig. 27).

We suggested that the active end-plate, in spite of its minute size, short-circuits the surrounding active fibre membrane, bringing its potential down, from 35 to 22 mV. In order to produce this effect, it is clear that the active end-plate must itself have a low resistance, of the order of 20,000 Ω. The presence of such a low-resistant sink must have an important influence on the further time course of the action potential, and it is possible to explain the appearance of the end-plate 'hump' without additional assumptions. It can be seen from Fig. 25 that the 'hump' is in reality due to a rapid fall of the active-membrane potential from its peak towards a lower level which is not far from zero, and to a delayed return from this to the resting level. The hump is probably due to a continued short-circuiting of the membrane which not only reduces the 'sodium-potential' of the active fibre membrane, but causes it to discharge quickly when the period of high sodium permeability comes to an end. Similarly, the continued leakage through the end-plate must delay the restitution of the membrane potential, and these effects are probably responsible for the characteristic 'hump' of the end-plate spike.

To summarize, the neuromuscular transmitter not only produces an e.p.p. which gives rise to a muscle spike, but it interacts with the further course of the spike by depolarizing the active membrane and holding its potential close to zero.

The relation between end-plate potential and resting membrane potential

It is possible to change the p.d. across the fibre membrane, by means of electric currents, over a fairly wide range without substantially altering the resistance or capacity of the membrane (Hodgkin & Rushton, 1946; Katz, 1948). This method helps one to distinguish between three conceivable mechanisms by which the e.p.p. may be produced. The e.p.p. might be the result of one of the following processes: (*a*) the transfer across the end-plate of a fixed number of ions (for example, by extrinsic current flow from the motor nerve);

(*b*) a shift of the membrane potential towards a fixed new level (determined, for instance, by a *selective* permeability change to one species of ions); (*c*) the establishment of a *non-selective* ion sink, equivalent to placing a fixed leak resistance across the membrane.

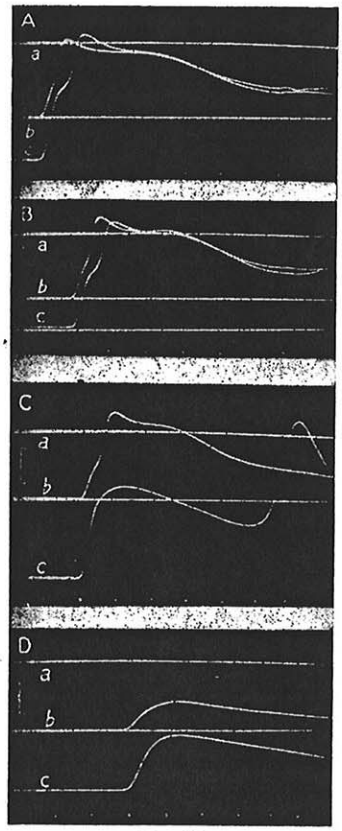

Fig. 28

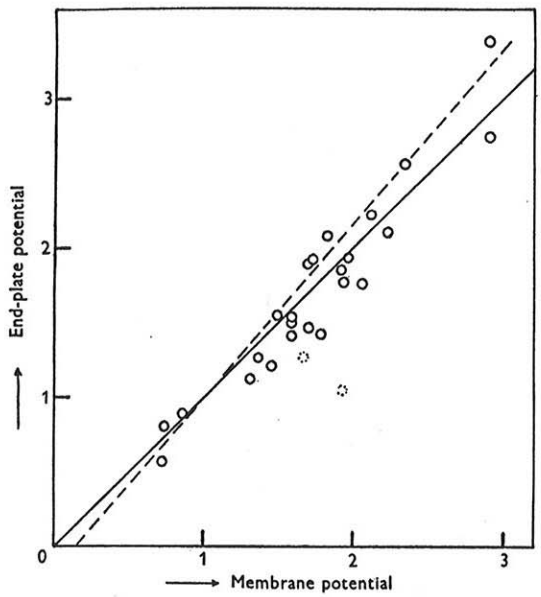

Fig. 29

Fig. 28. The effect of increased ('anodic') resting potential on the size of the e.p.p. Examples from four end-plates. *a*: zero p.d. across membrane; *b*: normal resting potential; *c*: resting potential has been increased by applied inward current. At C, transmission is blocked by the inward current, while at D transmission had failed at the normal level of the resting potential. Voltage scale: 50 mV., time marks: msec. (Record A(*b*) was taken with too high electrode resistance, giving some amplitude reduction; in all other records no appreciable distortion occurred.)

Fig. 29. Relation between size of end-plate potential and initial membrane potential. Normal resting and end-plate potentials are taken as unity. Normal resting potentials were: 90 mV. mean (65–104 mV.). E.p.p's were measured at a fixed time after their start (before the spike originated or, if transmission was blocked, at the peak of the e.p.p.). The full line indicates direct proportionality; the broken line intersects the horizontal axis at the theoretical junction p.d. between Ringer and myoplasm (see text).

Process (*a*) would give a constant amplitude, and constant charge, of the e.p.p., independent of the initial level of the membrane potential (provided resistance and capacity of the membrane remain constant).

Process (*b*) would result in a variable amplitude, but approximately constant final level of the e.p.p., rather like that attained by the peak of the spike whose level is only slightly affected by changes of resting potential (Fig. 28).

Process (*c*) would reduce the resting potential to a constant fraction and lead to a directly proportional relation between the size of the resting membrane potential and the amplitude of the e.p.p. (see Appendix II).

The experimental procedure was to raise the resting potential to a higher level by subjecting the membrane to an inward current. The e.p.p. was observed at the normal resting potential (mean value 90 mV.) and at the increased level (varying between 118 and 235 mV.), and its size was measured at a fixed interval after the start. Examples are shown in Fig. 28, and the results of twenty-six experiments have been plotted in Fig. 29. The mean increase of resting potential in twenty-one experiments (not including the five observations discussed below) was 87·5%, the corresponding increase of e.p.p. size was 84%. There is little doubt that the size of the e.p.p. is approximately proportional to the value of the initial membrane potential, a result which is consistent with the 'short-circuit' hypothesis (*c*), but not with the other hypotheses stated above.

The results of these experiments were obtained at junctions with widely different safety margins. At some the e.p.p. formed so large a proportion of the resting potential that no anodic block could be produced; at others transmission was readily blocked by an inward current (cf. Katz, 1939), and a pure e.p.p. produced, and finally at some end-plates, the e.p.p. was small and transmission failed even without the application of an inward current. The proportional relation between e.p.p. and membrane potential shown in Fig. 29 was found regardless of the condition of the individual junction.

It may be argued that even when the end-plate has become completely short-circuited, a junction potential of some 14 mV. (Nastuk & Hodgkin, 1950) would remain between the outside bath and the myoplasm. On this basis, the theoretical relation should follow the broken line in Fig. 29 rather than the full, 45°, line.

In Fig. 29, the results of three measurements are included in which the resting potential had dropped below the original level, owing to local injury. Under these conditions, the theoretical relation is no longer exactly linear, but the divergence is too slight to be noticed.

Another point requires comment: not all muscle fibres were able to withstand strong 'anodization', and at times there were signs of dielectric breakdown when the membrane potential had been raised to some 200 mV. This showed itself in a rapid decline of the membrane potential while the inward current was maintained. The two measurements shown in Fig. 29 as dotted circles were made in this unstable condition and should, therefore, be disregarded.

DISCUSSION

A large part of our results is 'descriptive' and deals with the intracellular recording of potential changes at the motor end-plate. These results may be briefly discussed in relation to previous work in which similar techniques or preparations have been employed.

The values of resting and action potentials of the 'muscle fibre', as distinct from its end-plate (Table 9), agree with those reported by Ling & Gerard (1949) and Nastuk & Hodgkin (1950). At the end-plate, the resting potential is the same, but the action potential is reduced, provided excitation occurs via the nerve and the intensity of the transmitter action is high. The general features of the normal end-plate response (the 'step-peak-hump' complex) agree very well with those recently reported by Nastuk (1950).

The composite nature of the end-plate response had previously been demonstrated in an admirable way by Kuffler (1942*a*) who applied an external recording electrode to an isolated nerve-muscle junction. The records obtained by Kuffler differ from our results in some important respects, and these differences require an explanation. Kuffler employed a special technique of 'interface recording' which amounts in effect to the application of a microelectrode to the surface of a muscle fibre in a large volume of saline. This method had certain advantages and was well suited to Kuffler's delicate preparation, but it must be realized that under these conditions the observed potential change follows the time course of the *membrane current*, not that of the *membrane potential* (see Bishop, 1937; Lorente de Nó, 1947; Brooks & Eccles, 1947). This leads to important differences in the shape of the e.p.p. and spike, for the membrane current depends upon d^2E/dx^2, the curvature of the surface potential gradient, and may be directed inward or outward through the membrane. The 'interface' method is, in fact, a differential recording technique which is very sensitive to changes in the local potential gradient, but its results cannot be directly interpreted in terms of the membrane potential. For example, when the interface electrode was moved along the muscle fibre, the response changed very critically, and at 0·5 mm. from the end-plate, the initial e.p.p. deflexion had not only declined, but reversed its sign. This reversal means that the position of the electrode has been moved from the end-plate 'sink' where current flows *into* the fibre, to an adjacent region where the current *leaves* the fibre. At the centre of the junction, a triphasic response was recorded the first phase of which consisted of a 'pure e.p.p.' not superseded by a spike. This naturally led to the supposition that the membrane potential during the e.p.p. may attain the same level as during the spike; but with this conclusion, the present results do not agree. In our opinion, the absence of a spike component in Kuffler's experiment merely implied that the inward current through the end-plate had reached a peak during the initial

e.p.p. and begun to decline when the spike originated. To explain this behaviour, we may refer to the results of Fig. 21 and Table 9, where it is shown that the size of the e.p.p. is *greatest*, while the size of the spike is *least* at the centre of the junction. Hence, during the transition from e.p.p. to spike, the electric response at the end-plate changes from the position of a spatial maximum to that of a spatial minimum, and during this process the curvature of the potential gradient, and the membrane current, reverse. The situation may

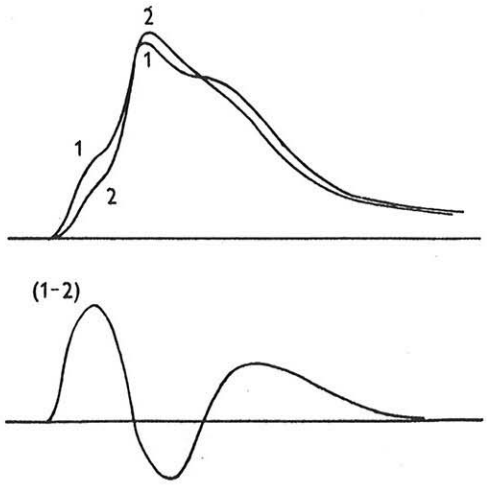

Fig. 30. Diagram explaining the derivation of 'interface' recording (cf. Kuffler, 1942*a*) at the centre of the end-plate. The upper part indicates the changes of membrane potential, (1) at the centre of the end-plate, and (2) a small distance from (1). The lower part illustrates a 'differential' record (1) minus (2), which resembles Kuffler's 'inter-face' recording.

be appreciated more easily by the simplified diagram in Fig. 30 in which Kuffler's relevant record has been reconstructed as differentially recorded between a point at, and slightly off, the centre of the junction.

To summarize, it would appear that the discrepancies between Kuffler's and the present results can be explained by differences in recording technique, remembering that we are concerned with changes of the membrane potential which are not faithfully recorded with an external microelectrode.

In its quantitative aspects the present paper provides strong support for certain views previously presented: for example, it has again been confirmed that the transmitter action at the nerve-muscle junction is a brief impulsive event, and that the characteristic spread and decay of the e.p.p. is largely determined by the resistance and capacity of the resting muscle fibre.

In addition, there have been two pieces of information which invite further comment: first, the determination of the quantity of electric charge which is transferred across the end-plate, and secondly, the fact that the end-plate

reaction leads to a smaller potential change, but apparently a larger change of conductance (per unit area), than the normal membrane spike.

It has been pointed out that the electric charge which flows across the normal end-plate during a single impulse requires the net transfer of at least $2–4 \times 10^{-14}$ mol. of univalent cations inward or anions outward and that this quantity becomes multiplied by a factor of about 50 in the presence of a cholinesterase inhibitor. The question arises how such a large flux of ions can be maintained across the presumably minute area of the motor end-plate, and what species of ions are involved. We are not in a position to answer this question, but certain, otherwise plausible, mechanisms are eliminated by the present results. It has recently been shown by Fatt (1950) that acetylcholine ions produce a substantial depolarization of the end-plate even when the external electrolyte content has been reduced to a small fraction and when no sodium is present in the outside fluid. Fatt considered the possibility that the flux of acetylcholine cations themselves might produce the necessary inward current. This did not seem a full, or very likely, explanation, but it remained conceivable under the conditions of his experiment. In view of our present results, we feel that this hypothesis has become untenable. If acetylcholine were to depolarize the end-plate by direct penetration, it would have to be released in quantities of some $1-2 \times 10^{-12}$ mol. per junction per impulse, for enough acetylcholine ions must be made available to produce and maintain the e.p.p. in the prostigmine-treated muscle. We have no adequate information concerning the surface or the volume of the nerve endings at which acetylcholine is released, but it is difficult to believe that they are large enough to contain this amount of acetylcholine. If we take Kühne's (1887) drawings of the terminal arborizations in frog muscle, we are likely to over- rather than under-estimate the size of the nerve endings (see Couteaux, 1947). The surface area of Kühne's nerve-endings tree may be as large as 10^{-4} cm.^{2}, and its volume as much as 2×10^{-8} cm.^{3}. Even if we were to assume that the intracellular cation content of all these structures is made up entirely of acetylcholine, at a concentration of 120 mM., the amount of acetylcholine inside the nerve would be only $2{\cdot}4 \times 10^{-12}$ mol, i.e. barely enough for one or two impulses. Hence, even with such extremely favourable, though unrealistic, assumptions we are led to the absurd result that practically the whole cation store of the nerve endings would have to be exchanged during a single impulse in order to produce an e.p.p.

The only reasonable alternative appears to be that small quantities of acetylcholine alter the end-plate surface in such a way that other ions can be rapidly transferred across it, not only sodium and potassium, but probably all free anions and cations on either side of the membrane. Apparently, we must think in terms of some chemical breakdown of a local ion barrier which occurs as soon as acetylcholine combines with it, and whose extent depends upon the number of reacting molecules.

An explanation of this kind fits the facts reasonably well: it helps us to understand why a depolarization by acetylcholine can still be produced in the absence of external sodium salts, and it explains why the action potential, as well as the resting potential, is short-circuited by the e.p.p. Finally, it satisfies the requirement for a very large amplification of ionic currents which must occur at the point where an impulse is transferred from minute nerve endings to the enormously expanded surface of the muscle fibre.

Our results suggest that the action of acetylcholine 'short-circuits' the muscle fibre at the end-plate and so reduces the active-membrane potential, but this effect does not occur when the muscle fibre is stimulated directly. It appears, then, that the action potential of the muscle fibre, if started elsewhere, sweeps past the end-plate region without stimulating its neuroreceptors, for if they were made to react in the way in which they respond to a nerve impulse, the active-membrane potential would be the same in either case. It is a characteristic property of nerve or muscle membranes to respond to an electric stimulus with a regenerative electrochemical reaction. This reaction is now known to depend upon a selective increase of sodium permeability (Hodgkin & Huxley, 1950), leading to rapid entry of sodium into the fibre with a consequent lowering of its surface potential and reinforcement of the initial electrical alteration. This reaction proceeds towards an equilibrium level which is near the potential of a sodium electrode (Hodgkin & Katz, 1949; Nastuk & Hodgkin, 1950; Hodgkin *et al.* 1949). Our evidence indicates that the end-plate receptors do not behave in this manner: they react to acetylcholine and various other chemical substances, but apparently not to the local currents of the muscle impulse; and if the end-plate does not respond to electric stimulation, then its electrical reaction to acetylcholine cannot be regenerative in the manner of the electric excitation of the surrounding membrane. Thus, it appears that the end-plate, i.e. the neuroreceptive area of the muscle fibre, differs from the surrounding fibre surface not only in its specific sensitivity to chemical stimulants, but in its lack of sensitivity to electric currents.

APPENDIX I

The solution of the problem considered here has been kindly provided by Mr A. L. Hodgkin.

In the special case in which a charge is placed instantaneously, at time $t=0$, on a point along the fibre, at distance $x=0$, the solution of the general differential equation for the leaky capacitative cable without net current, viz.

$$-\lambda\frac{d^2V}{dx^2}+\tau_m\frac{dV}{dt}+V=0$$

takes the form

$$V=\frac{q_0}{2c_m\lambda\sqrt{(\pi t/\tau_m)}}\exp\left(\frac{-x^2\tau_m}{4\lambda^2 t}-\frac{t}{\tau_m}\right), \tag{4}$$

where q_0 is the charge initially on the membrane and c_m is the capacity of the membrane per unit length of fibre. Taking the natural logarithm of equation (4) we obtain:

$$\log_e V=\frac{-x^2\tau_m}{4\lambda^2 t}-\frac{t}{\tau_m}+\log_e\frac{q_0}{2c_m\lambda\sqrt{(\pi t/\tau_m)}}. \tag{5}$$

Since x appears only in the first term on the right of equation (5), if for any given t, $\log_e V$ is plotted against x^2, a straight line will result with slope equal to $-\tau_m/4\lambda^2 t$, from which λ can be obtained. When applied to the curves of Fig. 9, for t greater than 3 msec., this method gives values of λ between 2·35 and 2·1 mm. For the peak of the potential wave at any position x, $dV/dt=0$; so from equation (5) by differentiating and equating to zero, one finds that

$$\frac{x^2}{\lambda^2}=\frac{4t^2}{{\tau_m}^2}+\frac{2t}{\tau_m}.$$

This equation provides a simple means of evaluating λ. Plotting $4T^2/{\tau_m}^2+2T/\tau_m$ against x^2 (T is the time of peak potential at distance x) a straight line is obtained with slope equal to $1/\lambda^2$. This method was used on p. 332, with the results shown in Table 3.

APPENDIX II

The 'short-circuiting' of the end-plate during neuromuscular transmission

It is suggested that acetylcholine short-circuits the end-plate and thereby discharges the surrounding muscle membrane and gives rise to a propagated spike. The short-circuit resistance thus placed across the end-plate surface must be low enough to produce the characteristic features of the normal end-plate response, viz. (i) to depolarize the membrane at an adequate rate and (ii) to shunt the active membrane effectively and reduce its reversed p.d. At the normal nerve-muscle junction, the resting potential is about 90 mV., and the

e.p.p. reduces it to one-half in about 0·5 msec. Experiments with anodic block (cf. Fig. 28) indicate that the maximum end-plate depolarization is reached at about 1·2-1·3 msec. (20° C.) and, in the absence of a muscle spike, amounts to about 70% of the resting potential.

The resting muscle fibre can be represented by the electrical cable model shown in Fig. 31. The quantities have been computed from Table 4 ($E_0 = 90$ mV., $r_m = 100{,}000$ Ω.cm., $r_i = 1{\cdot}6$ MΩ./cm., $c_m = 0{\cdot}3$ μF./cm.). If a short-circuit

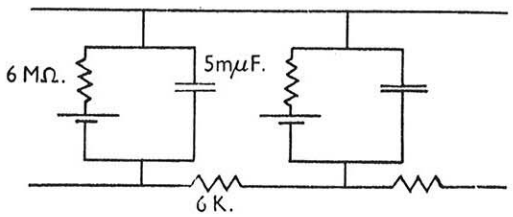

Fig. 31. Two sections of an artificial transmission line representing the passive properties of a muscle fibre. The input resistance at *one* end of the line is approximately equal to the resistance across the midpoint of a muscle fibre; the time constant is 30 msec. and the length constant is represented by thirty-two sections. The e.m.f. was replaced by a dry cell (1·58 V.).

resistance R is placed, at time $t=0$, across the mid-point of this transmission line (neglecting 'liquid junction potentials' and assuming the line to be longer than $8\sqrt{(r_m/r_i)}$) then the potential V at this point changes with time according to the following equation which has been kindly derived for us by Dr E. J. Harris:

$$\frac{V}{E_0} = 1 - a[1 - \exp t/\tau_1 . \operatorname{erfc}\sqrt{(t/\tau_2)}] + b \operatorname{erf}\sqrt{(t/\tau_3)}, \tag{6}$$

where

$$\begin{aligned}
\tau_1 &= R_0^2\, c_m/(r_i - R_0^2/r_m),\\
\tau_2 &= R_0^2\, c_m/r_i,\\
\tau_3 &= r_m c_m,\\
a &= r_i/(r_i - R_0^2/r_m),\\
b &= R_0\sqrt{(r_i/r_m)}/(r_i - R_0^2/r_m),\\
\operatorname{erfc}\sqrt{(t/\tau_2)} &= 1 - \operatorname{erf}\sqrt{(t/\tau_2)},\\
R_0 &= 2R.
\end{aligned}$$

Using equation (6), the depolarization at $t=0{\cdot}5$ msec. has been calculated for various values of R (Table 14). Another way of finding the potential changes,

TABLE 14. Relation between short-circuit resistance and 'end-plate potential.'

R (Ω.)	'e.p.p.' at $t=0{\cdot}5$ msec. (mV.)
15,000	65
20,000	56
30,000	46
50,000	32

at various points of the line, consists in constructing an electrical model similar to that of Fig. 31 and recording the changes of potential at the desired point. A line of 100 sections was used, each having the components shown in Fig. 31. This line had the same input impedance and time constant as an average muscle fibre taken from Table 4. The 'characteristic length' (i.e. about 2·5 mm.) was represented by thirty-two sections and the resting potential was replaced by a dry cell (1·58 V.). When a series of different short-circuit resistances was placed across the end of this line, a family of curves was obtained, plotted in Fig. 32. To depolarize this model at approximately the same rate as the normal end-plate (50% depolarization in 0·5 msec., 70% in 1·25 msec.) a short-circuit

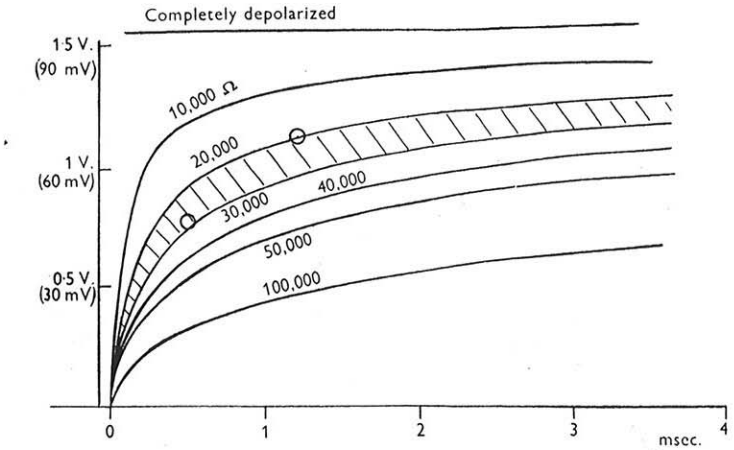

Fig. 32. Depolarization resulting from a short-circuiting of the transmission line. The two circles indicate the depolarization levels observed during the rise of the normal e.p.p. This corresponds to short-circuiting by 20,000–30,000 Ω. (shaded area). Ordinates: depolarization in volts (corresponding values for muscle fibre in brackets). Abscissae: msec.

resistance of 20,000–30,000 Ω. must be used. As the release and decay of the transmitter are gradual processes, one may assume that the resistance of the end-plate membrane falls, during the first msec., to a value rather less than 20,000 Ω. and then gradually recovers, but the average value during the rising phase of the e.p.p. appears to be about 25,000 Ω.

During normal impulse transmission we thus have, very roughly, an end-plate 'sink' with a leak resistance of the order of 25,000 Ω., in parallel with an active muscle membrane which—when *not* short-circuited by the active end-plate—produces a peak potential of 35 mV. and has a resistance of the order of 20,000 Ω. (Table 13). The presence of the end-plate sink reduces the active-membrane potential from 35 mV. to $35 \times 25{,}000/(25{,}000+20{,}000) = 19{\cdot}5$ mV. This drop of 15·5 mV. may be compared with the observed reduction of 13 mV. (Tables 9 and 12) and 16 mV. (Table 12).

An e.p.p. can be imitated in even more realistic fashion by placing a transient short-circuit, e.g. a series combination of resistance and capacity across the artificial line, with the result shown in Fig. 33. It is then a simple matter to reconstruct the experiments of p. 357, where an approximately linear relation

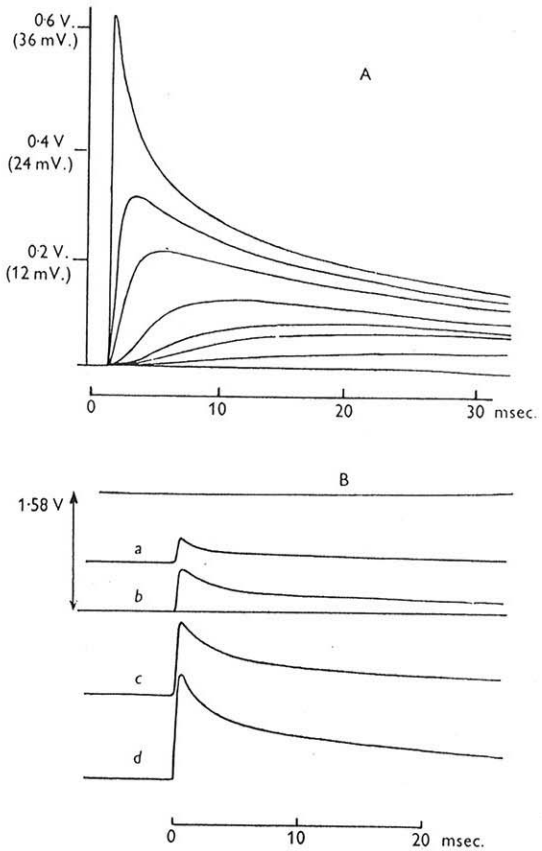

Fig. 33. A: artificial e.p.p.'s. Oscillograph tracings from various points along the artificial line, when a short-circuit of 20,000 Ω., in series with a 0·012 μF. condenser, was placed across it. The distances from the short-circuited points were, successively from above: 0 sections (corresponding to the end-plate centre); 5 sections (corresponding to about 0·4 mm. from the end-plate centre); 10 sections (0·8 mm.); 20 sections (1·6 mm.); 30 sections (2·4 mm.); 40 sections (3·2 mm.); 60 sections (4·8 mm.) Co-ordinates as in Fig. 32. B: relation between artificial e.p.p. and initial voltage level. *b*, 'normal' voltage and e.p.p.; *a*, line voltage reduced by partial short-circuit; *c* and *d*, line voltage increased by applied inward current.

between resting potential and e.p.p. was observed. As during the actual experiment, the resting potential of the model was increased by passing an inward current (through 10 MΩ.) into the line, and it was reduced by a steady shunt imitating the effect of local mechanical injury. Under these conditions,

a linear relation betwen e.p.p. and resting potential was obtained over a range from 40 to 240% of the normal level.

According to the present hypothesis, an end-plate which has been depolarized by applied acetylcholine should act as a partial short-circuit to the muscle fibre, and one would expect this to shorten the time course of a superimposed e.p.p. This prediction appears to conflict with experimental observations (e.g Fillenz & Hanafin, 1947) according to which the time course of such an e.p.p. remains unchanged. During a steady depolarization, however, additional factors must be considered which the present simple hypothesis does not take into account. The resistance of the surrounding muscle membrane does not remain constant, but has been found to increase or decrease during depolarization, depending upon the extent of the potential change (Katz, 1948). A moderate depolarization leads to a prolonged 'local response', associated apparently with entry of sodium ions into the fibre. This causes the initial potential change to build up to a higher level, and locally raises the resistance and time constant of the fibre membrane (cf. Hodgkin, 1947; Katz, 1948). A similar situation apparently occurs when acetylcholine is applied, for it has recently been shown (Fatt, 1950) that in the presence of sodium ions, the depolarization around the end-plates builds up to a higher maintained level than if sodium salts have previously been withdrawn. Fatt suggested that this is due to the regenerative action of sodium ions, which tends to spread and reinforce the depolarization in the surrounding region and thus increase the steady state resistance of the membrane. Hence, during steady depolarization by acetylcholine, we may have to consider a situation in which the end-plate 'sink' itself presents a low resistance, while the resistance and time constant of the surrounding fibre membrane are raised.

SUMMARY

1. The electrical properties of the 'motor end-plates' of frog muscle have been investigated with an intracellular recording electrode.

2. The resting potential of the end-plate membrane is about 90 mV. at 20° C.; it is the same as elsewhere along the muscle fibre and is unaffected by curarine.

3. When neuromuscular transmission is blocked, a simple end-plate potential (e.p.p.) is recorded which reaches 20–30 mV. in some fibres, but varies in amplitude over a wide range at different junctions. The e.p.p. rises sharply, reaches a peak in 1–1·5 msec. and declines to half in another 2 msec. The e.p.p. spreads electrotonically along a few mm. of the muscle fibre.

4. In a curarized muscle, the displacement of electric charge from the fibre membrane reaches a maximum at about 2 msec. after the start of the e.p.p., followed by a gradual replacement. The restoration of charge follows an exponential time course, with a time constant of 20–30 msec.

5. An analysis of the distribution of charge indicates that the active phase of neuromuscular transmission is a brief, impulsive, event lasting only a few msec., and that the prolonged spread and decline of the e.p.p. are determined by the resistance and capacity of the resting muscle fibre. The values of the membrane resistance and capacity determined from the properties of the e.p.p. are 4000 Ω.cm.^{2} and 6 μF./cm.^{2} Another series of measurements, using applied inward current and an analysis of the electrotonic potential, gives 4000 Ω.cm.^{2} and 8 μF./cm.^{2} respectively.

6. The net electric charge which is transferred across a curarized end-plate during one impulse is of the order of 8×10^{-10} coulomb, corresponding to 8×10^{-15} mol. of univalent ions.

7. In normal muscle, this value becomes 3–4 times larger. After treating the muscle with a cholinesterase inhibitor, the e.p.p. becomes greatly prolonged and the total amount of charge transferred through the end-plate increases by a factor of up to 50. Under these conditions a charge, equivalent to at least 10^{-12} mol. of univalent ions, passes through the 'end-plate sink', while building up and maintaining the depolarization of the surrounding fibre membrane.

8. During normal impulse transmission, the electric response of the end-plate differs from that of other parts of the muscle fibre in three respects: (i) the response is initiated by a large e.p.p., forming an initial half-millisecond step of about 40 mV. height; (ii) the peak of the spike is *reduced*, the reversed p.d. across the active membrane being about 20 mV., as compared with 35 mV., at other points of the fibre; (iii) during its fall, the action potential passes through a 'hump', at a level of the membrane potential which is not far from zero.

9. The height of the initial step signifies the threshold level at which the potential of the muscle membrane becomes unstable: a step of the same height is seen, when an action potential is set up by passing outward current through a muscle fibre, at or off the end-plate.

10. The subsequent characteristic features of the end-plate response (reduced peak, followed by 'hump') cannot be reproduced by an extrinsic current. Moreover, they are *not* seen when an action potential, produced by *direct* stimulation, is recorded at the end-plate, the active-membrane potential being then about 15 mV. larger than during neuromuscular transmission. Hence, the local action of the transmitter depolarizes not only the resting, but also the active surface of the muscle fibre.

11. A simple hypothesis is put forward to explain the features of the end-plate response, and also certain previous observations concerning the electromotive action of acetylcholine. Assuming that acetylcholine produces a large non-selective increase of ion permeability, i.e. a short-circuit, of the end-plate, then the production of the e.p.p., the diminution of the active-membrane potential, and the hump during the falling phase can all be explained, as well as the fact that acetylcholine depolarizes the end-plate even in the absence of sodium salts (Fatt, 1950).

12. A quantitative estimate, based upon two independent sets of measurements, indicates that the end-plate membrane is converted, during normal impulse transmission, into an ion 'sink' of approximately 20,000 Ω. leak resistance.

13. The size of the e.p.p., at normal or blocked junctions, can be varied over a wide range by increasing the resting membrane potential with anodic polarization. E.p.p. and resting membrane potential are found to be approximately proportional, as would be expected from the above hypothesis.

14. During the muscle spike, the membrane resistance falls to a small fraction, approximately 1%, of its resting value. The resistance change occurs in two phases, associated respectively with the rise and fall of the action potential, and probably corresponding to the separate phases of increased sodium and potassium permeability (Hodgkin & Huxley, 1950).

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