Depolarization of the motor endplate region by decamethonium and acetylcholine / by B. Delisle Burns and W.D.M. Paton.

[*Reprinted from the Journal of Physiology*,
1951, Vol. **115**, No. 1, p. 41.]
PRINTED IN GREAT BRITAIN

J. Physiol. (1951) 115, 41–73

DEPOLARIZATION OF THE MOTOR END-PLATE BY DECAMETHONIUM AND ACETYLCHOLINE

BY B. DELISLE BURNS AND W. D. M. PATON
From the National Institute for Medical Research, Mill Hill, London, N.W. 7

(*Received* 13 *March* 1951)

Decamethonium was first investigated because, like D-tubocurarine, it caused neuromuscular block. It was shown, however, that the action of decamethonium was not only unlike that of curare but, in many important respects, was similar to that of acetylcholine (Paton & Zaimis, 1949; Buttle & Zaimis, 1949; Zaimis, 1951). For instance, both compounds elicit a twitch from cat muscle if injected into the artery, both cause contracture of frog's rectus and of avian muscle, and both are antagonized by D-tubocurarine. Further, it has been found that decamethonium, like acetylcholine, depolarizes the muscle fibre; thus a dose of decamethonium, adequate to block neuromuscular transmission completely, reduced the injury potential of the cat's tibialis muscle by 30–50% (Brown, Paton & Vianna Dias, 1949). Such a depolarization, affecting the whole length of every muscle fibre in the body, seemed likely to cause measurable changes in the distribution of sodium and potassium. With this in mind, the uptake of ^{24}Na by muscles under the influence of decamethonium was investigated (J. L. Malcolm & W. D. M. Paton, unpublished). It was found, however, that the uptake of ^{24}Na by frog sartorii from Ringer containing this isotope was not significantly greater in the presence of decamethonium iodide (0·01%) than in its absence; further, the level of plasma ^{24}Na in a cat which had received an injection of sodium chloride rich in this isotope, was altered by less than 1% when a massive dose of decamethonium was injected which caused long-lasting neuromuscular block. Whether or not the argument on which these experiments were based was justified, the results made us suspect that the depolarizing effect of the drug was not uniform throughout the length of the muscle fibre, but was restricted to the region of the end-plate.

Most of the experiments described in this paper have been on the cat's gracilis muscle, in which the end-plate region is conveniently localized. The results show that the depolarization due to decamethonium is confined to the membrane of the muscle fibre within 3–4 mm. of the end-plate. As a consequence of this local depolarization, electrical excitability around the end-plates falls,

and at the same time there is a reduction of the end-plate potential produced by a maximal nerve volley; these are the factors immediately responsible for the observed failure of transmission from nerve to muscle. In fact, the consequences of an injection of decamethonium can be imitated by any prolonged depolarization of the end-plate region. Thus, an injection of acetylcholine in the presence of eserine, tetanic stimulation of an eserinized preparation, the application of cathodal current to the end-plate zone, or an injection of decamethonium all produce an acute and persistent depolarization beneath the end-plates, and all have the same effects upon neuromuscular transmission.

METHODS

Preparation of the gracilis muscle

A skeletal muscle ideally suited for determination of the electrical effects of drugs would: (1) Present a thin sheet of long, parallel running muscle fibres. (2) Have a minimal overlap at the interdigitation of fibres lying in series. (3) Have end-plates arranged with minimal scatter about straight lines running across the muscle surface. (4) Present at least one nerve-free end. (5) Provide a motor nerve which is easy to isolate and stimulate. (6) Have an arterial supply convenient for close arterial injection. Properties 1–3 are to some extent satisfied in the cat's sartorius, rectus abdominis, tenuissimus and brachioradialis and a few of our experiments have been carried out with these muscles. Nearly all of the results reported in this paper have, however, been obtained using the cat's gracilis, which proved generally more convenient than any of the other muscles tested.

In cats under chloralose anaesthesia, the gracilis was prepared for stimulation through its motor nerve and electrical recording from the muscle surface, in the manner described by Brown & Burns (1949). The motor nerve was freed from its fascial sheath, tied centrally, and stimulated with fine platinum wire electrodes. When close arterial injection was required a cannula was tied into the femoral artery just distal to the branches to gracilis, for retrograde injection from a syringe. It was usually possible to tie most of those branches from the artery to gracilis which supplied muscles lying deeper in the thigh. Drills were fixed in the upper and lower ends of the tibia, the neck of the femur and the ankle joint of the opposite hind-limb. The four drills were clamped, so that the gracilis lay in a horizontal plane at the bottom of a bath of liquid paraffin whose walls were made by pulling up the cut edges of the skin. The paraffin bath was kept warm by radiation from an electric fire. In some experiments the sciatic nerve was tied at the hip, to immobilize the leg. Intravenous injections were made through a cannula tied into the jugular vein. Pentamethonium and decamethonium were always given as iodides, and D-tubocurarine as chloride; doses are given in terms of the salts.

The positions of the end-plate zones in the muscle surface were determined by observation of the action potentials, recorded from a pair of exploring leads during stimulation of the nerve. These exploring leads were made to touch the muscle surface at two points about 1 mm. apart and in the line of the muscle fibres. When the pair of leads was moved across an end-plate zone, or across from one fibre to another, the polarity of the recorded action potential was inverted. End-plate zones could be distinguished from points at which the muscle fibres interdigitated by the greater latency of the action potential recorded from the tips of the fibres, compared with the latency of the action potential near the end-plate zone. Usually we recorded from the most distal group of muscle fibres, whose end-plates lie in a narrow band running fairly straight across the muscle surface parallel to the musculotendinous junction. In most areas the records obtained from the exploring leads were multiphasic and these areas were never used for an experiment, since a complex action potential always proved to be the consequence of scattered end-plates. It was nearly always possible, however, to find within the distal group of muscle fibres a site for recording which provided a simple diphasic action potential, indicating that the end-plates of the 'bundle' of muscle fibres in question were well localized.

Instruments

The recording leads were non-polarizable (Ag-AgCl) and contact with the muscle was through fine silk wicks soaked in 1 % agar-NaCl solution. The leads used for passing continuous current into the muscle were of the same type; but for direct electrical stimulation of the muscle we employed a pair of platinum wires, whose tips touched the muscle surface about 0·5 mm. apart in a line at right angles to the length of the fibres. The potential from the recording leads was fed through cathode followers into a direct-coupled amplification system, and records were photographed on film from a double-beam cathode-ray tube. In the records of slow potential changes, negativity of leads in the end-plate zone is shown as an upward deflexion of the trace. Beneath the records is drawn a diagram of the arrangement of electrodes, in which the position of the end-plate region is indicated by a black dot.

Records of the spatial distribution of potential down the length of the muscle fibres were obtained by leading from a fixed reference electrode and a mobile electrode whose wick could be traversed across the muscle surface. Since we wished to obtain complete records of the spatial distribution of potential within a few seconds, the process was mechanized, and the travelling electrode was fixed to the plunger of a large ground-glass syringe. Air was forced in and out of the barrel of this syringe from a mechanically driven pump, thus moving the plunger with its electrode, backwards and forwards, along the line of the muscle fibres. The electrode holder rested on a flexible metal template, which could be bent as required, to enable the electrode to follow precisely the contours of the muscle. Movement of the plunger was transmitted through an electrical system to the X-sweep of the cathode-ray tube, and we were able in this way to obtain a complete curve of potential plotted against space in 1–2 sec. The wick of the mobile electrode could slide up and down a group of muscle fibres once every 5 sec. for a whole afternoon, without producing visible damage or measurable change of electrical properties.

RESULTS

The site of action of decamethonium

In the experiments described below, we measured potential differences between a reference electrode placed on the musculotendinous junction of the cat's gracilis, and an electrode making contact with various points along the length of the most distal group of fibres in that muscle. The point of contact of the distal or 'reference' electrode was fixed, while the central or 'recording' electrode could be moved over the whole length of these muscle fibres. In control experiments, when the recording electrode was moved steadily from the tendinous end of the muscle, across the end-plate zone and down to the proximal tips of the muscle fibres, the fluctuations of potential observed were never more than $\pm 0{\cdot}5$ mV. and sometimes less than $\pm 0{\cdot}1$ mV. These 'base-line' fluctuations varied from one experiment to another, and certainly bore no relation to the known position of the end-plate zone (cf. Buchthal & Lindhard, 1934).

When the ends of the muscle fibres beneath the reference electrode were injured by burning, cutting or pinching, a potential (the injury potential) of about 30 mV. was recorded between this point and an electrode resting anywhere upon the undamaged part of the muscle fibres. In these circumstances, the reference electrode is put in close electrical contact with the interior of the injured muscle fibres, and the 'injury' potential so recorded gives a measure of the potential across the membrane beneath the other electrode. The fraction of

the membrane potential which is recorded in this way depends upon short-circuiting by interstitial fluid, but although variable from cat to cat (the extreme values obtained were 12 and 40 mV.) the value within one region of one muscle under paraffin varied little, falling slowly with lapse of time.

Intravascular injection of decamethonium caused an immediate reduction of injury potential which was restricted to the region of the end-plate. In the record of Fig. 1, the injury potential was measured simultaneously at two fixed points, one close to the musculotendinous junction (upper trace), the other at the end-plates. While an injection of decamethonium caused a fall of injury potential at the end-plate region, there was virtually no change of potential at the other electrode.

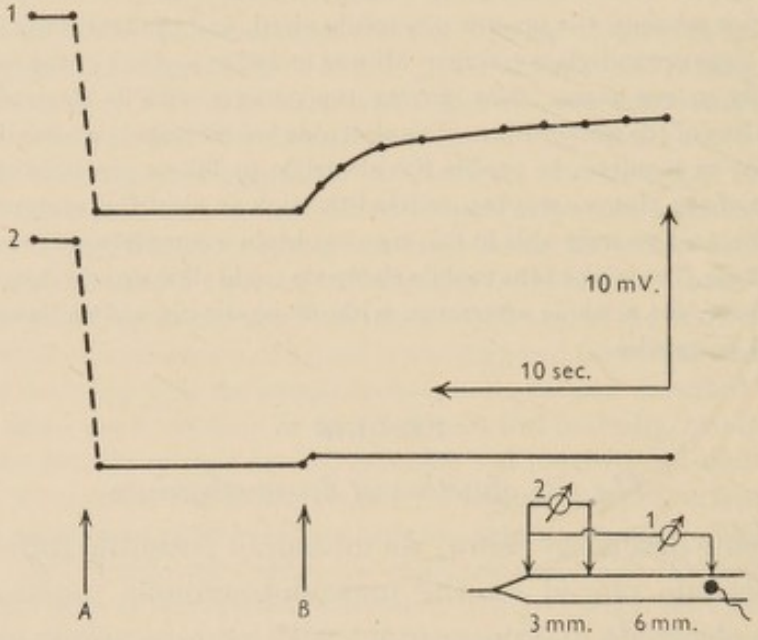


Fig. 1. Cat, chloralose. Gracilis. Graph of potential between electrodes on end-plate region (above) or 6 mm. away (below), and a common electrode on muscle fibre tip. In this and all other figures, upward deflexion means end-plate electrode or roving electrode becomes negative to end of muscle fibre. At *A*, muscle cut under common electrode. At *B*, 20 μg. decamethonium intra-arterially.

The same result can be shown using the spatial scanning technique. In Fig. 2, the continuous curve gives the distribution of potential round an injured region of the muscle and in the adjacent uninjured area. After the injection of 20 μg. decamethonium iodide intra-arterially, the dotted curve was obtained. Although the demarcation potential is quite unaltered, the end-plate regions are depolarized by about 30 % of the demarcation potential. This record also shows that, although the depolarization of the muscle is localized to the end-plate region, it does spread some way round the centre of the region.

The time course and magnitude of the depolarization

With intravenous administration of doses of the order of 30 μg./kg. the development of local depolarization was comparatively slow; the peak value

was reached only 3–5 min. after injection, and was followed by a steady repolarization to the normal value, taking 30–40 min. for its completion. Intra-arterial injection, on the other hand, caused an immediate depolarization of the end-plate region, and recovery was continuous from a few moments after injection. Provided sufficient time was allowed, the depolarization always passed off and the whole cycle of events could be repeated.

Since recovery never took less than half an hour it was not possible to carry out more than a few tests during the course of a day's work. Consequently, we have only rather incomplete data on the relation between magnitude of effect and the dose given, but our experiments have shown certain interesting facts. In the first place, the maximum depolarization produced by decamethonium was about 95% of the injury potential, and was achieved by doses of about 20 μg. intra-arterially; five times this dose produced no increase in local depolarization, while smaller doses had correspondingly smaller and briefer actions. In these experiments the parallel resistance of the tissues, which reduces the measured injury potential below the true membrane potential, should have affected the depolarizations due to injury and to decamethonium alike. Consequently we believe that decamethonium, in big enough dose, can abolish the membrane potential completely. We have never recorded a reversal of the membrane potential measured in the way we have described; but that such a reversal occurs cannot be excluded without more accurate knowledge about the spatial dispersion of the end-plates, with respect to the recording electrode.

The local depolarization produced by decamethonium was always bigger the first time the drug was injected than after later injections, even though the end-plate region had apparently returned to normal. Thus, in one experiment a peak depolarization, 95% of the injury potential, was produced by the first intra-arterial injection of 20 μg. decamethonium iodide; 90 min. later, when recovery appeared to be complete, doses as high as 100 μg. decamethonium iodide produced a depolarization of no more than 50% of the injury potential, although the absolute value of the latter was unchanged. These observations upon the results of arterial injection are consistent with our finding that the greatest depolarization we have produced by intravenous administration, when the drug reaches the muscle more slowly, have never exceeded about 40% of the maximal depolarization following arterial injection.

The spatial distribution of depolarization in the end-plate region

The fact that decamethonium was without effect upon the end of the muscle made it possible for us in subsequent experiments to use the uninjured musculotendinous junction as a point of reference potential; in this way we were able to avoid the unstable conditions associated with killing the ends of the muscle fibres. Fig. 3 was obtained in this way; potential differences were recorded between a fixed electrode on the musculotendinous junction and a travelling

electrode, which was traversed from end to end of the muscle fibre after an injection of 10 μg. decamethonium iodide intra-arterially. Although the depolarization was maximal in the end-plate region, the muscle fibre immediately adjacent to the end-plate was also affected. In Fig. 3*b* depolarization is plotted on a logarithmic scale against distance along the muscle fibre. It will be seen that the logarithm of the depolarization falls away in linear fashion on either side of a central plateau at the end-plates, but with a different slope on the two sides. From such experiments we have concluded that the spread of the depolarization falls exponentially from a central region of about 1–1½ mm.

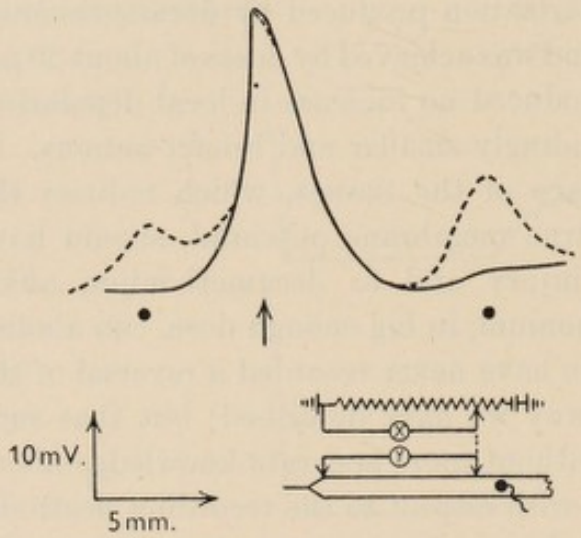


Fig. 2.

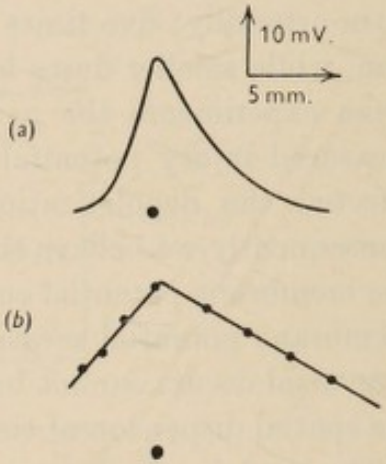


Fig. 3.

Fig. 2. Cat, chloralose. Gracilis. Spatial distribution of potential; continuous line before injection, dotted line after injection of 20 μg. decamethonium intra-arterially. Muscle cut at arrow; centres of end-plate regions marked by black dots. Ordinates, potential with respect to musculotendinous junction. Abscissae, distance along muscle fibre.

Fig. 3. Cat, chloralose. Gracilis. (*a*) Distribution of potential after intra-arterial injection of 10 μg. decamethonium; (*b*) same distribution with logarithmic scale for ordinates. Ordinates and abscissae as in Fig. 2.

breadth, with a space constant of about 1½–2 mm. This generalization is necessarily approximate for several reasons. First, the distribution of the depolarization is sometimes skew (as Fig. 3*a*). Secondly, an accurate determination of the distribution of depolarization would require an end-plate region more isolated from its neighbours than any we have yet found. It is usually easy to obtain a distribution of depolarization with a single peak from the tendinous end of the muscle, but the base-line of such a record is sloping, as a consequence of the depolarization of the large number of end-plates lying deep and more centrally; on the other hand, the end-plates in the central part of the muscle are usually irregularly distributed.

We have attempted to estimate the spatial distribution of the end-plates themselves and to compare this with the distribution of the depolarization. This

was done conveniently by recording end-plate potentials during stimulation of the motor nerve whilst recording the spatial distribution of depolarization. Under these conditions, since complete neuromuscular block was present, the end-plate potentials appeared superimposed on the record of the depolarization

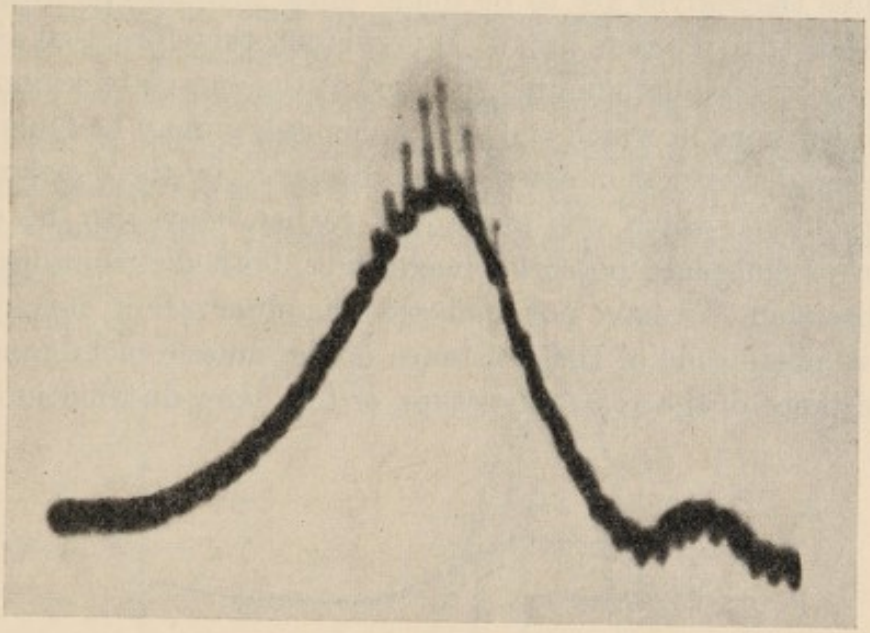


Fig. 4. Cat, chloralose. Gracilis. Distribution of potential after intravenous injection of 60 μg./kg. decamethonium. During whole of sweep, excitation of motor-nerve by maximal shocks at 33/sec.

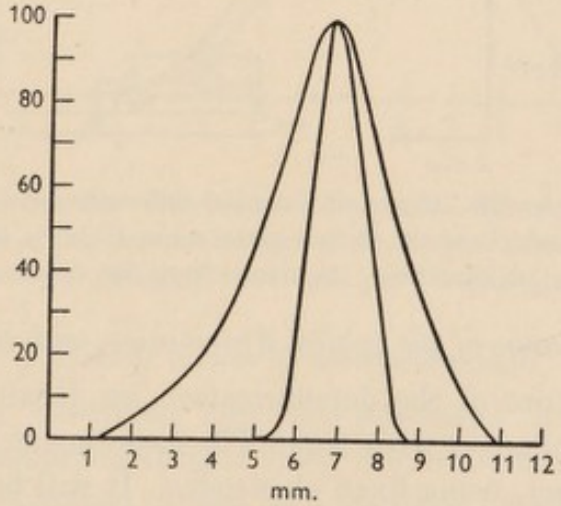


Fig. 5. Data from experiment of Fig. 4. Outer curve, distribution of depolarization; inner curve, spatial distribution of height of end-plate potential to nerve stimulation. Ordinates, % maximum height; abscissae, distance along muscle fibre in mm. Centre of end-plate zone at 7 mm.

(Fig. 4). The height of these potentials has been measured, and the curve in Fig. 5 constructed giving the variation of the peak end-plate potential with distance along the muscle fibre, together with the distribution of depolarization caused by an injection of decamethonium.

This experiment demonstrates incidentally the fact that the depolarization by decamethonium centres round the end-plate region. But more striking is

the rapidity with which the height of the end-plate potential falls away on either side of the central region, with a space constant of roughly 0·25 mm.; it is hardly detectable 1·5 mm. from the centre of the end-plate zone. Some of this distribution of potential must be by electrotonic spread. The band within which the end-plates themselves are to be found must therefore be even narrower, and may be less than 0·5 mm. broad. It is evident, therefore, that although the depolarization by decamethonium is centred at the end-plate region, it spreads well beyond the zone in which end-plates themselves may be found.

The occasional skewness of distribution displayed by the depolarization due to decamethonium, which was mentioned earlier, may also be seen in the distribution of end-plate potential magnitude, both distributions leaning in the same direction. We have not analysed this observation; it may be due to variations in magnitude of the resistance of the muscle membrane, or of the parallel resistance of the adjacent tissues, or to a skew distribution in space of end-plates.

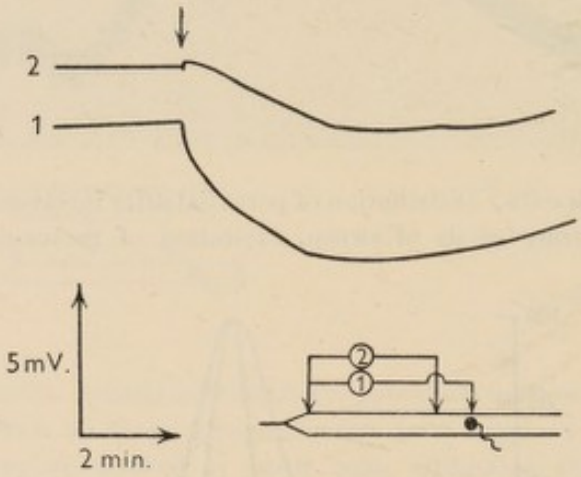


Fig. 6. Cat, chloralose. Gracilis. Graph of potential difference between electrode on musculotendinous junction and electrode on end-plate zones (below), or electrode 3·5 mm. from end-plate zone (above), against time. At arrow, 30μg./kg. decamethonium intravenously.

Change of the spatial distribution with time

An important feature of the depolarization by decamethonium is that its spatial distribution changes with time. The experiment from which Fig. 6 is taken shows this effect, using fixed electrodes. It will be seen that the initial effect of the drug is restricted to depolarization at the recording electrode on the end-plate region; for the first 30 sec. after the injection there was no significant change of potential at the other electrode 3·5 mm. away, although the potential at the end-plate electrode had fallen by 2 mV. After this latency, the potential at the more distant electrode began to fall and remained depressed until the whole effect of the drug was passing off. Had the depolarization remained restricted to one area, the potential falls at the two electrodes should have remained in constant proportion to each other; in this experiment, however, the ratio of end-plate depolarization to depolarization 3·5 mm. away,

fell from infinity immediately after the injection, to a value of about 2 several minutes later.

The spread of depolarization can also be demonstrated by comparing the distributions recorded by the spatial scanning technique at various times. Fig. 7 gives results from such an experiment, showing the distribution 0·5 and 20 min. after the injection of decamethonium. The spread is more easily appreciated if the distributions are corrected to the same peak height, and this has been done. A characteristic effect of the spread, which is seen particularly clearly when records are taken from an end-plate zone of complicated distribution, is the rounding and blurring with lapse of time of contours initially sharp and distinct.

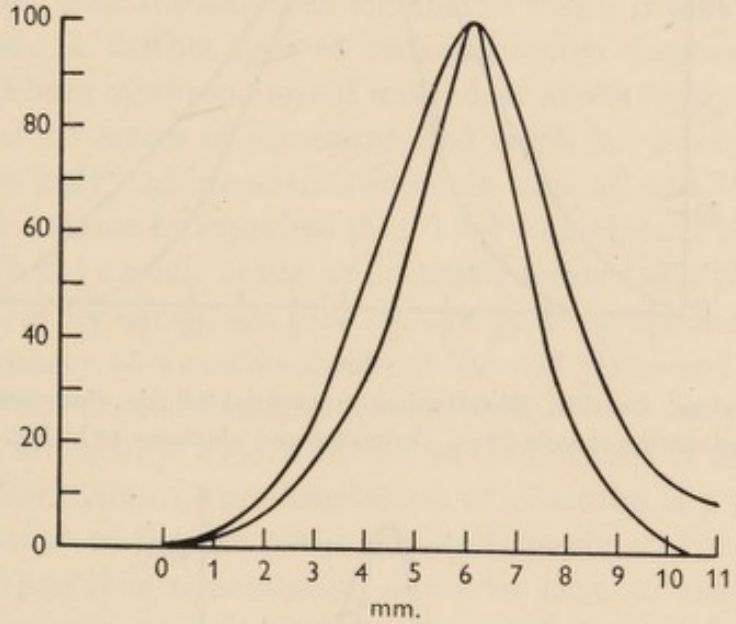


Fig. 7. Cat, chloralose. Gracilis. Distribution of potential 0·5 min. (inner curve) and 20 min. (outer curve) after 60 μg./kg. decamethonium intravenously. Abscissae, distance along muscle fibre in mm. Centre of end-plate region at 6 mm. Ordinates, % of maximum depolarization.

Site of action of decamethonium

We have already described how the prolonged depolarization by decamethonium can be recorded further from the end-plate region than can the transient end-plate potential caused by a maximal nerve volley; this difference becomes accentuated with time. These facts do not, however, necessarily imply that decamethonium attacks the muscle fibre elsewhere than at the end-plate region. An alternative theory is that such a discharge of the adjacent membrane potential is a necessary physical consequence of any persistent local depolarization. The latter hypothesis was tested by comparing the distribution of depolarization due to decamethonium with that surrounding the site of an injury of the muscle fibres. Fig. 8 shows the distribution of depolarization around a cut across the muscle fibres; and Fig. 9 shows the same round a localized burn. The space constant for spread of depolarization round these

injuries was approximately 1·5 mm., and there was no significant difference from that round an end-plate depolarized with decamethonium. Figs. 8 and 9 also show that depolarization caused by local injury spreads with time, in much the same way as that due to decamethonium. Such results were independent of the method by which the muscle fibre was injured; for we have tested local injuries produced by burning, cutting and pinching, and in every case the local

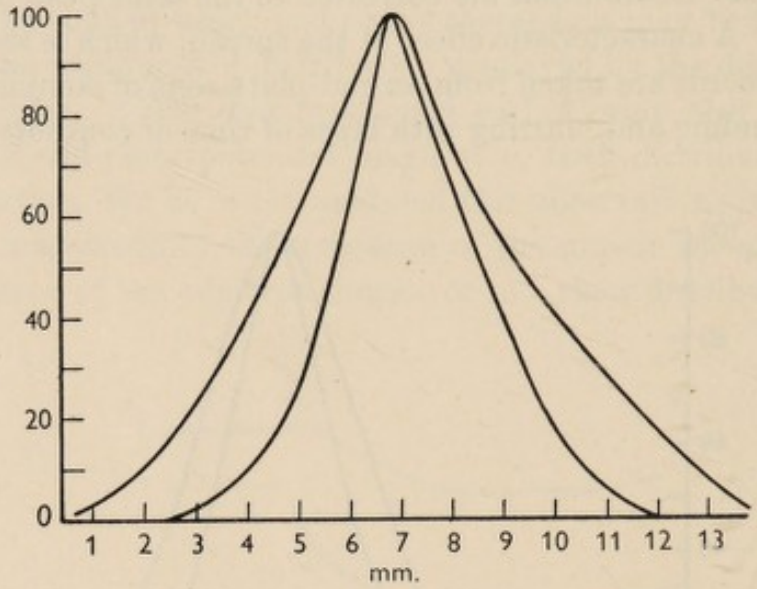


Fig. 8. Cat, chloralose. Gracilis. Distribution of potential 0·5 min. (inner curve) and 20 min. (outer curve) after cutting muscle fibre. Ordinates and abscissae as in Fig. 7; cut at 7 mm.

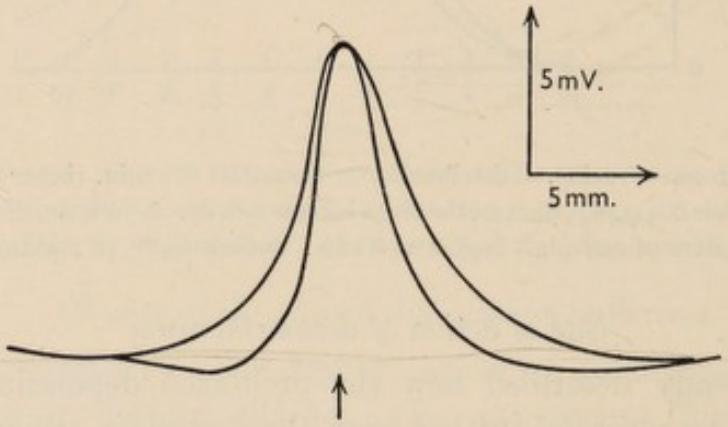


Fig. 9. Cat, chloralose. Gracilis. Distribution of potential 5 sec. (inner curve) and 20 min. (outer curve) after localized burn of muscle fibre at position marked by arrow.

depolarization was seen to spread. We have, therefore, been able to obtain a distribution of potential very similar to that due to decamethonium, by means which cannot involve the spreading action of a drug. It may be objected that the width of the distribution of depolarization round an injury and the spread with time demonstrate no more than a spreading death of the adjacent tissues. It would be remarkable if this process should coincide so closely with the spread due to decamethonium, whether the central injury was made by cutting, burning or pinching. Further evidence on this point is given later in the paper.

A localized depolarization of very similar distribution may also be obtained by the local application of cathodal current to the muscle; and this depolarization spreads spatially and with time in the same way, although there is no question of drug action or tissue necrosis (see Fig. 18).

Further evidence for this hypothesis suggested itself during the analysis of the antagonism to decamethonium by D-tubocurarine, described later. We found that the repolarizing action of D-tubocurarine is greater at the central region of the end-plate zone than in its immediate environs. The experiment of Fig. 16 illustrates this result; 5 mg. of D-tubocurarine chloride were given intravenously after 0·1 mg. of decamethonium iodide: the depolarization at once began to wane, but the centre repolarized considerably faster, so that it actually reached a lower potential than the adjacent membrane which it had (not long since) itself discharged. A further dose of decamethonium demonstrated that the point which had been most repolarized was indeed at the centre of the end-plate region, and that no errors of alinement had crept in. Since D-tubocurarine antagonizes not only the depolarization but also all the other actions of decamethonium, it must be supposed that the site at which it repolarizes is also the site at which the specific action of decamethonium takes place. This result therefore supports our conclusion that the spread of depolarization by decamethonium is secondary to a central action at the end-plate, and is incompatible with the belief that decamethonium acts diffusely.

The question arises why, even with the earliest records (a few seconds after a dose of decamethonium), the distribution of potential is a good deal more extensive than that of the end-plate potentials produced by stimulating the motor nerve. When it is remembered, however, that an end-plate potential lasts a few milliseconds, whereas even the mere performance of an intra-arterial injection may take 100 times as long, that the drug injected has then to diffuse to the end-plate region, and that the early stages of the spread of depolarization must be very rapid, it would be surprising if there were not considerable differences between the two distributions. Not until a record of the end-plate depolarization can be taken a few milliseconds after the application of decamethonium to the end-plate can a comparable distribution to that of the end-plate potential be expected.

It appears, therefore, that all the results so far described can be explained by assuming that decamethonium acts solely upon muscle fibre immediately beneath the end-plate. The consequence of an injection of decamethonium is an acute collapse of the membrane potential of the muscle fibre at this point; this depolarization immediately begins to spread to adjacent regions by discharging membrane not yet depolarized; the process thus ultimately extends well beyond the zone of end-plates, but never invades the whole of the muscle fibres.

The relation between depolarization and neuromuscular block

There is considerable evidence that depolarization of muscle membrane by potassium leads to a reduction of electrical excitability (Overton, 1904; Fenn, 1940). Such a loss of electrical excitability might therefore be expected to follow the prolonged depolarization which decamethonium causes, and would directly account for its blocking action. If neuromuscular block by decamethonium is, in fact, due to depolarization and consequent inexcitability in the end-plate zone, the muscle fibres exposed to decamethonium should also exhibit a lowered excitability to direct electrical excitation localized to the end-plate region. Further, we should expect conduction across the end-plate zone of a contraction wave started by direct stimulation at one end of the muscle fibre, to fail when neuromuscular transmission fails. Finally, it is possible that repolarization of the membrane in the neighbourhood of the end-plates relieves a partial neuromuscular block.

In the following experiments we have tested these points.

Electrical excitability of muscle after decamethonium

We arranged two electrodes, about 1 mm. apart, to stimulate a few muscle fibres on the surface of the cat's gracilis while recording the action potential from a pair of electrodes placed over the tendinous end of the same fibres. The pair of stimulating electrodes could be traversed from one end of these muscle fibres to the other, so that an excitation wave could be initiated from any point on their length. Excitability of the fibres in the region of the stimulating electrodes was measured in terms of that stimulus strength which would just produce a recorded action potential of a fixed but arbitrary size. It was usually possible, by using weak stimuli of long duration, to traverse the stimulating electrodes across the end-plate region without eliciting a nerve-stimulated action potential. Consequently, we sometimes were able to obtain values for direct excitability throughout the whole length of a group of normal muscle fibres (Fig. 10*a*). The spatial distribution of excitability was also obtained from the same muscle, after treatment with D-tubocurarine, when excitation through the nerve was blocked (Fig. 10*c*). These control results for normal muscle, and those for muscle paralysed by D-tubocurarine, were indistinguishable. Measured in this way the voltage required to excite at any point on the length of the untreated fibres lay between 1·5 and 2·5 V. × 0·5 msec.; such variations of threshold as we observed appeared to be due mainly to the presence of fascial thickenings at some points of the muscle surface; but these fascial fragments could not be removed without risk of damaging the muscle fibres.

Measurements made after the intravenous injection of 0·24 mg. decamethonium iodide showed a great decrease of excitability in the end-plate region. Fig. 10*b* gives the results of such an experiment. The two curves show the

excitability of the same muscle under the influence of decamethonium. It will be seen that excitability became least over the end-plate region, while the depression decayed in approximately exponential form with increase of distance from this point. The space constant of spread was about 1·5 mm., so that the muscle fibre 5 mm. or more away from the end-plate had an excitability indistinguishable from normal.

A considerable number of measurements must be made in determining an excitability curve such as one of those shown in Fig. 10. Consequently, we were

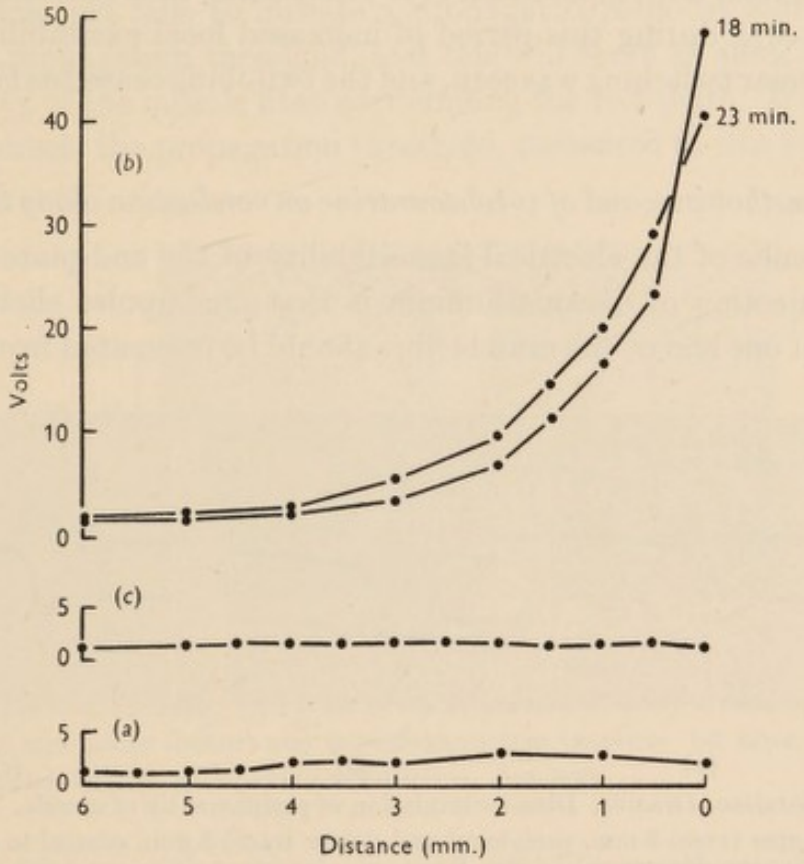


Fig. 10. Cat, chloralose. Gracilis. Graph of excitability measurements in (*a*) normal muscle; (*b*) 18 and 23 min. after 80 μg./kg. decamethonium intravenously; (*c*) after 0·7 mg./kg. D-tubocurarine intravenously. Ordinates, volts required to produce standard action potential by direct stimulation; abscissae, distance along muscle fibre of point of stimulation from centre of end-plate zone at zero.

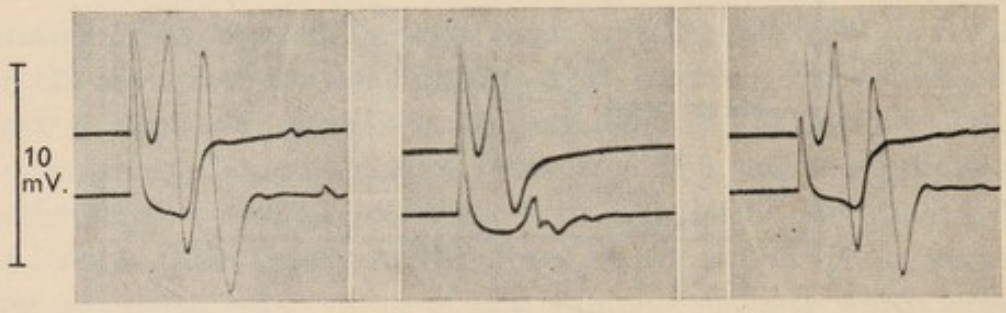
only able to get accurate information about excitability changes an appreciable time after the injection of decamethonium, when the rapid initial effects of the drug were complete and conditions were changing more slowly. As was expected from a consideration of our measurements of local depolarization, we found that the area of the region of depressed excitability increased with time; this spread continued even while the excitability at the end-plate region was beginning to recover. The excitability curves in Fig. 10*b*, obtained 18 and 23 min. after the intravenous injection of decamethonium, illustrate these points.

Although it was impossible to obtain the complete spatial distribution of

excitability during the initial phase of action of decamethonium, we were able to record the direction of change of this excitability at the end-plate region continuously. This was done by fixing the stimulating electrodes over the end-plate zone and stimulating once every second with a weak (submaximal) shock, while recording the magnitude of the action potential arriving at the ends of the muscle fibres, as before. After an intravenous injection of 0·12 mg. decamethonium the reduced excitability in the end-plate region, which we have already described, was preceded by a comparatively short period (about 15 sec.) in which the recorded action potential grew, indicating that excitability had been increased. It was during this period of increased local excitability that spontaneous muscular twitching was seen, and the twitching ceased as the excitability decreased.

Effect of decamethonium and of D-*tubocurarine on conduction along the muscle fibre*

A consequence of the electrical inexcitability of the end-plate region which follows an injection of decamethonium is that an impulse elicited by direct stimulation of one end of the muscle fibre should be prevented from crossing the



(*a*) (*b*) (*c*)

Fig. 11. Cat, chloralose. Gracilis. Direct stimulation of peripheral tip of muscle. Action potentials recorded (upper trace) 3 mm. peripheral and (lower trace) 3 mm. central to end-plate region. (*a*) normal muscle; (*b*) after 1 mg./kg. decamethonium intravenously; (*c*) after D-tubocurarine 1 mg./kg. intravenously. First upward deflexion in all records is stimulus artifact.

depolarized end-plate region; in the curarized muscle, however, such conduction from one end of the muscle fibre to the other should proceed normally. In the experiment of Fig. 11 the muscle was excited either directly by distal electrodes placed on the muscle near the tendinous end, or by shocks applied to the nerve to test the degree of neuromuscular block; records of action potentials were taken from pairs of electrodes on either side of the end-plate region. After D-tubocurarine, in a dose (1 mg./kg.) sufficient to cause complete neuromuscular block, conduction of the directly excited impulse from one end of the fibre to the other was still normal. But after the injection of a large dose of decamethonium iodide (1 mg./kg.), although an action potential still spread from the direct stimulating electrodes as far as the end-plate region, it failed to cross and reach the recording electrodes on the other side.

End-plate propagation thresholds with decamethonium and with D-*tubocurarine*

The evidence above has shown that the region surrounding the end-plate under the influence of decamethonium is no longer to be excited either by direct electrical stimulation or by the normal muscle action potential. It follows that it should be resistant to excitation by an end-plate potential. Now it is possible, by varying the dose of D-tubocurarine or of decamethonium, to vary continuously the size of an end-plate potential in a muscle treated with one of these drugs. By such means, we can determine the size of an end-plate potential which just fails to initiate a propagated response along the muscle fibre, i.e. the propagation threshold, and this will serve as another measure of the excitability of the muscle fibre surrounding the end-plate. If our argument hitherto is correct, the propagation threshold, measured in this way, should be

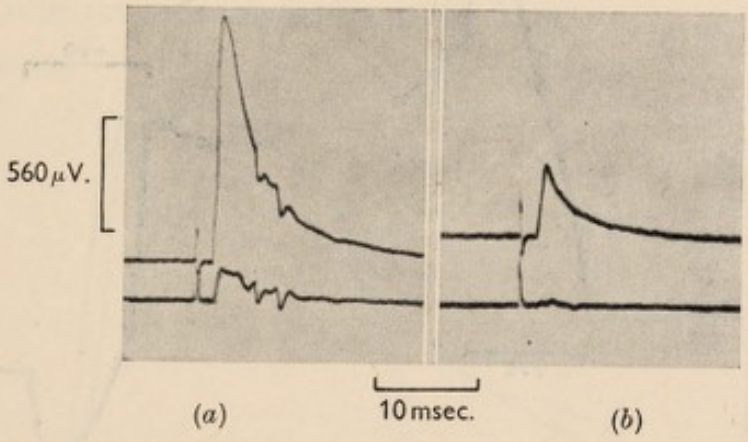


Fig. 12. Cat, chloralose. Gracilis. End-plate potentials recorded between centre of end-plate zone (above) or 2 mm. away (below) and musculotendinous junction. (*a*) after decamethonium; (*b*) after D-tubocurarine, with same amplification. See text.

substantially higher in the muscle treated with decamethonium than it is in a muscle poisoned with D-tubocurarine, where there is no change of electrical excitability in regions close to the end-plates.

In the experiment of Fig. 12, after the production of neuromuscular block by decamethonium, recovery was allowed to take place until the appearance of small irregular spikes on the end-plate potential, recorded 1 mm. away from the end-plate zone, indicated that propagation was imminent (Fig. 12*a*). D-Tubocurarine was injected under the same conditions, and the propagation threshold was again determined; Fig. 12*b* is a tracing of the end-plate potential with which propagation failed completely (very small abortive action potentials being just visible on the lower trace). From these records the propagation threshold after decamethonium is about $3\frac{1}{2}$ times that after D-tubocurarine.

This difference in propagation threshold presented itself in a striking way; if D-tubocurarine was injected while the muscle was under the influence of

decamethonium and the end-plate potential was close to propagation threshold, the first effect of the D-tubocurarine was to *initiate propagation* at the very moment that the end-plate potential itself was dwindling. Not until the end-plate potential had been reduced to one-third or less of its value initially did block return.

Effect of polarizing current at the end-plate region on neuromuscular block

If there is a causal relation between local depolarization and neuromuscular block, it might be possible to reinstate conduction by repolarization of the muscle membrane. We therefore applied polarizing currents through a saline wick supported in a coil of chlorided silver wire, and placed upon the end-plate

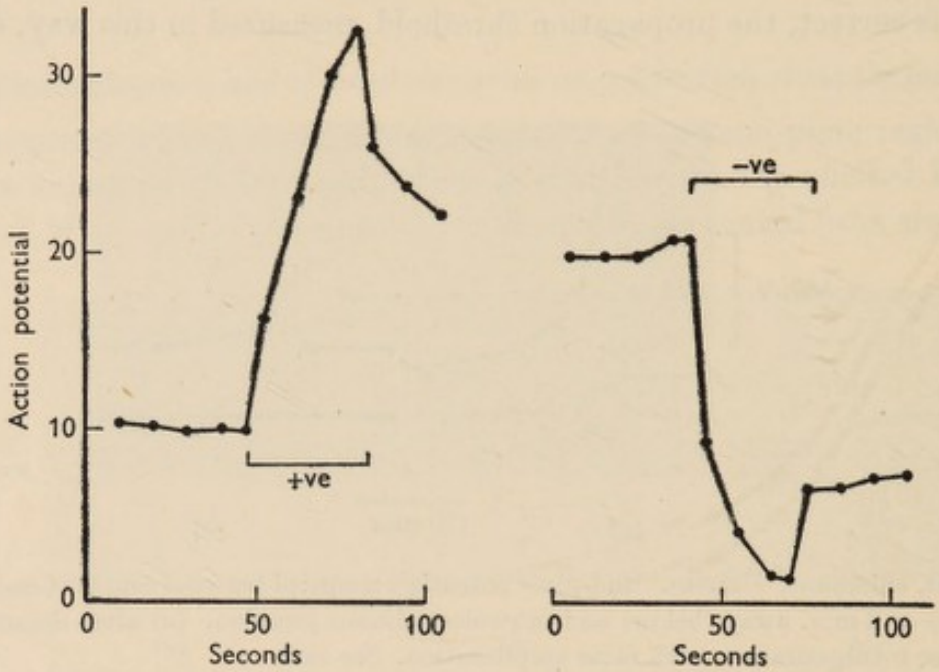


Fig. 13. Cat, chloralose. Gracilis. Graph of height of muscle action potential to nerve stimulation (in arbitrary units), after decamethonium 60 μg./kg. intravenously, with anodal and cathodal polarization of end-plate region.

zone; the circuit was completed through a diffuse, distant electrode, and potential was applied through a 10 MΩ. resistance, while the current passed was recorded with a microammeter. Anodal polarization for a period of 15 sec. with a current of 300–400 μA. clearly increased neuromuscular conduction in a preparation partly blocked by decamethonium (see Fig. 13). Conversely, in the same experiment cathodal polarization of the end-plate region practically abolished transmission from nerve to muscle.

These results may be epitomized by saying that, in muscles poisoned with D-tubocurarine, it is quite impossible to locate the end-plate region by measurements of excitability to direct stimulation. The same muscle treated with decamethonium presents a totally different picture; excitability in the end-plate

region may be decreased more than twenty-fold, and the end-plate zone is easily recognized as that part of the muscle length which will not respond to direct excitation. The end-plate region treated by decamethonium is thus one whose electrical inexcitability prevents any form of excitation from initiating a propagated response, and is to be contrasted with the curarized end-plate region which, though it is no longer to be stimulated chemically, retains its normal electrical excitability.

Our evidence thus far, that neuromuscular block as a result of decamethonium is a result of depolarization, consists of the following facts: (1) the course in time of the depolarization resembles that of the block (compare Fig. 6 with Paton & Zaimis, 1949, Fig. 1); (2) the muscle fibre will not respond to direct electrical stimulation in the end-plate region during the block; (3) conduction of a muscle action potential along the fibre is blocked at the end-plate region; (4) propagation threshold at the end-plate is raised; (5) artificial repolarization of the end-plate zone reverses the action of the drug.

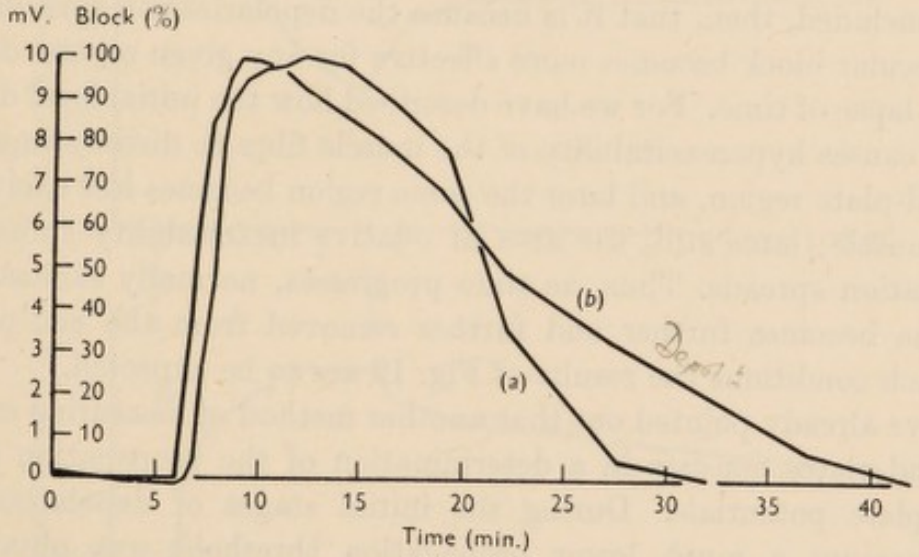


Fig. 14. Cat, chloralose. Gracilis. Graphs of (*a*) degree of neuromuscular block, as % reduction of nerve-excited muscle action potential; (*b*) depolarization of end-plate region (in millivolts), against time in minutes. At 6 min. 40 μg./kg. decamethonium intravenously.

The mechanism by which decamethonium produces block

We have so far made no distinction between the consequences of depolarization immediately at the end-plate and of depolarization of the adjacent muscle fibre length. Although there is a clear connexion between depolarization of the end-plate *region* and the advent of neuromuscular block produced by decamethonium, the experiment of Fig. 14 shows that the degree of block cannot be correlated directly with depolarization at the end-plate alone. Thus, as the depolarization at the end-plate progressed, neuromuscular block set in and was 50% complete when a recorded depolarization of 8·4 mV. had been attained, and the block increased rapidly as the depolarization continued, being virtually abolished at 10·0 mV. depolarization. During repolarization,

however, neuromuscular conduction did not reappear at the same value; but only when depolarization had fallen to 8·3 mV. did the muscle again respond to stimulation of its nerve, and 50% block did not return until the depolarization had fallen to 5·5 mV. The fact that neuromuscular block is not entirely dependent upon local conditions at the end-plate is also shown by the short period of superexcitability which occurs immediately after the injection of decamethonium. In the early stages of the fall of demarcation potential, a single nerve volley will elicit a repetitive response from the muscle fibres, and spontaneous twitching is nearly always visible without nerve stimulation. These phenomena are all present during the induction of neuromuscular block with decamethonium; the same degree of depolarization at the end-plate during recovery from decamethonium is invariably associated with depressed excitability of the muscle fibres and partial neuromuscular block.

We have already shown that the spatial distribution of depolarization in the end-plate region is more restricted during induction than during recovery. It can be concluded, then, that it is because the depolarization spreads that the neuromuscular block becomes more effective for any given central depolarization with lapse of time. For we have described how the initial local depolarization first causes hyperexcitability of the muscle fibre to direct stimuli applied in the end-plate region, and later the same region becomes less excitable than normal muscle; later still, the area of relative inexcitability spreads as the depolarization spreads. Thus, as time progresses, normally excitable muscle membrane becomes further and further removed from the end-plate zone. Under such conditions the results of Fig. 12 are to be expected.

We have already pointed out that another method of measuring excitability at the end-plates consists in a determination of the propagation thresholds for end-plate potentials. During the initial stages of depolarization with decamethonium a much lower propagation threshold was obtained than during recovery. The values obtained for induction and recovery were usually in the ratio of approximately 1 : 3.

These results demonstrate that at least two factors contribute to the neuromuscular block produced by decamethonium:

(i) the effect on the local reaction of the muscle membrane beneath the end-plate to a maximal nerve volley; this reaction (the end-plate potential) is reduced by decamethonium as the depolarization increases and is directly dependent upon local conditions at the end-plates;

(ii) the spread of the barrier of inexcitability between the end-plates and the nearest excitable membrane.

The action of antagonists to decamethonium

The antagonistic action of D-tubocurarine chloride and of pentamethonium to the actions of decamethonium has already been described elsewhere. But

it was observed by Brown *et al.* (1949) that the action of pentamethonium in restoring neuromuscular conduction was sometimes much more dramatic than its action in repolarizing the muscle fibre, so that it was interesting to see whether its repolarizing action could account for its unblocking action. These experiments also provided evidence that the action of decamethonium is restricted to the end-plates themselves, for the repolarizing effects of pentamethonium and D-tubocurarine are exerted chiefly there, and only indirectly on the adjacent discharged membrane.

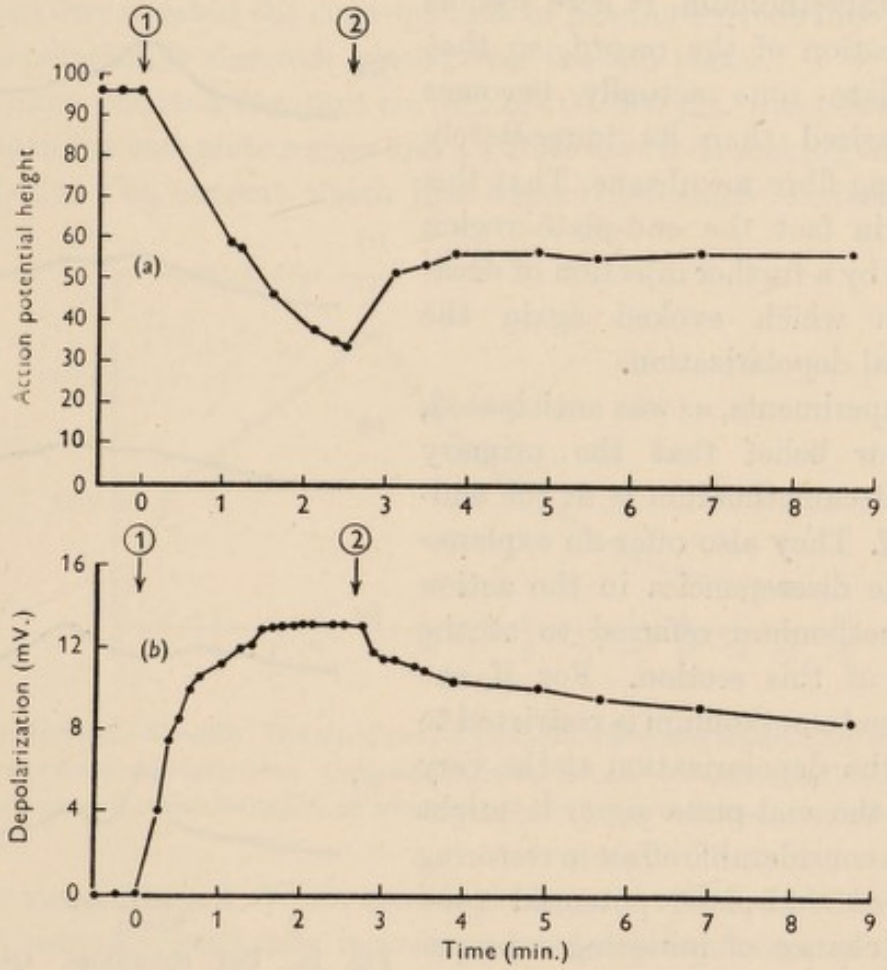


Fig. 15. Cat, chloralose. Gracilis. Graph (*a*) of height of muscle action potential to nerve stimulation (arbitrary units); (*b*) of end-plate depolarization (millivolts), against time in minutes. At (1), 35 μg./kg. decamethonium intravenously; at (2), pentamethonium, 5 mg./kg. intravenously.

As was the experience of Brown *et al.* (1949), pentamethonium is not strikingly effective in repolarizing the muscle under the influence of decamethonium; but the effect is certainly demonstrable (Fig. 15). In this experiment the depolarization of the fibre was recorded at the same time as the action potential height to nerve stimulation; 1 min. after 15 mg. pentamethonium iodide, the peak depolarization sank by 23 % and the action potential, which had been decreasing, increased from 32 to 56 % of its control height. A significant

point was the slight flattening of the depolarization curve, indicating that the action of pentamethonium is only at the centre of the end-plate zone, and that it does not affect the secondarily discharged adjacent membrane.

With D-tubocurarine, more striking effects can be produced. Fig. 16 consists of records from an experiment in which 50 μg. decamethonium iodide was followed by 5 mg. D-tubocurarine chloride. The repolarization is quite rapid in this case, by about 60% in 1 min. Further, the flattening mentioned above, due to pentamethonium, is here seen as an umbilication of the record, so that the end-plate zone actually becomes less depolarized than its immediately neighbouring fibre membrane. That this dimple is in fact the end-plate region was shown by a further injection of decamethonium which evoked again the typical local depolarization.

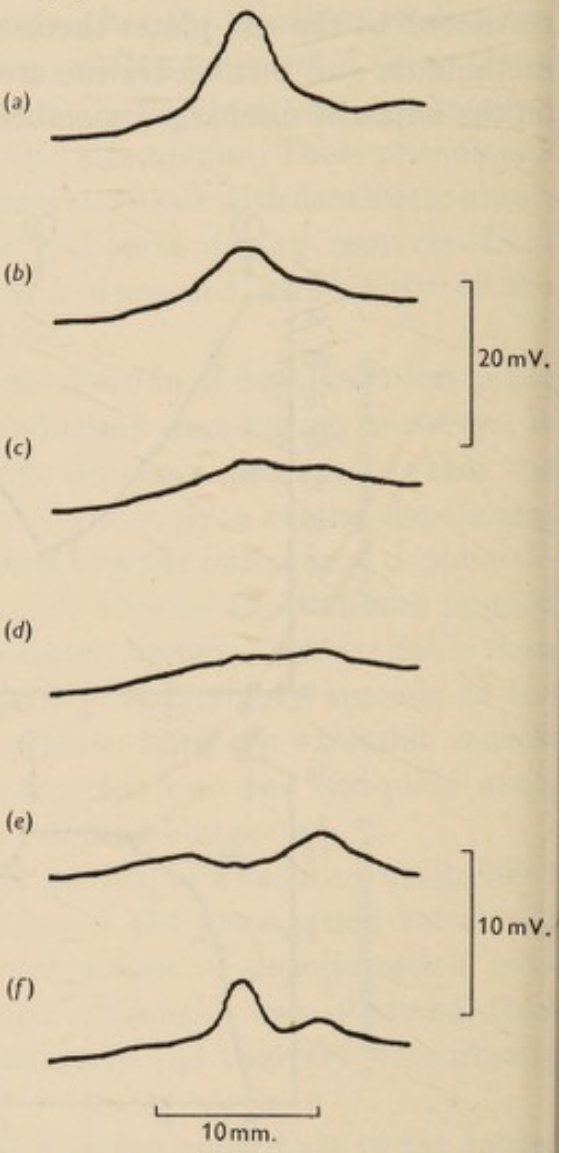


Fig. 16. Cat, chloralose. Gracilis. Intra-arterial injections. Distribution of potential. (*a*) 6 min. after decamethonium, 50 μg.; (*b*)–(*e*), 45 sec., 2½, 4 and 6 min. respectively after D-tubocurarine 2 mg. injected immediately after (*a*). Immediately after (*e*), decamethonium 200 μg. injected; (*f*) 20 sec. later. Amplification of potential increased 2 × for (*e*) and (*f*).

These experiments, as was anticipated, support our belief that the primary action of decamethonium is at the end-plate itself. They also offer an explanation of the discrepancies in the action of pentamethonium referred to at the beginning of this section. For if the action of pentamethonium is restricted to lessening the depolarization at the very centre of the end-plate zone, it might well have a considerable effect in restoring the reduced end-plate potential (and hence the chance of initiating a propagated response in the fibre) without much reduction in the total depolarization. Indeed, if the pentamethonium was given early after the dose of decamethonium (as it was in the experiments of Brown *et al.* 1949), the phase of spread might be still so rapid that total depolarization could increase while peak depolarization was decreasing; we have seen just such a phenomenon simply during the progress of depolarization after an intra-arterial dose of decamethonium. The changes of potential recorded from a muscle with irregularly distributed end-plates, using long wick

electrodes randomly placed, would necessarily be measures rather of such total depolarization than of the peak depolarization.

Experiments with polarizing currents

Since it appeared that all the effects of decamethonium could reasonably be explained as due to persistent depolarization at the end-plates, the ultimate spread of this depolarization to involve the adjacent muscle fibre being a secondary phenomenon, we felt that these effects should be imitated by any other agent which caused a prolonged and local depolarization in this region. We therefore investigated the consequences of passing current into the muscle from a non-polarizable electrode placed over the end-plates.

In these experiments a constant current of 100–300 μA. was passed between an electrode in the end-plate region and a diffuse electrode making contact with the cat's body. The current which just caused detectable excitation of the

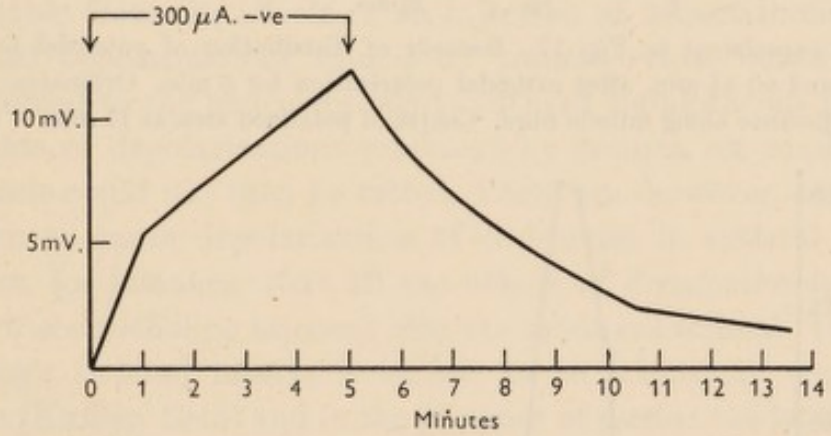


Fig. 17. Cat, chloralose. Gracilis. Graph of rise of potential difference between tip of muscle fibre and centre of zone of polarization. Cathodal current, 300 μA. Ordinates, millivolts; abscissae, time in minutes. Current switched off momentarily for observations during polarization.

muscle was about 110 μA. When the electrode over the end-plates was made negative, it caused a gradually increasing local depolarization of the muscle membrane and, when the current was switched off, this depolarization took a number of minutes to disappear. Fig. 17 shows the results of an experiment in which a cathodal current of 300 μA. was passed for 5 min. It will be seen that the induced depolarization both rose and decayed slowly; further, like the depolarization caused by decamethonium, the depolarization produced by cathodal current spread spatially round a central plateau of about the same width as the polarizing electrodes, with a space constant of about 2 mm., and this spatial spread increased with time (Fig. 18).

This depolarization could be annulled or reversed by applying anodal current for an appropriate time.

Cathodal current, however, is normally used for excitation, and such currents,

if too small to excite directly, have been shown by many workers to increase excitability in the region of its application rather than to cause block. Thus, Katz (1939) has shown that cathodal current applied to the end-plate region of a partly curarized muscle will increase neuromuscular conduction. We have repeated Katz's observations in preparations where neuromuscular conduction was held at a steady level of depression by an injection of 1·5 mg. D-tubocurarine,

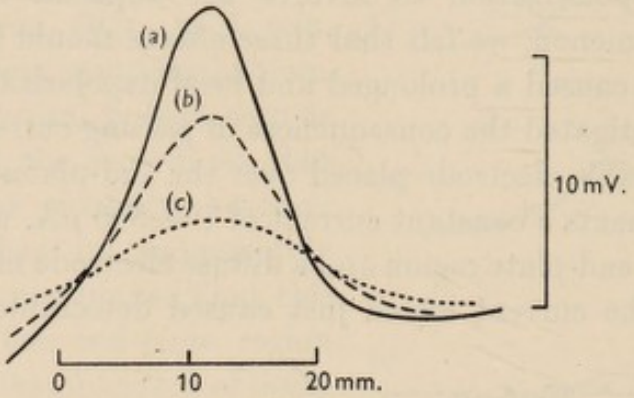


Fig. 18. Same experiment as Fig. 17. Records of distribution of potential (*a* immediately, (*b*) 95 sec. and (*c*) 4½ min. after cathodal polarization for 5 min. Ordinates, depolarization; abscissae, distance along muscle fibre. Centre of polarized area at 11 mm.

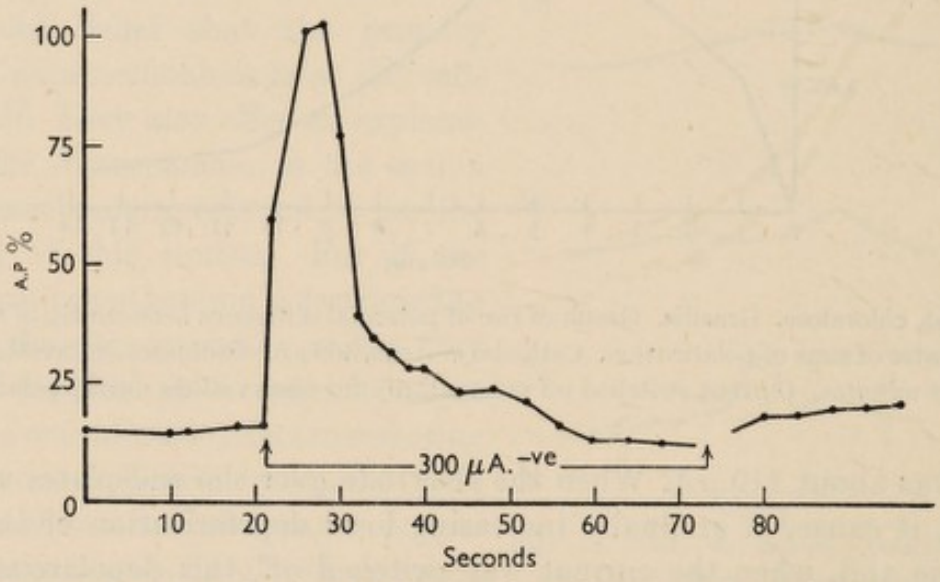


Fig. 19. Cat, chloralose. Gracilis. Graph of height of muscle action potential (as % normal) during infusion of D-tubocurarine intravenously. Between arrows, cathodal polarization of end-plate region, with current 300 μA.

followed by an infusion of the drug at 1·5–2 mg./hr. We used the action potentials recorded from the tips of a small group of muscle fibres in the gracilis as a measure of the number of muscle fibres responding to maximal nerve volleys. Fig. 19 shows the results of such an experiment during and after the application of cathodal current to the end-plate region. The first response to cathodal current is the dramatic increase of the muscle response, already described by Katz; as the current continues, however, the response wanes until it is ultimately

depressed beneath its original value. When the current is switched off the response returns slowly to the original level.

Similar results can be obtained in normal muscle. Cathodal current applied to the end-plate region will produce a steadily increasing state of neuromuscular block, which passes off slowly after the current is stopped; this neuromuscular block is reversed by anodal current. But in normal muscle the early phase of superexcitability cannot be so well demonstrated. In our experiments this phase showed usually as a very small temporary increase in action potential, presumably due to the recruitment of a small number of muscle fibres previously unable to respond to a single maximal nerve volley.

These experiments showed that all the fundamental features of neuromuscular block induced by decamethonium could be imitated by cathodal current applied to the end-plate region, provided that this current was passed for a sufficient time. They lead us to believe that any method of producing a local, acute depolarization of the end-plate region would have consequences indistinguishable from the effects of an injection of decamethonium. Unfortunately, local depolarizations caused by trauma—cuts, burns, pinching—cannot be applied to the end-plate region without simultaneous nerve injury, and the effects of depolarizations produced by trauma on conduction from nerve to muscle could not then be tested. There are, however, other chemical agents known to cause depolarization of end-plates in skeletal muscle. We should expect, for instance, that all the effects of decamethonium could be obtained with acetylcholine injected into the eserinzed animal. The end-plate region of frog's striated muscle is known to be selectively depolarized by acetylcholine (Kuffler, 1943) and in the presence of eserine the local depolarization should persist and spread.

Experiments with acetylcholine

An arterial injection of acetylcholine into the gracilis of a cat caused an immediate depolarization of the end-plate region (Fig. 20). Fig. 21 shows the effects of a similar injection into the brachioradialis, in which a larger number of poorly localized end-plate regions were being traversed by the moving electrode. In both these figures, the effects of an injection of decamethonium can be seen for comparison, and the areas depolarized and the distribution of the depolarization are almost identical, the only differences being in magnitude. The effect of acetylcholine was, of course, much more transient than that due to decamethonium, lasting not more than a few seconds. A previous dose of eserine (25–50 μg.), however, caused the depolarization to persist for several minutes, during which time the spread to adjacent regions was clearly recognizable.

The same technique as with decamethonium was used to determine whether this depolarization also caused a depression of the excitability of the muscle

fibre. Thus, after a dose of 100 μg. acetylcholine, injected into a muscle previously treated with eserine (50 μg.), the end-plate region became electrically inexcitable, although the regions remote from the end-plate were still normally excitable. Further, as with decamethonium, this inexcitable end-plate region formed a point of block to the propagation of a directly excited muscle action potential along the fibre (Fig. 22). In a few experiments an injection of acetylcholine was given in the presence of depolarization of the end-plate by

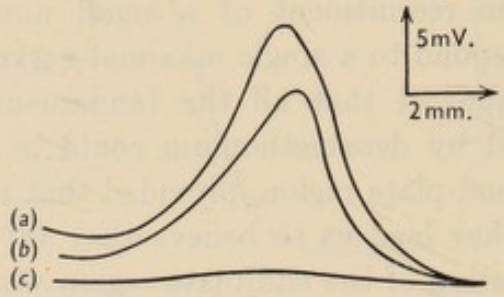


Fig. 20. Cat, chloralose. Gracilis. Distribution of potential after intra-arterial injections of (*a*) decamethonium 25 μg.; (*b*) acetylcholine 25 μg. (*c*) Record from muscle before injections.

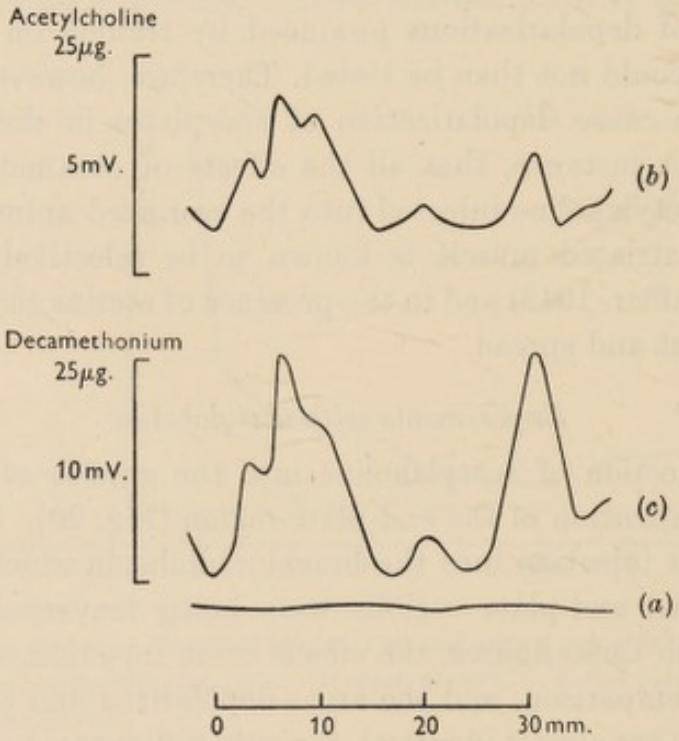
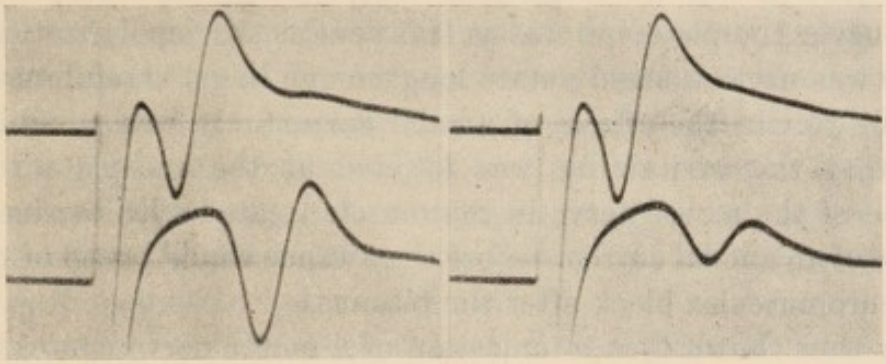


Fig. 21. Cat, chloralose. Brachioradialis. Retrograde intravenous injections. Distribution of potential (*a*) in normal muscle; (*b*) after acetylcholine 25 μg.; (*c*) after decamethonium 25 μg.

decamethonium. In these experiments a further depolarization was produced, intensifying that already existing, and appearing exactly as though an additional dose of decamethonium had been given.

Finally, we have tested the effect of anodal currents at the end-plate region during neuromuscular block due to acetylcholine. Fig. 23 shows the result of

such an experiment. After 30 μg. acetylcholine was injected, about 80% block was induced, which was still becoming slightly deeper. The passage of an anodal current of 50 μA., however, stopped the increase of block, and initiated recovery.



(*a*) (*b*)

Fig. 22. Cat, chloralose. Gracilis. Direct stimulation of peripheral tip of muscle. Action potentials recorded (upper trace) 3 mm. peripheral and (lower trace) 2 mm. central to end-plate region. (*a*) Normal muscle; (*b*) after intra-arterial injection of 50 μg. eserine followed by 100 μg. acetylcholine. First downward deflexion in all records is stimulus artifact.

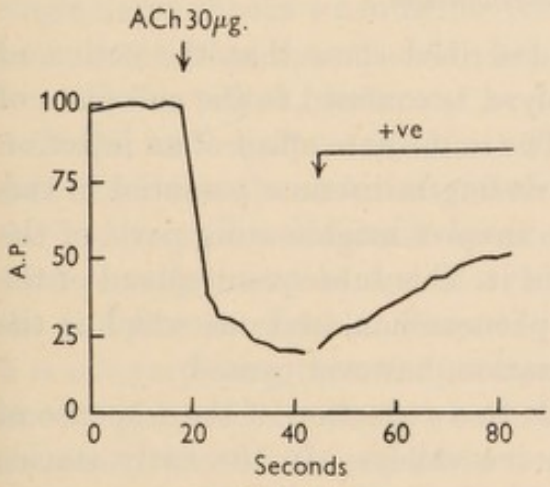


Fig. 23.

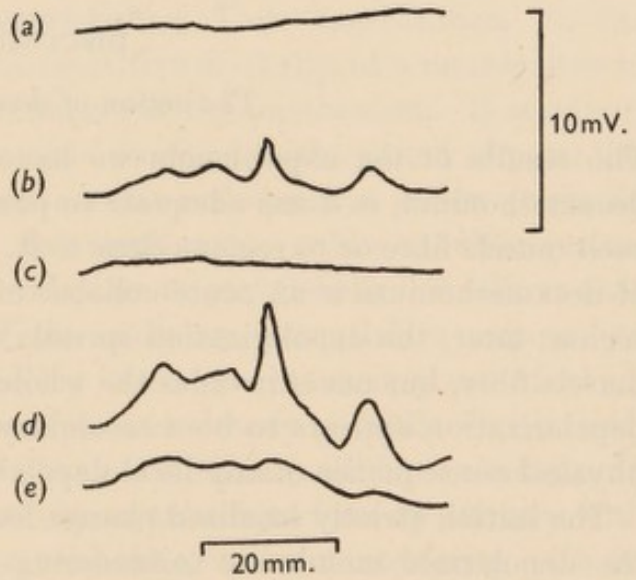


Fig. 24.

Fig. 23. Cat, chloralose. Gracilis. Graph of height of muscle action potential to nerve stimulation. Acetylcholine 30 μg., intra-arterially, followed by anodal polarization of end-plate region, current 50 μA.

Fig. 24. Cat, chloralose. Gracilis. Distribution of potential, (*a*) and (*c*) in resting muscle; (*b*) and (*d*) during tetanus to motor nerve at 690/sec. Between (*b*) and (*c*), 0·75 mg./kg. prostigmine methylsulphate intravenously. (*e*), 10 sec. after (*d*).

Experiments with tetanization of a motor nerve

In a normal gracilis, tetanization of the motor nerve for 5–10 sec. at a rate of 70–690/sec. produces a depolarization at the end-plate region which is small (not more than 1–2 mV.), sharply localized, and very transient, disappearing in

2–3 sec. (Fig. 24*b*). But after a dose of prostigmine, the depolarization is larger (Fig. 24*d*) and persists longer; it can now be seen to become less and less sharply localized as it passes off (Fig. 24*e*).

It was not easy to do satisfactory experiments under these conditions because the depolarization was never great or long-lasting, and the neuromuscular block was never complete and was as transient as the depolarization. Accordingly there was never a steady state long enough to get careful measurements of excitability or of the effects of anodal current. It was possible to show, however, that the excitability was lowered at the end-plate region after tetanization of the motor nerve in the muscle treated with eserine; and that the passage of an anodal current before the tetanus would lessen or prevent the onset of neuromuscular block after the tetanus.

We have thus shown that tetanization of a motor nerve causes a transient depolarization at the end-plate, which is intensified and prolonged by an anticholinesterase, causes neuromuscular block and depresses the direct excitability of the muscle, and that these effects may be reversed by the passage of an anodal current into the end-plate region.

DISCUSSION

The action of decamethonium

The results of the experiments we have described show that the action of decamethonium, in doses adequate to paralyse, is confined to the end-plate of each muscle fibre or to regions close to it. The immediate effect of an injection of decamethonium is an acute collapse of resting membrane potential in this region; later, the depolarization spreads to involve neighbouring parts of the muscle fibre, but never invades the whole of it. This subsequent spread of the depolarization appears to be a secondary phenomenon, and one which is the physical consequence of any local depolarization, however caused.

The initial, strictly localized change leads to a reduction of the response of the depolarized membrane to incoming nerve volleys. In the early stages, however, before the depolarization has had time to spread by discharging neighbouring areas, depression of the strictly local response may be more than offset by an increase of excitability in regions very close to the depolarized end-plate. The spread of depolarization is effected by the slow discharge of the membrane potential in regions surrounding the end-plate (a process distinct from the rapid discharge of the membrane capacity, complete in milliseconds). In this process, current must flow thence into the depolarized area; this current appears, by addition to currents aroused by the end-plate response to a nerve volley, at first to cause the net excitability of the system to rise above normal. Hence, for a short time after an injection of decamethonium, a single nerve volley will elicit a repetitive response of the muscle fibre, a rise in excitability

is detectable, and there may even be spontaneous contractions of the muscle fibre.

Soon, however, the depolarization spreads further, the excitability of membrane adjacent to the end-plate also falls, the end-plate potential can no longer excite the fibre, and neuromuscular transmission fails.

Thus, although the action of decamethonium is one continuous process, it is convenient to regard the failure of transmission from nerve to muscle as due to two factors: a reduction of end-plate response to the incoming nerve volley (although the resulting blocking effect may be temporarily lessened by an increased excitability of adjacent membrane), and a slowly spreading barrier of inexcitable tissue which separates the diminished end-plate potential from normally excitable muscle fibre.

It must be emphasized, however, that the first of these effects, the reduction of the end-plate response, is brought about in a totally different manner from the reduction of response caused by D-tubocurarine. For instance, the depolarizing action of decamethonium sums with that due to acetylcholine; similarly, on the frog's rectus preparation, in which both acetylcholine and decamethonium elicit a contraction, the simultaneous addition of decamethonium increases the action of acetylcholine. This 'sensitization' to the effects of acetylcholine causes also the repetitive discharge of a muscle fibre to single nerve shocks when under the influence of decamethonium. It is important to note that the repetitive firing is not confined to the period of potentiation of the twitch, but may be seen continuously as the action potential dwindles and disappears. If, then, decamethonium, unlike D-tubocurarine, does not lessen the sensitivity of the end-plate to acetylcholine, it follows that the reduction by decamethonium of the end-plate potential in response to a nerve volley must be due to a reduction in the maximum response of which the end-plate is capable. Since the end-plate is already partially depolarized, it is not surprising that the potential change which can still be evoked should also be reduced. In short, whereas D-tubocurarine interferes specifically with the sensitivity of the end-plate to acetylcholine, without interfering with its electrical excitability, decamethonium decreases the maximum local response to any form of stimulus, without reducing sensitivity to acetylcholine.

The differences between decamethonium and D-tubocurarine

It has already been argued that there is a fundamental difference in action between these drugs, despite the fact that they both produce a true neuromuscular block by classical tests (Paton & Zaimis, 1949), and analogies with acetylcholine have been emphasized (Buttle & Zaimis, 1949; Zaimis, 1951). Our experiments demonstrate that the fundamental basis for the difference from D-tubocurarine, and the likeness to acetylcholine rests in the ability of decamethonium to cause a persistent depolarization of the end-plate region.

The initially stimulant properties of the drug, most clearly seen on frog and avian muscle, but clearly demonstrable even in the cat, are a natural consequence of such depolarization. The antagonism to decamethonium of those substances which raise the threshold of the muscle to acetylcholine (such as D-tubocurarine or ether anaesthesia) is likewise to be expected. Most important of all, the failure of anticholinesterases to relieve block by decamethonium is explained; for these drugs can only prolong the action of released acetylcholine, thereby doing little more than to intensify the depolarization and inexcitability due to the decamethonium.

We may explain too, with one additional assumption, the differences in response of a muscle to a tetanus when treated with decamethonium and with D-tubocurarine. This assumption is that as tetanization proceeds, the acetylcholine released per nerve volley dwindles, as it is known to do in the cat's superior cervical ganglion (Brown & Feldberg, 1936). In normal muscle tetanized through its nerve at a moderate frequency, it may be supposed that the tetanus is sustained despite a dwindling acetylcholine output per nerve volley, because there is at the start a large excess of acetylcholine (as Brown, Dale & Feldberg (1936) showed), which provides sufficient safety margin. In curarized muscle this fall in acetylcholine output can be revealed, because the sensitivity of the muscle to acetylcholine is lowered. A suggestion of this sort was, indeed, tentatively made by Brown (1938). But the muscle treated with decamethonium, in which the sensitivity to acetylcholine is not depressed, must behave like normal muscle and sustain a tetanus as well as normal muscle does. Indeed, the fact that a tetanus is well sustained in the muscle treated with decamethonium, but not in that treated with D-tubocurarine, provides support for the belief that the failure by curarized muscle to hold a tetanus is related to the raising of its threshold to acetylcholine.

The action of acetylcholine

The properties of decamethonium at the neuromuscular junction assume a wider interest from the fact that they are shared by acetylcholine. The analogies between these two substances have been fully pointed out elsewhere. We may conclude, from the results of our experiments, that their likeness rests in a common ability to depolarize the end-plate region. The type of block which supervenes in eserinized muscle after a tetanus or after an injection of acetylcholine; the interference with this block by D-tubocurarine (Briscoe, 1938); the electrical inexcitability of the end-plate region of such a muscle; these and many related phenomena have their precise counterpart in block due to decamethonium.

We have shown that the block by decamethonium is due to the persistent depolarization at the end-plate to which it gives rise; and that there is no evidence that this block is due to the action of decamethonium anywhere else

on the muscle fibre. This makes it necessary to reconsider the conclusion by Brown *et al.* (1936) that the depressant effects of acetylcholine on a muscle are due 'to action on the muscle fibre as a whole and not on its end-plate'. The depressant effects which they observed were all in circumstances under which significant amounts of acetylcholine could persist at the end-plate region (oedema of the muscle, acetylcholine injected directly into the muscle, and the presence of eserine); no doubt acetylcholine did under these conditions leak away and come into contact with parts of the muscle remote from the end-plate. But there is reason to doubt that acetylcholine in concentrations not much higher than those just adequate to excite the end-plate would have any effect at all on the rest of the muscle fibres, since Kuffler (1943) showed that it needed 1000 times more to excite muscle away from the end-plate. Such persistence of acetylcholine in the end-plate region would, however, cause just that persistent depolarization which we have shown to cause block. There appears, therefore, to be no reason, in explaining the depressant action of acetylcholine, to suppose that acetylcholine then acts at any point other than the specifically sensitive end-plate region; but rather the depression is a consequence of the persistence of its depolarizing action, following the secondary depolarization and resulting inexcitablility of the adjacent membrane.

To obtain the stimulant action of acetylcholine, Brown *et al.* (1936) showed that it must be applied to the muscle as rapidly as possible; hence their adoption of the close arterial route for their injections. The same is true of decamethonium, with which a vigorous twitch of tibialis can be readily obtained by close arterial injection, but which causes a trivial contraction or none at all by the intravenous route. Our experiments showing how the depolarization, and with it, electrical inexcitability due to these drugs, spreads with time, provide a reasonable explanation for these phenomena. It is clear that the depolarization of the end-plate will be able to initiate propagated contractions of the muscle fibre only so long as the adjacent membrane is sufficiently excitable. If the depolarization rises rather slowly, the surrounding inexcitability, rising with it, may in fact prevent it from ever producing a normal excitation; or if a few contractions are evoked, they will presently be cut short. The failure of acetylcholine to cause contractions unless given very rapidly may, of course, be partly due to opportunities provided by a slow injection for the action of cholinesterase; but the similarity of the behaviour of decamethonium, in whose disappearance cholinesterase can play no part, suggests that the rate of rise of acetylcholine concentration at the motor end-plate plays a significant role in addition.

Finally, we have in these experiments adduced further evidence, if any is found necessary, that acetylcholine is the mediator between the nerve-impulse and the muscle fibre in mammalian muscle. We have shown that all regions of the muscle fibre are normally excitable to electrical stimulation even when

completely curarized. It is difficult to suppose that any process of electrical transmission could fail under conditions which would not reveal a local change of excitability to artificial direct local stimulation, particularly since profound changes of excitability are easily demonstrable when the muscle is paralysed not with D-tubocurarine but with decamethonium. On the other hand, we know that the curarized muscle is rendered insensitive to injected acetylcholine.

The analysis of the action of decamethonium has thus had a significance greater than might have been predicted. For it removes two possible difficulties in regarding acetylcholine as the normal transmitter of nerve excitation to muscle, the fact that acetylcholine may be depressant and the fact that special conditions are necessary to display its action in initiating propagated responses, by showing that these two properties are just those which necessarily follow from its excitant action. It is perhaps fortunate that natural curare does not produce block partly in the same way as decamethonium; had this been the case the electrical theory of neuromuscular transmission would have been much strengthened for a while by the existence of a powerful blocking agent, with which electrical inexcitability was also associated.

The mechanism of depolarization

Our experiments have not been designed to analyse those processes by which acetylcholine or decamethonium causes a depolarization of the end-plate region. There are, however, three observations which may ultimately throw some light on the problem. First, the end-plate potential evoked by a stimulus to the motor nerve has the same time course when transmission is blocked by decamethonium iodide as when block is due to D-tubocurarine. This fact may imply that decamethonium does not greatly alter the electrical constants of the muscle membrane; but this observation requires closer analysis with internal microelectrodes at the end-plate. Secondly, it is interesting that a preparation in which neuromuscular conduction is partly blocked with decamethonium is quite insensitive to either a burst of tetanic stimulation or to injected potassium chloride, while potentiation of the twitch in a partially curarized muscle is well known to follow both treatments. Thirdly, we have already remarked that successive doses of decamethonium produce progressively smaller depolarizations, despite apparent recovery of membrane potential after each dose. This result can be explained if the entry of decamethonium into the interior of the fibre is an essential part of the depolarizing process. Depolarization will then depend upon the concentration difference of the drug across the membrane so that apparent 'recovery' will take place as soon as there is the same amount inside as outside; but the decamethonium within a 'recovered' muscle can still reveal itself by lessening the rate of entry (and hence depolarization) of a subsequent dose of decamethonium.

It is interesting to note, if this process of depolarization by specifically rapid

entry of the drug is true for acetylcholine, that there is then an added reason for the presence of cholinesterase at the end-plate. Not only will it prevent the depolarizing action of acetylcholine giving rise to block of the kind we have described, but it will also prevent the persistence of acetylcholine inside the fibre from causing a progressive reduction of the effects of successive discharges of acetylcholine at the nerve endings. Whatever the correct interpretation of these results, we feel that they contain a key to the mechanism by which both decamethonium and acetylcholine depolarize the muscle membrane at the end-plate region.

The properties of a persistent cathode

The characteristics of block by decamethonium or by acetylcholine, which we have described above, are not specific to these drugs. Rather are they the property of any process which gives rise to a persistent localized cathode. Common to all these processes will be, for instance, the secondary spread of the depolarization to involve the adjacent membrane, as current flows from the surrounding regions into the end-plate region. It seems probable, from what is known of the movements of ions in nerve and muscle, that this flow of current will involve the passage of potassium ions from within the fibre outwards to the interstitial space, in amounts corresponding to the current which enters at the end-plate. In this connexion it is interesting that a preparation in which neuromuscular conduction is partly blocked with decamethonium is quite insensitive to either a burst of tetanic stimulation or to injected potassium chloride, while potentiation of the twitch in a partially curarized muscle is well known to follow both treatments.

Certain features of block due to a persistent cathode deserve emphasis; these are the initial excitation or increased excitability, the latency of onset while initial superexcitability passes into inexcitability, the spatial spread of inexcitability with time, the reversal of the block by an anode, and the lessening of the block by procedures which also lessen the associated excitation. A good deal of attention has been paid to analogies between the neuromuscular and other synapses, particularly those of the central nervous system. So far as such analogies are valid (and this is very far from established) it must be expected that block due to a persisting depolarization should exhibit the same characteristics when it appears as an inhibitory process in the central nervous system as at the motor end-plate; the same features should be displayed—of initial excitation, latency, spread, anodal reversal, and removal of excitation and inhibition together by antagonists. Satisfaction of these criteria would be demanded, for instance, of acetylcholine, if it were proposed as a mediator of central inhibitory processes. But the criteria described are of more general relevance than this, and would have to be met by any mechanism suggested, which was supposed to cause an inhibition of neural activity by means of a depolarization of the neuronal membrane.

SUMMARY

1. Decamethonium, injected intravenously or intra-arterially, causes a depolarization of the cat's gracilis muscle. The depolarization is centred round that part of the muscle which contains motor end-plates, and the demarcation potential of the muscle fibre remote from the end-plate zone is not diminished. In normal gracilis muscle, there is less than 0·5 mV. potential difference between the end-plate region and the rest of the muscle fibre.

2. The depolarization with intra-arterial injections may amount to 95% of the demarcation potential, but has never exceeded it. With intravenous injections, the depolarization rarely exceeds 30–40% of the demarcation potential. Successive doses produce progressively smaller depolarizations, even if time is allowed for full recovery from each dose.

3. The depolarization, although limited to the end-plate region, always extends slightly beyond the area in which end-plate potentials may be recorded. The extent of the spatial distribution increases with time. Other local depolarizations produced by injury of the muscle fibre by cutting, pinching, or burning it, or produced by the application of a cathode to the muscle, all have a spatial distribution resembling that of the depolarization produced by decamethonium, and this distribution spreads in the same way with time.

4. The end-plate region depolarized by decamethonium, after an initial transient increase of excitability which is associated with random spontaneous fasciculations, becomes inexcitable to direct electrical stimulation, although the muscle remote from the end-plate region remains normally excitable. The end-plate region now becomes a point of block to the propagation along the muscle fibre of a directly excited muscle action potential. After D-tubocurarine, on the other hand, the electrical excitability of the muscle remains unaltered, and propagation along the muscle fibre is unimpaired.

5. The magnitude of end-plate potential necessary to elicit a propagated action potential is much greater in the muscle under the influence of decamethonium than in the curarized muscle. The inexcitability of the muscle membrane around the point at which the end-plate potential is set up is therefore a principal cause of the neuromuscular block produced by decamethonium.

6. The intensity of neuromuscular block for a given degree of end-plate depolarization increases, and the propagation threshold rises, as the depolarization persists. During the early stages of a rapidly induced neuromuscular block by decamethonium, the propagation threshold is little higher than in the curarized muscle; the block is then largely due to a reduction of the local membrane response by the depolarization.

7. Removal of the end-plate depolarization, by passing an anodal current at the end-plate region, restores neuromuscular transmission in a muscle

blocked by decamethonium. Cathodal currents, on the other hand, intensify block by decamethonium.

8. Pentamethonium and D-tubocurarine will diminish or remove the end-plate depolarization produced by decamethonium, and the former will restore neuromuscular transmission at the same time. Both drugs, but particularly D-tubocurarine, repolarize the centre more rapidly than the outer part of the depolarized area.

9. All the principal features of block by decamethonium can be reproduced with acetylcholine, or by tetanization of the motor nerve in the presence of an anticholinesterase, or by a cathode applied to the end-plate region.

10. It is suggested that the characteristic features of block by decamethonium and acetylcholine at the neuromuscular junction are simply those of any persistent cathode, and that these features will be displayed at other synapses whenever a transmission block, due to a persisting excitatory process, occurs.

REFERENCES

Briscoe, G. (1938). *J. Physiol.* **93**, 194.
Brown, G. L. (1938). *J. Physiol.* **92**, 23*P*.
Brown, G. L. & Burns, B. D. (1949). *J. Physiol.* **108**, 54*P*.
Brown, G. L., Dale, H. H. & Feldberg, W. (1936). *J. Physiol.* **87**, 394.
Brown, G. L. & Feldberg, W. (1936). *J. Physiol.* **88**, 265.
Brown, G. L., Paton, W. D. M. & Vianna Dias, M. (1949). *J. Physiol.* **108**, 15*P*.
Buchthal, F. & Lindhard, J. (1934). *Skand. Arch. Physiol.* **70**, 227.
Buttle, G. A. H. & Zaimis, E. J. (1949). *J. Pharm. Pharmacol.* **1**, 991.
Fenn, W. O. (1940). *Physiol. Rev.* **20**, 377.
Katz, B. (1939). *J. Physiol.* **95**, 286.
Kuffler, S. W. (1943). *J. Neurophysiol.* **6**, 99.
Overton, E. (1904). *Pflüg. Arch. ges. Physiol.* **105**, 176.
Paton, W. D. M. & Zaimis, E. J. (1949). *Brit. J. Pharmacol.* **4**, 318.
Zaimis, E. J. (1951). *J. Physiol.* **112**, 176.