Anesthesiology
V 43, No 1, July 1975

Depolarization Block and Phase II Block at the Neuromuscular Junction

D. R. Waud, M.D., D. Phil., and B. E. Waud, M.D.†*

The effects of repeated doses of decamethonium or succinylcholine in muscles of the cat, dog, and rabbit have been examined. In particular, the relation of degree of neuromuscular block to intensity of the electrical change at the end-plate region has been found to be more consistent when the peak spatial gradient of depolarization is used as a measure of electrical effect than when the peak depolarization is used; the reason for this difference is discussed. A plot of twitch height against electrical change provides a convenient frame of reference for following the development of phase II block quantitatively. Examples presented show that the extent and kinetics of phase II block can vary considerably among species or among muscles in a given species. (Key words: Neuromuscular junction; Neuromuscular relaxants, succinylcholine; Neuromuscular relaxants, decamethonium.)

IT HAS BEEN KNOWN since the work of Zaimis[1] that depolarizing neuromuscular blocking agents have a "dual mode of action": with time, the neuromuscular block comes to resemble more that seen with tubocurarine than the original depolarization type of block. For example, a tetanus becomes less well sustained, and facilitatory agents like neostigmine may antagonize the block.

There has been considerable speculation as to the nature of this change in mode of action. Despite the resemblance to a competitive block, there seems little reason to believe that the mechanism is competitive at the molecular level. Phase II block has been attributed to intracellular accumulation of the depolarizing agent (see Taylor and Nedergaard[2]), but del Castillo and Katz[3] found no effect of intracellularly administered acetylcholine. Initially, it seemed possible that Paton's kinetic theory of a drug action[4] might provide an explanation, but the rate constants for the drug–receptor interaction are much too high.[5] It is conceivable that there might be a presynaptic contribution or that the ionic concentration gradients that generate the membrane potential might be running down, but both these views are difficult to test experimentally. Currently, most attention seems to be focussed on relating the development of phase II block to the phenomenon of desensitization. It has been known since the work of Katz and Thesleff[6] that the depolarizing action of applied acetylcholine is not well maintained at the neuromuscular junction despite continued presence of the drug. It is not unreasonable to suspect that such a loss of sensitivity to a depolarizing agent would extend to the transmitter and that interference with transmission of the signal from nerve to muscle could ensue. However, it seems prudent at present to distinguish clearly between desensitization and phase II block; that is, a definite connection between the two has not been demonstrated.

Because of the interest in Phase II block in the clinical context, it is worthwhile to characterize it further. It is conceivable that such a characterization could ultimately lead to definition of a link with desensitization, but an examination is justified if only to define clearly what phase II block is. To date, what we know of phase II block has been inferred from indirect indices of electrical events at the end-plate. For example, monitoring tetanic responses or the effect of neostigmine has been used to follow the rate

* Associate Professor of Pharmacology.
† Assistant Professor of Anaesthesia.

Received from the Department of Pharmacology, Harvard Medical School, and Department of Anaesthesia, Peter Bent Brigham Hospital, Boston, Massachusetts 02115. Accepted for publication February 4, 1975. Supported by USPHS research grant NS04618 from NINDS. Present address of Dr. D. R. Waud: Department of Pharmacology, University of Massachusetts Medical School, 55 Lake Ave. N., Worcester, Massachusetts 01605.

Address reprint requests to Dr. D. R. Waud.

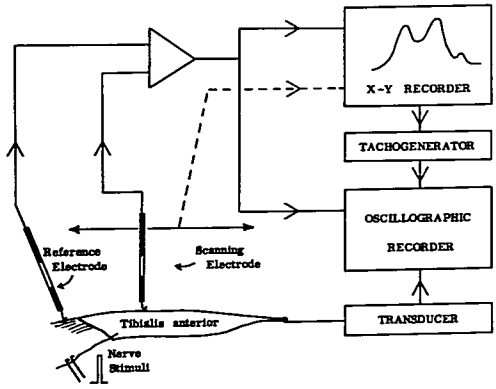

FIG. 1. Schematic diagram of the experimental arrangement. At the bottom is a conventional nerve–muscle preparation. In addition to this, a wick electrode was arranged to scan the surface of the muscle. The voltage recorded by this electrode and the twitch response were recorded against time on an oscillographic recorder. By means of an XY recorder, the voltage was also displayed against distance along the muscle. Finally, a voltage proportional to the spatial voltage gradient was obtained from a tachogenerator mechanically coupled to the Y axis of the XY recorder. This voltage was recorded on a third channel of the oscillographic recorder.

of development of phase II block. Such observations are difficult to quantitate, are of unknown reproducibility, and are rather far removed from the decrease in depolarization that, by definition, is associated with the change of mode of action of decamethonium or succinylcholine. As the first step in understanding phase II block, it would be convenient to have some index of its intensity. This would then make it possible to answer preliminary questions such as: How fast does phase II block come on or wear off? Do the kinetics depend on the muscle or species? This paper deals with some observations on possible indices that could be used to follow development of phase II block. A frame of reference for following development of phase II block is presented and used to show that different patterns of block can be distinguished in different muscles.

Early in the study we noticed an unexpected relationship between intensity of depolarization and intensity of neuromuscular block. Stimulation seemed to follow rather than precede depolarization block (*c.f.*, discussion below in connection with figures 4 and 5). This led us to examine other indices of intensity of the electrical effect, in particular, the spatial gradient of depolarization.‡

Therefore, in addition to presenting a measure of intensity of phase II block, this paper includes some empirical observations comparing depolarization per se with spatial gradient of depolarization as indices of the electrical effect of depolarizing neuromuscular blocking agents.

Methods

Most experiments were done on the tibialis anterior and soleus muscles of cats of unselected sex and weighing 2.5–4 kg. A few results were obtained in the tibialis anterior of the dog or rabbit. The preparation was similar to that of Paton and Waud.[7] The cats were anesthetized with chloralose, 80 mg/kg, iv, after ether induction, dogs, with pentobarbital, 25 mg/kg, iv, and rabbits, with urethane, 1 g/kg, iv, supplemented as necessary. The animals were ventilated to an extent sufficient to maintain arterial P_{O_2}, P_{CO_2} and *p*H levels of 85–100 torr, 24–42 torr and 7.3–7.5, respectively, values comparable to those found by Hughes.[8] Arterial pressure was monitored from the carotid artery. About

‡ Terminology is unfortunately somewhat clumsy here. The voltage measured on the muscle depends both on the position along the muscle and on time. Therefore, there are two slopes or gradients one can measure—that with distance and that with time. To refer explicitly to the former, we shall use the term "spatial gradient."

half a liter of lactated Ringer's solution with 5 per cent glucose added was infused over an eight-hour experiment to help maintain arterial pressure and urinary flow.

Both mechanical and electrical events were recorded from the muscle (fig. 1). The left lower extremity was fixed at the lower end of the tibia with a pin mounted in a myograph stand. The sciatic nerve in the thigh was stimulated supramaximally every 10 seconds and the resulting mechanical response recorded with an isometric transducer connected to the tendon of the tibialis anterior muscle. The skin over the surface of the muscle was incised and attached to a ring to form the walls of a paraffin pool. The paraffin was warmed to 35 C with an infrared lamp. An indifferent wick electrode was placed on the patellar tendon and a scanning wick electrode was arranged so it could be swept mechanically along the muscle fibers at a constant rate. The potential at the scanning electrode was recorded along the Y-axis of an XY recorder while the X-axis was connected so as to monitor distance along the muscle fibers. Thus, a plot of potential against distance along the muscle could be recorded automatically, in the manner of Burns and Paton.[9] Drugs could be injected intravenously through a catheter in the external jugular vein or intra-arterially through a catheter in a side arm of the femoral artery, as described by Waud and Waud.[10] A tachogenerator was mechanically coupled to the servomotor driving the Y-axis of the XY recorder. The output of this generator was recorded with a conventional oscillographic recorder to give a tracing proportional to the spatial gradient of potential along the muscle. This gradient could also be obtained by graphically measuring the slope of the plots generated by the XY recorder. Both methods gave the same value. Figures 2 and 10 illustrate the sort of record obtained on the oscillographic and XY recorders, respectively.

Drugs used were succinylcholine chloride and decamethonium iodide, both from K & K. All doses refer to the base.

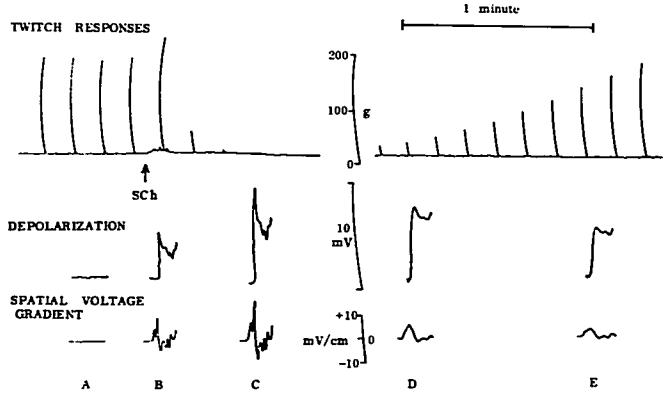

FIG. 2. Illustration of experimental results obtained. *Top tracing:* twitch response against time. *Middle tracing and lower tracing:* depolarization and spatial voltage gradient recorded as the scanning electrode passed along the muscle. Since both the electrode and the chart paper moved at a fixed rate, these last two records, though recorded against time, give voltage against distance along the muscle (records between scans have been masked out for clarity). After control records were obtained (*A*) succinylcholine 0.1 mg/kg was administered intravenously (at SCh) and the muscle was scanned twice (*B, C*) 15 and 45 seconds after SCh during onset of block. Later, during recovery, the muscle was scanned again (*D, E*) 525 and 583 seconds after the dose of succinylcholine.

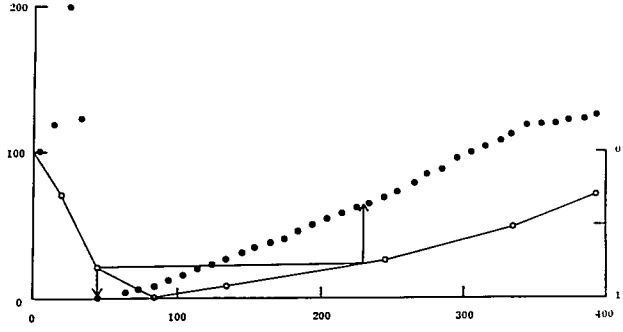

FIG. 3. Time course of effect of succinylcholine on the mechanical and electrical responses of a cat tibialis anterior. Single intravenous dose of 0.1 mg/kg succinylcholine. *Abscissae:* time (seconds) after injection. *Full circles:* twitch response (scale of ordinates on the left: tension as per cent of normal). *Open circles:* peak depolarization along muscle plotted to parallel depression of twitch response (scale of ordinates at right: 0 = no depolarization, 1 = greatest depolarization, 8.7 mV, during course of action of the dose of succinylcholine). *Horizontal line* joins points of equal intensity of depolarization during onset and offset. *Vertical arrows* indicate associated twitch responses.

Results

Relation of Intensity of Neuromuscular Block to Intensity of the Electrical Effect of a Depolarizing Blocking Agent

We begin by examining the time course of the responses to a single intravenous dose of succinylcholine (fig. 3). The twitch response (full circles) shows the usual initial stimulation followed by a block and gradual recovery. A negativity (open circles) develops over the end-plate region, reaches a maximum, and also recovers gradually. An interesting relation between the mechanical and electrical responses can be seen in figure 3. For example, focus on the intensity of depolarization indicated by the horizontal line. During onset this intensity of depolarization is associated with complete neuromuscular block. However, during offset it is associated with only about 35 per cent block. Thus, the mechanical and electrical effects do not seem to go hand in hand. This may be seen more clearly in figure 4, where the twitch response is plotted against peak depolarization. A hysteresis loop is obtained. Hysteresis was expected, because it is well known that depolarizing agents stimulate before they block, and thus one might expect a given intensity of depolarization to be associated with less block during onset than during offset. However, the curve obtained in figure 4 was surprising because the loop went the wrong way. If any additional factor such as desensitization were superimposed on the depolarization blocking mechanism a deeper block would be expected during offset as this shift to a phase II mechanism began to appear. For theoretical reasons (that subsequently seem too naive) we were led to plot twitch height against peak gradient of depolarization seen during a scanning of the muscle surface rather than peak depolarization. This gave the hysteresis loop in figure 5. Less block was associated with a given intensity of electrical effect (voltage gradient) during onset than during offset. This is in accord with what one would expect from conventional models of behavior of electrically-excitable membranes. With depolarization, one initially sees excitation, but this is followed rapidly by the development of accommodation, which in our experiments would appear as neuromuscular block.

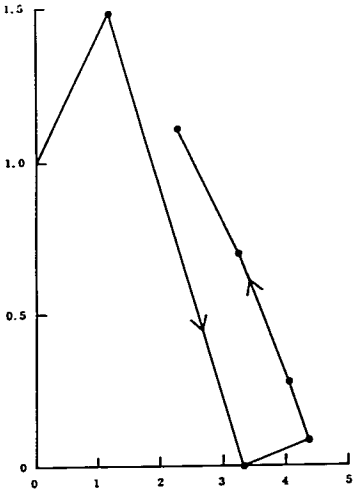

FIG. 4. Plot of twitch height against depolarization during the onset and offset of the action of succinylcholine. Same preparation as in figure 3. *Ordinates:* twitch height as fraction of control level. *Abscissae:* peak depolarization in mV. During onset there is more block for a given degree of depolarization than during offset.

Experiments such as those in figures 3-5 were repeated under more varied conditions. For example, the succinylcholine was given intra-arterially instead of intravenously, or the agent decamethonium was used, or the experiment was done in the dog or in the rabbit. The results can be summarized by saying that in the great majority of cases, the hysteresis loop was of the same form as that illustrated in figure 5 when twitch was plotted against potential gradient. When twitch was plotted against depolarization, the results were much more inconsistent. In some cases the hysteresis loop was in the same direction as that shown in figure 4; in other cases, the use of depolarization as an index gave a curve similar to that found when gradient of depolarization was used. Examples are shown in figure 6. In figures 4–9, the dose of depolarizing agent was chosen to produce a deep block. Analogous hysteresis loops were obtained with doses that produced neuromuscular blocks of lesser intensities. Thus, in the context of neuromuscular block, the spatial voltage gradient of depolarization appeared empirically to be more relevant than depolarization itself as an index of electrical action of a depolarizing agent.

OBSERVATIONS ON THE DEVELOPMENT OF PHASE II BLOCK

In the preceding section, the effects of only single doses are considered.

The next step was to examine a series of injections that might be expected to lead to the development of phase II block. The latter would be expected to appear as a progressive shift of the recovery limb of a hysteresis curve to the left, *i.e.*, the gradual development of more block for a given voltage

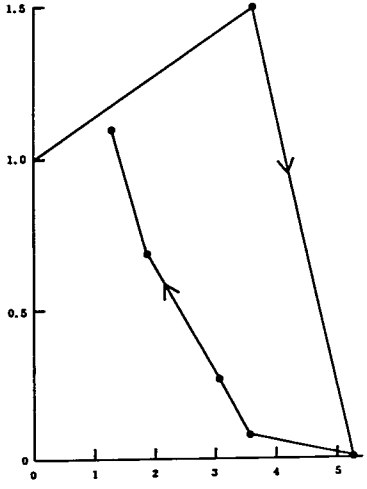

FIG. 5. Plot of twitch against peak spatial potential gradient along muscle. Same experiment as in figure 4. *Ordinates:* twitch height as fraction of control level. *Abscissae:* potential gradient in mV/cm. During onset there is less block for a given voltage gradient than during offset. This reflects initial excitation.

FIG. 6. Effect of decamethonium, and effects in dog and rabbit. *Upper row:* plots as in figure 4. *Lower row:* corresponding plots as in figure 5. The two pairs on the left represent responses to intravenous injection of decamethonium (0.1 mg/kg) in the cat tibialis anterior. In one case the upper plot reflects initial stimulation; in the other it doesn't. On the other hand, the lower plot gives a consistent picture. The third pair is from rabbit (succinylcholine, 0.3 mg/kg, iv) and the last two from dog tibialis anterior (succinylcholine, 0.1 mg/kg, iv, and decamethonium, 0.01 mg/kg, ia). Again, the upper plots show variable direction in the hysteresis loop, while the lower plots are consistent.

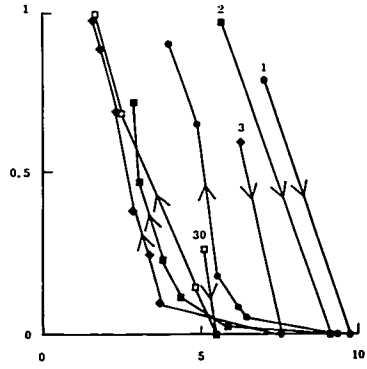

FIG. 7. Effect of repeated administration, cat tibialis anterior. Plot of twitch height against potential gradient as in figure 5. Responses to the first three and the last of a series of 30 doses of 0.1 mg/kg succinylcholine, iv (Succeeding doses were given upon recovery from the depolarization produced by the preceding dose.) The recovery limb shifts to the left between the first and second doses but then stays in roughly the same position.

gradient or a lower voltage gradient associated with any given level of block. The results of such an experiment in cat tibialis anterior are shown in figure 7. Initially, the recovery curve shifts to the left as anticipated. The last (thirtieth) curve is in essentially the same place as the second. Thus, some change occurs initially, but then a steady state, reminiscent of that described by Paton and Waud (their figure 3),[11] is reached.

In the tibialis anterior of the dog, a more marked development of phase II block was obtained (fig. 8). With repeated doses of succinylcholine, the block was associated with a lower and lower spatial potential gradient until finally neuromuscular block was associated with negligible electrical effect.

The tibialis is a fast, or white, muscle. The soleus was examined as an example of a slow, or red, muscle. An example is shown in figure 9. The hysteresis loop is similar in shape to that obtained with the tibialis, but the development of phase II block is more pronounced. This is particularly the case since the doses were spaced 3 hours apart in the soleus, whereas they were given as soon as the muscle recovered (*ca.* 20 minutes) in the tibialis.

Discussion

INTENSITY OR SPATIAL GRADIENT OF DEPOLARIZATION?

The experimental results suggested that the spatial gradient of depolarization was a more meaningful index of the effect of a depolarizing neuromuscular blocking agent than was depolarization per se. That is, a consistent picture was obtained when voltage gradient was used as the index, and that

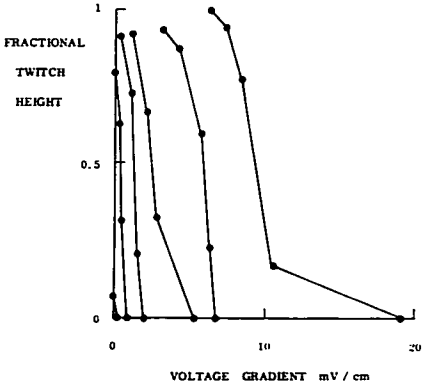

FIG. 8. Effect of repeated administration in the dog. Recovery limbs of a series of curves as in figure 7. From right to left, responses to first, second, fourth, sixth, eighth, and tenth intravenous doses of 0.1 mg/kg succinylcholine. Eventually block occurs, with little or no electrical concomitant.

picture was consistent with stimulation preceding block, whereas use of intensity of depolarization led both to much more variable results and to results that implied that stimulation followed the block.

This observation that depolarization and gradient of depolarization do not go hand in hand implies that the voltage profile along the muscle changes its shape. Such a change has already been described by Burns and Paton.[9] Their figure 7 shows clearly that the depolarization spreads with time. The same effect can be seen in the depolarization records of figure 2. Figure 10 shows the XY records from that experiment and demonstrates the phenomenon particularly well. We have noticed that the first response of the day shows the spreading phenomenon more prominently than subsequent responses. It appears that recovery to the point where a very sharp initial peak is obtained requires a considerable temporal separation of doses (of the order of four hours).

Portella *et al.*[12] have recently reported a spread of depolarization with time. They attributed it to the existence of two sets of receptors responding to quaternary compounds. This seems neither necessary nor appropriate because the depolarization response to the application of a cathode can spread similarly. Burns and Paton[9] show this in their figure 18, and this is confirmed in the experiment of figure 11. In this experiment, the anode of a 45-volt battery was connected to the metal ring supporting the skin around the muscle and a 20-gauge platinum wire connected to the cathode was laid across the middle of the muscle for 60 seconds and then removed. The voltage profile was recorded periodically afterwards. This experiment suggests that the change brought about under the cathode leads to changes in the adjacent membrane such that electrical signals spread passively further along the fiber. This might reflect an increase in the membrane resistance, since the internal and external resistances would hardly be expected to change; alternatively, the ionic concentration gradients may be altered by prolonged depolarization (see below).

The greater consistency obtained with spatial voltage gradient as a measure of intensity is interesting. In the model of depolarization block originally proposed by Burns and Paton,[9] the block results from inexcitability

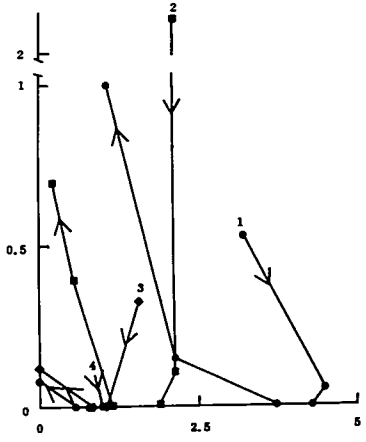

FIG. 9. Cat soleus. Results plotted as in figure 7 for four successive intravenous doses of 0.5 mg/kg succinylcholine. There is less block for a given voltage gradient during onset, as in the tibialis, but Phase II block develops more prominently.

produced around the end-plate (see also Waud[13]). Since the inexcitability was the result of the end-plate driving current outward through this electrically excitable membrane, and since the voltage along the outside of muscle fiber changes as current passes through the membrane into the extracellular space, we chose the spatial rate of change of extracellular potential, *i.e.*, the spatial voltage gradient, as an index of the intensity of the electrical event producing block. However, C. D. Thron (personal communication) pointed out that the argument was too glib. He argued that although outward current was the cause of the block, the Hodgkin-Huxley description[14] of the excitability process indicates that the inexcitability or accommodation is voltage-dependent, not current-dependent. In this case, a simple spreading of potential will not alter the intensity of block because the stimulating action of the transmitter will be affected as much as the blocking action of the depolarizing agent and no change in block will result.

Why, then, does the gradient work better?

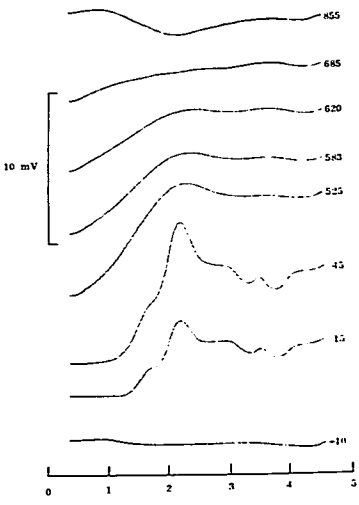

FIG. 10. Spreading of depolarization. Records from XY recorder in experiment of figure 2. *Ordinates:* potential recorded by the scanning electrode (left end of tracing represents zero, since at that point the electrode was beside the reference electrode). Successive tracings were moved upward to avoid overlap. *Abscissae:* distance (cm) along muscle (proximal end on the left). *Bottom tracing:* control 10 seconds before succinylcholine, 0.1 mg/kg, iv. Remaining tracings were taken 15–855 seconds after the injection; the first two came during onset of block, the rest during offset. Note the spreading during offset. The fine detail associated with various bands of end-plates is still visible in the 45-second record but has been completely obliterated by 525 seconds. (The hyperpolarization seen in the last tracing was also found by Burns and Paton, 1951).

An answer appears to be available in the studies of Jenkinson and Terrar.[15] These investigators have demonstrated that the spreading is due to a redistribution of chloride ions. At rest the chloride ions are distributed passively according to their electrochemical gradient, with fewer of these negative ions in the negative cell interior. In the presence of a depolarizing agent, the interior becomes more positive and chloride begins to shift into the cell. The important point is that while chloride ions are shifting inward, the voltage change produced by a depolarizing process is reduced, since some of the effect of sodium rushing inward is cancelled by chloride entering as well. However, when depolarization persists and chloride concentrations approach a new steady-state distribution, this shunting effect of chloride disappears. At this point the depolarization will be more marked and also will be able to spread further. Again, both the transmitter and the depolarizing drug will benefit, and their two effects will be roughly equal and opposite. However, a third factor is involved in excitation—activation of the sodium mechanism in the electrically excitable membrane around the end-plate. As the prolonged action of a depolarizing agent leads to a new steady-state distribution of chloride ions across this membrane, the inward sodium current of the action potential will also be opposed by less chloride current, so the threshold will fall. This would lead to less block.

Thus, the redistribution of chloride explains why voltage intensity alone does not tell the whole story. On the other hand, the measurement of spatial voltage gradient should be more successful, since it reflects both intensity of depolarization and spread. In particular, as chloride ions approach their new steady state, which will be associated with a decrease in the intensity of neuromuscular block, the recorded potential would spread, decreasing the spatial voltage gradient as well. In other words, spatial voltage gradient would be expected to change in the same direction as depolarization block.

Note that the preceding analysis does not imply that no other factors contribute to the hysteresis loop. The nature of the action of depolarizing agents is such that stimulation should precede block (in fact the action of the transmitter is so brief that only this phase is seen). The observations of Okamoto *et al.*[16] suggest that a presynaptic component also contributes to initial stimulation. Finally, onset of densensitization and/or phase II block will contribute to the location of the recovery limb.

In summary, although spatial voltage gradient was originally examined for the wrong reason, it does appear to possess properties such that it is a better index of the electrical events underlying depolarization block than that provided by intensity of depolarization.

However, the situation is complex, hard to quantitate and, therefore, of principal interest in terms of the insight the analysis provides into underlying mechanisms. In many, if not most, practical situations, the simplicity of measurement of peak depolarization will probably offset its slightly lower informational content.

Examination of the Development of Phase II Block

We turn now to consideration of a scale for assessing the extent of development of phase II block. First, it appears convenient to focus on the recovery limb of the hysteresis loop and thus avoid the period when initial stimulation complicates the picture. The frame of reference can then be a plot of twitch height versus intensity of the electrical effect at the end-plate. The choice of electrical effect could be depolarization or its gradient. Either could be recorded extracellularly or directly from measurements of transmembrane potential. However, the extracellular method will probably prove more practical, since the mechanical response of the preparation will also need to be recorded. In any case, a diagram such as the recovery limbs in figures 7 and 8 can provide a reasonably quantitative picture of the extent of development of phase II block—certainly a more concrete picture than the almost anecdotal reports available to date, and also a much better frame of reference than that provided by use of such indirect indices as fade of a tetanus or response to neostigmine. Since the spatial voltage gradient seemed to give a more consistent picture than peak depolarization, the gradient was used in figures 7 and 8. However, this advantage seems to apply particularly to the early part of the block, so that if one focuses on the recovery limbs of the hysteresis loops, the two electrical indices give similar results. Thus, the peak depolarization, which is more easily recorded than the spatial gradient, would not be an unreasonable choice for the index of electrical effect.

In any case, a plot of twitch height against electrical effect allows one readily to see a change in the contribution of depolarization to neuromuscular block. For example, figure 7 shows quite clearly that while there is some change in the mode of action of the drug in the cat tibialis anterior, the change does not proceed to completion. On the other hand, figure 8 shows a complete development of phase II block in the same muscle of the dog. If a numerical index of the extent of development of phase II block is desired,

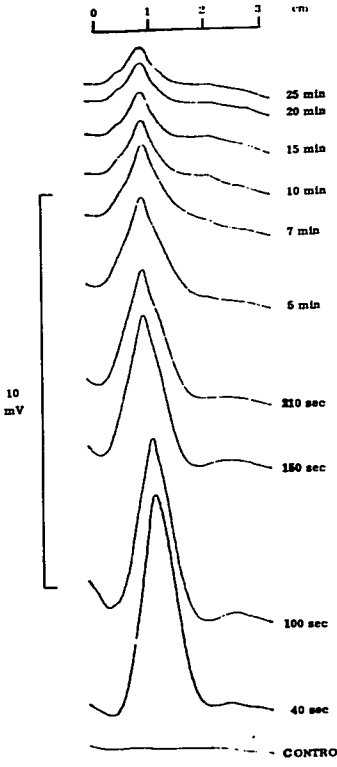

Fig. 11. Effect of a cathode. Tracings of the voltage profile in a cat tibialis anterior as in figure 10. After a control tracing (*bottom*) was obtained a 45-V cathode was laid across the muscle for 60 seconds. Then the cathode was removed and the spatial voltage profile recorded periodically thereafter. With time, the peak of the area of depolarization becomes blunter.

one might simply select some level§ of twitch height and compare the depolarization (or gradient thereof, as in figure 7 or 8) associated with that level. Thus, one could say that with a second dose only half as much depolarization was associated with a given level of block, *i.e.*, half the contribution seems to come from a phase II mechanism.

A competitive block can also be easily pictured in the proposed frame of reference. Like a fully developed phase II block, a competitive block would be represented by a path down and then back up the axis of ordinates. Note that this does not imply a similar mechanism of action.

The plot of twitch height against electrical effect also provides an interesting way to view facilitatory agents[17] such as neostigmine. Conventionally, such agents are noticed because of their ability to produce repetitive firing and thus an increase in the mechanical response to a single supramaximal nerve stimulus. However, in the proposed point of view, when twitch height is plotted against gradient of depolarization, facilitation would be reflected in the vertical distance between the two limbs of the hysteresis loop at a given gradient of depolarization. Thus, evidence of facilitation could be detected even when the twitch height has been reduced.

The specific examples given in figures 7–9 show that the rate of development of phase II block can vary considerably among species and even among muscles within species. Even these preliminary observations emphasize the need for quantitative measurement of the extent of phase II block.

This section may be summarized by saying that we propose a plot of twitch height against depolarization (or gradient of depolarization if that measurement is available) as a framework for viewing development of phase II block. In this frame of reference, phase II block will appear as a shift to the left of the relation between twitch height and depolarization.

§ The actual value obtained will depend on level of twitch height chosen since the recovery limbs are not generally found to be parallel. If a less arbitrary index were desired, one could use areas between the recovery limb and the scale of ordinates. In this case, the extent of development of phase II block could be expressed as unity minus the area observed at the time in question divided by the area observed with the first curve.

References

1. Zaimis EJ: Motor end-plate differences as a determining factor in the mode of action of neuromuscular blocking substances. J Physiol 122:238–251, 1953
2. Taylor DB, Nedergaard OA: Relation between structure and action of quaternary ammonium neuromuscular blocking agents. Physiol Rev 45:523–554, 1965
3. del Castillo J, Katz B: On the localization of acetylcholine receptors. J Physiol 128:157–181, 1955
4. Paton WDM: A theory of drug action based in the rate of drug–receptor combination. Proc R Soc Lond [Biol] 154:21–69, 1961
5. Waud DR: The rate of action of competitive neuromuscular blocking agents. J Pharmacol Exp Ther 158:99–114, 1967
6. Katz B, Thesleff S: A study of the "desensitization" produced by acetylcholine at the motor end-plate. J Physiol 138:63–80, 1957
7. Paton WDM, Waud DR: The margin of safety of neuromuscular transmission. J Physiol 191:59–90, 1967
8. Hughes R: The influence of changes in acid–base balance on neuromuscular blockage in cats. Br J Anaesth 42:658–668, 1970
9. Burns BD, Paton WDM: Depolarization of the motor end-plate by decamethonium and acetylcholine. J Physiol 115:41–73, 1951
10. Waud DR, Waud BE: A technique for intra-arterial injection of drugs. J Appl Physiol 30:140–141, 1971
11. Paton WDM, Waud DR: Drug–receptor interactions at the neuromuscular junction. Curare and Curare-like Agents. Ciba Foundation Study Group #12. Edited by AVS de Reuck. London, Churchill, 1962, pp 34–47
12. Portella A, Perez RJ, Vaccari J, et al: Muscle membrane depolarization by acetylcholine, choline and carbamylcholine, near and remote from motor end-plates. J Pharmacol Exp Ther 175:476–482, 1970
13. Waud DR: The nature of "depolarization block." ANESTHESIOLOGY 29:1014–1024, 1968
14. Hodgkin AA, Huxley AF: A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol 117:500–544, 1952
15. Jenkinson DH, Terrar DA: Influence of chloride ions on changes in membrane potential during prolonged application of carbachol to frog skeletal muscle. Br J Pharmacol 47:363–376, 1973
16. Okamoto M, Longenecker HE, Riker WF, et al: Destruction of mammalian motor nerve terminals by black widow spider venom. Science 172:733–736, 1971
17. Riker WF, Okamoto M: Pharmacology of motor nerve terminals. Annu Rev Pharmacol 9:173–208, 1969