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CHANGES IN MUSCLE CONTRACTION CURVES PRODUCED BY DRUGS OF THE ESERINE AND CURARINE GROUPS

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ESERINE in small doses acts as a stimulant to muscle. This paper describes the depressant effect of large doses on the contraction curves of normal mammalian skeletal muscle and compares the eserine type of depression with that seen in curarine poisoning. Reversal of normal grading and alteration in the initial curve of contraction are the main characteristics of changes caused by drugs of the eserine group. These effects, as seen in a mixed muscle, quadriceps of the cat, were briefly described in a preliminary communication [Briscoe, 1936*a*]. The well-known antagonism [Pal, 1900] between eserine and curarine was also demonstrated by the rapid removal of either type of depression by its antagonist, thus restoring the muscle curves to the normal shape [Briscoe 1936*c*]. In these experiments muscle contractions were recorded by an isotonic method. In the present investigation isometric recording has also been used, and in addition the effect of these drugs on a red muscle, soleus of the cat, and a white muscle, gastrocnemius, has been tested. In all essential features the results have been similar whether recorded by isotonic or by isometric methods.

METHODS

For isometric recording a flat steel spring $\frac{3}{8}$ in. wide firmly held in a robust steel frame has been used. The leg is first denervated by section of nerve trunks. The lower part of the muscle with its tendon and the piece of bone to which it is inserted is freed. Stout drills transfix the ischial tuberosity, the lower end of the femur and, when necessary, the tibia. These drills are clamped to steel pillars fixed to the operation table, the muscle being arranged to give a horizontal pull. The tendon is connected

by a steel hook to a short vertical rod attached to the flat spring. The changes in tension are recorded by a light aluminium lever moving in the vertical plane.

For isotonic recording the muscle tendon has not been freed, but the movement of the limb, whether extension of the knee or extension of the ankle, has been recorded by a thread passing from the limb to a light isotonic lever.

Both methods have their advantages and disadvantages. The isometric record gives a better picture of the changes occurring in the muscle during the initial stages of contraction, the isotonic curve being complicated by the overshoot of the limb which accompanies the sudden shortening of the muscle. On the other hand, the ease and quickness of the isotonic set-up allows simultaneous investigation of several muscles. In some experiments the effect of a drug on all three types of muscle has been shown in the same preparation. With suitable stimulation the isotonic preparation is practically unfatigable. The quadriceps can hold the leg in full extension for hours if allowed to shorten under its normal conditions of attachment [Briscoe, 1931], while under isometric conditions the contraction curve begins to give way in a few seconds (Fig. 2A). One reason for this difference is that the fusion rate under isometric conditions is higher than for isotonic recording. Owing to this greater liability to fatigue, the isometric preparation cannot be tested so frequently for its responses to different rates of stimulation. With drugs which act slowly this is no particular disadvantage, but with drugs like the onium salts which act in a few seconds and recover in a minute or two the isotonic method is useful.

For stimulation neon lamp discharges [Briscoe & Leyshon, 1929] have been used, a number of resistances with cut-out keys being inserted into the charging circuit to enable the stimulation rate to be varied in steps as desired without alteration in the strength of the stimuli. The nerves are placed on protected silver wire electrodes. Dial has been used as anæsthetic. Occasionally decerebrate preparations with preliminary ether and no dial have been used and similar results obtained. The circulation is kept intact. The drugs are injected into the jugular vein.

Results

Normal curves. In unpoisoned muscle a rise of stimulation rate (up to a certain point) produces an increased shortening or a greater rise of tension according to the method of recording employed. This increased

response to a higher frequency is referred to as normal grading. Still higher frequencies produce a deterioration in the responses and these have not been dealt with.

The first step is to find the lowest frequency compatible with fusion. (The variables upon which fusion rate depends have been discussed in a previous article [Briscoe, 1934].) The effect of increasing the stimulation rate is tested until improvement in the response ceases. This frequency is termed the optimum stimulation rate. It is usually in the neighbourhood of 100 per sec. for quadriceps and gastrocnemius and well below 100 per

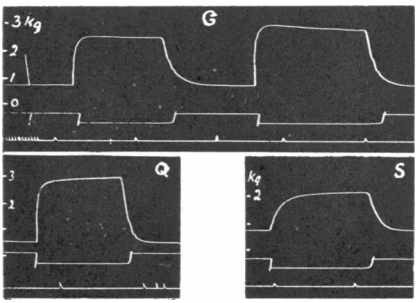

Fig. 1. Cat. Isometric curves of normal muscle. *G.* Gastrocnemius. Stimuli at fusion rate on the left, at optimum rate (90 per sec.) on the right. *Q.* Quadriceps. Rate (40 per sec.) just below fusion. *S.* Soleus. Rate (22 per sec.) just above fusion. Time interval, seconds. Tension calibration in kg.

sec. for soleus. In the controls responses to four or five different rates are recorded, but when quick changes are taking place, as in the restoration produced by the antagonism of curarine to eserine, it is advisable to test only one slow rate and one fast rate. Normal isometric curves are shown in Fig. 1.

Effect of drugs of the eserine group

Changes in the myogram produced by this group were first noticed after a depressant dose of prostigmin. It was established that eserine itself gave the same type of reaction and after this most of the experiments were carried out with prostigmin and a closely allied compound, substance 36 (methyl-phenyl-carbamic ester of 3-oxyphenyl-trimethyl-ammonium-methyl sulphate). Substance 36 can be tested without giving atropine. Atropinization (1–2 mg.) does not prevent the results described below.

The first stage of eserine poisoning is seen in the fast rate myograms. The response becomes smaller and the power of maintaining a contraction is diminished. As the poisoning deepens this deterioration in size and power

of maintenance becomes more marked, until eventually the magnitude of the response is equal to or smaller than the magnitude of the slow rate response. Normal grading is thus disturbed or reversed, the optimum rate of stimulation shifting from 100 per sec. (*circa*) to 50–75 per sec. in quadriceps and gastrocnemius; from 50–75 per sec. to 20–30 per sec. in soleus [Briscoe, 1936*b*, 1937]. Fast rates are occasionally tested before slow rates to show that the disturbance in grading is not the result of fatigue (Fig. 3B).

After the alteration in the fast rate myograms has been in evidence for a few minutes another change begins to show itself in the responses to the slow rate. The initial contraction which may or may not be diminished in size is followed by a brief period of depression which in turn is succeeded by a rise of tension or increase of shortening. This depression, followed by recovery, produces a notch in the myogram. In some preparations the notch appears early, soon after the first reduction in the size of the fast rate responses, in others it does not show until the fast rate (optimum for normal muscle) is producing smaller contractions than slow rates. If the stimulation is continued for more than a few seconds a gradual deterioration of the response sets in.

The course of events is illustrated in Fig. 2. In this experiment the drug acted quickly so that the first change is not shown. During the second minute after injection the fast rate response became smaller than the slow rate response. In the sixth minute there was the first indication of a notch and in the seventh minute this was obvious (Fig. 2C). This notch remained in evidence for nearly two hours except when it was temporarily removed in the manner described below. Two hours after injection the slow rate response was nearly normal but the fast rate response was still small and unable to maintain tension.

Varieties of deformed curves in different muscles are shown in Fig. 3. The abnormalities in these curves disappear gradually as the depressant drug is eliminated. The rate of disappearance varies greatly among different members of the eserine group. With substance 36 it is a matter of several hours and the course of elimination can be watched by recording the gradual restoration of the myograms. The curves can however be rectified rapidly by two methods: (*a*) very temporarily by repeating short closely spaced spells of stimulation, (*b*) more permanently by giving an antagonistic dose of curarine.

(*a*) In studying temporary rectification of deformed curves it is necessary to use a slowly eliminated drug like substance 36, so as to obtain a period when a given stimulation produces a constant effect.

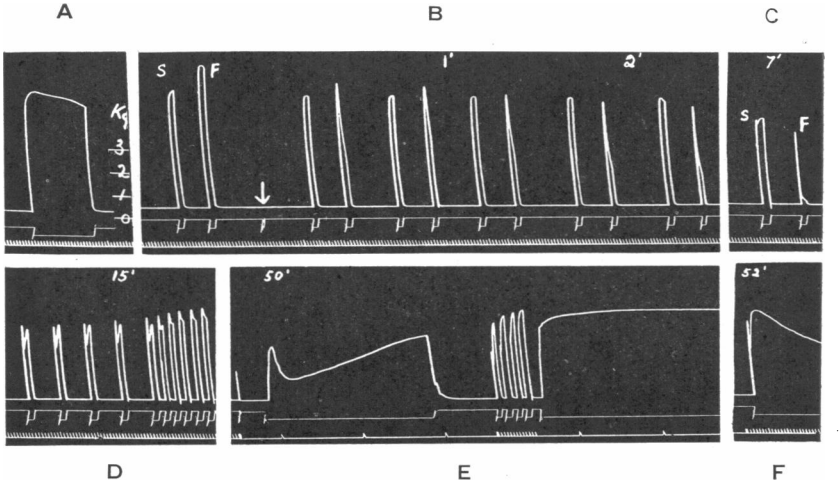

Fig. 2. Quadriceps. Isometric records. Figures above give interval after injection. Time, seconds. A. Effect of prolonged stimulation (20 sec.) at fusion rate, normal muscle. B. Slow and fast (50 and 125 per sec.) rates alternated in two-second spells. At arrow 0·5 mg./kg. substance 36 injected intravenously. In the second minute grading is reversed. C. In seventh minute notch in slow rate response. Lower line all at slow rate of stimulation. D. Effect of decreasing interval between spells of stimulation. E. After 8 min. inactivity; notched curve on fast drum. The second curve on the fast drum taken immediately after the four contractions shown on slow drum. F. One min. later, curve notched. Effect of prolonged stimulation.

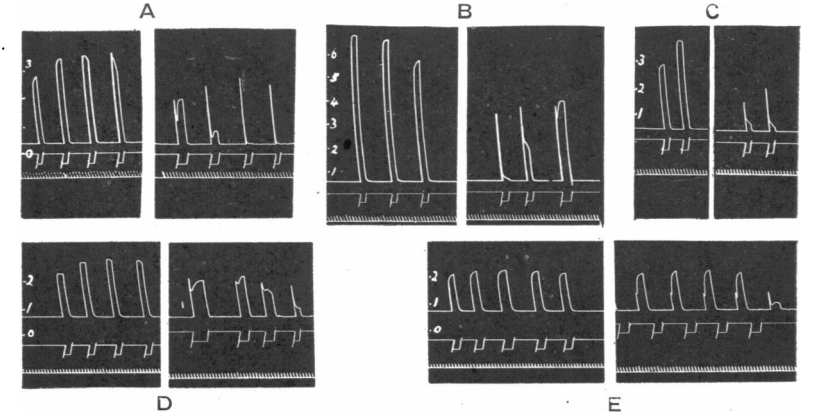

Fig. 3. Eserine and curarine types of depression. Time, secs. Upper line, quadriceps. All curves isometric. A. Rates 45, 75, 112, 160 per sec. Control curves first, then effect of substance 36. 1·0 mg./kg. Notch appears before reversal of grading is complete. B. Fast rates tested first (125, 80, 50 per sec.). Controls and then effect of substance 36. 0·5 mg./kg. C. Rates 40 and 100 per sec. Effect of curarine. 0·2 mg. Both responses proportionally depressed. Lower line. D. Gastrocnemius. Rates 35, 58, 90, 122 per sec. E. Soleus. Rates 22, 36, 55, 77, 110 per sec. Controls and then effect of substance 36. 1·5 mg./kg. D and E recorded in same experiment.

Short spells of stimulation lasting 1–2 sec. applied at intervals of 10–20 sec. are usually suitable for this purpose. When a series of uniform curves has been obtained the interval between the spells of stimulation is suddenly reduced to, say, 1 sec. The next response is different in that the notch is less well marked and the second or third response may show a normal curve with no initial depression and therefore no notch, though a slow depressant effect will appear if the stimulation is prolonged beyond a few seconds. After a short rest, abnormalities again appear, the notch becoming deeper the longer the period of inactivity (Figs. 2 and 5). The height of the initial contraction, if it has been reduced by the drug, is often improved by repeated activity and is reduced again by prolonged rest (Fig. 2E).

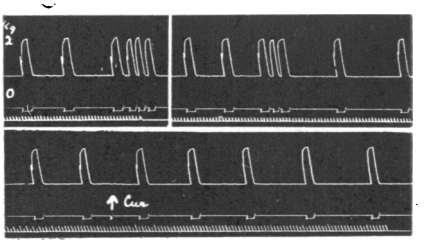

Fig. 4. Soleus. Stimulation rate 22 per sec. Time in sec. Curves deformed after substance 36. 1·5 mg./kg. Control curve shown on fast drum in Fig. 1. Upper line. Notch removed or restored by shortening or lengthening interval between spells of stimulation. Lower line. Notch permanently removed by curarine 0·15 mg. injected at arrow.

(*b*) A small dose of curarine is sufficient to abolish the notched curves seen in eserine poisoning. The rectification usually occurs in less than a minute and is long lasting. It is not easy to choose the dose of curarine which will correct the eserine effects with speed and exactitude and yet not give rise to its own characteristic depression. Fig. 4 shows the effect of 0·15 mg. of curarine chloride on the notched curve of soleus under isometric conditions. In Fig. 5 the abnormal quadriceps curve, recorded isotonically, is rectified in less than a minute by 0·6 mg. of curarine. The curves of soleus and gastrocnemius showed similar swift rectification in the same preparation. In both these figures (4 and 5) responses to a slow rate of stimulation are shown. With fast rate responses the antagonistic effect may be equally rapid; a figure showing the change, in less than a minute, from a small twitch-like response to a full size normal contraction curve has already been published [Briscoe, 1936*c*].

The chief difference between isometric and isotonic records is seen in the rapidity of the phases depression-recovery. If the muscle is contracting under isometric conditions this period may last from two to three seconds. Fig. 2E. If contracting isotonically the whole of the notch, depression and recovery, usually occurs in a second or less, though occasionally recovery is slow (Fig. 5). This applies to quadriceps and gastrocnemius. The choice of experimental method does not make much difference to the myograms of soleus, depression and recovery occur

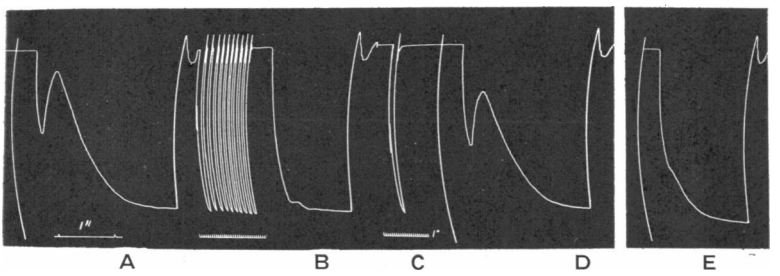

Fig. 5. Quadriceps. Effect of substance 36, doses of 0·5 mg./kg. (i.v.) given at 12·47, 1·45 and 2·52 p.m. A. 3·8 p.m. Response to rate of 35 per sec., strength maximal for full extension. B. 3·9 p.m. Notch removed by repeated spells of activity which are shown on slow drum. C. 3.10 p.m. After one minute's rest notch reappears. D. 3.11 p.m. Notch shown on fast drum. 3.25 p.m. Curarine 0·2 mg./kg. E. 3.26 p.m. One minute after, notch nearly gone. Isotonic records, contraction downwards.

slowly whichever method is used. In both the notch is a temporary interruption in the steady increase of contraction, rather than a diminution in the size of contraction (Fig. 4).

Other members of the eserine group. A number of members of this group have been tested and have caused changes in the contraction curves similar to those described above. They vary considerably in strength, in rates of action and elimination. Substance 36 takes longer to exert its action than prostigmin, but on the other hand it is eliminated more slowly and thus possesses some clinical advantage in the treatment of myasthenia gravis. Substance 38 (dimethyl carbamic ester of 8-hydroxy-methyl-quinolinium-methyl sulphate) is more active than prostigmin and more quickly eliminated. Preparation 3393 (dimethyl carbamic ester of *m* hydroxy-phenyl-diethyl methyl ammonium iodide) acts with great rapidity in very small doses. A dose of 0·01 mg./kg. (after 1 mg. atropin) produces about the same effect as 0·25 mg./kg. of eserine; it is rapidly eliminated. (I am indebted to the makers, Roche

Products, for samples of these three drugs.) Miotin hydrochloride, kindly sent me by Dr Stedman, produces marked depression with doses of 0·25 mg./kg. showing reversal of grading and notched curves.

Hordenine compounds

Schweitzer *et al.* [1938] have shown that compounds of hordenine vary in their effect on the central nervous system. Prof. Wright has kindly sent me samples of two compounds, hordenine hydrochloride

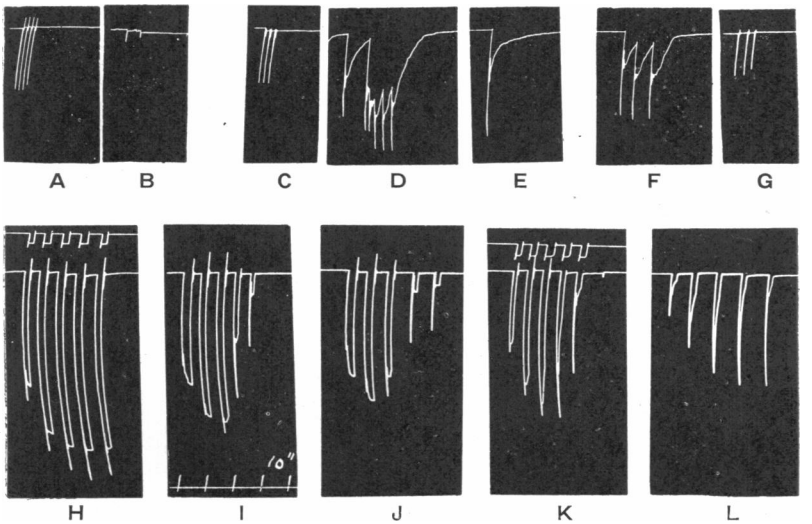

Fig. 6. Upper line, kneejerk. A. Control. B. Two min. after hordenine methyl sulphate 5 mg./kg. C. Control. D and E. Effect of hordenine hydrochlorine 8 mg./kg. F and G. Two min. and 27 min. after curarine. Lower line, quadriceps, isotonic record, contraction downwards. Both drugs give similar depressant effects peripherally. H. Control for rates (35, 58, 87, 122, 175 per sec.). I and J. Progressive depression after hordenine hydrochloride 8 mg./kg. Optimum rate shifts from 122 to 58 per sec. K. Two min. after curarine 0·45 mg. normal grading resumed. L. Twenty-five min. later. Typical curarine depression.

which is convulsant and hordenine methyl sulphate which is depressant for the C.N.S. The two drugs have identical anticholinesterase action *in vitro*, and the same peripheral effect, namely potentiation of single shock twitches, tested every 10 sec. I have tested the two drugs by the methods given above. Both produce peripheral depression of the eserine type, notching and reversal of grading being well shown (Fig. 6). On the other hand, they produce different effects on the kneejerk (tested in the opposite intact limb). Hordenine methyl sulphate (quaternary salt)

caused depression (Fig. 6*b*) or temporary abolition of the kneejerk, while hordenine hydrochloride (tertiary salt) caused excitement on the reflex side which was coincident with depression on the peripheral side. In one case the quadriceps became so rigid that it was difficult to obtain the "clasp-knife" reaction, there was tail movement, licking and swallowing; in another the kneejerk was nearly doubled and was followed by tonus (Fig. 6E). Curarine causes an immediate improvement in the peripheral depression.

Depressant effects of curarine on normal muscle

In mild curarine poisoning there is no reversal of grading, faster rates still produce larger contractions than slow ones, but magnitude and power of maintaining a contraction are reduced. With deeper poisoning the responses to all rates are twitchlike contractions much reduced in size but retaining their normal order relative to each other (Figs. 3C and 6L). Tests of different rates should not be too closely spaced otherwise fatigue effects supervene more readily than in normal muscle. They should therefore be taken occasionally in reverse and random order.

Discussion

The theory of chemical transmission of excitation assumes that the quanta of acetylcholine which are liberated by the arrival of impulses at the nerve endings act as direct stimulants to muscle fibres and are then rapidly destroyed by the cholinesterase at the nerve endings. It is known that eserine delays the normal swift destruction of acetylcholine, thus allowing of accumulations. If these accumulations are sufficient to cause reduction in size of contraction, it is to be expected that the responses to fast rates would be more deeply affected and at an earlier stage than the responses to slow rates of stimulation. Accumulations would reach the threshold for depression the more rapidly the faster the rate applied and this would account for the reversal of grading seen in deep poisoning.

Abnormal myograms of the same general type (contraction-relaxation-contraction) as those described here occur after eserinization in frog muscle [Cowan, 1936] and in the nictitating membrane [Brown & Feldberg, 1936; Cannon & Rosenblueth, 1937]. Brown & Feldberg measured the output of acetylcholine from the superior cervical ganglion perfused with eserine. During prolonged preganglionic stimulation the output starts at a high level and then falls rapidly to a steady low level. The slow recovery of contraction of the nictitating membrane after the initial depression occurs during the rapid fall in the output of acetylcholine. In skeletal muscle depression and recovery occur with

much greater rapidity, but the sequence of events is the same. Reduction in the size of initial contraction followed by a swift relaxation may be attributed to excess of acetylcholine. With fast rates of stimulation this relaxation persists. The recovery from the initial depression seen with slow rates may be explained on the ground that after the first excess has been worked off a more normal balance obtains between the formation and destruction of acetylcholine. The muscle begins to emerge from its depression causing a notch to appear in the myogram. This more normal balance may be due partly to a lowered output of transmitter, partly to the long interval between the stimuli, allowing more time for its destruction.

The disappearance of the notch after a short spell of activity is difficult to explain. If there is a drop in output with continued stimulation this condition may persist for a short period after stimulation ceases, so that a contraction occurring within this period would be normal because there was no excess of acetylcholine. The period of exemption is short, as depressant effects quickly reassert themselves. Brown & Feldberg discuss the possibility that acetylcholine is liberated from a pre-existing complex in which it is stored in an inactive form and protected from destruction. If this suggestion prove to be correct the exemption from depressant effects would be due to depletion of the depot and the rate of refilling could be gauged by their reappearance.

The differences seen in the myograms of eserine and curarine poisoning suggest that these two drugs produce their depressant effects by different mechanisms. The suggestion has been discussed in a previous publication [1936*c*] that curarine antagonizes eserine by raising its threshold for depressant action. If the dose of curarine is larger than is necessary to secure the antagonistic effect, the rectification of curves may occur within a few seconds, but is transient. After two or three minutes the curves again become abnormal, showing the curarine type of poisoning. With all rates there is loss of power to maintain a contraction and proportional diminution in magnitude, so that normal grading is resumed. It is difficult to form any conception of the mechanism by which the temporary release from depression is obtained. If a struggle took place for the same receptors, or if the drugs combined with receptors of different pattern, as suggested in the specific receptors theory of drug antagonism [Clark, 1937], why should there be a period of normal response? A rise of threshold for depressant effects would explain how the muscle can pass through a short period during which the curves are more or less normal before a rise in stimulation threshold brings about depression of a

different type. Something more than a simple rise of threshold is required to explain the selective action of the two groups of drugs. It is possible that they act at different sites in the nerve muscle junctions. Since both types of drugs, in large doses, depress the two functional activities of muscle, contraction and the maintenance of contraction, the selective action is largely a question of emphasis, and differences such as the thickness or the permeability of membranes may be of decisive importance.

It has been shown by Brown *et al.* [1936] and confirmed by Schweitzer & Wright [1937] that eserine potentiates the nerve muscle twitch when single shocks are applied at intervals of ten seconds. Records of muscle action potentials show that this effect is due to repetitive response of individual fibres to single nerve volleys [Brown, 1937]. With iterative stimulation as in the present experiments depression ensues after eserinization. It is clear that the type of response in peripheral muscle, whether potentiation or depression, can be varied by regulating the interval between the stimuli applied to the nerve. It follows that peripheral effects bear no necessary relation to the states of excitement or depression produced in the central nervous system by the various drugs.

Summary

Drugs of the eserine group produce abnormalities in the contraction curves of different types of mammalian muscle (mixed red and white). Normal grading is disturbed or reversed in that slow rates of stimulation produce larger contractions than fast rates.

Curarine produces abnormal myograms of a different type.

The abnormal contraction curves seen in eserine depression in response to slow rates of stimulation can be temporarily rectified by repeated activity of the muscle.

The antagonistic action of curarine to eserine is shown by the rectification of the responses to all rates. With correct dosage this rectification is permanent; if the dose is too large the period of normal response is followed by depression of the curarine type.

The possible mode of action of this antagonism is discussed.

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