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THE PERIPHERAL ACTION OF TETANUS TOXIN

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It was noted very early in the experimental work on tetanus toxin that when one of the extremities of an animal received an injection of the toxin subcutaneously, intramuscularly or intraneurally, the limb became rigidly extended after an interval of 1–4 days. This rigidity was found to occur in the absence of other manifestations, and, if the dose injected was not too large, the muscles eventually regained their normal state. This interesting phenomenon has been called local tetanus, and it can hardly be distinguished from the unremitting closure of the jaws which is so characteristic of the generalized disease in man.

Meyer and his co-workers [1903, 1916] believed that this state of continuous, but strictly localized, rigidity was brought about by the action of the toxin on central neurons, just as are the tactile reflex spasms and convulsions of the more generalized form of the disease. They supposed that the toxin was carried in the motor axis cylinders to the motor neurons of the central nervous system after its absorption by the motor nerve terminals of the skeletal muscles. These conceptions were quite generally accepted until the recent series of experiments carried out by Abel and his colleagues [cf. Abel, Firor & Chalian, 1938], which show quite clearly that there is no evidence that tetanus toxin reaches the central nervous system by any way other than the blood stream. In their view, the symptoms produced by tetanus toxin are due to a twofold action: (1) a peripheral action, in the region of the motor end-plates of the muscles, which the injected toxin reaches directly, resulting in an enduring, local muscular rigidity; and (2) a fixation to the anterior horn cells of toxin, reaching them through the circulation, resulting in the appearance of reflex motor tetanus without any constant rigidity. A full account of this controversy and the experimental facts relating to it may be found in the many papers from Abel's laboratory.

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Gumprecht [1894] concluded that local tetanus was a central effect, since the rigidity disappeared after the motor nerve was cut, and Ranson [1928] similarly found that it disappeared within 5 days after nerve section. That tetanus would not develop in a muscle, whose motor nerve had been cut beforehand and allowed to degenerate, was first observed by Courmont & Doyon [1899]. Gumprecht also pointed out that the response of the muscle, both to nerve stimulation and to direct excitation, was reduced when the continuous rigidity was present. No clear-cut evidence has yet been presented of the exact nature of this localized rigidity; whether it is a contraction due to impulses propagated along the muscle fibres and accompanied by oscillatory action potentials, or a contracture due to localized, non-propagated activity in the muscle fibres. Using the string galvanometer, most of the earlier investigators found no action currents accompanying local tetanus, either in man or in experimental animals [Fröhlich & Meyer, 1916; Semerau & Weiler, 1918; Liljestrand & Magnus, 1919]. Action-potential changes recorded by Fujita [cf. Kuré & Shmosaki, 1924] disappeared when the motor nerve was cut. Keller [1927*a*, *b*] also observed oscillatory potential changes, even when the animal was under deep ether anaesthesia or decerebrate, but in his experiments the muscle retained its connexions with the central nervous system.

The action of curare in this condition serves to localize the lesion to the region of the motor end-plates or nerve endings. Bremer, Titeca & Meeren [1927] showed in the rabbit that local tetanus of recent origin, and of extreme degree, could be completely abolished by 0·2–0·3 mg. of curare given subcutaneously. Interest in the use of curare for the treatment of tetanus in human beings, first described by Hoche, has recently been revived by West [1936]. It is noteworthy that the generalized convulsions of experimental tetanus in rabbits can be reduced in violence by the administration of curarine, but their number is not decreased unless doses which produce almost complete paralysis are given [Florey, Harding & Fildes, 1934].

Because of the long incubation period accompanying the action of this toxin, no direct experimental proof of the peripheral action has yet been advanced. No satisfactory functional examination of the muscles in local tetanus has yet been made. It seemed desirable to reinvestigate the condition with more modern myographic, electromyographic, and pharmacological methods. Direct proof has thus been obtained of the peripheral action and evidence concerning its nature. A preliminary report of this work has already appeared [Harvey, 1939].

Methods

In these experiments the method devised by Abel [1934] for producing a long-lasting local tetanus with very small doses of toxin has been used. The toxin (no. 641B), kindly supplied me by Dr W. M. Firor, contained in 1 c.c. 14,000 guinea-pig L.D. 50. One two-hundredth to one-fiftieth of the lethal dose for the cat, in 1 c.c. of 0·9% saline solution, was injected at 30–40 points into the tibialis anterior muscle of the cat, under ether or nembutal anaesthesia, by means of a small hypodermic needle.

For the acute experiments, the cats were decerebrated under preliminary ether anaesthesia, and the tibialis anterior muscle was prepared for arterial injections and for recording by the method described by Brown [1938]. Muscular contractions were recorded with an isometric lever of the flat spring type, either optically, or on a smoked drum by a light aluminium lever. The sciatic nerve, previously ligated high in the thigh, was left in position, and was stimulated through shielded electrodes. The stimuli were slightly supramaximal condenser discharges, timed by means of a gas discharge tube. For direct stimulation of the muscle a silver pin was pushed through the tendon, the drill piercing the condyles of the femur serving as the other electrode; in this instance, the stimuli were break induction shocks, timed by a Lewis rotating contact-breaker. In the experiments in which optical records were made, the stimuli to the nerve or muscle were delivered by a Lucas pendulum.

The lead-off electrodes for the action potentials were either silver pins, the earth lead being pushed into the belly of the muscle and the grid lead into the tendon, or a concentric needle electrode of the type described by Adrian & Bronk [1929] in the belly of the muscle. The action potentials were amplified by two or three resistance capacity-coupled stages, and recorded by a Matthews' oscillograph or a cathode-ray oscillograph.

The muscle temperature was measured by means of a needle thermocouple, the junction being steel and constantan wire. The cold junction was surrounded by melting ice, and the current was measured with a microammeter, calibrated to read in ° C.

Acetylcholine solutions were always freshly prepared in acid saline at *p*H 4, and those of eserine sulphate in a similar manner. The injected volume was always 0·25 c.c.

Results

The relation of the nerve supply to the development and maintenance of local tetanus

When $\frac{1}{200}$ to $\frac{1}{50}$ of the lethal dose of toxin is injected in the manner described, a sustained and extremely firm rigidity of the tibialis anterior and of many of the surrounding muscles, including the gastrocnemius, develops within a period of 3–4 days. The leg is then quite stiff at the ankle joint, and is held off the ground. The animal gives no evidence of pain when the leg is touched, and external stimuli do not have any effect on the degree of rigidity. This local tetanus may persist for several months, but, if the dose has not been too great, the animal finally regains its ability to use the muscles normally. The effect of the toxin is usually perceptible about 24 hr. after the injection, and before any permanent rigidity appears the muscles show persistence of activity on voluntary movement, and a well-defined clonus upon dorsiflexion of the foot.

The temperature changes in these muscles is very striking, and even to ordinary touch they feel, through the skin, very much warmer than those of the normal leg. When measurements are made with a needle thermocouple thrust into the muscle, the animal being under nembutal anaesthesia, this difference in temperature is found to be as great as 2–3° C. when the local tetanus is fully developed.

Section of the mixed nerve to the muscle decreases the rigidity slightly, probably because the proprioceptive impulses arising in the muscle itself are cut off. Complete relaxation, however, does not occur until 3–5 days later, when the nerve endings have had time to degenerate. On the other hand, the injection of tetanus toxin into a muscle, of which the motor nerve has been previously cut and allowed to degenerate, produces no perceptible effect. These effects of nerve section have always been thought to exclude the possibility of the toxin having a direct peripheral action, but the following type of experiment demonstrates clearly that local tetanus can develop in a muscle, even when the nervous connexion with the central nervous system has been severed:

Cat 2·9 *kg.* 29 *Nov.* 1938: Under nembutal anaesthesia $\frac{1}{100}$ of a lethal dose of toxin was injected at 30 points into each tibialis anterior. 30 *Nov.* 1938: The right sciatic nerve was cut high in the thigh exactly 24 hr. after the injection of the toxin. 1 *Dec.* 1938: There is beginning rigidity of both legs, with marked clonus on passive movement or stretching. This is slightly less on the right side (there is no reflex activity on this side). 2 *Dec.* 1938: Both legs now show definite local tetanus of the

tibialis anterior and the gastrocnemius, but the left leg is more rigid than the right. 3 *Dec.* 1938: The right leg is almost completely flaccid, while there is extreme rigidity of the left.

The interpretation of these various experiments, which in themselves leave no doubt of a peripheral action of tetanus toxin, will be considered in the discussion.

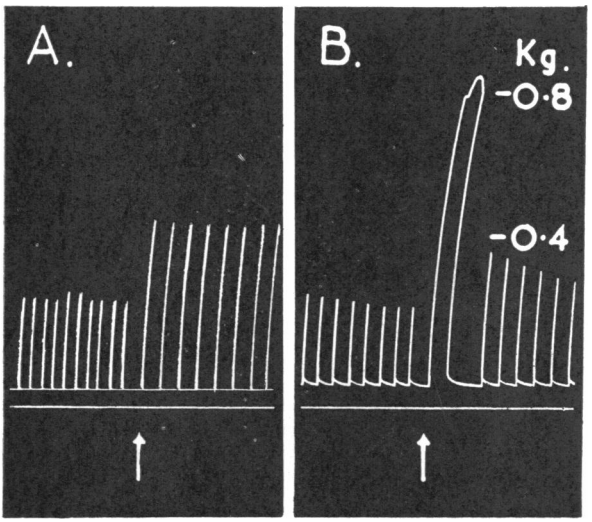

Fig. 1. Cat, 2·6 kg., decerebrate, isometric contractions at 10 sec. intervals of tibialis anterior muscle into which $\frac{1}{100}$ lethal dose tetanus toxin injected 5 days previously. A. At arrow change from maximal motor nerve volleys to maximal direct stimuli. B. Maximal motor nerve volleys. At arrow maximal motor nerve tetanus at 50 per sec. for 12 sec.

The response of the muscle to nerve stimulation and to direct excitation. A muscle which has been injected with tetanus toxin gradually loses its ability to respond to maximal stimulation of its motor nerve (Fig. 1). After a period of 4–5 days, the indirect twitch is only 50–60% as strong as that evoked by maximal direct stimulation of the muscle, the tension in the latter case being still almost as great as that developed by direct stimulation of the opposite and normal muscle. Two or three weeks after the injection, the response of the muscle to nerve stimulation is either very small or absent, and at this time the tension evoked by direct excitation is also considerably reduced. The direct twitches, though reduced in tension, always have a relatively normal appearance, the twitch-tetanus ratio not being reduced, and the tension developed by a

tetanic response being well maintained as long as the stimulation is continued (Fig. 1B).

The potentiation of the twitches following a period of tetanic stimulation [Brown & Euler, 1938] is quite normal (Fig. 1B). In many experiments there was a slow continuous development of tension, following a tetanic contraction, which lasted often for 20–30 sec., and during this the twitches in response to nerve stimulation were a little depressed. Both of these effects are seen also with direct excitation of the muscle, at a stage when the twitches evoked by motor nerve volleys are very small or absent.

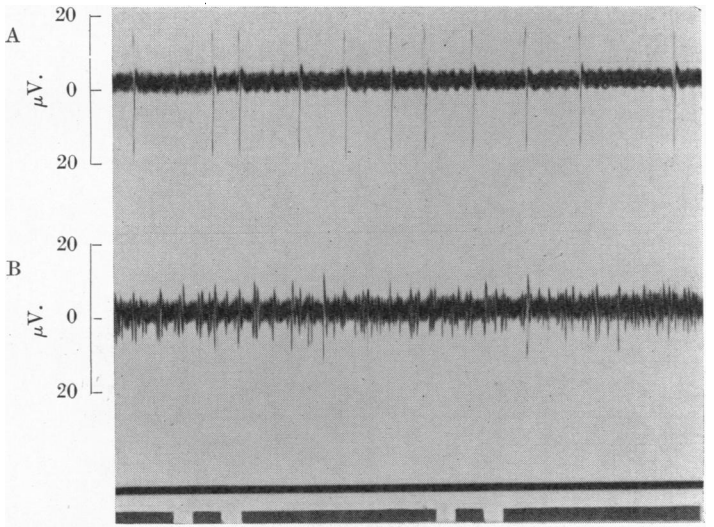

Fig. 2. Cat, 2·9 kg., decerebrate. Spontaneous activity in tibialis anterior after injection of $\frac{1}{100}$ lethal dose of tetanus toxin 23 days previously. Concentric needle electrodes. Sciatic nerve cut high in the thigh 30 min. before. A and B taken 5 min. apart with electrodes in same position. Time 0·5 sec.

The electrical response of the muscle

In the decerebrate animal, in which the central connexion of the motor nerve to the tibialis has been severed, a considerable degree of rigidity still remains. When concentric needle electrodes are passed through the skin into the muscle, oscillatory potential changes of varying frequencies and rhythms can be recorded (Fig. 2). The amount of spontaneous electrical activity varies from moment to moment. A group of fibres may respond at a rate of 1 per sec. for several minutes, and then suddenly increase its speed to 100 or 150 per sec. The activity may then cease for long periods. In other words, groups of fibres are intermittently active,

and during the periods of activity may show rapidly changing rates of discharge from 1 to 150 per sec. The potential changes are not like those accompanying the response of the fibres in a motor unit, but are similar to those recorded from the irregular fibre responses in a muscle whose motor nerve has been cut and allowed to degenerate.

In the decerebrate preparation with the motor nerve intact, this electrical activity is much greater, on account of the reflex activity of the proprioceptive end-organs in the muscle. The electrical activity in response to stretch is then much greater than in the normal muscle, as is also the degree of response evoked by shifting the position of the needle electrode within the muscle. The normal muscle in the relaxed state was found to be electrically "silent", even with the motor nerve intact, when, under similar conditions, the muscle in local tetanus was the seat of intense spontaneous activity.

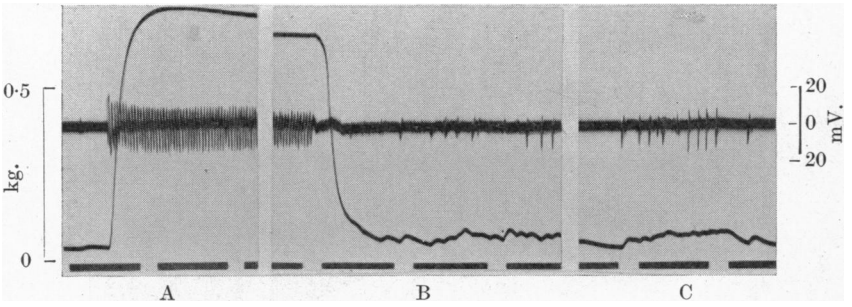

Fig. 3. Same experiment as Fig. 2 A. Onset of motor nerve tetanus at 60 per sec. for 7 sec. For previous 30 sec. almost no spontaneous activity. B. End of tetanus followed by increase in spontaneous discharge. C. 30 sec. later. Time 0·5 sec.

The effect of repetitive stimulation of the cut motor nerve upon this spontaneous activity of the muscle is very striking (Fig. 3). As soon as the stimulus is stopped, there is an outburst of asynchronous electrical activity, accompanied by an irregular twitching of the muscle fibres, which is readily visible in the mechanical record and on inspection of the surface of the muscle. This increased activity after repetitive stimulation of the nerve continues at a high level for periods up to 1 min., and then gradually wears off.

The action potential in response to stimulation of the cut motor nerve. The action potential of the whole muscle, with belly to tendon leads, in response to a single nerve volley, has a double-spiked negative deflexion, which contrasts with the clean cut, single deflexion of the normal tibialis anterior. The potential of the negative deflexion is usually quite small,

in one instance being only 14 mV., in contrast to a potential change of 26 mV. in the normal muscles of the same cat. When two nerve volleys are set up at intervals between 10 and 200 msec. apart, the relative sizes of the responses were the same as in a normal muscle.

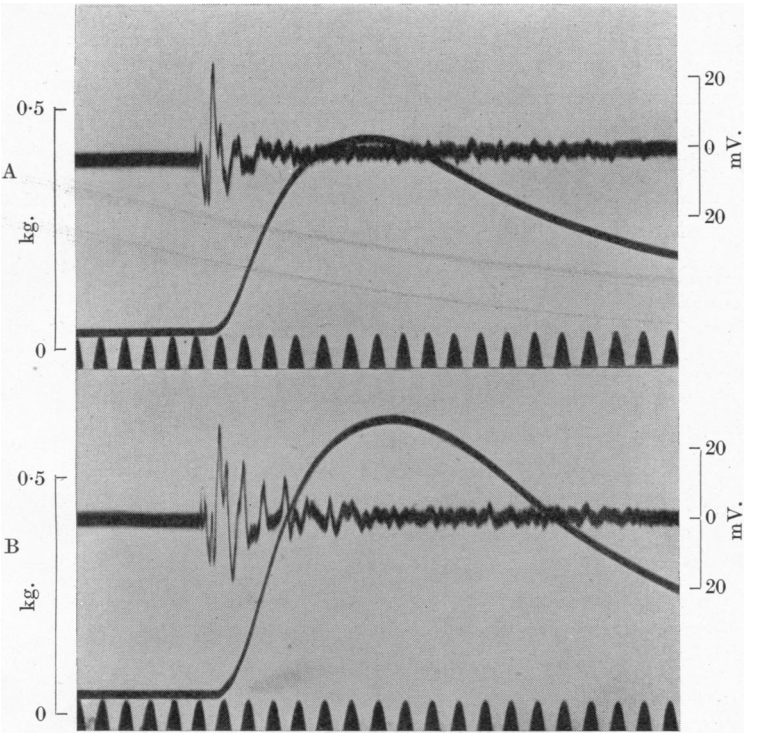

Fig. 4. Cat, 2·9 kg., decerebrate. Tibialis anterior. $\frac{1}{100}$ lethal dose of tetanus toxin injected 7 days previously. Concentric needle electrodes. A. Response to a single maximal motor nerve volley. B. Response to two maximal motor nerve volleys delivered 3 msec. apart. Time 10 msec.

Because of the peculiar shape of these potentials, and the fact that some of them were apparently repetitive in nature, records were taken, using concentric needle electrodes. The use of this more localized lead clearly reveals the repetitive nature of the response of the muscle to a single nerve volley. Oscillatory potential changes may persist as long as 25 msec. after the beginning of the electrical response, even in a muscle in which the tension response was greatly reduced (Fig. 4A). When two nerve volleys are set up at a short interval apart, the duration of the

repetitive discharge is increased, as might be expected from the effects of tetanic stimulation on the spontaneous discharge described above (Fig. 4B). This effect is greatest when the interval is about 50 msec.

The action of curarine

Although the action of curare in relation to the effects of tetanus toxin has been known since the work of Brunner [1892], its importance has never been sufficiently emphasized. The spontaneous electrical activity and rigidity of the muscle in local tetanus can be abolished by the administration of curarine intravenously to the decerebrate animal in doses which do not eliminate the response of the muscle to stimulation of the motor nerve. Partial curarization of the animal greatly reduces the duration of the repetitive potential activity, but the effective dose is one which results in a considerable reduction of the tension developed by the motor nerve twitch. That this repetitive response to a single nerve volley is due to some change in the process of neuromuscular conduction, or in the activity of the nerve endings in the muscle, and is not the result of an alteration in the response of the muscle fibres themselves, is shown by the fact that it is not present in the action potential of the completely curarized muscle when excited directly. It is very difficult in records taken with concentric needle electrodes, using direct excitation of a muscle of this size, to be certain of the differentiation between stimulation artefact and action potential. However, in the records taken before and after curarization, the difference is distinct enough to be reasonably certain that the repetitive discharge is no longer present in the fully curarized muscle.

The effect of acetylcholine by close arterial injection

When an injection of 5–10μg. of acetylcholine is made into a muscle in local tetanus there is an initial quick twitch-like response similar to that seen in normal muscle [Brown, Dale & Feldberg, 1936; Brown, 1937], but instead of immediately relaxing, the tension gradually decreases over a period varying from 10 to 30 sec. (Fig. 5). In many of the records there is, after the first contraction, a second increase in tension quite like that seen in a denervated muscle. During the period of response to acetylcholine the muscle contractions evoked by motor nerve stimulation are usually greatly depressed. The maximum tension reached by the twitch-like response to this drug is usually many times greater than that resulting from a maximal motor nerve volley. This shows that the reduction by

tetanus toxin of the response to nerve stimulation is not of the same nature as that produced by the administration of curarine.

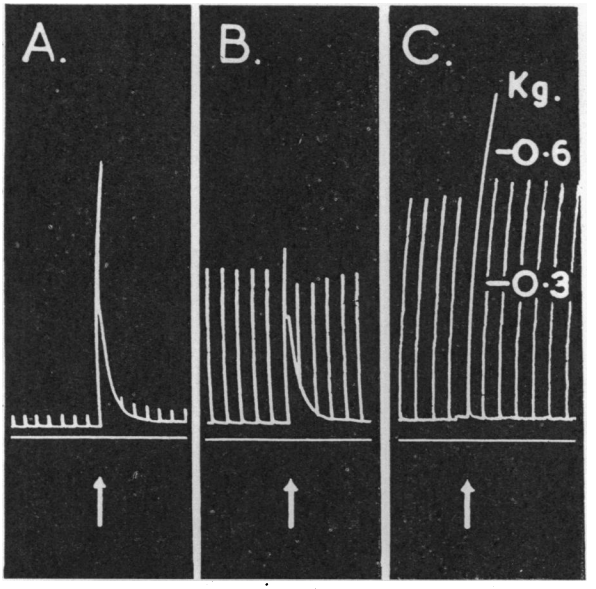

Fig. 5. Cat, 2·4 kg., decerebrate. Left tibialis 9 days after injection of $\frac{1}{100}$ lethal dose of tetanus toxin. A. Maximal motor nerve volleys at 10 sec. intervals. At arrow 10μg. acetylcholine intra-arterially. B. Maximal direct stimuli every 10 sec. At arrow 5μg. acetylcholine. C. Right tibialis anterior, normal. Maximal motor nerve volleys at 10 sec. intervals. At arrow 10μg. acetylcholine.

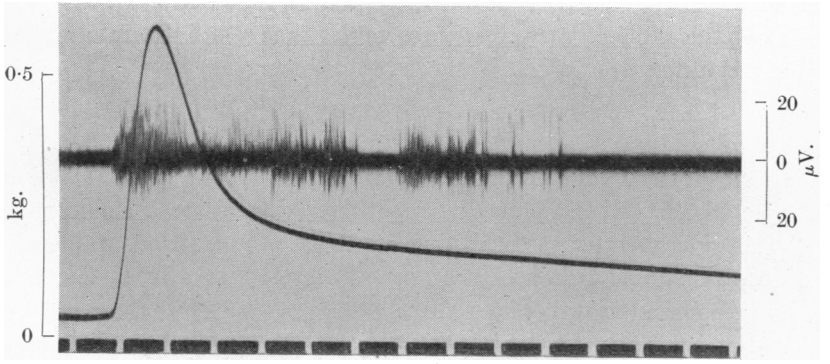

Fig. 6. Cat, 2·9 kg., decerebrate. Tibialis anterior 7 days after local injection of $\frac{1}{200}$ lethal dose of tetanus toxin. Acetylcholine 5μg. intra-arterially. Time 0·5 sec.

When the action potential of the response to acetylcholine is recorded by concentric needle electrodes, it is seen that there are oscillatory

potentials present throughout the greater part of the prolonged mechanical response (Fig. 6). Thus, the reaction to acetylcholine in these muscles is a prolonged response, but otherwise similar in nature to that seen in normal muscle. In some of these acetylcholine contractions, however, there may be an element of contracture, particularly when the local tetanus is one of long standing.

The duration of the acetylcholine response is greatly prolonged by the administration of eserine (Fig. 7). In this figure it is seen that the superimposed twitches in response to nerve volleys are greatly depressed.

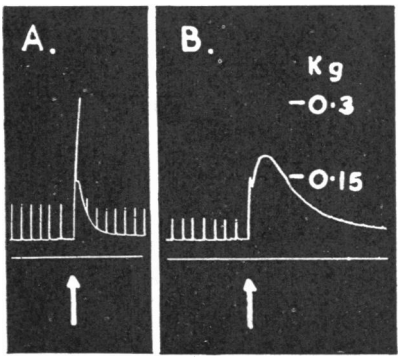

Fig. 7. Cat, 2·4 kg., decerebrate. Tibialis anterior 28 days after injection of $\frac{1}{50}$ lethal dose tetanus toxin. Maximal motor nerve stimuli at 10 sec. intervals. At arrows 10μg. acetylcholine intra-arterially. Between A and B 25μg. eserine intra-arterially.

There is almost certainly an element of contracture in such a case, and the mechanical effect very closely resembles that seen in a muscle whose motor nerve has been cut and allowed to degenerate.

The effect of eserine

In view of the very unusual response of the muscle in local tetanus to injected acetylcholine, and its repetitive response to a single nerve volley, it was of interest to observe its reaction to eserine, which also converts the single twitch of the normal muscle into a brief, declining tetanic response (Brown *et al.* 1936).

In the muscle in local tetanus, 25μg. of eserine administered intra-arterially caused a slight increase, or none, in the tension of the single nerve twitches, while the same dose in the opposite normal leg may almost double the tension developed in response to a single nerve volley (Fig. 8). The degree of eserine potentiation seemed to diminish in pro-

portion to the duration of the local tetanus, but not enough experiments were done to be certain of this point.

When action potential records are taken with concentric needle electrodes, eserine causes a slight increase in the amount of repetitive discharge in response to a nerve volley, but not nearly as much as appears in the normal leg receiving the same dose of the drug. A similar contrast is seen when eserine 0·3 mg./kg. is given intravenously. This dose produces generalized muscular twitchings, but apparently has no effect on the spontaneous activity of the muscle injected with tetanus toxin.

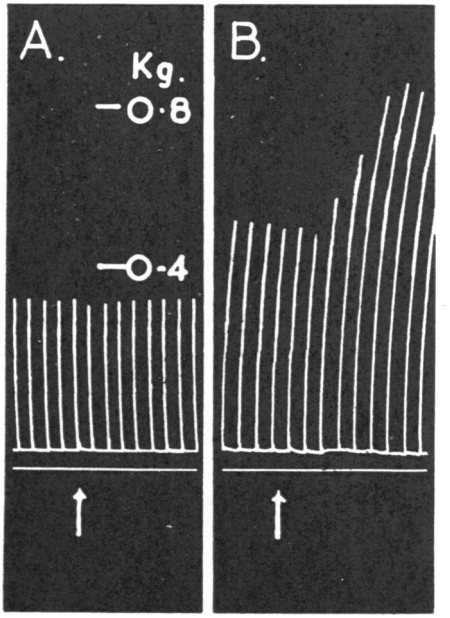

Fig. 8. Same experiment as Fig. 5. A. Maximal direct stimuli at 10 sec. intervals. Left tibialis anterior with local tetanus. B. Maximal motor nerve volleys at 10 sec. intervals. Right tibialis anterior, normal. At arrows 25μg. eserine intra-arterially.

Discussion

Though the researches of Abel and his co-workers had shown clearly that local tetanus was not due, as had been supposed, to the spread of toxin by the motor nerve fibres to the corresponding motor nerve cells in the spinal cord, they had provided no direct proof of the alternative peripheral action on the muscles to which the toxin had direct access, and no data concerning the mechanism of such a local effect.

Ranson [1928] had shown, indeed, that the rigidity of the muscles, after local injection of the toxin into them, persisted for some days after section of their motor nerves; in my own experiments also it has still been recognizable for 3–5 days after such nerve section. On the other hand, since the appearance of the local tetanus had not been observed when the toxin was injected after the nerve section, it was natural to suppose that functional connexion with the spinal cord was necessary for the development of the tetanus, even though the condition persisted for some time after this connexion had been severed. My own experiments exclude such an interpretation, and show that the determining factor is the integrity of the motor nerve endings, and not connexion with the spinal cord. I have shown that, if the toxin is injected into the muscle soon enough after the nerve has been cut, the rigidity is still produced, though it fades and disappears at the time when the motor nerve endings lose their transmitting function, and when the muscle fibres are functionally intact. This location of the effect at the nerve endings is confirmed by the action of curarine, which immediately abolishes the rigidity, before or after section of the nerve, leaving the muscle fibres normally responsive to direct stimulation.

There were facts, indeed, which appeared to support the earlier attribution of local tetanus to abnormal excitability of the motor nerve cells. In general tetanus in which the toxin is carried in sufficient concentration by the blood, the effect on the nerve centres must contribute to the total effect, though, as Firor & Jonas [1938] have shown, it is an action of a different type, producing by itself only a spasmodic reflex activity of the muscles innervated by the affected segments of the spinal cord, and not a persistent rigidity. When the toxin has been administered locally in excessive dosage, it is not improbable that a minor degree of the central action, due to carriage of toxin in the blood stream, often complicates the local effect on the muscle. Even with very small doses such as I used, impulses from the cord undoubtedly contributed to the local tetanus earlier in its development, section of the motor nerve causing at that stage an immediate weakening of the rigidity. Such an effect, however, also involves the action of the toxin on the motor nerve endings, which is clearly shown later to become the dominant factor in the local tetanus, by the effects following nerve section after the condition is fully developed. Even in general tetanus, involving muscles remote from the site where the toxin is produced or injected, the persistent contraction of muscles such as those closing the jaws, almost certainly involves an effect of the toxin on their motor nerve endings, of the kind

here described, as well as on the corresponding motor nerve cells or synapses.

Electrical records from single motor units show that the rigidity is not wholly due to a condition of contracture, the muscle fibres being involved in continuous, asynchronous, propagated contractions, with different and fluctuating rhythms. The effects following nerve section seem to indicate that the stimuli causing this activity arise somehow from the nerve endings. The condition is in several ways similar to that seen in an unpoisoned muscle in the earlier stages of the degeneration of the motor nerve after section, with the difference that the activity of the muscle in local tetanus is much more intense, and sufficient to produce a definite tension. The response of the muscle in local tetanus to single motor nerve volleys is much reduced, even when, in the earlier stages, the response to shocks directly applied is not obviously diminished. The reduction is, therefore, not due merely to the refractory condition at any moment of a proportion of the muscle fibres. Electrical records with a localized lead show, however, that the diminished response to a single nerve volley is repetitive in its nature. On the other hand, it is not due to a curare-like block of transmission, for the tetanic response to repetitive stimulation of the nerve has a tension which, though lower than the normal, is well maintained. The response to injected acetylcholine also, which curare easily abolishes, is at least as great as normal, and is in some respects like that of a denervated muscle. After a period of tetanic stimulation, through the nerve or directly, the muscle in local tetanus shows a great enhancement of its irregular activity. This effect is presumably attributable to the lowering of the threshold of excitation at the motor end plates after a period of tetanus [Brown & von Euler, 1938] which apparently renders them also abnormally excitable to the irregular stimuli continuously affecting them in local tetanus. Eserine, on the other hand, produces in the muscle with well developed local tetanus practically none of its effects on the normal muscle; the weakened response to a single nerve volley which, as we have seen, is already repetitive in nature, showing little or no enhancement by any dose of eserine. In the repetitive response to a single volley, the condition of the muscle in local tetanus resembles, indeed, that of a muscle which has already been treated with eserine.

On the conceptions of neuromuscular transmission which have been conventional until recent years, it would be difficult to find an interpretation of the different features of the condition of local tetanus, as thus revealed. On the more recent suggestion that the transmission is mediated

by the liberation by an impulse of a small charge of acetylcholine from the nerve ending, this acting as the direct stimulant of the muscle fibre at its motor end-plate, the observed facts can be brought into coherent relation if certain simple assumptions are made. Evidence obtained largely from the analogous case of the synapses in a ganglion [Brown & Feldberg, 1936; Kahlson & MacIntosh, 1939] shows that, when acetylcholine is liberated from the depot at a nerve ending, it is replaced by synthesis, the quantity in the depot being normally maintained at a remarkably constant level. In a living system it may be supposed that such an equilibrium is dynamic, and that, even in the absence of discharge of impulses, acetylcholine would be always escaping from such a depot in small quantities and regularly replaced by synthesis. This small leakage, however, would normally be rendered ineffective as regards stimulating action, by the cholinesterase which is also deposited in close relation to the nerve endings [Nachmansohn, 1939; Brücke, 1937]. Such a balance could be upset in various ways. Eserine, by depressing the cholinesterase, would enable the normally leaking acetylcholine to persist long enough to produce irregular twitching of muscle fibres [Rosenblueth & Morison, 1937], and would cause repetitive response to the sudden discharge evoked by a nerve impulse [Brown *et al.* 1936]. The action of eserine, however, would not interfere with synthesis or deplete the depot, so that the response to an impulse would be not only repetitive, but correspondingly increased in tension. The degenerative changes in synaptic endings, following early after nerve section of a preganglionic nerve, cause disappearance from the synapses of both acetylcholine [MacIntosh, 1938] and cholinesterase [Brücke, 1937]. If the same happens after motor nerve section, we should expect the leakage of acetylcholine from the degenerating nerve endings, though not replaced by synthesis, to cause while it lasts fibrillary activity of the muscle, facilitated by the concurrent disappearance of esterase, which would also cause the muscle to become abnormally sensitive to injected acetylcholine. Both of these effects do, in fact, follow motor nerve section. We have seen that there are features of the condition of local tetanus recalling the effects of eserine, on the one hand, and those early following nerve section on the other.

The irregular spontaneous activity of the muscle in local tetanus is, however, much more pronounced than that in eserine poisoning, or in early nerve degeneration, and it may be continued for months. On the other hand, the muscle in local tetanus shows a weakened, though repetitive response to a nerve volley, and some prolongation of its response to injected acetylcholine. Tetanus toxin might be supposed in

some way to affect the motor nerve ending, so that acetylcholine is liberated with abnormal readiness from its depot. There is reason to suppose [Kahlson & MacIntosh, 1939] that depletion of the depot leads immediately to accelerated replacement by synthesis which, at the lower level of storage due to the action of the toxin, would be continually in action. Under such conditions, acetylcholine would be continually leaking at an abnormal rate from a depot which would never be more than partially replenished. If we further suppose that the effect of the toxin on the nerve ending causes a lowering of the normal concentration of cholinesterase, we provide a scheme which satisfactorily summarizes the various features of the complex of effects seen in local tetanus. The constant accelerated leakage of acetylcholine from the nerve ending, maintained by unchecked synthesis, with weakened control of the effect of such leakage by cholinesterase, would maintain the persistent irregular activity of the muscle fibres which is the central feature of local tetanus. The concurrent depletion of the acetylcholine depots and weakening of the concentration of cholinesterase would render the response of the muscle to a single volley weaker, though at the same time repetitive. Degeneration of the nerve endings after nerve section would cause a gradual, curare an immediate, disappearance of the local tetanus; and the points of resemblance and of contrast between the conditions observed in local tetanus on the one hand, and those which occur in eserine poisoning and in the early stages of nerve degeneration on the other, would find ready explanation. As to the intimate nature of the action of the toxin on the nerve endings, the facts available afford no clue. The toxin might act on them as a continual irritative stimulus, or it might cause in them changes of the kind appearing early after nerve section, but not progressing beyond that point. It may be added that the location at the nerve endings, and not in the muscle fibres, of the effects of the toxin in producing local tetanus, may raise the question whether its action in the central gray matter is on the motor nerve cells, or on the synaptic endings of sensory fibres in relation to them.

Summary

1. Local tetanus was produced in the tibialis anterior muscle of cats by the injection at a number of points of a total of $\frac{1}{200}$ to $\frac{1}{50}$ of the lethal dose of tetanus toxin.

2. The rigidity is not due to a central action of the toxin, but is dependent upon the integrity of the motor nerve endings in the muscle.

3. It is caused by constant irregular contraction of the muscle fibres as shown by the oscillatory action potentials which can be recorded. This spontaneous activity is greatly increased by a brief tetanic stimulation of the motor nerve.

4. During the early stages of a local tetanus there is a large reduction in the tension response to a maximal motor nerve volley, while the twitch evoked by direct stimulation of the muscle is normal. This is not due to a "curarization" as the tension response to a motor nerve tetanus is well maintained.

5. The response to a single nerve volley is repetitive in nature, being a brief, asynchronous tetanus.

6. The usual quick, twitch-like contraction of the muscle in response to acetylcholine is followed by a prolonged increase in tension lasting 30–60 sec.

7. Eserine has little or no potentiating effect on the nerve twitch tension and hardly increases the existing repetitiveness of the muscle action potential.

8. These experiments afford direct experimental evidence of a peripheral action of tetanus toxin in the region of the neuromuscular junction. The possibilities concerning the nature of this action of the toxin are discussed.

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