THE LOCALIZED ACTION ON THE SPINAL CORD OF INTRAMUSCULARLY INJECTED TETANUS TOXIN

By GEORGE H. ACHESON, M.D., OSCAR D. RATNOFF, M.D., AND
EMANUEL B. SCHOENBACH, M.D.

(*From the Departments of Physiology and of Bacteriology and Immunology, Harvard Medical School, Boston*)

(Received for publication, January 10, 1942)

The pathogenesis of local tetanus has been the subject of much dispute. Until recently, the most generally accepted view was that of Marie and Morax (1902) and Meyer and Ransom (1902–03). They believed that local tetanus is the result of the action of tetanus toxin on the anterior horn cells of the spinal cord, the toxin reaching the central nervous system by way of the axons of the motor nerves. On the other hand, Vaillard and Vincent (1891) and Zupnik (1905) held that local tetanus is the result of the action of the toxin directly on the muscle, and that the toxin reaches the central nervous system through the blood stream rather than along peripheral axons, causing there generalized rather than local tetanus.

No one doubts the susceptibility of the central nervous system to tetanus toxin. If toxin is injected directly into the spinal cord in small amounts, a typical local tetanus may result (Fröhlich and Meyer, 1915). The question then remains whether local tetanus following intramuscular injection of small amounts of toxin is due to the action of the toxin on the segments of the spinal cord which innervate the site of injection, or on some peripheral structure. If the former is true, it remains to be demonstrated how the toxin reaches the spinal cord.

In addition to the immunologic evidence for the nerve transport of tetanus toxin recently reviewed by Friedemann, Hollander, and Tarlov (1941), certain other experiments have supported this theory. Courmont and Doyon (1894) injected tetanus toxin into the right hind paw of a dog. When the animal had developed stiffness limited to the right hind leg, they cut the spinal cord at the union of the lumbar and thoracic levels and sectioned all the dorsal roots. Stimulation of the dorsal roots with an inductorium was now effective in producing a reflex contraction with a current strength on the tetanic side one-fourth that necessary on the normal side. After removal of the spinal cord, stimulation of the ventral roots was effective at the same strength of stimulus on both sides. They therefore concluded that the action of the toxin is not on the peripheral motor elements of the reflex arc and must be either on the sensory nerves or on the spinal cord.

An experiment reported by Sherrington (1905) further clarifies the situation by excluding the sensory nerves as the seat of action of tetanus toxin. Normally, stimulation of the central end of the hamstring nerve produces inhibition of extensor activity in the opposite limb. If the stimulus is applied to the hamstring nerve of a limb which exhibits local tetanus as a result of the peripheral injection of toxin, the muscles of the opposite, unaffected limb undergo the normal inhibition of activity. In contrast to this, stimulation of the corresponding nerve of the uninjected limb produces excitation of the extensors of the injected limb. The altered response is therefore not dependent on a change in the afferent neuron. These experiments of Courmont and Doyon and of Sherrington demonstrate by exclusion that the phenomena of local tetanus with which they dealt have their origin in the spinal cord itself.

Although the above experiments seem conclusive in proving that the toxin acts on the spinal cord in local tetanus, this conclusion has been challenged by Abel and his collaborators (1934, 1935, 1938), whose views have had wide acceptance (Green, 1937; Reimann, 1941). Abel revived the theory of Vaillard and Vincent and of Zupnik that local tetanus results from an action of the toxin on muscle. He denied the possibility that intramuscularly injected toxin can travel up the nerve to the spinal cord, as had been previously accepted.

Certain data suggest that the tetanus toxin may have some effect in muscle. Ranson (1928) studied a phenomenon of local tetanus familiar to older investigators, namely, the shortening of the affected muscles which remains after section of the motor nerve, and hence is a muscular, rather than a nervous phenomenon. And Harvey (1939) studied the electrogram of cat muscles with local tetanus and reported "that after single condenser shocks delivered to the peripheral end of the cut motor nerve there was repetitive response of motor units."

The present study is an attempt to clarify the pathogenesis of local tetanus by analysis of the components of the reflex arc through use of electrographic methods not hitherto applied to the problem. Any or all of the components of a reflex arc—receptor, afferent nerve, spinal cord, efferent nerve, and effector—might be the site of action of tetanus toxin. The experiments to be reported were designed to exclude the peripheral components of the arc and then test whether or not the spinal cord itself is affected by intramuscularly injected tetanus toxin.

Method

Stabilized tetanus toxin, part of a lot P 74-V, was prepared on March 13, 1941, on a casein-hydrolysate, protein-free medium, and filtered through Berkefeld filter V. Each cubic centimeter contained 160,000 minimal lethal doses for mice, and 60,000 M.L.D. for guinea pigs. The L+ dose was 0.01 cc., when tested against a standard serum obtained from the National Institute of Health, and there were 10 Lf units per cc. The toxin was first used 10 weeks after its preparation; repeated tests showed that the minimal lethal dose for guinea pigs remained 60,000 M.L.D. per cc. The toxin was diluted as needed in aqueous solution of ½ per cent Witte peptone and ½ per cent sodium chloride; and the diluted toxin was stored at 4 to 8°C.

Cats of either sex, weighing between 1.4 and 4.1 kilos, were anesthetized by intraperitoneal injection of a solution of sodium pentobarbital (veterinary nembutal, Abbott), 0.65 cc. = 42 mg. per kilo of body weight. Fifty to 200 guinea pig M.L.D. of tetanus toxin in a volume of 0.4 to 1.0 cc. were injected through a No. 26 gauge needle into the lower part of the calf in the approximate vicinity of the gastrocnemius muscle, usually of the right leg, with care to avoid the biceps femoris muscle. Assuming that the M.L.D. for a cat weighing 3 kilos is approximately 10,000 guinea pig M.L.D. (Fildes, 1929), the usual dose of toxin was approximately 1/100 cat M.L.D.; in most instances this dose was contained in a volume of 0.4 cc. There was no apparent difference in the clinical appearance of cats whether the injection was made in one or up to a dozen points in the muscle.

The changes in the electric potentials of muscle and nerve were studied 1 to 9 days after injection of the toxin. The spinal cord was sectioned under ether anesthesia at either the 1st or 12th thoracic level, and the animal then permitted to recover from the effects of the anesthetic. The spinal cat was supported comfortably, and its legs were symmetrically extended at hip, knee, and ankle, and firmly fastened upon boards arranged to give about 45° of abduction. At the conclusion of the observations the animal was destroyed by a rapid intravenous injection of 5 cc. of ether.

Nerves were stimulated with condenser discharges through shielded silver wire electrodes. Records were taken from nerve with shielded silver wire electrodes, and from muscle with steel needle electrodes, one pushed through the skin into the belly of the muscle, the other at the ankle. The responses were amplified with a capacity-coupled push-pull amplifier, and recorded photographically from a cathode ray oscillograph. For most purposes, the amplifier was used with the largest coupling condensers (time constant, ½ second); but when high amplification was employed to detect asynchronous discharge the condensers were so arranged as to emphasize waves of high frequency and to eliminate slow waves.

For isometric mechanical records from the calf muscles, an optical, frictionless, torsion-spring myograph was employed. It was attached by a linen thread to the Achilles tendon, which had been freed from the os calcis and surrounding fascia through a small skin incision.

In general, the appearance of cats with local tetanus was that described by earlier observers. The incubation period was usually at least 36 hours. Muscular stiffness, hyperreflexia, and muscular shortening then appeared. Anesthetization with ether, cutting the motor nerves, or death of the animal resulted in a complete relaxation of the local tetanus early in its course, but if the disease had progressed for several days, stiffness of the ankle and to some extent of the knee persisted in these circumstances as if the calf muscles had been shortened.

The tetanus produced by injection of 1/100 cat M.L.D. of toxin into the calf muscles was limited to the injected leg. It was therefore possible to control each experiment by comparing the electrograms of the corresponding muscle or nerve of each side. To simplify the comparison of records, the degree of amplification of the electrogram of nerve was often so adjusted that the amplitude of the maximal action potential of A fibers following a single stimulus was equal on the right and left. By increasing the amplification proportionally on the two sides, comparable records could be obtained at higher amplification.

RESULTS

(*a*) *The Muscular Response.*—The animal with well developed local tetanus exhibits a striking picture suggestive of reflex hyperactivity. A weak stimulus to the affected limb produces a vigorous increase in the extension of the limb. This type of response might depend upon any or all of a variety of factors. For example, long lasting repetitive responses of the nerves, myoneural junctions, or muscles, to single stimuli, could account for it, independent of any change in the spinal mechanisms. The experiments of this and the succeeding section were designed to test this possibility.

The augmented response of the tetanic leg to a weak stimulus, described above, was studied in a series of cats by recording the electric and mechanical responses of the calf muscles. The stimulus used was either a sudden tap on the Achilles tendon or a shock to the sciatic nerve. The difference between the tetanic and the control legs was apparent, as can be seen in Fig. 1, where A and B represent the tetanic side and D and E represent the control. The increased mechanical response of the tetanic leg is accompanied by a fusillade of muscle action potentials.

If this augmented response depended upon an altered response of the motor nerve or of the muscle, the difference between the sides should persist after section of the sciatic nerve central to the stimulating electrodes. As may be seen in Fig. 1, C and F, when this was done the electromyograms of the two sides were indistinguishable and the mechanograms differed only in amplitude.

The augmented response of the leg with local tetanus does not depend, therefore, upon a change at the myoneural junction, in the muscle, or in the motor nerve. This is in agreement with the experiments of Courmont and Doyon (1894) and others. Repetitive responses of the muscle to single stimuli applied to the cut motor nerve, as described by Harvey (1939), were not observed by us. Differences in technique may account for the differences in result. Although our experiments do not exclude changes in the muscle due to tetanus toxin, muscular changes do not explain the phenomena described.

(*b*) *The Peripheral Nerve.*—The following experiments demonstrate that the augmented response after a single excitation of the sciatic nerve, described above, is not due to repetitive activity of either the motor or the sensory components of the nerve trunk.

After section of the posterior tibial and peroneal nerves in the popliteal space, recording electrodes were placed on the branch of the posterior tibial nerve which innervates the lateral head of the gastrocnemius muscle; the portion of the nerve under the distal electrodes was crushed in order to secure

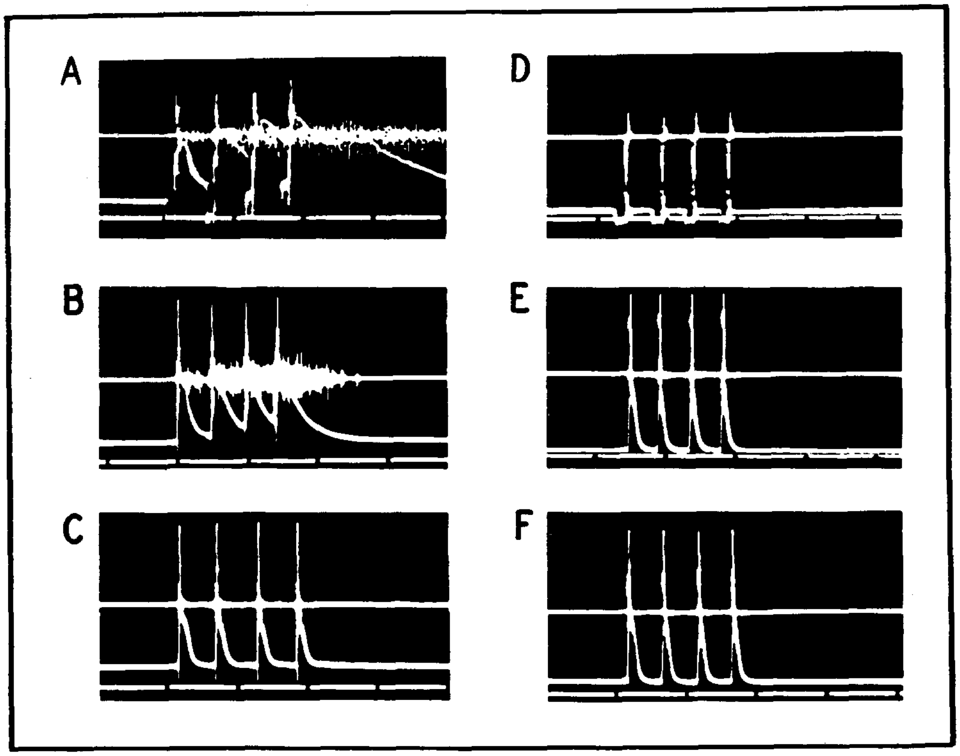

FIG. 1. The augmented response of the tetanic leg and its dependence on the reflex arc. Mechanical and electric records from the calf muscles of a cat with unilateral local tetanus, 4 days after injection of 1/133 cat M.L.D. of toxin. The spinal cord had been sectioned at the level of the 12th thoracic vertebra 5 hours before the records were taken. In each record, the uppermost line is the electrogram taken from needle electrodes, one in the belly of the muscles, the other at the ankle. The second line is the mechanogram obtained with an optical, frictionless torsion-spring myograph. The peak of each contraction curve obscures part of the corresponding initial electrogram. The bottom line is a time signal marking intervals of 1 second.

A. Responses on the tetanic side to taps on the Achilles tendon.

B. Responses on the tetanic side to brief condenser discharges applied to the intact sciatic nerve at the hip.

C. Responses on the tetanic side as in B after section of the sciatic nerve central to the stimulating electrodes.

D, E, and F. The same as A, B, and C, respectively, on the unaffected side.

monophasic records. Stimulating electrodes were placed on the sciatic nerve at the hip. The nerve was cut central to the stimulating electrodes and excited with maximal, brief condenser discharges.

Of 14 animals studied, 10 showed no repetitive response. Repetitive responses of equal degree were present on both sides in one instance; in two others there was a repetitive response limited to the side with local tetanus; and in one, there was a repetitive response on each side, but predominantly on the side with local tetanus.

The excitability of the branch of the posterior tibial nerve to the lateral head of the gastrocnemius muscle was studied *in situ* in 8 animals with the preparation described above. Strength-duration curves were determined by the method of Hill (1936). There was no significant variation in either the rheobase or the time constant between the nerve of the tetanic and that of the unaffected leg.

The amplitudes of the maximal action potential of the A fibers of the nerve to the gastrocnemius muscle of the tetanic and control sides were compared in 22 animals. In 12, the spike amplitude of the tetanic side exceeded that of the control; in 6 there was less than 10 per cent difference between the two sides; and in 4, the spike of the control exceeded that of the tetanic side. Differences in the degree of shunt around the nerve and recording electrodes may account in part for the difference in the spike amplitude observed.

Thus occasionally there may be changes in the peripheral nerve in local tetanus. Yet the typical responses of local tetanus were present in many experiments in which repetitive response to single stimuli did not occur in the peripheral nerves. In every instance in which repetition was demonstrated in the peripheral nerve by the technique outlined above, the presence of the abnormal response was obvious before the special tests were made. In order to avoid confusion of peripheral and central effects, the experiments of the succeeding sections relate to animals in which there was electrographic evidence that the sciatic nerve, when severed from the central nervous system, did not respond repetitively to single stimuli.

(*c*) *Changes in the Spinal Cord.*—Since the augmented activity of the calf muscles associated with local tetanus is, as demonstrated above, independent of changes in the muscle, neuromuscular junction, and peripheral nerve, its origin must by exclusion be either in the dorsal root ganglia or in the spinal cord. The following experiments were devised to study these possibilities.

The spinal cord of 18 cats with local tetanus limited to one hind leg was sectioned either at the first, or more frequently the 12th thoracic level. Stimulating electrodes were placed on the sciatic nerves at the hip, and the peroneal and posterior tibial nerves, the nerves to the biceps femoris and hamstring muscles, and in some instances the femoral nerves were sectioned. Recording electrodes were placed on the central end of the divided branch of the posterior tibial nerve which innervates the lateral head of the gastrocnemius muscle; the portion of the nerve under the distal electrode was crushed in order to secure monophasic records.

The electrograms obtained from this preparation on stimulating the ipsilateral sciatic nerve differ strikingly on the tetanic as compared to the control side. A single strong condenser discharge is followed by 3 negative waves. The 1st of these is similar on the two sides and represents the volley of impulses initiated by the stimulus at the hip and descending the nerve. The 2nd wave begins 3.5 to 4 milliseconds after the stimulus. Since it is abolished by section of the sciatic nerve central to the stimulating electrodes, it may be called the early reflex response. This wave is small in comparison to the amplitude of the 1st wave resulting from maximal activity of the A fibers of the nerve. If the strength of stimulus is increased from subthreshold to supramaximal values by steps, the early reflex response undergoes a series of changes: it increases in amplitude until it reaches a maximum at a strength of stimulus still submaximal as judged by the amplitude of the 1st wave, and then decreases with stronger stimuli. The early reflex response resembles the *s* wave described by Renshaw (1940) in the reflex responses from ventral roots.

If the sciatic nerve is stimulated repetitively at a frequency of 4 to 8 per second, the early reflex response undergoes a decrease in amplitude which may be interpreted as either fatigue or inhibition. This decrease is regularly less pronounced on the side which is subject to local tetanus than on the control side. Fig. 2 illustrates this difference. In other respects the early reflex response is similar on the two sides.

About 7 to 11 milliseconds after the stimulus a 3rd wave occurs. Like the 2nd wave, this disappears on section of the sciatic nerve central to the stimulating electrodes and hence may be called the late reflex response. It is irregular in form on both the tetanic and the control sides, but more strikingly so on the tetanic. It probably represents the fusion of the responses of several groups of neurons firing asynchronously over a period of 6 to 16 milliseconds. This wave starts earlier, lasts longer, and is usually of greater amplitude on the tetanic than on the normal side, as may be seen in Fig. 3. Exceptionally the amplitude of the late reflex response of the tetanic side is no greater than that of the control.

If the sciatic nerve is stimulated at the rate of 4 to 8 shocks per second, the amplitude of the late reflex response on the control side either diminishes or remains unchanged. The same procedure on the tetanic side usually leads to an increase in the amplitude of the late reflex response. The latency of this response usually shortens with repetitive stimulation, almost invariably more on the tetanic than on the control side (Fig. 3).

If the response is examined with high amplification and with the coupling condensers of the amplifiers arranged most favorably to record activity of high frequency, considerably more after-discharge is seen to follow the 3 waves described on the tetanic side than on the control. Exceptionally the two sides showed no difference in this respect. Repetitive stimulation at 4 to 8 shocks per second invariably results in an increase of the amplitude and often of the duration of the after-discharge on the tetanic side, which may last as long as 3 seconds after the final stimulus. This is in sharp contrast to the electrogram of the control leg, in which summation is uncommon and never as great as may be seen in the injected leg. Fig. 4 illustrates the after-discharge on the two sides of an animal with local tetanus.

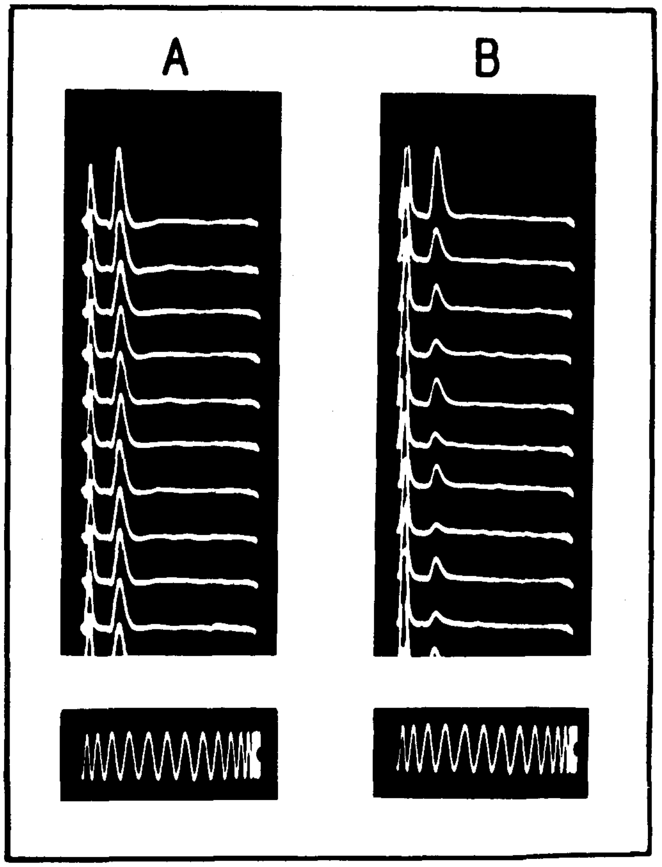

FIG. 2. Greater decrease of the early reflex response to repetitive stimulation on the control side. Electrograms from the nerve supplying the lateral head of the gastrocnemius muscle, 3 days after injection of 1/100 cat M.L.D. of tetanus toxin into the right calf, in a cat in which the development of symptoms of local tetanus was prevented by extensive denervation of the legs 19 hours after the injection.

A. Response of the nerves of the tetanic side to 8 stimuli per second to the sciatic nerve at an intensity which produces a maximal amplitude of the 2nd wave.

B. The same as A on the unaffected side.

Tetanus toxin injected into the calf muscles therefore produces a clearly

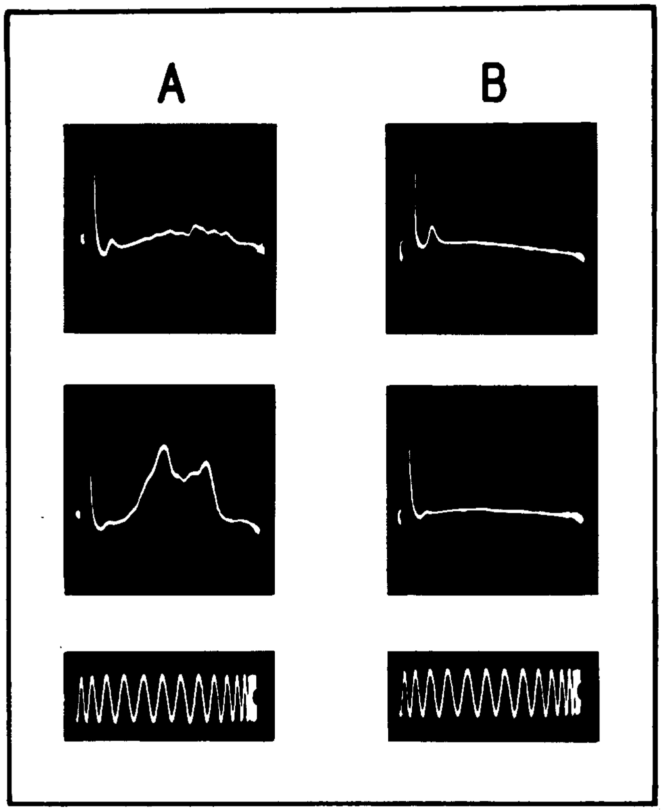

FIG. 3. Differences in the late reflex response of tetanic and control legs. Electrograms from the nerve supplying the lateral head of the gastrocnemius muscle 4 days after injection of 1/100 cat M.L.D. of tetanus toxin into the right calf, in a cat in which the development of symptoms of local tetanus was prevented by extensive denervation of the legs 18 hours after the injection.

A. Upper picture: response on the tetanic side to a single strong stimulus to the sciatic nerve. The 3 waves described in the text are shown, from left to right. Middle picture: response to the last of 8 stimuli delivered within 1 second, showing summation and change of latency of the late reflex response, and decline of the early reflex response. Lower picture: time signal; each cycle represents 2 milliseconds.

B. The same as A on the unaffected side. There is no summation in the late reflex response and the decline of the early reflex response is more marked than on the tetanic side.

recognizable alteration in the pattern of nerve impulses which are discharged by the spinal cord in response to a stimulus applied to the sciatic nerve.

(*d*) *Effect of Section of Dorsal Roots on Electrogram of Nerve.*—Three further possible mechanisms might explain the augmented response of the tetanic leg. It might be due to an altered state of the spinal cord itself, or of the dorsal root ganglion, or to a change in the activity of peripheral sensory mechanisms. The latter two possibilities were tested in the following experiments by complete deafferentation of the spinal cord by section of the dorsal roots central to the dorsal root ganglia.

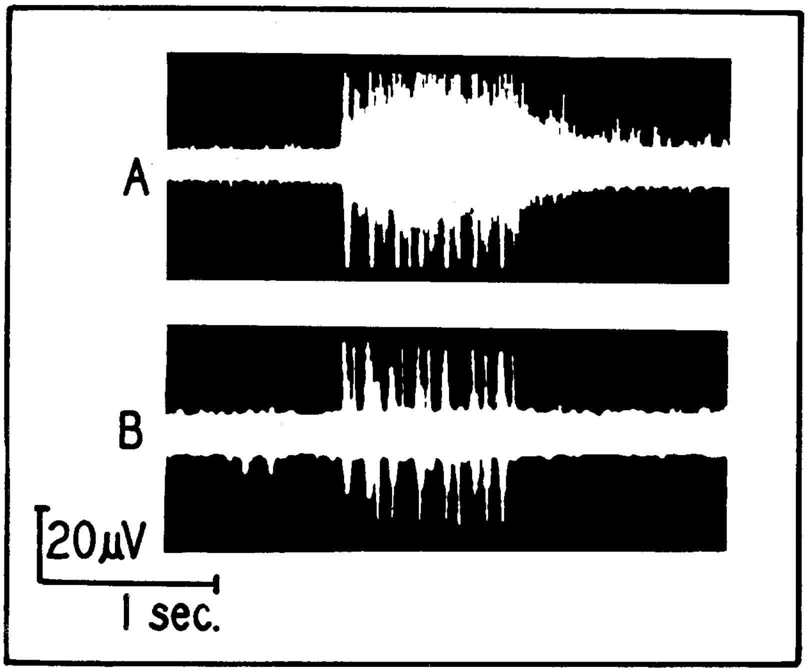

FIG. 4. Augmented after-discharge on the tetanic side. Electrograms from the nerve which innervates the lateral head of the gastrocnemius muscle, following stimulation of the 7th lumbar dorsal root, in a cat with unilateral local tetanus 9 days after injection of 1/75 M.L.D. of tetanus toxin. The spinal cord had been sectioned at the level of the 12th thoracic vertebra 5 hours before the records were taken, and all the dorsal roots below this level cut bilaterally. These records were taken at high amplification, with the coupling condensers arranged to emphasize asynchronous discharge of the nerve.

A. Electrogram of the affected side upon repetitive stimulation of the sciatic nerve.

B. The same as A on the unaffected side.

The effect of section of the dorsal roots was studied in 11 cats with tetanus limited to one leg. The spinal cord was sectioned under ether anesthesia at the level of the 12th thoracic vertebra, and the lamina of the lumbar vertebrae removed. The animal was then allowed to recover from the effects of the anesthesia, and was symmetrically fixed as in earlier experiments. Stimulating electrodes were then placed bilaterally upon the sciatic nerve, and recording electrodes on the nerve to the lateral head of the gastrocnemius muscle. In each case the presence of tetanus limited to one leg was confirmed by recording the electric response of the nerve supplying the gastrocnemius on stimulation of the sciatic nerve. Four or 5 hours after the section of the spinal cord, the dura mater was laid open, and the dorsal roots of the lumbosacral segments cut central to the dorsal root ganglia. The cauda equina was sectioned at the upper border of the sacrum. The dorsal roots were then stimulated through silver wire shielded electrodes; stimulation at the 7th lumbar level was usually most effective.

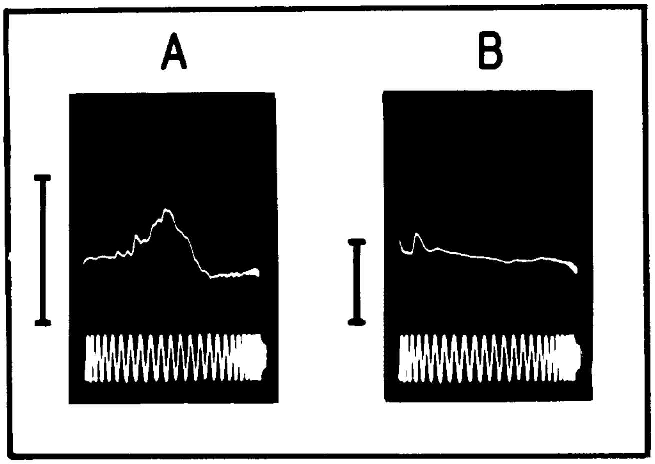

FIG. 5. Reflex response to dorsal root stimulation. Electrograms from the nerve which innervates the lateral head of the gastrocnemius muscle, following stimulation of the 6th lumbar dorsal root, in a cat with unilateral local tetanus, 7 days after injection of 1/75 cat M.L.D. of toxin. Five hours before the records were taken, the spinal cord was cut at the level of the 12th thoracic vertebra. All the dorsal roots below this level were sectioned bilaterally. The vertical lines at the left of the pictures represent the relative heights of the maximal spikes of the A fibers of the respective nerves; the amplitude of the response of the unaffected side must therefore be doubled to compare it with that of the tetanic side.

A. Upper line: electrogram from the nerve of the injected leg after a single strong stimulus to the dorsal root. Lower line: time signal; each cycle represents 2 milliseconds.

B. The same as A on the unaffected side. In this experiment the early reflex response was greater on the control than on the tetanic side. The late reflex response showed the usual predominance on the tetanic side.

In 7 of 11 experiments, the condition of the spinal cord after this procedure seemed satisfactory as judged by the responses elicited by stimulating the dorsal roots. In 2 of these 7, the responses of the left and right sides were equal. In the remaining 5, the electrogram of the tetanic side showed the changes typical of local tetanus as outlined in Section (*c*). A single strong condenser shock evoked 2 negative waves, corresponding to the 2nd and 3rd waves, the reflex responses, seen following stimulation of the sciatic nerve. In all 5 experiments, repetitive stimulation of the dorsal root of the tetanic side at the rate of 4 to 8 shocks per second resulted in little or no decline of the early reflex response, and an increase in the amplitude of the late reflex response. When the corresponding dorsal root of the control side was stimulated, the initial magnitude of the response was smaller (Fig. 5), and after repeated stimulation there was either no change or a decrease in the magnitude of the late response. In 4 of these 5 animals, repetitive stimulation of the dorsal root elicited an after-discharge lasting about 0.4 second on the injected side when examined with high amplification as in earlier experiments (Fig. 4). An after-discharge was never seen in the nerves of the control side under similar conditions.

The origin of the augmented activity of the tetanic gastrocnemius muscle after stimulation of the sciatic nerve is therefore within the spinal cord, and limited to the same side as the local tetanus.

(*e*) *Effect of Denervation after Injection of Toxin on the Development of Local Tetanus in the Spinal Cord.*—It is conceivable that the changes in the spinal cord described above are dependent on changes in the peripheral structures. For example, changes in the muscle following intramuscular injection of tetanus toxin might result in a prolonged continuous bombardment of the spinal cord by afferent impulses which might produce the observed changes. This possibility can be excluded by sectioning the nerves of the injected area before the appearance of local tetanus.

After the injection of 1/100 cat M.L.D. of tetanus toxin into the gastrocnemius muscle neither the physical signs nor changes in the electrogram of nerve characteristic of local tetanus are present within the first 24 hours. The right calf muscles of 3 animals were injected with 1/100 cat M.L.D. of tetanus toxin. Eighteen to 20 hours later, at a time when none of these animals showed physical signs of tetanus, the following operations were performed bilaterally under ether anesthesia. The femoral and obturator nerves were cut in the groin, and the nerve to the hamstring muscles at the hip. The popliteal space was cleared of fat, and all the branches of the peroneopopliteal nerve were cut—namely, the nerve to the biceps femoris muscle, and the peroneal, posterior tibial, and sural nerves. For later identification, a black silk suture was tied to the central end of the cut branch of the posterior tibial nerve which innervates the lateral head of the gastrocnemius muscle. Three, 4, and 5 days after injection, when control cats injected at the same time with the same amount of toxin exhibited typical local tetanus, there were no signs of tetanus in the animals upon which operations had been performed. The hips, knees, and ankles of the latter were flaccid, and both upper legs were held abducted. Under ether anesthesia the spinal cord was cut at the level of the 12th thoracic vertebra, after which the animals were permitted to recover from the effects of the anesthesia. On each side, stimulating electrodes were placed on the sciatic nerve and recording electrodes on the branch of the posterior tibial supplying the lateral head of the gastrocnemius. The pattern of the electrogram of the nerve to the gastrocnemius muscle of the injected leg agrees in all respects with that previously described for the nerve when the muscular picture of local tetanus is present. The electrogram of the control side is likewise the same as that of the uninjected leg in animals in which the innervation is intact. Figs. 2 and 3, which have been used to illustrate the characteristic waves, are from animals in which the leg had been denervated.

In local tetanus, therefore, changes develop in the spinal cord even though section of the peripheral nerves 18 to 20 hours after injection of the toxin has prevented the appearance of the peripheral signs of local tetanus.

(*f*) *Effect of Denervation before Injection of Toxin on the Development of Local Tetanus in the Spinal Cord.*—The localization of the spinal effects of intramuscularly injected toxin to the region supplying the innervation of the injected area is difficult to explain unless the toxin reaches the cord by of way the nerve trunks. In this case section of the nerve trunks supplying the area of injection before the injection of the toxin should prevent the spinal effects. The following experiments were devised to test this.

The procedure was the same as that described in the preceding section except that the section of nerve trunks was performed shortly before the injection of the toxin. Three animals so prepared received 1/100 cat M.L.D. of tetanus toxin into the right calf muscles. Three, 4, and 5 days later none of the animals showed signs of local tetanus. The spinal cord was cut under ether anesthesia and the electrograms from the nerves supplying the lateral head of the gastrocnemius were studied as in the previous sections. None of the changes characteristic of local tetanus was present on either side in these animals.

The localized action of tetanus toxin on the spinal cord when the toxin is injected into the calf muscles can therefore be prevented by cutting the nervous pathways which connect the area of injection with the cord.

(*g*) *Effect of Local Trauma on the Localization of Tetanus.*—It has been suggested that the tetanus toxin reaches the central nervous system through the circulation, but that the trauma of injection determines its localization to that part of the spinal cord from which the innervation of the site of injection arises. In 5 cats, 0.4 cc. of tetanus toxin which had been inactivated by boiling was injected into the left calf muscles at the same time that active toxin was injected into the muscles of the right calf. In no instance did signs of local tetanus appear in the left leg, nor did oscillographic records from the nerves of the left leg 2 to 4 days after injection reveal the changes characteristic of local tetanus. The trauma of injection, therefore, played no rôle in the localization of the tetanus. Gumprecht (1895) excluded this possibility in a more drastic fashion. He injected tetanus toxin into the right hind leg and croton oil into the left foreleg of mice. Local tetanus appeared in the former but not the latter leg.

DISCUSSION

The experiments reported in Sections (*a*) to (*d*) demonstrate that local tetanus in the cat resulting from the intramuscular injection of toxin is associated with a characteristic response originating in that region of the spinal cord from which the innervation of the injected area arises. The experiments of Section (*e*), in which the nerves of the legs were sectioned after the injection of toxin but before the appearance of any signs of tetanus demonstrate further that the change in the spinal cord is not dependent upon changes in the muscle or neuromuscular junction but represents an action of the tetanus toxin on the spinal cord itself.

How, then, does the toxin injected into the leg reach the corresponding segment of the cord? If it were carried from the site of injection to the spinal cord only through the blood stream, it is difficult to understand why its action is limited to this small segment. With the doses of toxin used the contralateral portion of the spinal cord at the same level remains unaffected. The localization of tetanus is more understandable if the toxin be supposed to ascend the peripheral nerves to the spinal cord. This view is supported by the experiments of Section (*f*), in which the action of the intramuscularly injected toxin on the spinal cord was prevented by section of the nerves of the leg before the injection of the toxin. This theory of the path of transport, as well as the logical steps leading up to it, were probably first proposed by Gumprecht (1894, 1895). The present observations establish facts which are adequately explained by this theory and by none other yet advanced. It may be concluded that in local tetanus the toxin is carried to the spinal cord by way of peripheral nerves.

The experiments reported give no hint as to which element of the peripheral nerve may be the pathway of the toxin to the central nervous system. Gumprecht believed that the tissue spaces of Key and Retzius were the route. Marie and Morax (1902) and Meyer and Ransom (1902) suggested that the ascent was through the axis cylinder itself.

Abel (1934, 1935, 1938) argued that the peripheral nerves cannot serve as pathways for diffusable substances to the central nervous system. He questioned the technique of investigators who had reported the passage of dyes up nerves to the spinal canal. Perdrau (1937), however, used techniques free from the objections raised by Abel, and confirmed the previous results. He demonstrated that the central end of the cut sciatic nerve of anesthetized cats is capable of absorbing dyes from solutions into which the nerves are dipped, and that these dyes diffuse up the axis cylinder as far as the spinal cord at a rate sometimes as fast as a centimeter per hour. Perdrau pointed out that the passage of substances through the axis cylinder is compatible with de Renyi's observation that the consistency of the axis cylinder is that of a soft jelly.

The suggestion that tetanus toxin reaches the central nervous system by way of the peripheral nerves is not unique. Strong evidence indicates that the viruses of the poliomyelitis (Bodian and Howe, 1941) and of rabies (di Vestea and Zagari, 1889), for example, reach the central nervous system in this way.

SUMMARY

Local tetanus limited to one leg was studied in cats after intramuscular injection of tetanus toxin.

1. The electric and mechanical response of the affected muscle after a single stimulus to the intact sensory-motor nerve is greater in amplitude and duration than the response of the corresponding muscle of the unaffected leg (Fig. 1).
2. This augmented response of the muscle is associated with an augmented response arising from the ipsilateral portion of the spinal cord, while the contralateral part of the cord is unaffected, as demonstrated by electrographic records from the motor nerves (Figs. 2 to 5).
3. The augmented muscular response is abolished when the reflex arc is broken, but the augmented response in the spinal cord is independent of changes in the muscle, the neuromuscular junction, the afferent and efferent peripheral nerves, and the dorsal root ganglia.
4. The augmented spinal response develops in the absence of the peripheral signs of local tetanus. Hence the pathogenesis of the altered state in the spinal cord is independent of the peripheral effects of the toxin.
5. In local tetanus, therefore, the toxin injected intramuscularly acts selectively upon the segments of the spinal cord which supply the innervation of the injected area.
6. The augmented spinal response may be prevented by section of the nerve trunks supplying the area of injection prior to the injection of the toxin.
7. It is concluded that in local tetanus the toxin is carried to the spinal cord by way of peripheral nerves.

BIBLIOGRAPHY

Abel, J. J., *Science*, 1934, **79**, 122.

Abel, J. J., Evans, E. A., Jr., Hampil, B., and Lee, F. C., *Bull. Johns Hopkins Hosp.*, 1935, **56**, 84.

Abel, J. J., Firor, W. M., and Chalian, W., *Bull. Johns Hopkins Hosp.*, 1938, **63,** 373.

Abel, J. J., Hampil, B., and Jonas, A. F., Jr., *Bull. Johns Hopkins Hosp.*, 1935, **56,** 317.

Bodian, D., and Howe, H. A., *Bull. Johns Hopkins Hosp.*, 1941, **68,** 248.

Courmont, J., and Doyon, M., *Arch. physiol. norm. et path.*, 1894, **16,** series 5, 391.

di Vestea, A., and Zagari, G., *Ann. Inst. Pasteur*, 1889, **3,** 237.

Fildes, P., in Fildes, P., and Ledingham, J. C. G., A system of bacteriology in relation to medicine, London, His Majesty's Stationery Office, 1929, **3,** 298.

Friedemann, U., Hollander, A., and Tarlov, I. M., *J. Immunol.*, 1941, **40,** 325.

Fröhlich, A., and Meyer, H. H., *Arch. exp. Path. u. Pharmakol.*, 1915, **79,** 55.

Green, C. C., *Ann. Surg.*, 1937, **105,** 998.

Gumprecht, F., *Deutsch. med. Woch.*, 1894, **20,** 546.

Gumprecht, F., *Arch. ges. Physiol.*, 1895, **59,** 105.

Harvey, A. M., *J. Physiol.*, 1939, **96,** 348.

Hill, A. V., *Proc. Roy. Soc. London, Series B*, 1936, **119,** 440.

Marie, A., and Morax, V., *Ann. Inst. Pasteur*, 1902, **16,** 818.

Meyer, H. H., and Ransom, F., *Arch. exp. Path. u. Pharmakol.*, 1902–03, **49,** 369.

Perdrau, J. R., *Brain*, 1937, **60,** 204.

Ranson, S. W., *Arch. Neurol. and Psychiat.*, Chicago, 1928, **20,** 663.

Reimann, H. A., *Arch. Int. Med.*, 1941, **68,** 325.

Renshaw, B., *J. Neurophysiol.*, 1940, **3,** 373.

Sherrington, C. S., *Proc. Roy. Soc. London, Series B*, 1905, **76,** 269.

Vaillard, L., and Vincent, H., *Ann. Inst. Pasteur*, 1891, **5,** 1.

Zupnik, L., *Deutsch. med. Woch.*, 1905, **31,** 1999.