FUNCTIONAL CHANGES IN THE CENTRAL NERVOUS SYSTEM IN TETANUS AND THE MODE OF ACTION OF THE TETANUS TOXIN

III. THE CENTRAL AND PERIPHERAL ACTION OF THE TETANUS TOXIN*

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Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 42-48, January , 1960

Original article submitted June 12, 1959

Despite the number of years for which it has been studied, much still remains unexplained concerning the mode of action of tetanus toxin.

There are three possible interpretations of its action. According to one [23, 24] the toxin acts on the motoneurones of the spinal cord which it reaches by passing along the motor nerves. Abel and his followers [14, 15, 25] deny that the toxin uses this path and consider that it enters the brain directly from the blood. A. V. Speranskii [12] and his co-wokers [2, 3, 4, 7, 8, 9] do not deny that the toxin enters the central nervous system but consider that its principal action is reflex.

One of the methods most widely used for determining the mechanism or action of the toxin is that of interrupting the sensory innervation of tissues into which the toxin is injected. This may be done either by injecting local anesthetic or by sectioning the corresponding dorsal roots in the spinal cord. The use of local anesthetic has given equivocal results [1, 7, 8, 13]. In addition, the results obtained are complicated by the extensive side effects of the drug on the nervous system.

Equally ambiguous results have been obtained when the sensory supply has been interrupted by a section of the dorsal roots [1, 13, 17-22, 26]. This is chiefly to be ascribed to the considerable methodological difficulties of carrying out this kind of experiment. On account of the structure of the spinal cord of the usual laboratory animals the operation of deafferentation inevitably causes considerable trauma and interference with the blood supply, so that when the operation is successfully performed various complications including locomotor disturbances or even complete paralysis of the hind limbs may occur. For this reason almost all the investigations referred to were carried out on a limited number of animals, and several authors [17, 18, 22] have used only one.

We decided to experiment on white rats which we had previously deafferented, and in which we had sectioned the motor roots to the hind limbs. In these animals, owing to the compact lumbar enlargement and the long dorsal roots of the spinal ganglia it is possible to carry out the operation without causing much trauma. The animals survived the operation quite well, and after section of the dorsal roots they usually showed no traumatic locomotor disturbances. This result is extremely important, because it enables us firstly to avoid any possible complications in the course of the disease which might produce misleading results in the experiment, and secondly to use a sufficiently large number of animals in the experiment.

METHOD

In all cases we deafferented the left hind limb. For this purpose, after removing the spinous processes of lumbar vertibrae I-III we removed the anterior articular process of vertibra III, the anterior and posterior articular processes of the II lumbar; and the posterior articular process of I lumbar; we also divided the pedicles between their origin and the base of the spinous processes (including the latter). In this way we exposed the left half of the lumbar enlargement and the central fissure of the spinal cord over only a limited area, and thus protected the cord from excessive trauma. We divided seven spinal roots: L_1–L_6, and S_1; in many cases, for purposes of control, we also divided the 13th thoracic and 2nd sacral roots. When the operation was correctly carried out, sensation was lost over the whole

* The principal results were presented at the Conference on Pathology of the AMN SSSR Institute of Normal and Pathological Physiology in April, 1955.

TABLE 1 The Action of Tetanus Toxin when Injected Intramuscularly into Normal Rats and Those with Deafferented Limbs

Number of group	Conditions of experiment	Number ofanimals in experiment	Number of animals affected with local tetanus
1	Toxin in gastrocnemius muscle of the deafferented left hind limb.	15	15
2	Toxin injected into gastrocnemius muscle of opposite (right) hind limb (left hind limb deafferented).	15	15
3	Toxin injected into gastrocnemius of mock-operated rats (spinal cord exposed).	15	15
4	Toxin injected into gastrocnemius muscle of left hind limb of unoperated rat (control for toxin)	15	15

limb from the digits to the thigh (as far as the inguinal fold).

The motor outflow was interrupted also on the same side by dividing the anterior roots of the same segments. When the operation had been correctly carried out, sensation was preserved over the whole limb, but there was a complete paralysis. Tests for sensation and mobility were applied twice, once a few days after the operation, and again before injecting the toxin. If there was any suspicion that some sensation remained after deafferentation, or movement after interrupting the motor outflow, the animals were not used for further experiments.

In the first set of experiments we studied the effects of deafferentation on the course of the illness (local tetanus) caused by injection of tetanus toxin. We used 1/20-1/25 of the M.L.D.(minimum lethal dose) contained in a volume of 0.05 ml. The toxin was injected into the gastronemius muscle 1-1½ weeks after the operation. In addition to this principal form of the experiment in which the toxin was injected into the deafferented muscle, we also carried out additional and control experiments to determine any possible effect of spinal cord trauma or of sensory loss on the course of the disease (Table 1).

In the second set of experiments we compared the effects of deafferentation and deafferentation on the course of tentanus induced by a lethal dose of toxin. The latter was given as two injections, one into the gastrocnemius muscle (1M.L.D. in 0.05 ml) and the other into the posterior group of the thigh muscles of the left leg (2 M.L.D. in 0.1 ml). To prevent the spread of toxin via the blood intravenously, we also injected antitetanus serum. The dose of the serum was 0.02 A. E. (antitoxic units) which was equal to about 2½ times that required to neutralize the total amount of toxin (3 M.L.D.) when injected directly into the blood. Because division of the motor nerves leads to muscular atrophy, it was shown in preliminary control experiments that the tetanus toxin injected into muscles of a deefferented limb does not become inactivated in the atropic muscles.

A special set of experiments was made to record the electrical activity in the left and right sciatic nerves. The record was made by a string oscillograph connected to the output of a two-channel amplifier. The potentials were led off from needle electrodes. These experiments were made at the end of the 1st and 2nd days after infection. In this last set of experiments 35 animals were used. The results were consistent, so that only the general result need be presented.

RESULTS

As is shown by the results presented in Table 1, injection of sublethal doses of tetanus toxin into the muscles of a deafferented limb caused a local tetanus in all cases without exception.

In the experimental group, the symptoms developed more slowly and were less severe than in the control animals. In all three control groups (Nos. 2, 3, and 4) the disease was at its height at the 3-4th day; there was no mobility in any of the joints of the limbs and all the muscles were rigid; in the experimental animals for the whole period of observation the mobility was preserved to some extent in the knee and hip joints. At the height of the disease, which was reached on the 4-6th day, there was a marked rigidity of the muscles of the lower leg, particularly of the gastrocnemius muscle into which the toxin was injected. Sometimes the muscles of the lower leg showed rigidity while those of the thigh were atonic, like a typically deafferented muscle.

These experiments showed therefore that division of the sensory portion of the reflex arc does not prevent the development of tetanus but only modifies its intensity owing to the changed tone in the deafferented muscles.

Injecting a lethal dose of tetanus toxin into the muscles of a deafferented limb in all cases caused an ascending tetanus to develop: This was at first local with a characteristic rigidity of the limb muscles; it then became segmental, and finally it became general and in most cases fatal (Table 2, Fig. 1).

There was no appreciable difference in the severity of the condition or in the number of animals dying (Group 1) and the corresponding controls (Group 3). We found merely that there was some lag in the development of the local tetanus in the experimental group on the 1st day.

The condition of animals receiving tetanus toxin into the muscles of the deafferented limb (Group 2) was very different. Not one animal in this group was affected by either local or general tetanus. Both before and after the injection of the toxin the deafferented limb remained in a paralyzed condition. No symptoms developed in the animals of the 2nd control group (No. 4) who received toxin injections into the blood together with the antiserum.

These results agree with those of other authors [20] who carried out a similar set of experiments.

Thus the interruption of the sensory innervation of a limb into which the toxin was injected does not prevent the development of either local or general tetanus. The development of the latter cannot be ascribed to the spread of toxin through the blood, because this was excluded by the failure of the disease to develop not only in control group No. 4 but in the experimental group with cut motor roots (Group 2). Complete protection from general tetanus was afforded only by section of the motor roots.

We must emphasize the total agreement between all experiments. There was no individual variation from one animal to another. This shows that the data obtained is completely reliable and that they demonstrate the conditions under which the disease can develop.

The result of the tests of the electrical activity of the sciatic nerves in the experimental and control groups (Fig. 2) agrees entirely with the results of the experiments described above. In the control rats which were not subjected to any operative measure, and also in those with a deafferented "tetanized" limb, during the 1st and 2nd days there was an increase in the electrical activity of the nerve in the injected limb. In rats with a deafferented left hind limb, neither left nor right sciatic nerve showed any increased electrical activity on either the 1st or 2nd day. In the left sciatic nerve the normal background potentials were absent.

The results of these experiments confirm those which we obtained previously [5, 6] and those of other authors [1, 2, 3, 16, 19, 27]; they show that the increased electrical activity in nerves during tetanus is of central origin and associated with the excitation of the corresponding motor neurones.

The results of all the experiments indicate therefore that the elimination of the sensory section of the reflex arc does not prevent the development of tetanus. It follows that the disease is not brought about by re-

TABLE 2 Animals Sick or Killed by Injecting Tetanus Toxin into the Muscles of Deafferented and Deefferented Limbs while Giving a Simultaneous Injection of Antitetanus Serum Intravenously

No. of group	Conditions of experiment	Number of animals in experiment	Number of animals affected by tetanus	Number of animals dead from tetanus	Average length of life of animals which succumbed (days)
1	Toxin in muscles of deafferented limb, serum into vein	20	20	15	6.1
2	Toxin in muscles of deafferented limb, serum into vein	20	0	0	–
3	Toxin in muscles of normal limb, serum into vein	20	20	18	6.5
4	Toxin into vein, serum into vein	20	0	0	–
5	Toxin in muscles of a normal limb	20	20	20	2.3

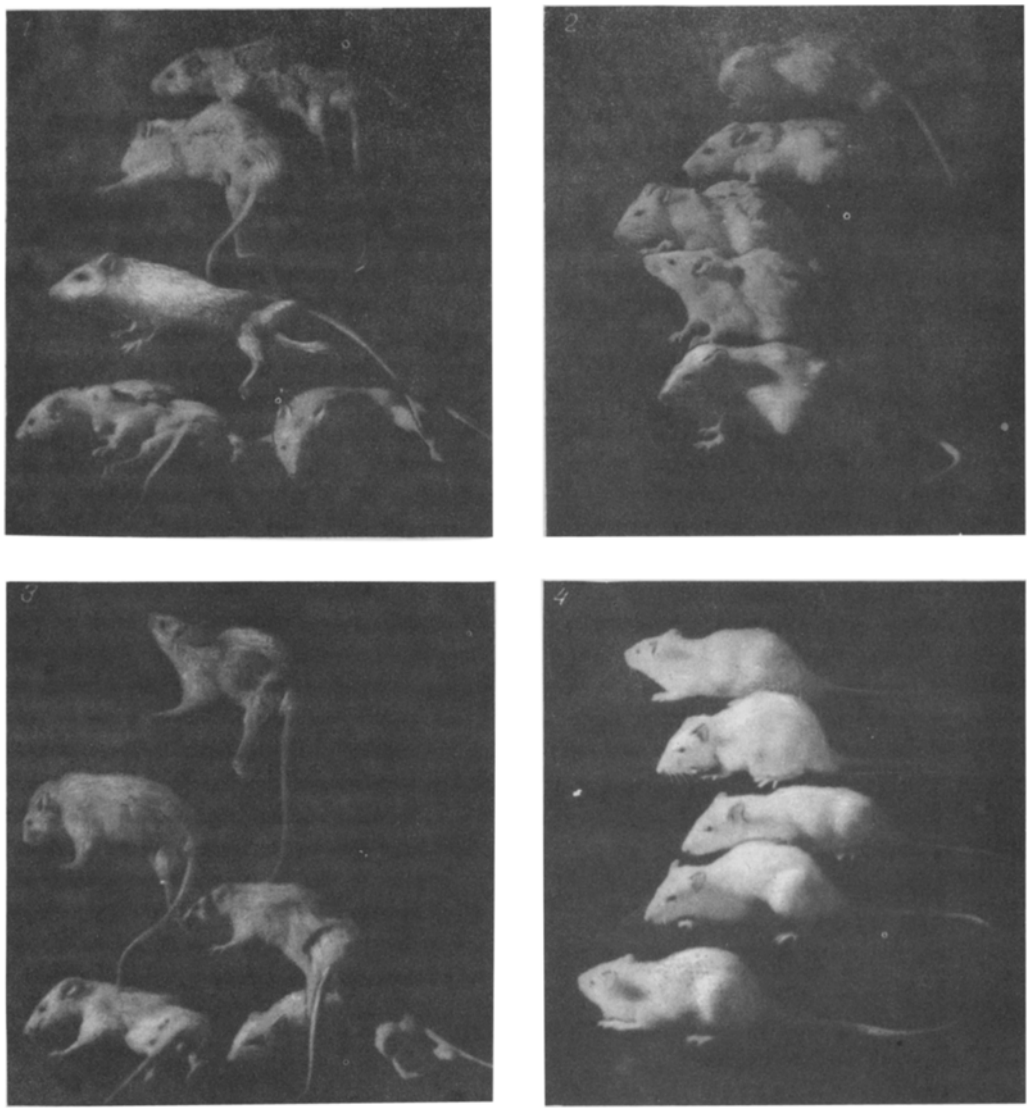

Fig. 1. Condition of animals on 4th day after injecting 3 MLD of tetanus toxin into muscles of a deafferented (1), a deafferented (2), and a normal (3) left hind limb, and after injecting toxin into the blood (4) while giving a simultaneous injection of antitetanus serum intravenously.

flex action. Our results confirm that an essential part of the action of the tetanus toxin is on the central nervous system. The fact that general tetanus is prevented by section of the anterior roots allows us to join with other authors [20] in saying that the main path of access of the toxin to the spinal cord is through the motor fibers.

Recently, in this laboratory, we and our associates have been able to obtain a direct proof of the fact that under normal conditions tetanus toxin enters the spinal cord principally if not exclusively by the ventral roots. A description of these experiments will be published separately and they agree entirely with those of the present work and confirm all the conclusions.

The role of the peripheral mechanisms in tetanus toxin may be to determine certain of the clinical features of the disease including changes in the reactivity of central nervous structures. This problem, however, requires to be investigated separately, especially because the published results are very much at variance.

It is important to emphasize that the fact that the toxin is able to move centrally along the nerve trunks is not denied by the supporters of the theory of its reflex action. A. D. Speranskii and his co-workers [10, 11, 12] not only pointed out that the tetanus toxin could enter the spinal cord, but also described many details of how this process was brought about.

Finally we must note that Abel's view [14, 15, 25] that general tetanus may develop only through the penetration of the toxin into the central nervous system from the blood contradicts much of the work which has been published and has not been confirmed by our experiments. The humoral pathway of the toxin may be of importance in determining the course and outcome of

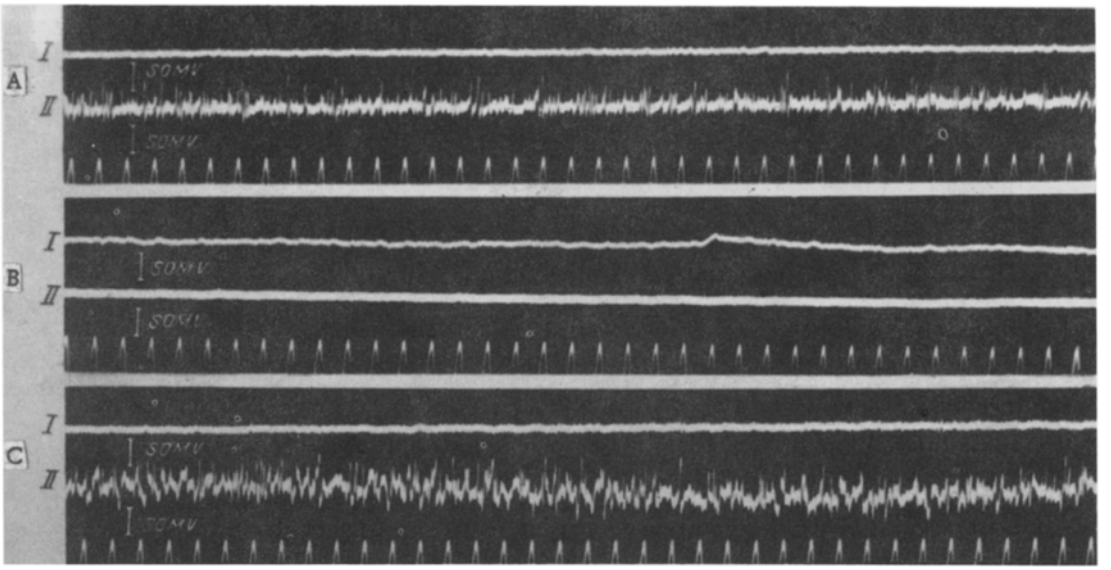

Fig. 2. Electrical activity in the right (I) and left (II) sciatic nerves one day after injecting toxin into the deafferented (A), deefferented (B), and normal (C) left hind limb while simultaneously injecting antitetanus serum into the bloodstream. Time marker (20 msec).

the disease, but is evidently not a necessary condition for its development. It is possible that the importance of this factor in the pathogenesis of the disease is different in different animals.

SUMMARY

Experiments were performed on white rats in which the sensory and motor roots were divided subdurally.

Deafferentation of one limb failed to prevent the development of a local tetanus following the injection of small doses of toxin into the muscles.

Lethal toxin doses injected into muscles of a deafferented limb while the humoral route of the toxin is blocked by antitetanus serum causes not only local but also general and fatal tetanus.

With the humoral route again blocked in the same way, lethal doses of toxin injected into the muscles of a deafferented limb do not cause a general tetanus.

In a deafferented injected limb, action potentials could be recorded from the sciatic nerve, but not from that of the deafferented limb. It is concluded that central mechanisms play a very important part in both local and general tetanus.

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