ROUTES OF TETANUS TOXIN ENTRANCE INTO THE CENTRAL NERVOUS SYSTEM AND SOME PROBLEMS IN THE PATHOGENESIS OF EXPERIMENTAL TETANUS

REPORT I. EXPERIMENTS ON ALBINO RATS

G. N. Kryzhanovskii, L. A. Pevnitskii, V. N. Grafova and A. A. Polgar

From the Laboratory of Infectious Pathology (Head – Corresponding Member of the Akad. Med. Nauk SSSR Prof. A. Ya. Alymov) of the Institute of Normal and Pathological Physiology (Dir. – Active Member of the Akad. Med. Nauk SSSR Prof. V. V. Parin) of the Akad. Med. Nauk SSSR, Moscow. Director of the work – Candidate of Medical Sciences G. N. Kryzhanovskii
(Presented by Active Member of the Akad. Med. Nauk SSSR V. V. Parin)
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The data in the literature on the advancement of tetanus toxin along nerve trunks to the region of the central nervous system are rather contradictory. Although many authors regard this pathway as the basic mode of entrance of the toxin into the central nervous system [1, 14, 15, 18, 20, 21, 23-27], a number of investigators categorically reject the very possibility, as well as the importance, of movement of the toxin along a nerve in the mechanisms of the illness' development [11, 12, 21]. In addition to this, the opinion exists [17] that the toxin can enter the nerve passively, by means of simple impregnation of the nerve trunks from the depot at the site of its administration or formation. The controversy in the published data on this problem is made more difficult by the differing conditions in the investigative methods of the various authors, by the tendency to draw wide conclusions on the basis of results from experiments performed on some individual species of animals, by a definite artificiality in the basic forms of the experiments (for example, the introduction of the toxin directly into a nerve, bypassing the peripheral tissue), and sometimes also by defects in the methods (survey see [12]).

In a previous work [8] we showed that introduction of tetanus toxin into white rats by injection into the muscles of previously deafferentiated extremities, with simultaneous blockade of the vascular routs of toxin dissemination by the use of tetanus antitoxin, results in the development of an initially local, and subsequently generalized, ascending tetanus. Inasmuch as the possibility of toxin dissemination via the blood was excluded in these experiments by the presence of antitoxin in the blood, the development of the illness could not be related to the toxin's gaining access to the central nervous system by means of the vascular system. In addition the injection of toxin into the muscles of the deafferentiated extremity under the same conditions of blockade of the vascular routes by antitoxin did not cause any of the characteristics of the illness. The results of these experiments, coinciding with comparable data of other authors [18], made it possible to postulate that the basic route of advance of tetanus toxin in the development of ascending tetanus is via the anterior roots of the spinal cord which provide the motor innervation of the muscles that were injected with the toxin.

This hypothesis, however, required direct proof. There are no indications in the literature of the possibility of demonstrating tetanus toxin in the spinal cord roots. Authors that have studied the question of the role

of nerve trunks as possible conductors of the toxin have limited their investigations to only peripheral nerves [10, 14, 15, 16, 20, 21, 23, 24, 26].

All the foregoing induced us to undertake systematic investigations on one of the basic questions in the pathogenesis of tetanus – the question of the routes of entry of tetanus toxin into the central nervous system. For its elucidation it was also necessary to study the mechanisms of action of the toxin. We felt it was important to perform the investigation on a comparative level, with the use of animals of varying species, since the clinical forms of the illness are not uniform in different animals, and this difference might be related to characteristics of the routes of advancement and mechanisms of action of the toxin in the different animals. In order to study these questions on a comparative level it was necessary to obtain supplementary data for elucidation of the basic principles in the development of the illness, and, to a certain degree, to aid in descriminating between the contradictions which abound in the literature on the pathogenesis of tetanus. In this work we present the results of experiments carried out for the purpose of studying the aforementioned questions in albino rats – the animals in which those previous investigations were performed [8] which have served as the starting point for this project.

EXPERIMENTAL METHOD

The experiments were carried out on animals 200-230 g in weight. A glycerine solution of dried tetanus toxin (series 587 of the IÉM of the Akad. Med. Nauk, SSSR) was diluted with physiological saline so that 0.15-0.2 ml contained 10-15 DLM for rats. This dose was introduced into the muscles of the left lower leg (anterior and posterior groups) by one or serveral injections. The latter means of toxin injection was adopted for the purpose of simultaneous involvement in the process of as large a number of muscles as possible, and thus, of the corresponding nerve conductors which enter into the composition of the sciatic nerve. At various intervals following injection of the toxin the animals were sacrificed by exsanguination. For the investigation, we then took the sciatic nerves, spinal ganglia, posterior and anterior roots related to the sciatic nerve (L_5 and L_6), on both the side of toxin injection and the opposite side. In order to obtain an adequate amount of material for investigation in the experiment we simultaneously used 6-10 rats. The most distal portion of the sicatic nerve was excluded from the sample, i.e., the portion surrounded by the fatty tissue of the popliteal fossa, since, as has been shown experimentally, in this part the toxin may enter immediately after injection, apparently by passive means. In determining the rate of advancement of the toxin along the nerve conductors, the sciatic nerve, minus its most distal portion, was divided into three parts. The branches constituting the lumbar plexus entered into the upper part; the anterior roots were divided into two portions. A 1% solution of peptone (for better preservation of the toxin) was added to the material to be investigated, on the basis of 0.1 ml of the peptone solution per 10 mg of tissue. The tissue was ground up under refrigerated conditions, using a glass homogenizer. Antibiotics (penicillin and streptomycin) were added to the homogenate to prevent bacterial contamination, and the suspension was injected into mice, 16-18 g in weight, in the muscles of the left posterior extremity. The amount of toxin contained in the homogenate was evaluated by the degree to which the mice were stricken with tetanus. Serum from the rats, taken at the time they were bled, was titered by the standard method.

At the same time that we determined the toxin in the nerves and roots, we performed special experiments on a portion of the animals, investigating the electrical activity of the left and right gastrocnemius muscles every 3 hours following the moment of injection of the toxin into the left gastrocnemius. The muscle biopotentials were drawn off by concentrated needle electrodes. Amplification and recording were performed with the aid of a cathode electromyograph of the "Diza-elektronik" type.

EXPERIMENTAL RESULTS

As can be seen from Table 1, significant amounts of tetanus toxin could be observed in the sciatic nerve of the injection side in all the experiments without exception. The same was true for those anterior roots of the spinal cord related to the sciatic nerve and on the same side as the toxin injection. In addition, there was no toxin either in the nerves or in the roots of the opposite side. This is evidence that its presence in the nerves and roots on the side of the injection is not related to vascular dissemination, but rather to the advancement of the toxin from the muscles along the nerve trunks. Attention is merited the fact that the anterior roots contained more toxin that the sciatic nerve if figured on a weight basis. This may be explained by the presence of formations in the nerve trunk which are not related to the transport of toxin or are able to do so in very small amounts.

TABLE 1

Tetanus Toxin Content in the Spinal Cord Roots, Spinal Ganglia, and Nerves of the Posterior Extremities in Albino Rats Following the Injection of Toxin into the Muscles of the Left Lower Leg

Material investigated	Time elapsed between toxin injection and sampling of material (in hours) and experiment number																	
	9		12										18				24	
	1		2		3		4		5		6		7		8		9	
	amount of material (in mg)	clinical observation	amount of material (in mg)	clinical observation	amount of material (in mg)	clinical observation	amount of material (in mg)	clinical observation	amount of material (in mg)	clinical observation	amount of material (in mg)	clinical observation	amount of material (in mg)	clinical observation	amount of material (in mg)	clinical observation	amount of material (in mg)	clinical observation
Side of toxin injection																		
Anterior roots of the sciatic nerve	25	++	32	Ex_7	32	Ex_7	40	Ex_5	46	Ex_7	48	Ex_6	40	Ex_4	40	Ex_5	40	Ex_5
Posterior roots of the sciatic nerve	n/d		40	–	40	–	40	–	43	–	48	–	40	–	40	–	40	–
Spinal ganglia	26	–	n/d		23	–	n/d		n/d		n/d		18	+	40	+	18	+
Sciatic nerve	40	++	40	++	40	++	40	++	40	++	45	++	40	++	40	++	40	++
Femoral nerve	n/d		40	⊥	40	±	n/d		n/d		n/d		40	±	40	±	n/d	
Obturator nerve	n/d		38	±	n/d		n/d		n/d		n/d		22	±	30	±	n/d	
Opposite side																		
Anterior roots of the aciatic nerve	n/d		n/d		n/d		40	–	40	–	48	–	n/d		40	–	40	–
Posterior roots of the sciatic nerve	n/d		n/d		n/d		40	–	40	–	48	–	n/d		n/d		40	–
Spinal ganglia	n/d		n/d		n/d		n/d		n/d		n/d		n/d		40	–	14	–
Sciatic nerve	n/d		n/d		n/d		40	–	40	–	45	–	n/d		n/d		40	–
Concentration of toxin in the serum	4DLM/ml		n/d		4DLM/ml		n/d		n/d		n/d		2DLM/ml		4DLM/ml		n/d	

Arbitrary symbols for clinical impressions of the illness in mice: ⊥ barely noticeable elevation in the extensor tonus; ± small, but distinct elevation in the extensor tonus; + manifest extensor tonus; ++ local tetanus, up to segmental symptoms; +++ local tetanus with systemic symptoms; ++++ systemic tetanus; Ex_4, Ex_5, Ex_6 – expired on the 4th, 5th or 6th days, respectively; n/d – determination not performed.

TABLE 2

Tetanus Toxin Content in Various Sections of the Spinal Cord Roots and the Sciatic Nerve of Albino Rats at Different Time Intervals Following the Injection of Toxin into the Muscles of the Lower Leg

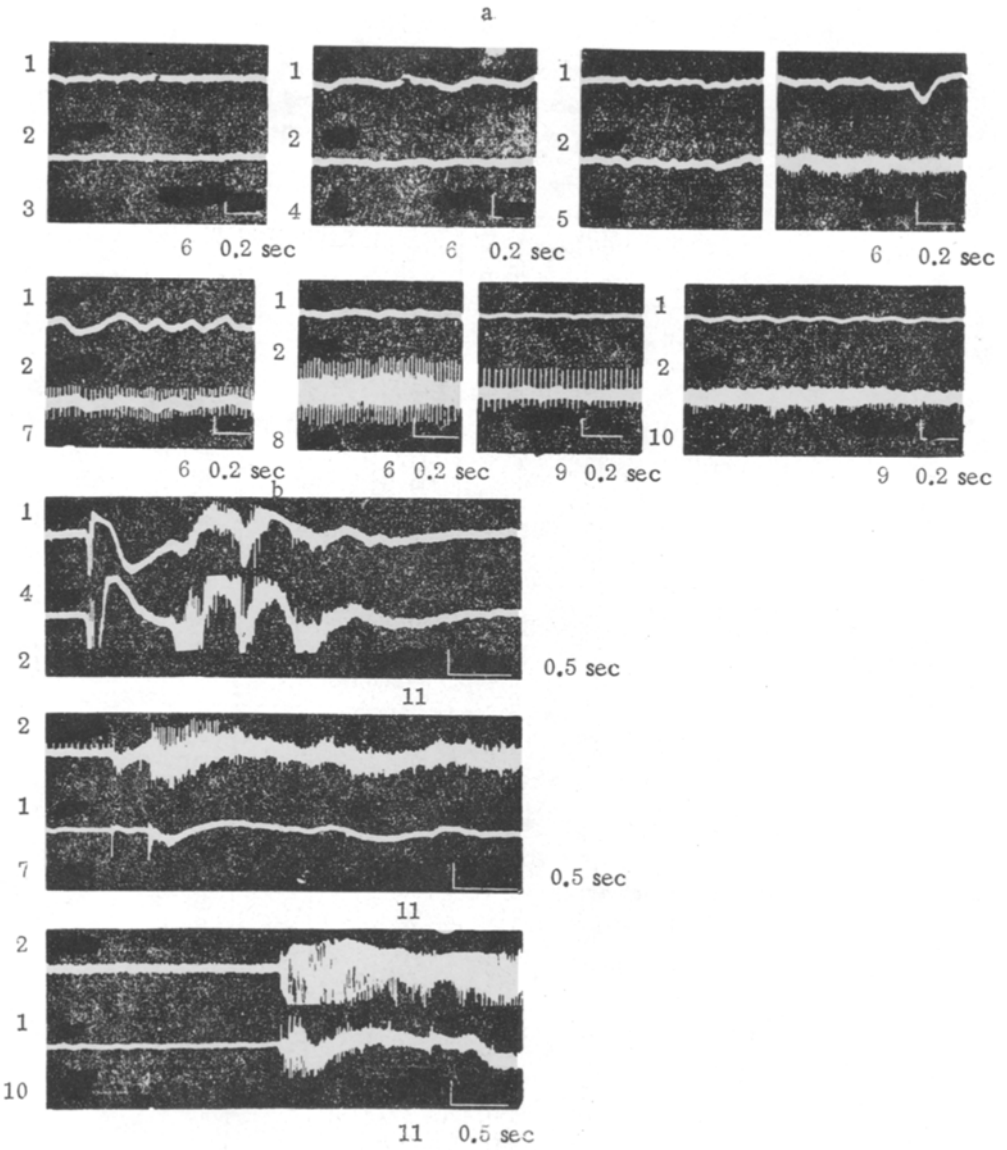
Material investigated		Time elapsed between toxin injection and sampling of material (in hr)	Experiment No. 1: amount of material (in mg)	Experiment No. 1: clinical observation	Experiment No. 2: amount of material (in mg)	Experiment No. 2: clinical observation
Sciatic nerve	Lower third	3	39	++	40	++
		6	35	Ex_6	40	Ex_6
		9	39	++	40	Ex_4, Ex_6
		12	36	Ex_7, Ex_9	40	+++ + Ex_6
	Middle third	3	42	—	40	—
		6	34	+	40	++
		9	33	++	40	Ex_3, Ex_6
		12	33	++, Ex_8	40	Ex_6, Ex_7
	Upper third	3	44	—	40	—
		6	36	—	40	+
		9	38	++	40	Ex_6, Ex_7
		12	36	+++	40	+++
Anterior roots	Distal portion	3	26	—	19	—
		6	22	—	24	—
		9	24	++	24	++
		12	21	Ex_6	25	++++
	Proximal portion	3	22	—	17	—
		6	22	—	20	—
		9	22	+	24	++
		12	19	Ex_5	25	Ex_6
Concentration of toxin in the serum		3	>1 DLM/ml		2 DLM/ml	
		6	>6 »		4 »	
		9	6 »		4 »	
		12	6 »		n/d	

Note: Arbitrary symbols are the same as in Table 1.

The results of the experiment justify concluding that the epineuria and its lymphatic pathways are not the formations which secure the advancement of toxin to the spinal cord, since the roots are devoid of this covering and do not bear lymphatic vessels and nevertheless the greatest amount of toxin is specifically noted in these roots. As far as the presence of toxin in the spinal ganglia on the side of the injection, its entry here may be explained by advancement either along bundles of sensory fibers or along the lymphatic pathways of the nerve.

The question of the toxin's pathways of access to the spinal ganglia, however, remains open and demands special investigations. Our further investigations (the data of which will be the subject of a special report) showed, in particular, that in animals of certain species (for example, in dogs, donkeys, etc.) the toxin finds it way to the ganglia mainly from the blood. Apparently, the specific importance of the different pathways of the toxin's entry into the spinal ganglia varies for different animals.

The data presented also indicates that the posterior roots are in no way an essential pathway for the entry of the toxin into the spinal cord: we were unable to detect determinable amounts of toxin in the posterior roots



Electrical activity in the left and right gastrocnemius muscles of rats at different time intervals following the injection of toxin into the left gastrocnemius muscle. a) Background activity (in the resting state of the animal); b) background activity and activity during and after a provoked general motor reaction. Key: 1) rt.; 2) lt.; 3) 3 hours; 4) 6 hours; 5) 9 hours; 6) 50 microvolts; 7) 12 hours; 8) 15 hours; 9) 150 microvolts; 10) 18 hours; 11) 75 microvolts.

of the albino rats in a single case. The possibility has not been excluded that the spinal ganglia can act as a unique barrier in the pathway of the toxin's progress along afferent nerve fibers (if this movement takes place).

Finally, there is still another result of these experiments which demands attention: the presence of toxin not only in the sciatic nerve, but in other nerves of the extremity as well, e. g., the femoral. Inasmuch as the toxin did not find its way into other nerves via the blood, it must be postulated that it arrived here as a result of penetration from the injection site into neighboring muscles. It is possible that the overlap in the innervation zones of the separate nerves in the extremity may have a certain importance here.

Thus, the data of the experiments performed completely coincides with the results of our previous investigations [8] and serves as direct proof that the anterior roots of the spinal cord are a fundamental passage route of the toxin from the muscles to the spinal cord.

Having obtained these data, we decided to compare the time interval for the entrance of tetanus toxin into the spinal cord with the times of the appearance of the first signs of change in the electrical activity of the muscles, this being one of the characteristic signs of local tetanus [1-4, 6-9, 13, 16, 27].

As can be seen from Table 2, within 6 hours after injection into the gastrocnemius muscle the toxin could be observed throughout the entire length of the nerve, but, as a rule, was absent from the anterior roots. After 9 hours it could already be demonstrated in both halves of the anterior roots, but in greater amounts in the lower (distal) half. After 12 hours the amount of toxin was very high in the distal as well as the proximal portions of the anterior roots. Thus, 9 hours after injection into the gastrocnemius muscle the toxin had already reached the anterior horns of the spinal cord.

The pictures of the electrical activity (EA) are presented in the figure for the left and right gastrocnemius muscles at the indicated intervals following the toxin injection. Three to six hours after the injection the character of the EA was unchanged; the initial background of the EA in the left gastrocnemius muscle was the same as in the right (see figure, a). A general motor reaction of the animal was accompanied by short-duration bursts of activity in both muscles, which disappeared after cessation of the general reaction (see figure, b). After 9 hours, as rule, it was possible to observe changes in the EA of the left gastrocnemius muscle; after a general motor reaction the burst did not disappear immediately, but was followed by additional activity that varied in length in the different cases. Sometimes this activity lasted comparatively long, as a result of which it acted like the initial background. After 12 hours this "after-activity" increased in strength and became so prolonged that one could consider the background activity as "spontaneous" activity, a form described by a number of authors [1-4, 6-9, 16, 27]. Against this background the general motor reaction manifested an intense fulminant activity in the "tetanic" muscle. As this time the appearance of the first clinical signs of the illness could also be noted, in the form of a small elevation in the extensor tonus.

As can be seen from the presented data, the appearance of the first signs of changes in the EA coincided in time with the intervals at which the toxin gained access to the spinal cord. In a series of experiments involving more detailed investigations of these parameters, it was possible to note changes in the EA within the "tetanic" muscle 7-8 hours after the injection of toxin, and, at that same time, to detect small amounts of the toxin in the proximal portions of the anterior roots.

Thus, a change in the character of the EA for a muscle that has been injected with toxin only occurs after the entrance of the toxin into the spinal cord. Despite the fact that the toxin is found in the muscle for several hours, the EA of that muscle does not change if the toxin does not reach the spinal cord. As far as the first clinical signs of the illness are concerned, they are seen to follow the change in the EA.

The data of these experiments bear evidence that development of local tetanus, with all its accompanying phenomena (change in the EA and tonus of the muscles), is related to the action of tetanus toxin on central nervous formations. This conclusion is in agreement with the impressions of other authors [1, 16, 17, 20, 21, 25, 27], and justifies repudiating the point of view that local tetanus is a result of the peripheral action of tetanus toxin only on the myoneural synapse or directly on the muscle tissue [11, 12, 19].

SUMMARY

As shown in experiments on albino rats, the principal route of entry into the spinal cord for tetanus toxin is through anterior roots. Apart from the latter, the toxin is detected in the nerves of extremity and spinal ganglia on the side of toxin administration. The toxin was undetectable in the posterior roots, and could not be revealed in the nerves, ganglia and roots of the contralateral side, after injecting it in a dose of 10-15 DLM. The data obtained coincide with the results of previous investigations. Both demonstrated that only preliminary deafferentation (as distinct from deafferentation) of the extremity, into the muscles of which the toxin was injected, prevented the development of general tetanus with the circulatory path of the toxin spread blocked by means of tetanus antiserum. The changes in the electric activity of the muscle (EA) into which the toxin was injected were examined at various intervals after its administration. As demonstrated, the changes in the EA occur only after the toxin reaches the anterior horns of the spinal cord. No changes in the EA were noted prior to this period, even with the toxin present in the muscle for several hours.

The results of the above-mentioned investigations confirm former conclusions and are in accordance with the inferences of other authors, namely that the main pathogenetic mechanism involved in the development

of local and general tetanus is the central and not the peripheral action of the toxin. The possible routes of toxin progress along the nerve are discussed.

LITERATURE CITED

1. N. V. Golikov, Sci. Records of Leningrad Univ. Biological Series 16, 6 (1949).
2. E. A. Gromova, in the book: The Problem of Reactivity in Pathology (Moscow, 1954) p. 61.
3. E. A. Gromova, Zhurn. Mikrobiol., Epidemiol. i Immunobiol. 61 (1959).
4. E. A. Gromova, Theses and References from the Reports of the Symposium on the Pathogenesis of Tetanus (Moscow, 1959) p. 6.
5. D. A. Zhdanov, The General Anatomy and Physiology of the Lymphatic System, (Moscow, 1952) p. 186.
6. G. N. Kryzhanovskii, in the book: The Problem of Reactivity in Pathology (Moscow, 1954) p. 52
7. G. N. Kryzhanovskii, Byull. Éksp. Biol. i Med. 11, 38 (1959).
8. G. N. Kryzhanovskii, Byull. Éksp. Biol. i Med. 1, 49 (1960).
9. G. N. Kryzhanovskii, L. A. Pevnitskii, V. N. Grafova, et al., Theses and References from the Reports of the Symposium on the Pathogenesis of Tetanus (Moscow, 1959) p. 21.
10. S. I. Levedinskaya, Arkh. Biol. Nauk 41, 3, 115 (1936).
11. J. J. Abel, Science 79, 121 (1934).
12. J. J. Abel, et al., Bull. John. Hopk. 56, 84, 317 (1935); 57, 343 (1935); 59, 307 (1936); 62, 522, 610 (1938); 63, 375 (1938).
13. G. H. Acheson, O. D. Ratnoff, and E. B. Schobenbach, J. exp. Med. 75, 465 (1942).
14. J. H. Baylis, J. Mackintosh, R. S. Morgan, et al., J. Path. Bact. 64, 33 (1952).
15. H. Bromeis, Otsch. Z. Chir. 250, 402 (1938); 252, 285 (1939).
16. J. R. Davies, E. A. Wright, et al., Arch. intern. Physiol. 62, 248 (1959).
17. R. Doerr, S. Seidenberg and Fl. Magrassi, Z. Hyg. Infekt-Kr. 118, 92 (1936).
18. U. Friedemann, A. Hollander, and I. M. Tarlov, J. Immunol. 40, 325 (1941).
19. A. M. Harvey, J. Physiol. 96, 348 (1939).
20. A. Marie and V. Morax, Ann. Inst. Pasteur 16, 818 (1902).
21. H. Meyer and F. Ransom, Arch. exp. Path., Pharmak. 49, 369 (1903).
22. W. Penitscha, Arch. Klin. Chir. 274, 435 (1953).
23. P. G. Raafe, Science 105, 180 (1947).
24. F. H. Teale and D. Embleton, J. Path. Bact. 23, 50 (1919-1920).
25. G. P. Wright, Pharmacol. Rev. 7, 434 (1955).
26. E. A. Wright, R. S. Morgan, and G. P. Wright, Brit. J. exp. Path. 32, 169 (1951).
27. E. A. Wright, R. S. Morgan, and G. P. Wright, Lancet, 1, 316 (1952).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
