ROUTES OF ENTRANCE OF TETANUS TOXIN INTO THE CENTRAL NERVOUS SYSTEM AND CERTAIN QUESTIONS IN THE PATHOGENESIS OF EXPERIMENTAL TETANUS

IV. ON THE PATHOGENESIS OF ASCENDING AND DESCENDING TETANUS

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In previous investigations [14-17], it was shown that in animals of different species—white rats, guinea pigs, rabbits, cats, dogs, and apes—tetanus toxin infiltrates through the muscles into a nerve trunk, advances along the trunk in the central direction, and enters the spinal cord via the anterior roots.

In all the enumerated animal species, the experimental tetanus followed the pattern of the so-called ascending type: initially, local tetanus arises in the extremity which was injected with the toxin, following which the systematic symptoms develop in the form of spasm of the muscles in the trunk, neck, extremities, and fits of generalized convulsions. However, in man [10, 18, 19, 20] and certain agricultural animals [3, 9], tetanus usually follows the pattern of the so-called descending type. In this form, the illness immediately begins with trismus and generalized rigidity; the symptoms of local tetanus are not observed. The question arises as to whether the indicated pathway of the toxin's entrance into the central nervous system takes place in those cases where the tetanus develops according to the descending type. Resolution of this question can be of major importance in understanding the pathogenesis of tetanus in man. This thus determined the basic purpose of the present work.

The goal of this investigation was to compare the characteristics of the clinical forms of the illness, and the routes of entrance of the tetanus toxin into the central nervous system.

METHOD

The investigations were carried out on donkeys, in whom, as in the case of horses [3, 22], the injection of a lethal dose of toxin leads to the development of tetanus of the descending type. Toxin (series 589, IÉM Akad. Med. Nauk SSSR), in a dose of 15 DLM (equal to 0.005 mg/kg, a total of 2 to 10 mg per animal) and a volume of 2 ml, was injected into the posterior muscle group of the left shank. 24-48 hours after the injection of toxin, the animals were sacrificed by either electrocution or exsanguination. For tetanus toxin determination we selected the following nerve structures: anterior and posterior roots of the spinal cord (L_5 and L_6 and C_5-C_8) on both sides, the spinal ganglia of the same segments, the sensory portion of the left sciatic nerve (the bundle of fibers taking their beginning from the spinal ganglia L_5 and L_6), the distal, middle and proximal portions of the left, and distal portion of the right, sciatic nerve, the sensory and motor (innervating the masticatory muscles) portions of the trigeminal nerve, the sensory roots of the trigeminal nerve entering the medulla oblongata, and the Gasserian ganglia. From the tissue of these structures, we prepared homogenates which we injected into mice in the muscles of the posterior extremity (60 mg of tissue per 0.6 ml of homogenate). The concentration of toxin in the homogenates was judged by the severity of the illness in the mice. In addition, using the method of biological titration in the mice, we investigated the concentration of toxin in blood drawn at the time of the animals' death. A more detailed description of the method used in the experiments has been given in an earlier report [15].

Besides determining the toxin in the indicated tissues, we studied the clinical course of the illness. In special experiments, we recorded the electrical activity in the muscles of the posterior extremities, using a myograph ("Elema" company) in which two channels were amplified by supplementary cascades. Conduction of the biocurrents was done via needle electrodes.

RESULTS

Donkeys are comparatively sensitive to tetanus toxin: the minimum lethal dose (DLM) for them, causing death on the fourth day, was 70 mice-DLM/kg of body weight, according to our data. However, animals died of tetanus after the injection of 1/10–1/20 DLM, and even smaller doses of toxin. The sensitivity of donkeys to tetanus toxin, apparently, approximates the sensitivity of horses [3, 24].

Following the injection of a lethal dose of toxin (1/3–2/3 DLM) into the muscles of the posterior extremity, symptoms of generalized rigidity arise in the animal after several days (usually after 2–4 days, depending on the dose of toxin), along with difficulty in locomotion and mastication. No signs of local tetanus are observed (Fig. 1a). The symptoms then rapidly progress, the trismus is intensified, and there is marked tension in the muscles of the neck, ears, trunk and extremities (the latter are held as straight as sticks) (Fig. 1b). Against this background, there arise paroxysms of generalized seizures. Thus, with the injection of sufficiently large doses of toxin, the illness, in donkeys, progresses along the lines of the descending type, with no manifest signs of local tetanus.

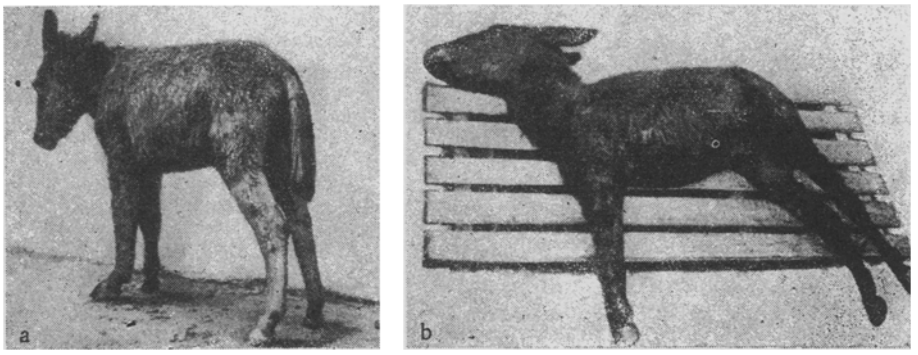


Fig. 1. Clinical picture of descending tetanus in the donkey, following the injection of 2/5 DLM of toxin into the muscles of the left shank. a) Appearance of general rigidity (6th day of illness); b) generalized tension in the muscles of the trunk, neck, ears, and extremities (7th day of illness). Death occurred on the 9th day following injection of the toxin.

Determination of tetanus toxin in various divisions of the peripheral nervous system showed (Table 1) that the toxin is found, with sufficient constancy, in the anterior roots and the sciatic nerve on the side of the injection. Tetanus toxin is only observed in the anterior roots and sciatic nerve of the contralateral side in isolated cases (apparently, the result of some unaccounted conditions or of peculiarities in the animals; the difference in the results of these determinations was statistically significant). These data indicate that the presence of toxin in the nerve and the anterior roots on the side of the injection is not related to its infiltration from the blood into the indicated structures, but to its advancement from the muscles along the nerve trunk

In addition, the toxin is gradually observed in the spinal ganglia, both on the side of injection and on the contralateral side; it also gradually appears in the ganglia of the cervical segments, and in the Gasserian ganglion. This indicates that the tetanus toxin enters the ganglia from the blood. A similar mechanism takes place in dogs [17]. The detection of tetanus toxin in the posterior roots on the same side as the injection, in half the number of cases, compels one to conclude that in certain animals it can also enter the posterior roots. In the majority of cases (in 5 out of 7), it was possible to detect toxin in the motor portion of the trigeminal nerve innervating the masticatory musculature. These data are proof that the toxin can enter the motor portion of a mixed nerve from the blood, and advance along bundles of motor fibers.

Thus, the experiments involving determination of the tetanus toxin in various divisions of the peripheral nervous system showed that in donkeys, just as in other animals [15, 16, 17], the tetanus toxin enters from the muscles into the corresponding nerve, and, subsequently, into the anterior roots of the spinal cord. However, the question

TABLE 1. The Concentration of Tetanus Toxin in Various Divisions of the Peripheral Nervous System in Donkeys, Following Its Injection into the Muscles of the Left Shank

No. of the animal	Weight (in kg)	Time of sampling of the material (in hr)	Clinical picture of the illness in mice after injection of material taken from: the side of injection of the toxin — roots: anterior	roots: posterior	sensory portion of the sciatic nerve	spinal ganglia	sciatic nerve: lower third	sciatic nerve: middle third	sciatic nerve: upper third	the contralateral side — roots: anterior	roots: posterior	spinal ganglia	lower third of the sciatic nerve	the cervical division of the spinal cord (both sides) — roots: anterior	roots: posterior	spinal ganglia	both trigeminal nerves: motor portion	sensory portion	posterior roots	Gasserian ganglia	Conc. of the toxin (in mice-DLM) per 1 ml of blood serum
1	26	24	⊥	—	H/O	⊥	±	⊥	—	⊥	—	+	H/O	—	⊥	Ex3	++	+	⊥	++++	>10<15
2	38	24	±	⊥	H/O	—	++	++	—	±	—	++	—	⊥	—	++	±	⊥	H/O	Ex7	>20<25
3	60	24	+	+	Ex4	—	+	—	—	—	—	++	—	—	Ex2	+++	H/O	H/O	H/O	H/O	25
4	66	24	Ex7	Ex7	—	+	Ex7	Ex3	Ex3	—	—	+++	—	—	±	+++	±	—	—	Ex3	>35<40
5	84	24	Ex5	+	H/O	Ex5	±	Ex2	+++	—	—	±	++	—	—	++	—	—	—	Ex6	>10<15
6	60	25	±	—	±	±	Ex7	±	—	—		Ex6	⊥	—	—	Ex5	⊥	±	—	Ex5	30
7	60	45	±	—	⊥	+	Ex6	—	+	—	—	Ex5	—	—	—	Ex6	H/O	H/O	H/O	H/O	⩾25
8	72	45	—	—	H/O	—	+	+	—	-	—	++	—	—	—	+++	—	—	—	Ex4	>20<25
9	100	45	Ex3	±	H/O	+	++	Ex3	++	±	—	Ex2	+	Ex4	Ex2	Ex5	H/O	H/O	H/O	H/O	>15<20
10	116	48	±	—	+	—	Ex3	—	—	—	—	Ex5	—	±	Ex3	++	+	±	⊥	Ex5	⩽30

Symbols for the clinical picture of the illness in mice: – absence of signs of the illness; ⊥ barely noticeable elevation in the extensor tonus; ± small, but definite, elevation in the extensor tonus; + manifest extensor tonus; ++ complete local tetanus, up to segmental symptoms; +++ symptoms of systemic tetanus; ++++ systemic tetanus; Ex2, Ex3, Ex4, etc.) death from systemic tetanus on the 2nd, 3rd, 4th day, etc. In each case, we presented the maximum manifestations of the illness; H/O) determination not performed.

remains as to whether this route of entrance of the tetanus toxin into the spinal cord plays any sort of important role in the pathogenesis of the illness, if local tetanus is not observed in donkeys.

In order to answer this question, we carried out experiments with blockade of the vascular pathway to dissemination of the toxin, using tetanus antiserum. For this purpose, serum (Diaferm-3,IEM Akad. Med. Nauk SSSR), in specially selected doses, was injected into the muscles of the right shank, and toxin-into the muscles of the left shank, the two injections being done simultaneously. Four animals were used in the experiments, weighing 70-80 kg; the serum and toxin were injected into the different animals in varying dosages (Table 2).

TABLE 2. Concentration of Toxin and Antitoxin in the Blood of Donkeys at Various Intervals Following Injection of the Toxin and Serum

No. of the animal	Doses of toxin and serum injected in each animal		Time of sampling of the blood, and results of the determination			
	toxin (in DLM)	antitoxin (in AE)				
1	15	30	2nd day <0.002 AE <1/5 DLM for mice	4th day <0.002 AE <1/5 DLM for mice	6th day <0.002 AE <1/5 DLM for mice	8th day <0.002 AE <1/5 DLM for mice
2	15	36	not determined	not determined	not determined	8th day 0.002 AE
3	30	60	1st day >0.002 <0.005 AE	4th day ≥0.002 AE	6th day < 0.002 AE	not determined
4	45	90	4 hr >0.005 <0.02 AE	3rd day 0.01 AE	5th day >0.005 <0.01 AE	not determined

Note. 1/5 DLM-minimum amount of toxin causing sufficiently clear picture of the illness in white mice.

The characteristic sign of the illness, under these conditions, was a clearly manifested local tetanus in the extremity which was injected with the toxin (Figs. 2a and 3a). Local tetanus arose in all the animals. Its first signs consisted of clumsiness in walking, limping, stretching the leg backward, and standing on the edge of the hoof, and were recorded on the 3rd day following the injection of toxin. The symptoms then progressed; in walking, the extremity did not bend, and a pronounced tension arose in all muscles of the leg. Recording of the electrical activity (EA) in both gastrocnemius muscles showed its significant increase in the muscles of the "tetanic" extremity. Qualitatively, the changes in the EA were analogous to those which are known in the literature [4, 5, 6, 23, 25], and have been described by us earlier [11, 14, 15, 17] in studying local tetanus within laboratory animals (in whom this form of the illness is usual): prolonged, slowly extinguishing, spikes of EA, with an appreciable aftereffect, appearing like a constant background of an elevated EA (Fig. 3c).

In animal No. 4, the development of the illness was limited to local tetanus, and vaguely manifested segmental symptoms in the form of minimal rigidity of the muscles in the posterior girdle (the donkey was sacrificed one month after the beginning of the experiment). In the remaining 3 animals, the disease progressed; on the 4th-6th day, symptoms of systemic tetanus appeared in the form of tension in the muscles of the trunk. Then, the animals could not stand, and lay on their backs. Characteristically, the muscles of the anterior extremities, neck, and masticatory group were not involved in the tonic spasms (Fig. 2), the anterior extremities remained mobile, the animal raised its head, and could eat and drink. Gradually, the tension in the muscles of the trunk, abdomen, and right posterior extremity, rose, but, during the tetanic seizures, the right posterior extremity bent, in contradistinction to the left. Step by step, the generalized reflex excitability increased, and we found it progressively easier to produce a generalized excitation by the application of a stimulus to the "tetanic" extremity. This latter phenomenon is characteristic of ascending systemic tetanus, and has been described by us earlier in other animals [12, 13, 17]. At the final stage of the illness, it was possible to observe enhancement of the trismus, and tension in the respiratory musculature with a major disturbance in respiration. Death of the animals occurred on the 11th-13th days.

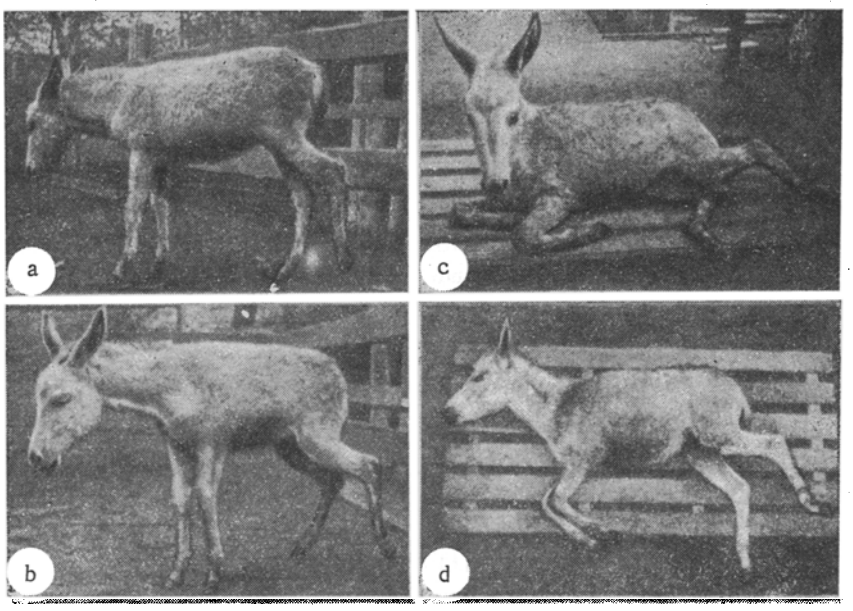


Fig. 2. Clinical picture of ascending tetanus in donkey No. 2 (see Table 2), following the injection of 15 DLM of toxin into the muscles of the left shank, and 36 AE of tetanus antiserum into the muscles of the right shank. a, b) Local tetanus (4th and 5th day of the illness); c) initial symptoms of systemic tetanus (6th day of the illness); d) systemic tetanus (9th day of the illness). Death of the animal occurred on the 13th day following the injection of the toxin.

It should be noted that the tonic spasm of the muscles in the trunk, abdomen, extremities, neck and masticatory group was manifested more strongly in donkey No. 1. In this animal, at the end of the illness (death occurred on the 11th day), the trismus was rather considerable, and the right posterior extremity was extended in the same fashion as the left. The phenomenon, manifested in the beginning of the illness, of generalized excitation with application of a stimulus to the "tetanic" extremity, was not observed at this time. In this case, therefore, the illness progressed in the pattern of a mixed type: initially it belonged to the ascending form, then to the descending. The results of tests with the serum (see Table 2) justify postulating that, in the course of time, toxin appeared in the blood of this animal in small quantities, difficult to determine by the usual techniques of biological assays, but fully sufficient to demonstrate their activity.

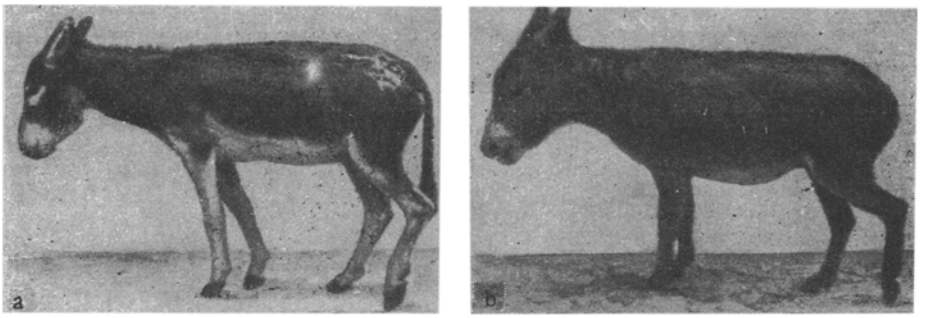
In the next experiments, we attempted to cause local tetanus, using small doses of toxin. For this purpose, 3 animals were injected with 1/10, 1/20, and 1/50 DLM respectively, into the muscles of the left posterior extremity. On the 4th-5th day, we were able to observe mild limping in all the animals, with clumsiness in walking and a barely noticeable stretching of the left posterior extremity. However, in the first 2 donkeys, the symptoms of systemic tetanus rapidly developed, and the two animals died on the 9th and 12th days. In the third donkey the symptoms of local tetanus progressed, and the illness took the typical form (Fig. 3b). It must be noted that in this animal too, however, symptoms then appeared of minor generalized rigidity, particularly evident in the muscles of the posterior girdle, but trismus and opisthotonus were not observed. The animals died 31 days after the injection of toxin of unknown causes. A substantial fall in body weight was noted (by 12 kg).

The investigations performed provide evidence that not only descending tetanus can arise in donkeys, but also ascending tetanus with all its symptoms. Basic to the development of this form of the illness is advancement of the tetanus toxin to the spinal cord via a nerve trunk and the anterior roots. A necessary condition for its development is the absence of any significant quantity of toxin in the blood.

Comparison of the concentration of toxin in the blood with the amount of toxin injected (see Table 1) shows that almost the entire amount of toxin injected into the muscles (95-98%) circulates in the blood of donkeys. In this, donkeys differ from other animals, the majority of whom, according to our previous investigations [15, 16, 17], show

no more than a third of the entire amount of toxin injected into the muscles in their blood. In connection with this, a relatively smaller amount of toxin is contained in the anterior roots of the donkeys. These two factors determine the peculiarities in the clinical form of the illness characteristic for donkeys: the local tetanus does not have time to develop before the systemic. In order for it to appear, it is necessary to either blockade the vascular pathway of toxin dissemination, or to inject doses of toxin which cannot cause rapid development of systemic tetanus.

It may be postulated that analogous mechanisms take place during development of the illness in man. Lately, descriptions of local tetanus in human beings are encountered more and more frequently [8, 10, 17-21]. As a rule,



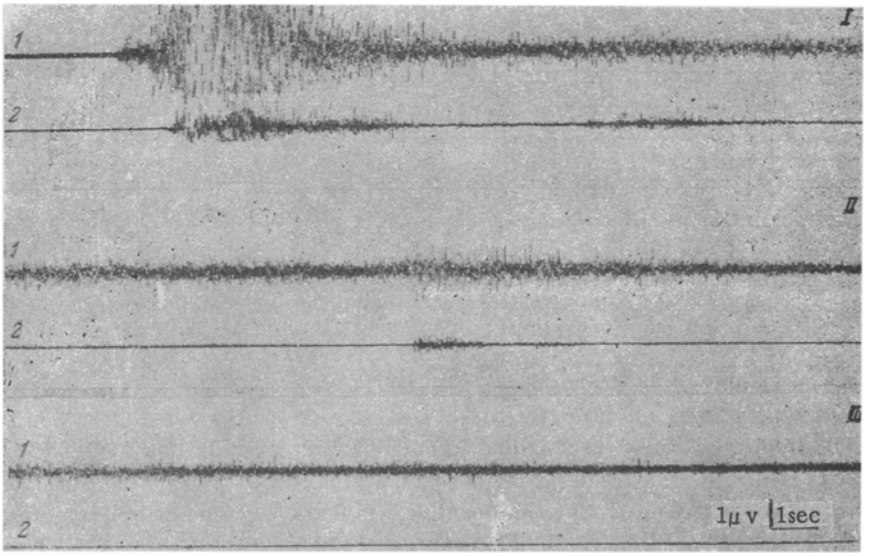


Fig. 3. Clinical picture of the illness, involving local tetanus, in donkeys, following the injection of toxin. a) (45 DLM) into the muscles of the left shank, and tetanus antiserum (90 AE) into the muscles of the right shank (donkey No. 4; 10th day of the illness); b) $^{1}/_{50}$ DLM into the muscles of the left shank (11th day of the illness); c) electromyograms of the muscles in the left (1) and right (2) shanks of donkey No. 4, recorded on the 11th day of the illness (original background and a provoked spike of electrical activity); I, II, III) consecutive fragments of the same tracing.

local tetanus is observed in patients who have been actively or passively immunized [1, 2, 7, 8, 20, 21], i.e., in those cases where dissemination of the toxin by the blood was excluded or essentially limited.

Comparing the data of this investigation with those which were obtained in previous works [15, 16, 17] one may infer that advancement of the tetanus toxin to the spinal cord via the motor roots takes place in all animals, regardless of which type of tetanus—ascending or descending—develops in them. This mechanism, therefore, is universal. The results of all the investigations performed by us [14-17] provide a basis for regarding the question of the natural pathway of entrance of the toxin into the central nervous system as finally resolved.

In addition, in all animals, along with movement of the toxin along the nerve, its entrance is observed into the blood. The peculiarities in the clinical form of the illness are determined, in each concrete case, by the relationship between the amount of toxin entering the blood and the amount gaining access to the regional nerve trunks.

However, the route by which the tetanus toxin travels from the blood to the central nervous system remains unclear.

In special, model experiments, carried out on white rats and involving the intravenous injection of tetanus toxin, we observed that the toxin can penetrate into the muscles from the blood, and, from there, move along the usual route, reaching the posterior roots and, then, the spinal cord. In addition, with a sufficiently large blood concentration, the toxin infiltrates into the nerve trunk, the posterior and anterior roots directly from the bloodstream. The results of this and previous works [17] show that the toxin enters from the blood into the spinal ganglia and the Gasserian ganglia. This also justifies concluding that the toxin may enter nerve structures directly from the blood. Apparently, the importance of this mechanism varies for different animals, just as, for example, the degree to which toxin enters the spinal ganglia from the blood differs: in rodents, cats, and lower apes, under usual conditions (with injection of several DLM), toxin does not enter the spinal ganglia from the blood [15, 16, 17]; in dogs [17] and donkeys, it enters these structures only from the blood. The question of penetration of the toxin into the central nervous system via the hematoencephalic barrier requires special investigations.

SUMMARY

In donkeys as well as in other animals tetanus toxin injected into the muscles of the shank passes along the sciatic nerve and reaches the spinal cord through the anterior roots (Table 1). In the usual conditions, after the administration of the toxin (lethal doses) into the muscles of extremities the course of tetanus in these animals is of the so-called descending type (Fig. 1). After blocking the circulatory route of the toxin spread by means of tetanus antiserum, tetanus develops according to the ascending type (Fig. 2), starting with local tetanus with increased electrical activity of the muscles into which the toxin was injected (Fig. 3c). Local tetanus may also be induced by minimal doses of the toxin (1/50 DLM) administered into the muscles of extremities (Fig. 3b). Ascending tetanus is based upon toxin spread from the site of administration along the regional nerve and the anterior roots into the spinal cord, whereas descending tetanus is based upon toxin spread by way of circulation.

When the main mass of toxin enters the circulation and a relatively small amount of it passes along the nerve general tetanus develops before local tetanus has time to appear. This mechanism takes place in donkeys. It is suggested that the mechanism of descending tetanus in other cases, including man, is analogous to this.

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