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

COMMUNICATION III. EXPERIMENTS ON MONKEYS AND DOGS

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Previous investigations [5, 6] carried out on white rats, guinea pigs, rabbits and cats showed that tetanus toxin injected into the muscles of an extremity is found in the apposite nerves and in the anterior roots of the spinal cord which provide the motor innervation of the muscles into which the toxin was injected. This article presents the results of experiments on monkeys and dogs.

The experiments on dogs were particularly interesting due to the fact that the works carried out by a number of authors [9] in order to prove that tetanus toxin enters the central nervous system through the blood rather than through the nerves were performed on dogs. These works are one of the main experimental bases for the theory of the circulatory route of tetanus toxin entrance into the spinal cord. The studies on monkeys are important because of the biological proximity of these animals to the human organism.

EXPERIMENTAL METHOD

The toxin (series 589 IEM AMN) was administered by several injections into the anterior and posterior muscle groups of the left leg in a dose of 15 M.L.D. 1-2 ml of fluid to the monkeys and in a dose of 3-4 M.L.D. in 2.5-4 ml of fluid to the dogs.

Tetanus toxin was determined in the anterior and posterior roots, the spinal ganglia on the left and right, the sensory portion of the left sciatic nerve (the bundles of fibers branching out directly from the spinal ganglia), the distal, medial and proximal parts of the left sciatic nerve and the distal portion of the right sciatic nerve. In each case, we examined two (sometimes three) anterior and posterior roots with fibers entering the sciatic nerve: L_6 and L_7 (sometimes S_1) in the monkeys and L_6 and L_7 in the dogs; the experimental ganglia belonged to these same segments. Homogenates made from the nerve tissue of the monkeys (dose – 0.4 ml) and dogs (dose – 0.6 ml) were injected into the muscles of the left posterior extremity of mice, each 0.1 ml of the homogenate containing 10 mg of nerve tissue. As well as determining the toxin in the neural conductors, we used the method of biological titration on mice to determine its content in the blood. The toxin content of the experimental tissues and the blood serum was estimated according to how sick the mice became. A more detailed description of the method used to determine toxin in the tissue of the neural conductors can be found in the previous communication [5].

The monkeys used in the experiments were Indian and Chinese Macaca rhesus and lapunders, the sensitivity of which to tetanus toxin is almost identical. Besides determining the toxin in the experimental tissues, we studied the clinical course of the disease and the electrical activity in the different muscles (using a "Diza-electronik" electromyograph). The biological currents were led off by concentric needle electrodes.

EXPERIMENTAL RESULTS

Experiments on Monkeys. Monkeys are animals comparatively sensitive to tetanus toxin [23]; according to our data, the minimal lethal dose for them, causing death on the fourth day is 30 mouse M.L.D./kg. The injection of a lethal dose of the toxin into the gastrocnemius muscle induces the ascending type of tetanus, in which local tetanus

of the injected extremity developed first: the tension of the muscles fixed the leg in a position in which it was drawn back a little and half bent (Fig. 1, a), with the digits curved; the digits showed no grasping reflex or active movements, and increased electrical activity was recorded in the muscles of the affected extremity (Fig. 1, c). Then general tetanus developed (Fig. 1, b); we observed general rigidity, tension of the trunk and tail muscles, opisthotonus, trismus and periodic convulsions. The phenomenon of facilitated generalization of excitation, which we observed earlier with other animals [3], was clearly recorded in response to stimulation of the injected extremity (Fig. 1, d). When larger doses of tetanus toxin were administered, there was less of an interval between the development of local and general tetanus, and the animal might die very soon after signs of general tetanus became apparent.

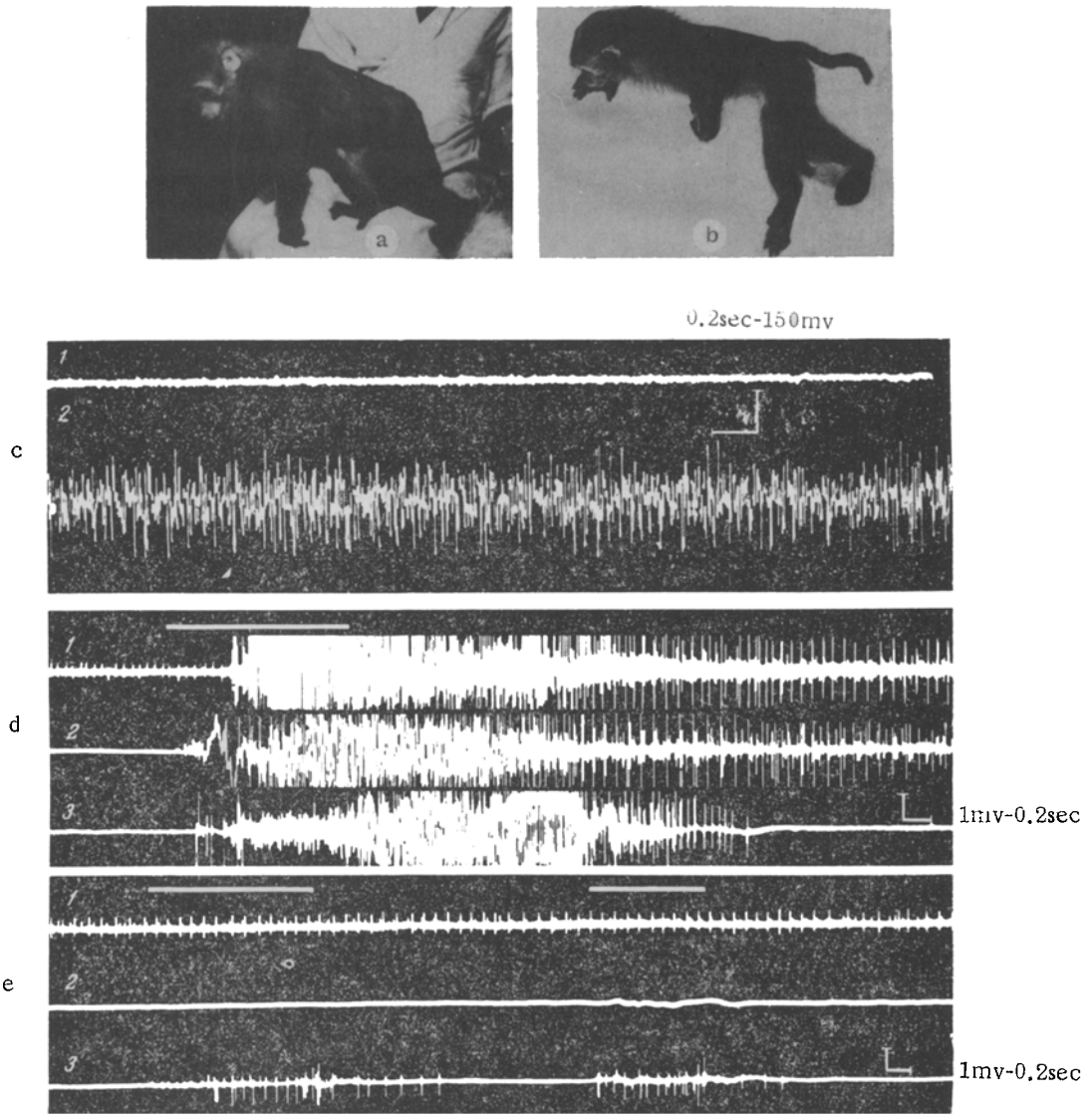

Fig. 1. Ascending tetanus in monkey after injection of the toxin into left gastrocnemius muscle. a) Local tetanus; b) general tetanus; c) electromyogram of right (1) and left (2) gastrocnemius muscles at the local tetanus stage; d) electromyograms; neck muscle (1); left gastrocnemius muscle (2); right gastrocnemius muscle (3) with stimulation [left foot pinched (white line on EMG)] applied to "tetanic" extremity at general tetanus stage (phenomenon of generalization of excitation); e) electromyogram of the same muscles with analogous stimulation applied to the opposite extremity.

TABLE 1. Tetanus Toxin Content of Spinal Cord Roots, Spinal Ganglia, Sciatic Nerves and Blood of Monkeys Following Injection of Toxin into Muscle of Left Leg

Monkey No.	Weight (in kg)	Time material was taken after toxin injection (hrs)	Clinical picture of disease in mice injected with material taken from: side of toxin injection: roots: anterior	roots: posterior	sensory portion of sciatic nerve	spinal ganglia ($L_6 - S_1$)	sciatic nerve: lower third	sciatic nerve: middle third	sciatic nerve: upper third	opposite side: roots: anterior	opposite side: roots: posterior	opposite side: spinal ganglia ($L_6 - S_1$)	opposite side: sciatic nerve (lower third)	opposite side: spinal ganglia of supralumbar region	Toxin content in 1 ml blood serum (in mouse M.L.D.)
1	1.8	24	+	−	−	−	±	±	±	−	−	−	−	−	≦ 10
2	1.55	24	++	−	−	−	+	⊥	±	−	−	−	−	−	8
3	1.75	24	+	−	−	−	++	±	±	−	−	−	−	−	8
4	1.65	24	+	−	−	−	++	+	+	−	−	−	−	−	> 8 ≦ 10
5	2.6	24	++	−	−	−	++	±	⊥	−	−	−	−	−	> 8 < 10
6	1.95	24	−	−	−	−	Ex_7	±	⊥	−	−	−	−	−	> 5 < 8
7	2.75	36	+	−	−	⊥	±	⊥	−	−	−	−	−	−	n/d
8	2.15	36	+	−	⊥	−	±	±	⊥	−	−	−	−	−	> 5 < 8
9	3.2	42	+	−	−	⊥	−	±	−	−	−	−	±	−	n/d
10	2.17	42	+	−	−	±	+	−	±	−	−	−	−	−	10

Clinical picture of disease in mice: −) No signs of disease; ⊥) barely noticeable increase in extensor tonus; ±) small but noticeable increase in extensor tonus; +) pronounced extensor tonus; ++) full local tetanus up to segmentary syndrome; Ex_7) death from general tetanus on 7th day; n/d) no determination. The maximal degree of the disease is given in all cases.

TABLE 2. Tetanus Toxin Content of Spinal Cord Roots, Spinal Ganglia, Nerves of the Posterior Extremities and Blood of Dogs after Injection of Toxin into the Muscles of the Left Leg

Dog. No.	Weight (in kg)	Time material was taken after toxin injection (hrs)	Clinical picture of disease in mice injected with material taken from: side of toxin injection: roots: anterior	roots: posterior	sensory portion of sciatic nerve	spinal ganglia ($L_6 - L_7$)	sciatic nerve: lower third	sciatic nerve: middle third	sciatic nerve: upper third	femoral nerve	opposite side: roots: anterior	opposite side: roots: posterior	opposite side: spinal ganglia ($L_6 - L_7$)	opposite side: sciatic nerve (lower third)	opposite side: spinal ganglia (thoracic region)	Toxin content of 1 ml blood serum (in mouse M.L.D.)
1	11.7	42	++	−	+	Ex_5	Ex_2	Ex_7	++	+	+	−	Ex_5	±	Ex_5	> 50 < 100
2	7.6	42	⊥	−	−	Ex_6	Ex_2	−	⊥	−	−	−	Ex_5	−	Ex_5	≧ 60
3	6.8	43	−	Ex_4	Ex_2	Ex_2	Ex_6	Ex_2	+	±	−	−	n/d	−	+	≧ 40
4	10	48	++	−	−	+++	Ex_2	Ex_3	++	±	±	−	Ex_5	−	+++	> 40 < 60
5	10	48	−	−	−	Ex_4	Ex_1	Ex_2	++	−	−	−	+++	+	Ex_7	60
6	12	48	++	−	n/d	Ex_4	Ex_2	+++	+	±	−	−	Ex_4	⊥	Ex_7	> 40 < 60
7	14	48	++	−	±	Ex_3	Ex_3	Ex_7	++	±	−	−	Ex_3	⊥	Ex_4	40
8	12.8	48	±	−	−	Ex_4	Ex_2	⊥	±	−	−	−	Ex_4	−	Ex_8	≦ 40
9	14	48	++	++	−	Ex_3	Ex_4	+	Ex_5	±	Ex_8	−	Ex_3	−	Ex_3	≧ 80
10	12.7	48	−	−	−	Ex_6	Ex_3	⊥	±	⊥	⊥	+	Ex_4	+	Ex_3	≦ 80

Symbols the same as in Table 1.

TABLE 3. Toxin and Antitoxin Determined in the Blood of Dogs Suffering from Tetanus with Tetanus Antitoxin Used to Prevent Circulation of the Toxin in the Blood

Dog. No.	Weight (in kg)	Antitoxin dose (in A.E./kg)	Time blood was taken after toxin and antitoxin administration									Clinical picture and outcome of disease	Life duration of animals which died (in days)
			1/2 hr	1 hr	3 hrs	5-6 hrs	1 day	2 days	3 days	4 days	5 days		
			Result of determinations										
1	8.3	10	n/d	−(A)	−(A)	−(A)	−	−	+	−	n/d	Ascending general tetanus. Death.	6
2	9	15	−	n/d	−	−	−	−	−	−	n/d	The same	9
3	11	20	n/d	−(A)	−(A)	−(A)	−	−	−	−	n/d	" "	10
4	14	20	−	n/d	−	−	−	−	−	−	n/d	" "	9
5	10.5	25	Not determined						−(A)	n/d	−(A)	" "	7
6	11.5	30							−(A)	−(A)	−(A)	" "	8
7	10	30	n/d	n/d	−	n/d	−	−	−	n/d	n/d	" "	7

Key: −) No toxin in blood; +) toxin present in blood; in case marked (A), antitoxin was also determined. In every case, its titer was over 0.005 A. E.; n/d) no determination of toxin or antitoxin. In dogs No. 5 and 6, the titer of antitoxin after death was over 0.002 A.E.. In dog No. 1, the toxin content of the blood was 1 mouse M.L. D. on the third day.

Determination of toxin in the neural conductors (Table 1) showed it to be present in every animal in the sciatic nerve and in the anterior roots on the side of the injection. No toxin could be found in the posterior roots, however. The fact that no toxin was present in the neural conductors on the side opposite the injection suggests that it enters the neural conductors from the muscle rather than from the blood. Only at the late stages were small amounts of the toxin found in the spinal ganglia on the injection side. On this basis, one can assume that a negligible amount of the toxin enters these ganglia from the regional tracts and remains there.

In monkeys (Macaca rhesus, lapunders, therefore, as in the other animals studied [5, 6], the main and direct route by which the toxin passes into the spinal cord during the development of ascending tetanus is the anterior roots.

Experiments on Dogs. Dogs are animals comparatively resistant to tetanus toxin [23]. It is difficult to establish exactly the M.L.D. for them; according to our data, it is about 7000 mouse M.L.D./kg (death on the 4th day).

In dogs, the disease develops as ascending tetanus: after the injection of a lethal dose of the toxin into the muscles of an extremity, first local, then general tetanus develop, showing all the typical characteristics of the two stages.

Determination of the toxin in the neural conductors (Table 2) showed it to be present in every case in large quantities in the sciatic nerve on the injection side and, in most cases, in the analogous anterior roots. In a few cases, the toxin was found in the anterior roots on the opposite side and in the posterior roots on the injection side. We were struck by the fact that tetanus toxin could be found in many cases in the sciatic nerve on the opposite side and, in all cases, in the spinal ganglia of different sections (lumbar, thoracic). This suggests that the toxin enters these formations through the blood.

The fact that there was a higher toxin content in the lower and middle parts of the sciatic nerve on the side of the injection than in the upper part and the anterior roots suggests that the toxin may enter certain other, auxiliary

formations which serve as receptacles for outflowing fluid. These formations could be the lymph tracts of the nerve, which empty into the regional lymph nodes [2, 13]. Passive impregnation of the lower part of the nerve with toxin is also a possibility. It should be mentioned that the toxin could also be found in other nerves of the extremity – the femoral, for example. This is in accordance with the data obtained in our previous investigations on other animals [5, 6].

Toxin determination in the different formations of the peripheral nervous apparatus of dogs, therefore, showed that the toxin is found all along the nerve and in the anterior roots providing motor innervation of the muscles into which it was injected in these animals also.

In order to determine the importance of these routes of the toxin's entrance into the spinal cord to the pathogenesis of tetanus in dogs, we performed experiments in which we excluded the circulatory route of toxin spread by means of tetanus antitoxin. In these experiments, selected doses of tetanus antitoxin were injected into the right gastrocnemius muscle while the toxin (10 M.L.D.) was being injected into the left. Blood was taken from the animals during the experiment in order to determine whether the toxin or antitoxin were present. The clinical picture of the disease was recorded at the same time. The results are shown in Table 3.

As Table 3 shows, the toxin was found in the blood of only one animal three days after its injection, and this animal had received a comparatively small dose of the antitoxin. No toxin was found in the blood of any of the other animals; the antitoxin, however, was found to be circulating in the blood in a rather high titer (> 0.005 A.E.).

The course of the disease in the animals was that of ascending tetanus (Fig. 2, a-d), attended by the characteristic phenomenon [3] mentioned above of facilitated generalization of excitation in response to stimulations applied to the "tetanic" extremity (Fig. 2, e, f). Certain features of the development of ascending tetanus induced by large doses of the toxin into the muscles, with the sanguiferous route of toxin circulation blocked, should be noted: the presence of marked symptoms of "algesic tetanus," the absence or mildness of the symptoms of general rigidity and constant tension of the trunk and other muscles which are observed during the course of the disease under ordinary conditions, when the blood contains toxin.

The results of these investigations indicate, therefore, that in dogs, as in monkeys and other animals [5, 6], neural conductors, specifically the motor part of the mixed nerve and the anterior roots, play a large part in the transportation of tetanus toxin to the central nervous system. When the toxin is prevented from circulating with the blood, these formations are not only the main, but indeed the sole route by which the toxin can enter the spinal cord. The data obtained allow one to conclude that circulation of the toxin with the blood is not necessary for the development of general tetanus, so that contrary to the opinion of several authors [8, 9, 19], the passage from the blood through the blood-brain barrier cannot be considered the only route by which the toxin can enter the central nervous system.

As we have already mentioned, the experiments of Abel and his disciples [9] on dogs constitute the experimental basis for the theory that a nerve cannot serve to conduct the toxin. These experiments demonstrated that the injection of pathogenic doses of the toxin into the sciatic nerve does not induce local tetanus, while the injection of the same or smaller doses of the toxin into the muscles of the extremity does induce the disease. Abel's data have recently been seriously criticized theoretically and experimentally by Wright and his co-workers [10, 22, 23, 24], who demonstrated the passage of toxin along a nerve in a series of investigations. Wright's studies, however, were conducted on rabbits, so that his results cannot be directly compared with Abel's.

A serious fault of Abel's experiments is the fact that they were conducted under artificial experimental conditions: tetanus toxin was injected into the nerve, by-passing the peripheral tissues, i.e., by-passing the usual route by which the toxin leaves the muscle. Moreover, the intraneural method of administration is subject to methodic errors. Special investigations employing radioactive substances [11] have shown that a substance injected into a nerve does not always enter the same formation of the nerve trunk, so that, depending on the injection site, it can pass either into the spinal cord or out into the blood. It should be noted in this connection that the experimental results obtained by Abel and his co-workers were extremely heterogeneous: the injection of tetanus toxin into the sciatic nerve of dogs induced disease in some cases, but had no result in others. The authors concluded that in the case in which the disease developed, the toxin had escaped from the nerve into the surrounding muscles. In the light of the material cited concerning the intraneural method of injecting substances [11], the heterogeneous nature of the data obtained by Abel and his co-workers can be otherwise interpreted.

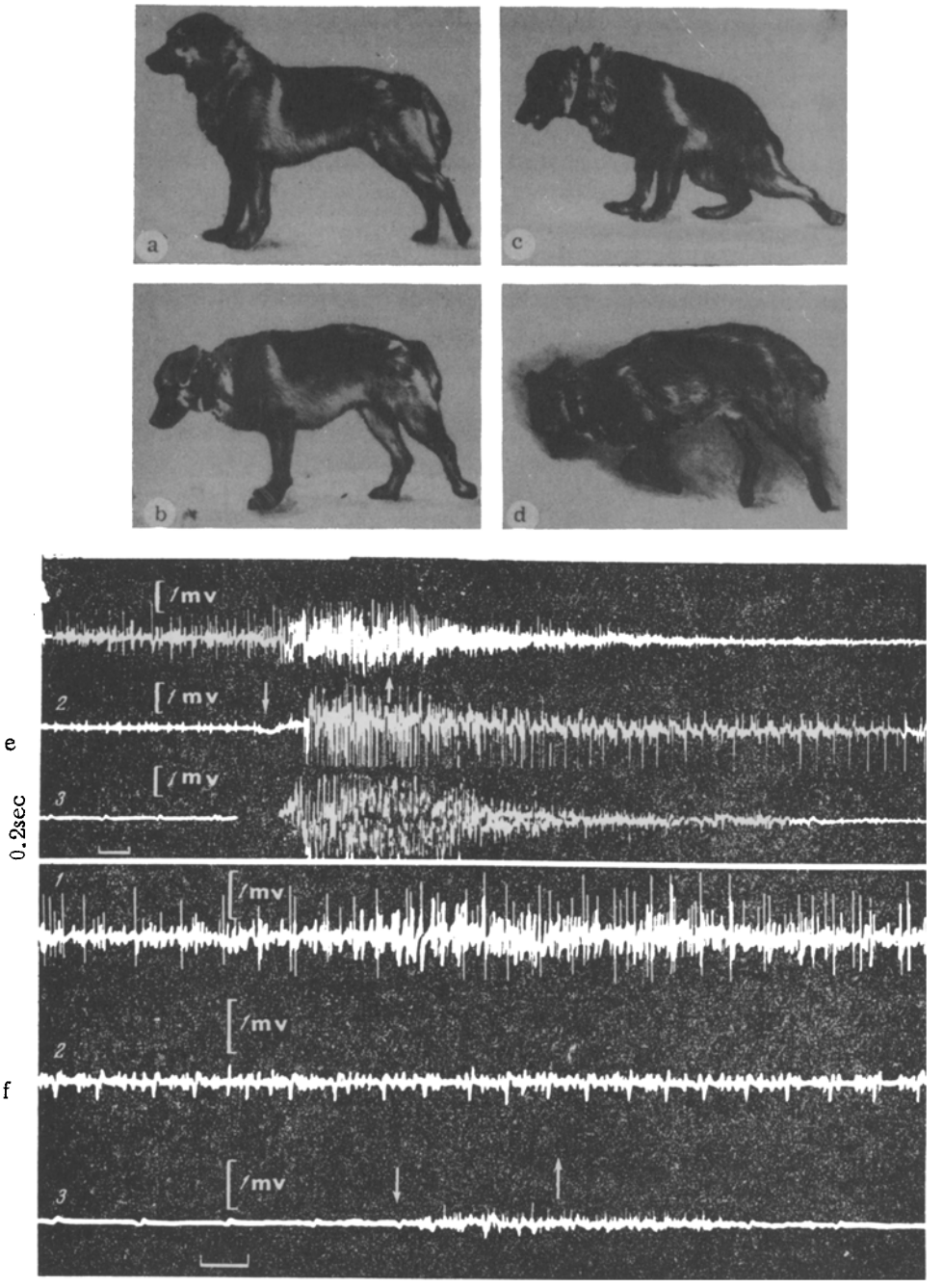

Fig. 2. Ascending tetanus in a dog with circulatory route of toxin spread blocked by tetanus antitoxin. a, b, c, d) Stages in the development of ascending tetanus in the animal after the injection of 10 M. L. D. toxin into the left gastrocnemius muscle and the injection of 30 A. E. tetanus antitoxin into the right (dog No. 6, Table 3); e) electromyograms of different muscles with stimulations [pinching (↑ – beginning, ↓ – end) of left foot] applied to "tetanic" extremity during the general tetanus stage (phenomenon of generalization of excitation); f) electromyograms of the same muscles with analogous stimulations applied to opposite extremity. Curves show: 1) Back muscles; 2) left gastrocnemius muscle; 3) right gastrocnemius muscle.

It is evidently correct, however, that it is more difficult to induce the disease in dogs, by injecting the toxin into the nerve than it is in other animals [16]. It may be that the supposed species characteristics of nerve trunk structure, specifically those of a dog's nerve, become especially significant under conditions of the intraneural method of administration and in some way hinder the entrance of the toxin into the spinal cord.

However that may be, it is important to stress that neither Abel nor his adherents attempted to determine the toxin directly in the neural conductors. As for the works of other authors who have studied this question, their experiments, unfortunately, were limited to attempts to demonstrate the toxin in the nerve trunk [1, 7, 12, 14, 17, 18, 21], where there are many elements which have no direct bearing on the mechanism conducting the toxin to the spinal cord; none of these investigations determined toxin in the neural conductors above the spinal ganglia.

The results obtained in this work and in previous investigations [5, 6] allow one to regard the question of the neural route of the toxin's passage into the spinal cord during the development of ascending tetanus as decisively solved. The data presented concur with the results of our previous investigations [4] and with those of other authors [16], according to which preliminary transection of the anterior roots prevents the development of ascending general tetanus after the injection of the toxin into the muscles of the de-efferentized extremity, when the circulatory route of the toxin's spread is blocked by tetanus antitoxin.

SUMMARY

As shown by experiments on monkeys (table 1) and dogs (table 2), the main and direct route of passage of the tetanus toxin from the muscles into the spinal cord are the anterior spinal roots. This result coincides completely with the data of previous investigations on albino rats, guinea pigs, rabbits and cats [4,5,6]. After blocking the circulatory route of spread of the toxin by means of antiserum, the administration of toxin into the muscles of the posterior extremity causes a fatal ascending tetanus in dogs (Fig.2); by the time of the animal's death the antitoxin may circulate in the animal's blood (table 3). Thus, the spread of the toxin with the blood is not a required condition for the development of general fatal tetanus. However, this factor plays an important role in determining the clinical form and the outcome of the disease. Both in the monkeys (Fig.1, d,f) and in dogs (Fig. 2, f, e) there is present a phenomenon of generalization of excitation in the central nervous system upon stimulation of the extremity into which the toxin has been introduced; this phenomenon is characteristic of the ascending general tetanus and was described by the authors earlier (3). A discussion is given on the clinical forms of the disease and the route of the tetanus spread of toxin in connection with the current concepts on the pathogenesis of tetanus.

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