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

PART II: EXPERIMENTS ON MICE, GUINEA PIGS, RABBITS AND CATS

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In a previous report [5] we were able to show that the anterior roots represent the main route of entrance of tetanus toxin from the muscles into the spinal cord. Within a certain time after the injection of the toxin into the corresponding muscles, considerable quantities of toxin can be found in the tissue of the anterior roots whereas at the same time no appreciable quantity of toxin could be found under similar experimental conditions in the posterior roots. These findings fully coincide with the results of our previous investigations [2,3,4] and also with the investigations of other authors [11] in which it was established that transection of the anterior roots which latter supply the motor innervation of the posterior limbs prevents the development of ascendent tetanus after the injection of toxin into the muscles of the corresponding limb, provided the spread of the toxin through the blood is prevented by anti-tetanus serum. Transection of the posterior roots had no such effect: after injection of the toxin into the muscles of a deafferentiated limb and simultaneous blockage of the hematogenous route by antitoxin, typical ascending tetanus developed. All our previous investigations had been carried out on white rats. To establish whether our findings represent general principles governing the development of ascendent tetanus or represent only special features characterizing the development of that illness in white rats we had to carry out similar experiments concerning the estimation of the amount of toxin present in the nervous tissues and roots of other laboratory animals.

TABLE 1. Content of Tetanus Toxin in the Sciatic Nerves of White Mice 18 Hours after Injection of 15 Mild MLD Toxin into the Muscles of the Leg

Material investigated	Clinical course of the illness in mice after injection of the material			
	Number of experiment			
	1	2	3	4
Sciatic nerve at the site of injection of the toxin	±	++	$Ex_{6,7}$	$Ex_{6,7}$
Sciatic nerve on the opposite side	-	-	-	-
Content of toxin in the blood serum	1MLD/ml	1MLD/ml	2MLD/ml	4MLD/ml

Conditions characterizing the clinical course of the illness in mice for the above and the subsequent Tables: ± sign, slight but marked increase of the extensor tonus; one + marked extensor tonus; ++ local tetanus, +++ local tetanus with symptoms of general tetanus; ++++ general tetanus; Ex_4, Ex_5, Ex_6: Death on the 4th, 5th and 6th day respectively.

TABLE 2. Content of Toxin in the Spinal Roots, Spinal Ganglia and Nerves of the Posterior Limbs in Guinea Pigs after Injection of Toxin into the Leg Muscles.

Material investigated	Number of experiment							
	1		2		3		4	
	Quantity of material injected (in mg)	Clinical picture	Quantity of material injected (in mg)	Clinical picture	Quantity of material injected (in mg)	Clinical picture	Quantity of material injected (in mg)	Clinical picture
Site of injection of toxin								
Anterior roots	17	+	40	Ex_4	20	+++	40	Ex_6
Posterior roots	24	-	34	-	40	-	40	-
Spinal ganglia	n.i.		36	Ex_4	40	+	40	+
Sciatic nerve, Upper half	n.i.		40	++++	40	++++	40	+
Sciatic nerve, Lower half			40	Ex_4	40	Ex_4	40	Ex_6
Femoral nerve	n.i.		40	±	40	+	40	±
Opposite side								
Anterior roots	25	-	36	-	40	-	40	-
Posterior roots	23	-	34	-	40	-	40	-
Spinal ganglia	n.i.		32	-	40	-	40	-
Sciatic nerve	n.i.		40	-	40	-	40	-
Content of toxin in the blood serum	n.i.		4MLD/ml		2MLD/ml		8MLD/ml	

Remark n.i.: Not investigated.

As the mechanism of action of tetanus toxin and its route, the routes of its entrance into the central nervous system have not yet been finally established and as the literature concerning these questions [2,5] is of extremely contradictory character the above investigations are, from a comparative point of view, of great importance.

In the present paper we report the results of studies concerning the content of tetanus toxin in nerves, roots, spinal ganglia and in the blood of the most important species of laboratory animals (guinea pigs, rabbits, mice and cats) after injection of the toxin into the muscles of the posterior limb.

EXPERIMENTAL METHODS

Those anterior and posterior roots of the spinal cord were selected for the investigation, the fibers of which are included into the trunk of the sciatic nerve: In guinea pigs - L5 and L6, in rabbits - L7, S1 and in cats - L7 and S1. The spinal ganglia in which the presence of toxin was investigated corresponded to the roots mentioned above. In the experiments on rabbits and cats we further studied the content of toxin in a small area of the sensory part of the lumbar plexus, the branches of which originate directly from the spinal ganglia. The area in question was thoroughly separated from the motor pathways at such a distance from the spinal ganglia which enabled us to carry out free preparation of the motor and sensory part of the nerve respectively. For the sake of shortness the areas of the sensory part of the branches of the lumbar plexus, investigated by us, which are included into the sciatic nerve, were in the Tables simply referred to as the sensory part of the sciatic nerve. Due to the small size of the ganglia and roots in mice in these animals the toxin was estimated only in the sciatic nerve.

In the experiments on cats we investigated material taken separately from each animal. In the experiments on other animals the material was, in view of its inadequate quantity, treated summarily: In rabbits material was taken from two animals, in guinea pigs from four animals and in mice from 10-15 animals.

TABLE 3. Content of Tetanus Toxin in the Spinal Roots, Spinal Ganglions and Nerves of the Posterior Extremities in Rabbits after Injection of Toxin into the Leg Muscles.

Material investigated	Number of experiment 1		2		3		4		5		6	
	Quantity of material injected (in mg)	Clinical course	Quantity of material injected (in mg)	Clinical course	Quantity of material injected (in mg)	Clinical course	Quantity of material injected (in mg)	Clinical course	Quantity of material injected (in mg)	Clinical course	Quantity of material injected (in mg)	Clinical course
Side of injection of the toxin												
Anterior roots	40	+	40	+	40	+	40	++	40	+	40	+
Posterior roots	40	-	40	-	40	-	40	-	50	-	40	-
Spinal ganglia	40	-	40	-	40	-	40	+	50	+	60	±
Sciatic nerve, Upper half	40	±	40	±	40	+	40	++	40	+	40	Ex_4
Sciatic nerve, Lower half	40	++	40	++	40	+	40	Ex_5	40	++	40	Ex_3
Sensory part of the sciatic nerve	n.i.		40	-	40	-	40	-	45	-	40	-
Femoral nerve	40	-	40	-	40	-	40	-	40	-	40	-
Opposite side												
Anterior roots of the sciatic nerve	40	-	40	-	40	-	40	-	40	-	40	-
Posterior roots of the sciatic nerve	40	-	40	-	40	-	40	-	40	-	40	-
Spinal ganglia	n.i.		n.i.		40	-	40	-	50	-	50	-
Sciatic nerve	40	-	40	-	40	-	40	-	40	-	40	-
Sensory part of the sciatic nerve	n.i.		n.i.		40	-	40	-	50	-	40	-
Concentration of toxin in the blood serum	<1MLD/ml		<1MLD/ml		<1MLD/ml		<1MLD/ml		n.i.		n.i.	

The toxin was injected into the left M. gastrocnemius in the following doses: For guinea pigs about 100 MLD, for rabbits 25 MLD, for cats $2\frac{1}{2}$ MLD and for mice 15 MLD. In some cases the toxin was injected at several points into the M. gastrocnemius and into the anterior group of crural muscles to secure simultaneous involvement of the greatest possible number of muscles and the corresponding nerve fibers into the process. The material used for investigation was collected from mice and guinea pigs 15-18 hours and from rabbits and cats within 24 hours after the injection of the toxin.

The details of the experiments were fully described in our previous communication [5].

EXPERIMENTAL RESULTS

The results of the experiments are set forth in Table 1-4. The Tables show that tetanus toxin was found in considerable quantities in the sciatic nerve of all animals and in guinea pigs, rabbits and cats in which the corresponding investigations were carried out. The toxin was also found in the anterior roots at the site of administration. The results of these experiments fully coincide with the results of similar investigations on white rats [5] and thus show that the progression of the toxin along the nerve and its entrance into the spinal cord through the anterior roots represents a general rule. The high degree of consistency in all experimental results deserves attention: In all cases and in all animals toxin was found in the trunk of the sciatic nerve and in the anterior roots. This fact underlines the universal character of the phenomenon discovered by us and confirms our earlier conclusions [2] that the entrance of the toxin into the spinal cord along the anterior roots constitutes a prerequisite for the development of ascending tetanus.

TABLE 4. Content of Tetanus Toxin in the Spinal Roots, the Spinal Ganglia and the Nerves of the Posterior Extremity in Cats after Injection of the Toxin into the Leg Muscles.

Number of cat	Clinical course of the illness in mice after injection of the material investigated										Concentration of toxin in the blood serum (in MLD/ml)
	Material from the side of injection of the toxin						Material from the opposite side				
	Anterior roots	Posterior roots	Spinal ganglia	Sensory part of the sciatic nerve	Sciatic nerve	Femoral nerve	Anterior roots	Posterior roots	Spinal ganglia	Sciatic nerve	
1	++	-	-	-	Ex_2, Ex_5	+	-	-	-	-	>4
2	±	-	±	-	Ex_2, Ex_3	-	-	-	-	-	>5
3	++	-	±	n.i.	Ex_2, Ex_4	+	-	+	±	Ex_2	10
4	+	±	±	n.i.	Ex_3	+	-	+	+	-	n.i.
5	+	-	+	n.i.	Ex_5	-	-	-	+	-	n.i.
6	+	-	±	n.i.	Ex_2	-	-	-	+	-	>10
7	Ex_6	-	Ex_7	n.i.	+	-	-	-	-	-	>6<8
8	+	-	-	-	+	-	-	-	+	-	80
9	++	-	++	-	+	-	-	±	+	-	>80
10	+	++	+	+	+	-	-	-	-	-	n.i.
11	++	-	-	-	Ex_2	-	-	-	-	-	20
12	++	-	+	-	Ex_2	±	-	-	-	-	40
13	++	-	+	-	Ex_2	+	-	-	-	-	50
14	+	-	+	-	Ex_3	-	-	-	-	-	n.i.

At the same time tetanus toxin was never found in the posterior roots of guinea pigs and rabbits. The fact that in rare cases it could be found in cats in the posterior roots (see Table 4) cannot be regarded as a regular and characteristic phenomenon for these animals as statistical evaluation of the findings shows that the results obtained are insignificant and the detection of toxin in the posterior roots in the above cases must be due to individual features of some of the animals.

This conclusion is consistent with the results obtained in that part of the experiment which concerned the content of toxin in the sensory part of the sciatic nerve: here toxin was as a rule not found. Its detection in a single case (the result is statistically insignificant) shows that some temporary factors may possibly play a role.

The results of the experiments concerning the toxin estimation in the spinal ganglia at the site of injection showed exactly the opposite: here toxin was found regularly. From this point of view the result of the above experiments coincides with the results of similar investigations on white rats [5].

With regard to the route of entrance of the toxin into the ganglions various assumptions could be raised. Inasmuch toxin is not found in the sensory part of the nerve which enters directly into the ganglion it might be thought that the bundle of afferent pathways does not represent the main route of entrance of the toxin into the ganglions. It is, however, possible that toxin enters along the afferent pathways in small quantities and is retained in the ganglions where it accumulates. It can also be assumed that toxin enters the ganglion along the sheaths of the nerve which are continued over the ganglion or by way of certain connections with the nearby bundle of efferent fibers (anterior root).

As far as the possible entrance of the toxin into the ganglion from the blood is concerned this question deserves particular attention. In white rats [5], guinea pigs (see Table 2) and rabbits (see Table 3), tetanus toxin is found after injection in selected doses into the leg muscles only in the spinal ganglia at the side of injection. This fact indicates the existence of regional mechanisms governing its entrance into the ganglia. In cats (see Table 4) toxin can be found, in addition to its regular occurrence in the spinal ganglia at the side of injection, in almost one half of the cases in the ganglia of the opposite side. It can consequently be assumed that in cats the hematogenic route of entrance of toxin into the ganglion may play a role in addition to the regional mechanism.

It is finally a fact deserving attention that in guinea pigs (see Table 2), cats (see Table 4), and also in white rats [5], toxin can be found not only in the sciatic nerve which innervates the leg muscles, i.e., the site of injection but also in other nerves of the extremity, e.g., the femoral nerve. Inasmuch as toxin does not enter the nerve trunks through the blood (as shown by the results of investigations of the nervous fibers at the side opposite the side of toxin injection) it can be assumed that toxin entered the other nerves of the "tetanus" extremity due to its infiltration into neighboring muscle from the site of injection. In this case the overlapping of the innervation of various muscles in the limb may also play a part.

Comparing the results of our investigations with the existing data in the literature it must be emphasized that the possible transport of the toxin along the motor nerves was shown and postulated by numerous authors [1,6,9,11,12, 13,15-19]. The conclusions concerning this route of transport of the toxin, however, were based on the fact that the latter was found only in the trunk of peripheral nerves. In this context the term motor nerve was practically identified with the term muscular nerve. A systematic and differentiated investigation of various parts of the peripheral nervous system, including attempts to estimate the toxin directly in the spinal roots, was never carried out. This fact and also the well-known methodical difficulties and omissions [8] committed by the authors in addition to the publication of papers which denied the progression of the toxin along the nerves and the importance of this mechanism in the pathogenesis of tetanus [7,8,19,14] rendered the problem in question the object of vivid discussions.

The results of our investigations including the findings of our previous studies [2,5] warrant the conclusion that tetanus toxin does indeed move along the muscular nerves and show that the anterior roots represent the main route of entrance of the toxin into the spinal cord during the development of ascendent tetanus.

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