THE RELATIONSHIP OF TOXIN AND ANTITOXIN INJECTION SITE TO TETANUS DEVELOPMENT IN THE RAT*

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Despite continuous clinical and experimental investigations, there is lack of agreement on some of the most important aspects of tetanus. The main interest centers around three problems: the mode and site of action of tetanus toxin, the dissemination in the body, and the type of rational treatment to be adopted in order to alleviate the persistent high mortality rate in non-immunized patients.

Information gained from investigations of tetanus has thrown much light on the pathogenesis of other neurotropic diseases (Wright, G.P., 1956). It is apparent from reviews of the literature (Pelloja, 1951, Fedinec and Matzke, 1958) that considerable controversy exists over the route followed by the toxin from the site of injection or infection to the central nervous system. The following pathways have been suggested by various workers: the nerve fibers, the endoneurial tissue spaces, the perineurial lymphatics, the vascular stream, and a combination of any two or more of the above. Previous experiments (Fedinec and Matzke, 1958, 1959 a, and Schellenberg and Matzke, 1958) have supported the theory that the tissue spaces of the peripheral nerve trunks are the most likely route of tetanus toxin dissemination. The spread of the toxin via the circulatory system, however, was not excluded.

The present investigation was undertaken in an attempt to demonstrate the relationship of the site of tetanus toxin injection to the degree of intoxication in an experimental animal, and the relationship of the same sites of injection to the effectiveness of antitoxin blockage of intramuscularly injected toxin. The evaluation of these results in the light of recent experimental data should be indicative of the most likely route of tetanus toxin dispersal in the body.

Materials and Methods

Adult rats from a highly inbred Sprague-Dawley strain were used in all the experiments. Diet, temperature, and space allowed for movement were kept uniform.

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A 5 per cent solution of evipal soluble administered intraperitoneally was used as the anesthetic. The dosage was 0.3 ml./100 gm. body weight. This anesthetic does not interfere appreciably with the development of tetanus symptoms. The tetanus toxin used had a potency of 600,000 MLD/ml., and the antitoxin, 4,500 u.s.v./ml. Dilutions were prepared with triple distilled water and used within ½ hour. An “agla” micrometer syringe mounted with either a 33 or 31 gauge hypodermic needle was used in all toxin and antitoxin injections. Experimental animals were observed hourly.

The direct method of graded dilutions was used to determine the minimum dose of the toxin used which would kill 100 per cent of the animals injected in 144 hours (MLD). Seven groups of 12 animals each were injected intramuscularly (IM). The MLD was found to be 0.001 ml./100 gm. body weight of a 1:25 dilution of toxin.

Five groups of 10 animals each were injected with 1 MLD of toxin into the right femoral vein. Immediately 0.001 ml./100 gm. body weight of various dilutions of antitoxin was injected into the left femoral vein. In this way the “minimal protective dose” (MPD) of antitoxin that would prevent any symptoms of tetanus was determined. The MPD of antitoxin for 1 MLD of toxin was found to be 0.001 ml./100 gm. body weight of a 1:1000 dilution of antitoxin.

EXPERIMENTAL PROCEDURES AND RESULTS

Experiment 1.—Relative Effectiveness of Tetanus Toxin Injection Sites.—

Seven groups of 10 animals each were injected with 3 MLD of tetanus toxin at the following sites: femoral vein, gastrocnemius muscle, endoneurium of the sciatic nerve, epineurium of the sciatic nerve, spinal cord, cerebrospinal fluid, and subdural space. All injections were made on the right side.

The femoral vein was surgically exposed in the femoral triangle. The gastrocnemius muscle was injected through the skin. Except for intramuscular and intravenous injections, all injections were made with the aid of a binocular microscope.

Two days before endoneurial and epineurial injections the femoral, genitofemoral, and obturator nerves were cut in the following manner: an incision was made through the linea semilunaris parallel to the fibers of the rectus abdominis muscle; the posterior abdominal wall was observed and the nerves exposed with blunt dissection. The nerve to the hamstring muscles was ligated and cut at the time of injection, leaving the sciatic as the only intact nerve in the limb. Endoneurial and epineurial injections were made at the mid-femoral level of the sciatic nerve. The small amount of fluid used, and the fact that it was injected slowly and directed peripherally, prevented distention of the nerve fascicle and rupture of its perineurium.

The spinal cord was visualized by performing a partial laminectomy in the lower thoracic region. The needle was then introduced through the translucent dura into the dorsal funiculus of the spinal cord. The spinal level ranged between thoracic segments 7 and 10.

A similar surgical approach was made for cerebrospinal fluid and subdural injections. Usually there was a noticeable difference in the ease with which an injection was made into the cerebrospinal fluid as compared to the slight resistance encountered in subdural injection. Extreme care was taken to prevent leakage. The tip of the needle was always kept in place for a few minutes after injection. Upon withdrawal of the needle a piece of thin filter paper and gelfoam sponge were held in place over the puncture hole for several minutes. Animals with noticeable escape of fluid were discarded.

Fig. 1 presents an organization of the experimental groups according to the length of latent, symptomatic, and survival periods. Table 1 presents the time of appearance of symptoms of the various forms of tetanus and the survival times. The latent period is the
Fig. 1. Relative effectiveness of tetanus toxin injection sites.

Abbreviations used in Figs. 1 and 2.

Injection sites

V. —Femoral vein.
MS. —Gastrocnemius muscle—single injection.
MM. —Gastrocnemius muscle—multiple injection.
ENN. —Endoneurium of the sciatic nerve.

EPN. —Epineurium of the sciatic nerve.
SCT. —Spinal cord thoracic segments 7 to 10.
SCL. —Spinal cord lumbar enlargement.
CSF. —Cerebrospinal fluid.
SDS. —Subdural space.

TABLE I

Effect of Tetanus Toxin Injection at Various Sites

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Toxin injection site</th>
<th>Local tetanus</th>
<th>Bloodborne tetanus</th>
<th>Tetanus dolorosus</th>
<th>Dorsal tetanus</th>
<th>Survival time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>Vein</td>
<td>—</td>
<td>37</td>
<td>—</td>
<td>—</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>Muscle</td>
<td>19½</td>
<td>—</td>
<td>56</td>
<td>56</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>Endoneurium‡</td>
<td>22½</td>
<td>—</td>
<td>92</td>
<td>78</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>Epineurium‡</td>
<td>24½</td>
<td>44½</td>
<td>—</td>
<td>—</td>
<td>6½</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>Spinal cord</td>
<td>—</td>
<td>—</td>
<td>20½</td>
<td>20½</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>Cerebrospinal fluid</td>
<td>—</td>
<td>48</td>
<td>72½</td>
<td>72½</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>Subdural space</td>
<td>—</td>
<td>57</td>
<td>53½</td>
<td>53½</td>
<td>—</td>
</tr>
</tbody>
</table>

Dosage: 3 MLD (0.003 ml./100 gm. body weight of 1:25 dilution).

* Hours after injection; averages calculated to the nearest ½ hour.

‡ Leg denervated except for sciatic nerve.
time elapsed between the toxin injection and the appearance of the first symptoms. The symptomatic period is the period of time between the onset of initial symptoms of tetanus and death. The survival period comprises the total number of hours between toxin inoculation and death.

**Group 1.**—The term “blood-borne tetanus” is here used to designate the condition which follows intravascular (IV) injection of toxin. In the literature this is frequently referred to as “general tetanus.” It seems more appropriate, however, to consider general tetanus as the final stage in several forms of tetanus.

The first symptoms of blood-borne tetanus appeared 36 to 40 hours after IV toxin injection. This was manifested in mild spasticity of the axial musculature and a cephalic syndrome consisting of trismus, immobilization of vibrissae, depression of the ears, and slight contraction of orbital and facial muscles. Within 41 to 43 hours the condition became generalized and the animals assumed the posture of emprosthotonus. Locomotion was limited and the extremities extended, but usually not spastic. Splanchnic manifestations were seldom pronounced. Blood appeared in the nostrils and eyes; marked dyspnea accompanied by wheezing (respiratory distress) ensued, and the animals died with general convulsions in 45 to 58 hours.

From the beginning of general involvement convulsions could be elicited by pinching or even touching the animal at any point on the body. As the disease progressed the frequency, duration, and severity of the convulsions were increased. During convulsions the animal assumed the position of opisthotonos.

**Group 2.**—Following intramuscular (IM) injection of tetanus toxin local tetanus appeared in 17 to 28 hours. The affected leg was held in flexion and touched the table only occasionally when the animal walked. The toes were extended. Pronounced local spasticity appeared first in the inoculated muscles; as other muscle groups became involved the leg was rigidly extended at all joints.

Within 29 to 38 hours the tail, extremity, and hip deviated to the injected side. This lumbar scoliosis was temporary, disappearing as the musculature of the opposite side was affected. Both extremities assumed a parallel extended position after the involvement of the dorsal and thoracic musculature became more marked.

Spasticity of the abdominal and thoracic musculature and flexor muscles of the spine and neck produced emprosthotonus in 42 to 49 hours. As the disease progressed the animals became very sensitive. A “hunch-back” or “buffalo neck” posture was typical of the final stage of ascending tetanus. The moribund animal presented a cephalic syndrome (trismus, blood in nostrils, etc.) similar to that observed in blood-borne tetanus. Splanchnic manifestations, such as urinary incontinence, defecation, and ejaculation...
in males, were present. Tachycardia, dyspnea, cyanosis, and convulsions were observed. Death occurred in 47 to 68 hours.

In the early stages of ascending tetanus, convulsions were more easily elicited from the inoculated extremity. In the generalized stage, however, convulsions could be produced by touching the animal at any site.

**Group 3.**—The sequence of symptoms of ascending tetanus following endoneurial toxin injection resembled that which followed IM inoculation. The latent period was 20 to 27 hours, and the survival period 80 to 104 hours.

**Group 4.**—Local tetanus appeared 22 to 25 hours after epineurial injection of toxin. Shortly after the appearance of lumbar scoliosis in 35 to 47 hours, blood-borne tetanus was superimposed upon the ascending tetanus (38 to 62 hours). The animals died with general convulsions in 69 to 90 hours.

**Group 5.**—Following toxin injection into sensory areas of the thoracic spinal cord, signs of tetanus dolorosus appeared within 4 to 8 hours. The exaggerated hypersensitivity which persisted from the beginning of symptoms produced an animal which had to be isolated and handled with extreme caution. They attacked any animal or objects with which they came in contact. Hypersensitivity appeared to be referred most intensely to the dermatomes innervated by the inoculated spinal segments.

There was no motor involvement of the head and neck musculature. General convulsions appeared before death, which occurred within 12 to 25 hours. Mild local symptoms in the extremities and axial spasticity, suggesting motor involvement, appeared only in the three animals that survived for 23 to 25 hours.

**Group 6.**—The inoculation of toxin into cerebrospinal fluid was followed by the appearance of blood-borne tetanus in 40 to 59 hours. The sequence and nature of the symptoms resembled the blood-borne tetanus observed after IV toxin injection in Group 1. All animals died within 64 to 81 hours.

**Group 7.**—Injection of toxin into the subdural space resulted in the appearance of “dorsal tetanus” in 44 to 62 hours, and also blood-borne tetanus in 52 to 64 hours in all animals. The dorsal tetanus manifested itself in a board-like stiffness of the axial musculature. When blood-borne symptoms became superimposed, the typical signs of trismus, paralysis of vibrissae, and depression of ears appeared. The terminal stage of the condition resembled, in all respects, the general stage of blood-borne tetanus. The animals died within 54 to 109 hours in general convulsions and respiratory distress.

**Experiment 2.—Relative Effectiveness of Tetanus Antitoxin Injection Sites.**—

A control group consisting of 10 animals was injected with 3 MLD of tetanus toxin and 3 MPD of antitoxin each in opposite femoral veins. No symptoms of tetanus developed, and all animals survived.
Eighty experimental animals were divided into 7 groups. All groups consisted of 10 animals each, except that the intramuscularly and intraspinally inoculated groups of 15 animals each. Each group represented one of the following sites of antitoxin injection: femoral vein, gastrocnemius muscle, endoneurium of the sciatic nerve, epineurium of the sciatic nerve, spinal cord, cerebrospinal fluid, and subdural space.

The IM injected group was subdivided into Group 2 A, 10 animals receiving the antitoxin in a single injection; and group 2 B, 5 animals that received the same amount of antitoxin in multiple injections administered within a period of 2 minutes.

The intraspinally injected animals comprised Group 5 A (10 animals) which received the antitoxin in the dorsal columns of thoracic segments 7 to 10, and Group 5 B (5 animals) which received the antitoxin in the lumbar enlargement.

The exposure of sites of antitoxin injection was similar to the procedures described in Experiment 1. The volumes of toxin and antitoxin given were identical: 0.003 ml./100 gm. body weight. The toxin dosage was 3 MLD in all cases, and was administered into the gastrocnemius muscle. Three MPD of antitoxin were given homolaterally within 1 to 5 minutes after toxin injection, at the sites outlined above.

Fig. 2 represents an organization of the experimental groups according to the length of latent, symptomatic period and death, and indicates which groups survived beyond 220 hours after injection. Table II presents the time of appearance of symptoms of the various types of tetanus, the survival times, and the last symptoms of those animals which survived.

**Group 1.**—Injection of antitoxin into the left femoral vein following toxin injection into the right gastrocnemius muscle did not prevent development of tetanus symptoms. Local tetanus appeared within 18 to 25 hours. A slowly progressing ascending tetanus became arrested in 116 to 165 hours at emprosthotonus. All animals survived.

**Group 2 A.**—When a single IM antitoxin injection was administered approximately into the same region of IM toxin injection, local tetanus developed in 19 to 26 hours. Further development of tetanus was arrested in 50 to 91 hours, when the animals exhibited a spastic leg extended at all joints. The tail deviated to the side of the involved extremity.

**Group 2 B.**—After multiple antitoxin injections into the region of IM toxin injection, the first signs of local tetanus were delayed to 26 to 30 hours. A spastic, extended extremity was produced in 102 to 128 hours, with no deviation of the tail. There was no further development of ascending tetanus. All the animals in Groups 2 A and 2 B survived.

**Group 3.**—The first signs of local tetanus appeared within 21 to 31 hours following antitoxin injection into the endoneurium of the sciatic nerve and IV toxin injection. Gradually emprosthotonus developed. At this time (80 to 103 hours) blood-borne tetanus became superimposed upon the ascending tetanus. All animals died in general tetanus within 114 to 196 hours.

**Group 4.**—Antitoxin inoculation into the epineurium after IM toxin injection was followed by the first signs of local tetanus in 19 to 21 hours. Slowly developing ascending tetanus became generalized, and all animals died within 186 to 251 hours.
TABLE II

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Antitoxin injection site</th>
<th>Local tetanus</th>
<th>Blood-borne tetanus</th>
<th>Survival time</th>
<th>Last symptoms of surviving animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>Vein</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2 A</td>
<td>10</td>
<td>Muscle, single injection</td>
<td>22 1/4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2 B</td>
<td>5</td>
<td>Muscle, multiple injection</td>
<td>28</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>Endoneurium‡</td>
<td>26 1/4</td>
<td>86 1/4</td>
<td>151 1/4</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>Epineurium‡</td>
<td>20 1/4</td>
<td>-</td>
<td>214 1/4</td>
<td>-</td>
</tr>
<tr>
<td>5 A</td>
<td>10</td>
<td>Spinal cord, thoracic segments 7-10</td>
<td>23 1/4</td>
<td>-</td>
<td>(8) 196 1/4</td>
<td>(2) Scoliosis</td>
</tr>
<tr>
<td>5 B</td>
<td>5</td>
<td>Spinal cord, lumbar enlargement</td>
<td>26 1/4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>Cerebrospinal fluid</td>
<td>28 1/4</td>
<td>-</td>
<td>105 1/4</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>Subdural space</td>
<td>24 1/4</td>
<td>-</td>
<td>78 1/4</td>
<td>-</td>
</tr>
</tbody>
</table>

Toxin dosage: 3 MLD (0.003 ml./100 gm body weight of 1:25 dilution; injected intramuscularly).
Antitoxin dosage: 3 MPD (0.003 ml./100 gm body weight of 1:1,000 dilution).
* Hours after injection; averages calculated to the nearest 1/4 hour.
‡ Leg denervated except for sciatic nerve.
( ) Number of animals from total of 10.
In the animals of Groups 3 and 4 all nerves to the injected extremity, excepting the sciatic, were cut as described in Experiment 1.

**Group 5 A.**—Following IM toxin injection all animals were injected with antitoxin intraspinally at thoracic levels 7 to 10. Local tetanus appeared in 21 to 26 hours. The progress of ascending tetanus was halted at the approximate spinal segment level at which the antitoxin was injected. At this time, 69 to 97 hours, all animals exhibited lumbar scoliosis. In most animals dorsal tetanus developed and severe splanchnic manifestations were present below the injected spinal region. The general condition rapidly deteriorated. Emprosthotonus was not observed; however, a few animals had blood in the nostrils, trismus, and paralysis of vibrissae. Eight animals died in 119 to 278 hours; the surviving animals continued to maintain lumbar scoliosis.

**Group 5 B.**—Injection of antitoxin in the lumbar enlargement after IM toxin injection rendered a better protection than antitoxin injection at thoracic levels. Although all animals developed local tetanus in 25 to 28 hours, ascending tetanus did not proceed beyond spastic extension of the extremity in 42 to 48 hours; all animals survived.

**Group 6.**—Following cerebrospinal fluid injection of antitoxin and IM toxin injection local tetanus developed in 24 to 32 hours. The ensuing ascending tetanus became generalized and all animals died within 79 to 143 hours.

**Group 7.**—Subdural antitoxin injection and IM toxin injection were followed by local tetanus in 22 to 27 hours. Ascending tetanus developed, and all animals died in general tetanus within 64 to 85 hours.

**DISCUSSION**

Comparative injection of tetanus toxin at various sites in the body has been performed by numerous authors. As determined by them, the following sites may be ranked from greatest to least sensitivity: medulla oblongata, spinal cord, peripheral nerves, muscle, and blood. There are, however, no conclusive data on injections of toxin into the cerebrospinal fluid and subdural space (Firor, Lamont, and Shumacker, 1940; Wright, E. A., 1953; and Imbriano, 1950 b). Blocking injected toxin with antitoxin, injected at various sites in the body, has led to controversial conclusions which have served to fortify diverging theories on the tetanus toxin dispersal in the body (Meyer and Ransom, 1903; Permin, 1914; Teale and Embleton, 1919–20; Abel, Hampil, and Jonas, 1935; Friedemann and Traub, 1949; Wright, E. A. et al., 1951; Yokoi, 1954; Málek et al., 1957; Fedinec, 1958; and others).

**Toxin and Antitoxin Dispersal in the Central Nervous System.**—In accordance with generally accepted views that the CNS is the site of tetanus toxin action, it was observed in Experiment 1 that the shortest latent period followed intraspinial injection of tetanus toxin. The symptoms of tetanus dolorosus appeared in approximately 6 hours and death ensued within 12 to 25 hours. The appearance of tetanus dolorosus was similar to that described by Meyer and Ransom (1903) and Fletcher (1903).
Brooks, Curtis, and Eccles (1957) concluded that the spread of toxin across the cord is substantially slower than its longitudinal spread. The sequence of symptoms in ascending tetanus produced by IM toxin injection provides further evidence for this conclusion, for spinal segments cephalad to the lumbar region became involved before the contralateral extremity.

Following toxin injection into the dorsal funiculus of the cord, we observed, as did Firor and Jonas (1938), that early death prevents development of motor symptoms. The animals developed sensory tetanus and died in general convulsions. Death was probably due to respiratory failure resulting from longitudinal spread of the toxin to the vital centers. The rigid extremities and generalized spasticity so characteristic of motor tetanus were not present. Only in the 3 animals surviving 23 to 25 hours was there motor involvement that indicated partial diffusion of the toxin into the motor areas of the cord.

Perrin (1914) found that the spinal cord was the most effective site of antitoxin injection in order to prevent death. He reported that local tetanus could be prevented following toxin injection of the hamstring muscles if antitoxin were injected into the homolateral ventral horn cells within 7 hours. The effectiveness of intraspinal injection of antitoxin was further attested by Friedemann and Traub (1949), who pointed out that the quantity of antitoxin needed to save an animal injected subcutaneously with tetanus toxin is considerably reduced if the antitoxin is introduced into the spinal cord rather than into a vein. Since the work of Meyer and Ransom (1903), however, it has been generally accepted that once a lethal dose of tetanus toxin is fixed by the nervous tissue no circulating antitoxin can neutralize it.

In the present experiments, antitoxin injection into the dorsal funiculus of the spinal cord at thoracic levels, 7 to 10 did not prevent local tetanus after homolateral toxin injection into the gastrocnemius muscle (Experiment 2, Group 5 A). The average latent period of 26 hours did not differ markedly from the latent period in all the other groups injected with antitoxin (Fig. 2). Probably the ascent of the tetanus toxin to the cord and its fixation in the lumbar region was not blocked.

The survival period, however, was prolonged, and 8 out of 10 animals died within 119 to 278 hours. Splanchnic and motor symptoms below the antitoxin inoculated segment were pronounced.

In contrast, the animals receiving the same dose of antitoxin in the lumbar enlargement (Group 5 B) developed only local tetanus and all survived. The progress of tetanus manifestations were considerably slowed or arrested at the site of antitoxin administration.

It appears that antitoxin injection into the dorsal funiculus of the thoracic spinal cord does not prevent death for the same reason toxin injected into the dorsal funiculus of the thoracic cord does not produce motor tetanus: the injected medium diffuses only very slowly dorsoventrally in the cord. The antitoxin is of higher molecular weight, and probably diffused even more slowly than the toxin, both longitudinally and dorso-ventrally. The lumbar injections of antitoxin were more effective because the toxin, injected into
the muscles of the extremity, was transported via the peripheral nerve trunks into the lumbar region of the cord.

Dispersal of Toxin and Antitoxin from the Periphery.—

Matzke and his coworkers (Matzke and Fedinec, 1955; Matzke, Fedinec, and Riggs, 1957; Schellenberg and Matzke, 1958; Fedinec and Matzke, 1958, 1959 a, 1959 b; and Fedinec, 1958) have investigated the spread of tetanus toxin in white rats and concluded that the tissue spaces of the peripheral nerve trunks are the most likely routes involved.

Evidence presented by Wright, G. P. (1953), Röblitch and Weiss (1955), Lehmann (1957), and others suggest that the tissue spaces within peripheral nerves are filled with tissue fluid and have no lymphatic vessels. True lymphatics are present in the epineurium (Defrise, 1930).

The tissue fluid within the endoneural space is surrounded by a perilemmic barrier which prevents, or greatly delays, the outward diffusion of substances. It does not, however, affect the inward diffusion of some substances. This is, then, a selectively permeable membrane. The tissue fluid within the nerve fascicle possibly forms a continuum with tissue fluid present in the spaces of the muscles served by that nerve. Centripetal movement of the tissue fluid is dependent on the pressure applied to the tissue fluid system by muscle contraction. These anatomical considerations should be kept in mind in interpreting the results of the experiments discussed below.

Intramuscular Injection of Toxin and Antitoxin.—In Experiment 1 local tetanus appeared following intramuscular, endoneurial, and epineurial toxin injection with average latent periods of 19, 22, and 24 hours, respectively. It seems probable that IM injected toxin is in the most favorable position to ascend to the CNS via the tissue spaces of the nerve trunks of the extremity.

Apparently, toxin is also absorbed from the muscle by lymphatic channels and finally emptied into the general blood circulation. The involvement of the head musculature in generalized ascending tetanus resembles the cephalic syndrome seen in blood-borne tetanus. This suggests that in generalized ascending tetanus where no circulating antitoxin is present, additional toxin from the bloodstream reaches the CNS, shortening the symptomatic and total survival periods. Possible factors in absorption of circulating toxin are discussed below.

In Experiment 2 local tetanus appeared after IM toxin injection despite IM antitoxin injection in the same region. This is not surprising if we realize that complete mixing of separately injected, equal volumes of toxin and antitoxin is not to be expected in muscle tissue under physiological conditions. Multiple IM injections of antitoxin were slightly more effective than single injections, and proved to be the most effective of all injection methods used. All animals given antitoxin IM survived, whereas those given endoneurial or epineurial injections of antitoxin died.
Endoneurial Injections of Toxin and Antitoxin.—Endoneurial injection of tetanus toxin produced the same sequence of symptoms of local and ascending tetanus as did IM toxin injection in Experiment 1. While the average latent period was approximately 3 hours longer than that following IM injection, the symptomatic and survival periods were extended by 34 and 37 hours, respectively (Fig. 1).

The explanation for this may be that the perilemmic barrier of the injected nerve markedly reduces (to an ineffective amount) the outward diffusion of the toxin so that little of it can be absorbed by the lymphatics and carried to the circulation. After endoneurial injection the bulk of the toxin travelled in the direction of minimal resistance; i.e., centripetally along the nerve trunk to the spinal cord and in the cord to the vital centers of the brain. The symptomatic and survival periods in endoneurially induced ascending tetanus were not shortened since additional toxin did not diffuse into the circulation.

The results of Experiment 2 agree to a certain extent with the observations of Teale and Embleton (1919-20) and Abel and his coworkers (1935) that endoneurially injected antitoxin of low concentration is ineffective in blocking IM injected toxin, and does not prevent the development of tetanus symptoms. To explain this peculiar result, an assumption is made that the endoneurially injected antitoxin cannot diffuse through the perilemma, and, as it is a larger molecule than the toxin, its centripetal movement in the tissue spaces of the nerve trunk is slower than the rate of ascent of tetanus toxin.

Two other possibilities may be considered in explaining the appearance of local tetanus. All the antitoxin was injected into one fascicle of the sciatic nerve; the injections were made very slowly and while the fascicle would swell, the restraining perilemmic membrane remained intact as far as could be determined by observation with the binocular microscope. Thus it is conceivable that some of the toxin could ascend the fascicle which contained no antitoxin and reach the cord before the antitoxin. Another possible explanation is that some of the toxin was picked up by epineurial lymphatics and entered the nerve above the level at which the slowly ascending antitoxin was confined, i.e., the perilemmic barrier permits a one way (inward) diffusion of toxin, and very little or no outward diffusion of antitoxin.

Part of the IM injected toxin was presumably absorbed by the circulatory system, whereas the endoneurially injected antitoxin was confined within the nerve with little access to the circulation. Therefore, unblocked circulating toxin was available in sufficient amount to produce superimposition of blood-borne tetanus upon the ascending tetanus. The symptomatic and survival periods were considerably shorter than in animals receiving antitoxin epineurially, furnishing further evidence that the toxin absorbed by the circulation plays an important role in the prognosis of tetanus.

Epineurial Injection of Toxin and Antitoxin.—Epineurial injection of tetanus toxin resulted in the appearance of both local and blood-borne tetanus. The
appearance of local tetanus within approximately 24 hours indicates the spread of the toxin to tissue spaces in the surrounding muscle, with subsequent ascent along the sciatic nerve. It is also conceivable that toxin may pass directly from the epineurium into the endoneurial spaces by permeating the perilemmic barrier.

The blood-borne tetanus is to be expected if it is realized that the epineurium possesses true lymphatic vessels which can quickly absorb the toxin and carry it to the circulation. The shorter symptomatic and survival periods, as compared with endoneurial injections, and the relatively early superimposition of blood-borne tetanus, are indicative of partial dissemination of toxin via the circulation following epineurial injection.

Antitoxin injected into the epineurium following IM toxin injection prevented the development of blood-borne tetanus but not that of local tetanus. It appears significant that the latent period for local tetanus was shorter when toxin was injected IM and antitoxin epineurally than when toxin alone was injected epineurally. This further supports the assumption that a more direct absorption of toxin into endoneurial spaces takes place from the muscle. Epineurally injected antitoxin probably cannot permeate the perilemmic barrier, and therefore can have little effect on preventing local tetanus. Antitoxin injected into the epineurium is apparently absorbed by the lymphatics, and carried into the circulation, where it successfully neutralizes any toxin reaching the bloodstream from the toxin-injected muscle. Thus, following epineurial antitoxin inoculation and IM toxin injection, fatal ascending tetanus developed, uncomplicated by blood-borne tetanus. The symptomatic and total survival periods were considerably longer than those following endoneurial antitoxin inoculation.

On the basis of the results cited in the present experiments the axonal theory of toxin transport is not substantiated. Nevertheless, the excellent descriptions of the progress of the disease by Meyer and Ransom (1903) show similarities to these observations. In view of new physiological data and the present experiments, it does not seem likely that tetanus toxin has a separate peripheral and central action as is postulated by Abel and coworkers (1935) and Penitschka (1953).

These experimental results are in agreement with most of the conclusions of Wright and coworkers (Wright, G.P., 1953 and 1956), suggesting the spread of tetanus toxin via the tissue spaces of the peripheral nerve trunks. It should be emphasized that further clarification is necessary to explain the role of the lymphatics and blood vessels in local tetanus (e.g., see Málek et al., 1957).

The Fate of Toxin and Antitoxin Introduced into the Blood, Cerebrospinal Fluid, and Subdural Space.—The average latent periods for groups injected with tetanus toxin in the vein, spinal fluid, and subdural space were the longest from all groups (37, 48, and 53 hours, respectively). Blood-borne tetanus
developed in all groups. The subdurally injected group had, in addition, symptoms of “dorsal tetanus.”

When antitoxin was given in the vein, spinal fluid, or subdural space following IM toxin injection, only animals receiving antitoxin IV survived.

Intravenous Injections and the Blood-Brain Barrier.—Meyer and Ransom (1903) noticed that following IV injection of tetanus toxin the first muscles to show involvement were the muscles of head and neck, and the axial musculature of the trunk. Shortly after, the condition became generalized. Since these authors supported the axonal carriage of toxin, they suggested that the absorption of toxin by the short axons from muscles lying close to the cerebrospinal axis will result in a shorter period of incubation. When toxin travelling up the long axons from distant muscles reaches the appropriate spinal segments the condition becomes generalized. Meyer and Ransom were also the first to suggest the presence of a blood-brain barrier for tetanus toxin.

The pattern of symptoms in blood-borne tetanus does suggest a muscular absorption of toxin from the circulation. There is, however, little information regarding a possible direct action of toxin on the blood-brain barrier. It is conceivable that the development of symptoms could be due to a difference of permeability or degree of vascularity of certain regions of the central nervous system.

Freidemann (1942) believed that the permeability of the blood-brain barrier to a toxin is dependent on its electrical charge. Tetanus toxin ions, being negatively charged, cannot pass. The permeability of this barrier to antitoxin is not hindered because the antitoxin molecule is amphoteric. Further, Broman and Lindberg-Broman (1945) have demonstrated that tetanus toxin has no injurious effect on the blood-brain barrier.

Yokoi (1954) believes there is a direct spread of tetanus toxin into the central nervous system by the circulation. Abel and Chalian (1938) concluded that tetanus toxin must be able to permeate the blood-brain barrier because antitoxin, which is of greater molecular size, will freely enter the central nervous system, a view recently supported by Penitschka (1953). Teale and Embleton (1919–20), on the other hand, were unable to detect horse serum in the CNS following intravenous injection.

Actually the postulation of a CNS barrier for tetanus toxin or its disproof is made only by inference. Development of local tetanus following intramuscular injection of toxin does not prove that toxin present in the circulation cannot enter the CNS directly. If the enzymatic action of the toxin (Imbriano, 1950 a) and the alterations in permeability of the blood-brain barrier resulting from enzymatic action (Beiler, Brendel, and Martin, 1956) are considered, such a possibility cannot be ignored.

The present experiments do not provide direct evidence either for or against a CNS barrier for tetanus toxin. In our opinion, however, the data presented suggest that circulating toxin is transported first to the muscles of the body.
and then to the CNS via the endoneurial tissue spaces of the peripheral nerves. Further research is needed to clarify this question.

Although the average latent period following IV injection was 6 times as long, the average symptomatic period was actually 2 hours shorter than comparable periods following intraspinal toxin injection. (Fig. 1). This indicates that (a) toxin diffuses slowly in the spinal cord; and (b) IV injected toxin reaches the CNS in high concentration and in close proximity to vital centers. Intravenous injection of antitoxin after IM toxin injection prevented death in all cases. Ascending tetanus progressed slowly to emprosthotonus, before it was arrested. The fact that local tetanus follows IM injection of toxin in the presence of circulating antitoxin does not prove that toxin is fixed to the end plates before antitoxin can reach it and neutralize it, as Abel and his coworkers asserted; nor that absorption along the axis cylinders has already taken place, as suggested by Ransom and by Meyer. The possibility that IM injected toxin ascends to the CNS via the endoneurial tissue spaces was discussed above. It was found in Experiment 2 that epineurially injected antitoxin apparently can diffuse only very slowly through the perilemmic barrier. It is suggested that toxin ascending in the endoneurium is protected from circulating antitoxin by the perilemmic barrier. The circulating antitoxin after IV inoculation probably reaches the toxin-injected muscle before all of the toxin is absorbed, thus halting the ascending tetanus short of death.

**Cerebrospinal Fluid Injections.**—Injection of toxin into the cerebrospinal fluid resulted in blood-borne tetanus with latent period approximately 12 hours longer than the following intravenous injection. This prolonged latent period provides support for the conclusion of Ramon and Descombes (1931) and D'Antona (1951) that toxin injected into the cerebrospinal fluid is absorbed into the blood and subsequently transported to the CNS.

Antitoxin injected into the subarachnoid space prolonged the symptomatic period, but did not prevent death. In contrast, intravenously injected antitoxin halted the progress of ascending tetanus at the stage of emprosthotonus. It seems apparent that the antitoxin was absorbed from the cerebrospinal fluid into the blood, rather than directly into the CNS. The time required for this absorption was sufficient to allow a lethal amount of the intramuscularly injected toxin to ascend to the spinal cord. Antitoxin did reach the blood in time to neutralize circulating toxin, prolonging the survival period.

Ransom (1917) found that even in highly immunized animals the antitoxin in the cerebrospinal fluid is more than 100 times less per cc. than in the blood. He also found that the ascending tetanus produced by intramuscular injection of toxin could not be prevented by administration of antitoxin into the cerebrospinal fluid, and that following toxin injection into the circulation even in large doses, none can be detected in cerebrospinal fluid.

Sherrington (1917), however, found that intrathecal injection of antitoxin in...
monkeys offered more protection than subcutaneous, intramuscular, intravenous, or cerebral inoculations, once symptoms of tetanus had appeared. A relatively large dose of 2000 U.S.P. units of antitoxin per kilo of body weight was used. Firor (1940) injected 2 MLD of toxin intravenously in dogs, and at various intervals after generalized tetanus had appeared, injected 680 U.S.P. antitoxin units intravenously or into the cerebrospinal fluid. He also found that intrathecal administration of the antitoxin was more effective than intravenous.

These studies cannot be compared directly with the present experiments because of the difference in the amount of antitoxin used, and the fact that in our series the antitoxin and toxin were administered almost simultaneously rather than after the development of symptoms.

**Subdural Injections.**—The injection of toxin into the subdural space resulted in dorsal tetanus (in approximately 53 hours) and blood-borne tetanus (approximately 57 hours). Since the appearance of motor involvement in the back region required a much longer incubation period than that following intramuscular injection, it seems unlikely that any toxin was absorbed directly into the CNS from the subdural space. Possibly the toxin was first absorbed by the lymphatics and carried to the periphery, where it penetrated the peripheral nerves and was carried to the spinal cord, resulting in intoxication of the corresponding spinal segments. Most of the toxin, however, reached the blood circulation and produced symptoms of blood-borne tetanus. Here, again, the prolonged latent period, as compared with that following intravenous or subarachnoid injections, indicates that the toxin could not penetrate directly into the spinal cord.

Subdural administration of antitoxin prolonged the symptomatic period, but was even less effective than subarachnoid injection. The animals died in approximately 79 hours (Fig. 2).

**SUMMARY**

The pattern of development of symptoms was studied following injection of 3 MLD (minimum lethal dose) of tetanus toxin in each of the following sites in the rat: vein, muscle, endoneurium, epineurium, spinal cord, subarachnoid space, and subdural space. Similar observations were made when 3 MPD (minimum protective dose) of antitoxin were injected into each of the above sites a few minutes after the intramuscular injection of toxin.

Local tetanus followed intramuscular, endoneurial, and epineurial injection of the toxin; blood-borne tetanus also appeared in the latter instance. Tetanus dolorosus followed intraspinal injection of toxin. Both dorsal tetanus and blood-borne tetanus developed after subdural administration of toxin. Blood-borne tetanus, alone, appeared following intravenous and subarachnoid injection of toxin.

In no case did injection of antitoxin at the various sites listed above prevent
local tetanus from developing after intramuscular injection of toxin. Of the various sites injected with antitoxin, the intramuscular, intraspinal, and intravenous were the most effective.

It is suggested that the endoneurial tissue spaces serve as a conduit for tetanus toxin from the muscle to the CNS. The perilemma of the peripheral nerve trunk may act as a selectively permeable membrane which permits tetanus toxin to pass from the epineurium to the endoneurium. Outward diffusion of toxin from the endoneurial spaces is apparently markedly reduced. The antitoxin probably is prevented from permeating this barrier in either direction. The importance of absorption of tetanus toxin by the lymphatic and blood circulation is discussed.

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BIBLIOGRAPHY


A. A. FEDINEC AND H. A. MATZKE


Fletcher, W. M., Tetanus dolorosus and the relation of tetanus toxin to the sensory nerves and the spinal ganglia, Brain, 1903, 26, 383.


Imbriano, A. E., Actividad enzimatica de la toxina tetanica, Semana med., Buenos Aires, 1950 a, 57, 185.


Ransom, F., Modern view of tetanus, Lancet, 1917, 2, 928.


DEVELOPMENT OF TETANUS IN THE RAT


