

# PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

## THE EFFECT OF REPEATED ADMINISTRATION OF SUBLETHAL DOSES OF TETANUS TOXIN ON THE ORGANISM

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(Received October 29, 1957. Presented by Active Member AMN SSSR L. A. Zil'ber)

E. Behring [8] noted that when horses were immunized with small doses of tetanus and diphtheria toxins, the animals died after administration of toxin in a dose much smaller than the lethal one. Sometimes the animals died when enough antitoxin to effect neutralization of the toxin was present in the blood.

Further investigations showed that development of increased sensitivity to small doses of bacterial toxins played an important role in the pathogenesis of toxic infections. S. M. Minervin and E. N. Kotliarovskaia [5] established the importance of sublethal doses of botulin toxin in the pathogenesis of botulism. The role of the Behring phenomenon in the pathogenesis of diphtheria intoxication was shown by A. T. Kravchenko and N. V. Galanova [2], as well as K. I. Matveev, T. I. Bulatova and A. P. Gindin [4] who observed death and development of pathomorphologic changes in the organs when guinea pigs were subjected to repeat administration of sublethal doses of diphtheria toxin. K. I. Matveev [3] obtained botulism development in animals which received daily injections of botulin toxin in doses amounting to hundredth and thousandth parts of the lethal dose. O. Ia. Ostryi, Z. I. Sobieva and A. N. Aliev [7] have shown experimentally that the process of summation of pathogenic nervous stimuli resulting from administration of subthreshold doses of toxins produced by gas gangrene pathogens can lead to a fatal outcome when the injections are given over microintervals of time.

In order to study the pathogenesis of tetanus we investigated the action of repeated administration of sublethal doses of tetanus toxin on the animal organism.

### EXPERIMENTAL METHOD

Experiments were carried out on guinea pigs weighing 300-350 g, on mice weighing 16-18 g and on rabbits weighing 1200-1500 g. First the lethal dose of dry tetanus toxin was titrated for each species of animal on intradermal injection; it was 0.0005 mg for mice, 0.004 mg for guinea pigs and 0.05 mg for rabbits. During the experiments the weighed toxin was dissolved daily in a definite volume of physiologic solution to give a concentration of 1 Mld in 1 ml. After this one lethal dose was diluted 50, 100, 250, 500 and 1000 times. Various groups of animals were given daily 1/50, 1/100, 1/250, 1/500 and 1/1000 Mld subcutaneously in a hind limb or back. Antitetanus serum was used to treat some of the animals when they developed the disease. The activity of the tetanus toxin used in the experiments was controlled by daily titration using mice.

### EXPERIMENTAL RESULTS

The first series of experiments was carried out on 67 guinea pigs of which 33 served as controls (see Table).

The animals which received 20/50, 3/100, 4/250, 4/500 and 6/1000 Mld of the toxin showed the first symptoms of tetanus on the 3rd-7th day. On further administration of the toxin a typical picture of local and then (on the 17th-21st day) of general tetanus was observed. As soon as the first symptoms of tetanus made their appearance the animals were given a toxin injection subcutaneously in the back in order not to traumatize

TABLE

The Effect of Sublethal Doses of Tetanus Toxin Administered Daily to Guinea Pigs

No. of experiments	Experiment (repeated administration of toxin)						Control (single administration of toxin)					
	No. of guinea pigs	Single dose of toxin (in Mld)	No. of toxin injections	Total amount of subcutaneously injected toxin	result			No. of expt. pigs	Single dose of toxin (in Mld)	result		
					No. death occurs	fatal	Recov-ery, no. of guinea pigs			no. day of death	fatal	Recov-ery, no. of guinea pigs
1	4	1/50	20	20/50	—	—	4	1	20/50	—	—	3
2	6	1/100	12-19	12/100-19/100	3	12-19- $\dot{a}$	3	2	7/100	—	—	3
3	5	1/100	10-13	10/100-13/100	5	10-13- $\dot{a}$	—	3	3/250	—	—	3
4	3	1/250	30	30/250	3	30-31- $\dot{a}$	—	4	4/500	—	—	3
5	4	1/250	15-24	15/250-24/250	4	15-24- $\dot{a}$	—	5	20/1000	—	—	3
6	4	1/500	18-25	18/500-25/500	4	18-25- $\dot{a}$	—	6	3/100	—	—	3
7	3	1/500	26-27	26/500-27/500	1	27- $\dot{a}$	2	7	4/250	—	—	3
8	5	1/1000	19-20	19/1000-20/1000	5	19-20- $\dot{a}$	—	8	4/500	—	—	3
								9	19/100	—	—	3
								10	30/250	—	—	3
								11	26/500	—	—	3
total	34				25		9	33	—	—	—	33

the leg in which the toxin was first injected. The animals died when the clinical picture of tetanus was pronounced. When sublethal doses of toxin were given daily the animals received a total of only 12/100-19/100, 24/250-30/250, 18/500-27/500, 19/1000-20/1000 Mld in the course of several days.

The clinical picture of tetanus was very characteristic in guinea pigs which received 1/1000 Mld toxin daily. Local tetanus of first degree appeared in this group on the 12th-14th day. Further injections of the toxin were made subcutaneously in the back. Local tetanus persisted until the 16th-17th day, then disappeared and kyphosis of the spine made its appearance. On the 21st-22nd day fulminating general tetanus developed and the animals died within 2-3 days. In rare cases the guinea pigs recovered.

Of 34 experimental guinea pigs 25 died from general tetanus, 9 animals had severe tetanus but recovered. Of 33 control animals which received the total dose in one injection, 18 animals remained quite healthy and 15 showed mild, transient manifestations of tetanus of first degree. Blood specimens taken on the 13th, 15th and 21st day from the beginning of toxin injections did not show even 1/1000 active units of antitoxin.

The second series of experiments was carried out on 20 mice and 6 rabbits. When the mice were given 1/25 and 1/50 Mld tetanus toxin daily they developed tetanus of the first degree on the 3rd-4th day. Further injections caused rapid development of general tetanus and all the mice died after receiving a total of only 6/25-7/50 Mld of toxin. Ten control mice which received the total dose in a single injection developed first degree tetanus but recovered after 9 days.

In L. N. Morgunov's and V. V. Khatuntsev's experiments [6] on mice the animals died from tetanus on daily injection of 1/10-1/25 Mld of tetanus toxin. Daily injection of 1/50 Mld of toxin led to the development of the clinical picture of tetanus but the animals did not die.

Six rabbits which received daily 1/1000 Mld toxin developed first degree tetanus on the 9th day; they were subsequently given the toxin subcutaneously in the back and by the 14th day they developed third degree tetanus. After 18 injections further administration of toxin was stopped since the rabbits began to recover. The rabbits were perfectly healthy after 39-40 days; no antitoxin was found in their blood.

The rabbits thus did not contract fatal tetanus when they were given sublethal doses of tetanus toxin.

Other investigations were devoted to antitetanus serum treatment of animals which contracted tetanus with daily injections of sublethal doses of tetanus toxin.

In 2 experiments 12 guinea pigs received 1/1000 Mld tetanus toxin daily. On the 21st day, i. e. after the appearance of general tetanus, they were treated. Each was given from 750 to 1000 active units of antitoxin serum subcutaneously. All the animals died from tetanus within the same periods of time as those which had not had serum treatment. In the third experiment 6 guinea pigs received 1/250 Mld toxin every day. On the 5th day from the start of injections, i. e. prior to the appearance of the signs and symptoms of tetanus, 3 animals were given 750 active units of serum and 3 animals were left untreated. The animals which had received the serum remained healthy, while those which had not been given the serum died from tetanus on the 15th-17th day.

These experiments show that the therapeutic action of the serum depends, with daily injections of sublethal doses of tetanus toxin, on the time of administration. Early administration of serum, prior to the appearance of tetanus manifestations, protects the animals from death; late administration of serum has no therapeutic effect and the animals die from tetanus. A similar picture is seen when serum therapy is used for man and animals following tetanus infection under natural conditions (wounds).

The investigations carried out suggest that when tetanus is contracted by man or animals following wounding the tetanus pathogen undergoes gradual multiplication in the wound during the incubation period and produces small, sublethal doses of toxin, without an immediate effect on the organism. The length of the incubation period depends on the amount of toxin produced by the pathogen and on the resistance of the organism. Consequently in cases of tetanus following wounding gradual action of sublethal doses of toxin takes place and leads to the development of the disease and finally to a fatal outcome.

I. N. Morgunov and V. V. Khatuntsev [6] have shown that the rate of development of the disease is directly related to the magnitude of the single dose of toxin and inversely proportional to the intervals of time between the appearance of the toxin.

P. F. Zdrodovskii [1] explains the increased sensitivity to repeated administration of sublethal doses of bacterial toxins by summation of stimulation, drawing on electrophysiology for this concept.

Since repeated administration of small doses of tetanus toxin to animals leads to gradual development of the clinical picture of tetanus similar to that seen with wound tetanus, with impairment of important vital processes in the nervous and muscular systems, we consider that this phenomenon represents not summation of stimuli but a summation of lesions. The gradual action of very small doses of tetanus toxin on the organism also suggests that it may be an antimetabolite subserving impairment of enzymatic processes in the nervous, muscular and other tissues of the body, with no clearly defined histologic changes in these tissues.

#### SUMMARY

When tetanus toxin is repeatedly administered to guinea pigs and mice in sublethal doses it causes an attack of tetanus resulting in death. This takes place when the sum total dose of administered toxin is considerably lower than the lethal dose. In single administration of the whole dose of the toxin (used formerly in several injections) the animals remain healthy or sustain an attack of tetanus from which they soon recover. The therapeutic serum was found to be ineffective in late treatment of tetanus which developed as a result of daily administration of sublethal doses of the toxin to guinea pigs.

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