EFFECT OF TETANUS AND BOTULINUS TOXINS ON THE CHRONAXY OF IMMUNE AND NON-IMMUNE RABBITS

M. M. Gromakovskaya

From the Immunity Laboratory (Head - Professor K. T. Khalyapina) of the Department of Experimental Pathology of Infections and Immunity (Head - Active Member of the Academy of Medical Sciences of the USSR, P. F. Zdrodovsky) of the N. F. Gamaleya Institute of Epidemiology and Microbiology (Director - Active Member of the Academy of Medical Sciences of the USSR, Professor V. D. Timakov) of the Academy of Medical Sciences of the USSR, Moscow.

Received March 18, 1955. Submitted by Active Member of the Academy of Medical Sciences of the USSR, Professor G. V. Vygodchikov)

The investigation of the differences in the clinical and immunological reactions of the immune and non-immune system to the administration of antigen has been described in the literature sufficiently. However, the physiological mechanisms which determine these differences, including the parameters of the excitability of the tissues and systems, have been studied little [1, 2, 3, 4]. In this connection, a comparative investigation of the chronaxy of the various tissues of the immune and non-immune animal during antigen administration represents considerable interest.

EXPERIMENTAL METHODS

The experiments were carried out on rabbits which were immune to tetanus and botulism and on non-immune ones, on which were made determinations of the subordinate chronaxy of the tibial muscle and of the sciatic nerve on the side on which the toxin was administered during several days before and after the administration of toxin; in addition, the chronaxy of the spinal cord and of the motor zone of the cerebral cortex were determined. The work was carried out on 34 rabbits - 13 immune ones and 21 non-immune. The chronaxy of the spinal cord was determined either through the skin after preliminary (several days in advance) excision of the spinous process and arch of one of the lumbar vertebrae, or with the help of needle electrodes which touched each other through the intervertebral space. Silver needle electrodes were used to determine the subordinate chronaxy of the muscles. The chronaxy of the motor zone of the cerebral cortex was determined either through the skin after the preliminary excision of the corresponding area of the skull bone, or with the help of platinum electrodes (0.3 mm in diameter), which were enclosed in a plastic sleeve passed through the trephined opening.

A condenser chronaximeter was used in the experiments. The chronaxy determination was carried out unipolarly. The inert electrode was inserted in the animal's rectum.

The experiment was carried out in the following manner. For several days prior to the administration of toxin, the chronaxy of the rabbits was determined, after which 0.25 - 0.3 ml of toxin was administered into the tibial muscle and the chronaxy was determined again during the hour. Later the chronaxy was determined daily. The rheobase was measured in volts, the chronaxy in microfarads with subsequent conversion into sigmas.

Experiments on rabbits not immune and immune to tetanus (series 1). The following doses of toxin were administered to the non-immune animals per 1 kg of weight.

10 mouse MLD	-	1 rabbit
75 mouse MLD	-	5 rabbits
100 mouse MLD	-	4 rabbits
150 mouse MLD	-	2 rabbits

The administration of 10 mouse MLD of tetanus toxin into the anterior tibial muscle caused the development of a weakly evidenced localized tetanus. The administration of 75 and 100 MLD per 1 kg of weight also led to the appearance of localized tetanus which changed into the generalized form only in some rabbits. The administration of 150 MLD per 1 kg of weight caused general tetanus with a subsequent fatal outcome.

The extent of the changes in the chronaxy level and the nature of the development of the clinical picture of the disease in the animal depended on the dosage of the administered toxin. Thus, with the administration of 10 mouse MLD of tetanus toxin per 1 kg of weight, large fluctuations of the chronaxy were not observed at any of the determined points. With the administration of 75 or 100 MLD per 1 kg of weight, however, considerable changes in chronaxy were noted. In the majority of experiments (9 out of 12), the rheobase and chronaxy changed in the same direction, i.e., when the rheobase increased, the chronaxy increased and vice versa.

In the first minutes after the administration of toxin, a slight shortening of the chronaxy developed, chiefly of the subordinate chronaxy of the muscle and nerve. In 24 hours, when there were still no signs of tetanus, a lengthening of the chronaxy was observed, which continued to increase in the subsequent days, reaching a considerable size in comparison with the normal. When the illness remained at the stage of localized tetanus, the chronaxy indicators returned to the original level 9-10 days after the administration of the toxin. In the case of generalized tetanus, the chronaxy suffered considerable changes, typified by an initial shortening with a subsequent sharp lengthening of it.

With the appearance of generalized tetanus, changes also appeared in the motor zone of the cerebral cortex which, as a rule, were absent from the phenomena of local tetanus. This fact indicates that, with the local form of tetanus, the change in the level of the process of excitability spreads chiefly to the motor neurons of the corresponding segments of the spinal column, while in generalized tetanus, the motor zone of the cerebral cortex is also attacked.

The immunization of the rabbits against tetanus was carried out by the administration of 1 ml of precipitated tetanus anatoxin subcutaneously, and 3-4 months later revaccination was carried out by the subcutaneous administration of the same amount of anatoxin. In the experiment shown in Fig. 1, the tetanus toxin was administered 8 months after revaccination. The AE titre of this rabbit $2\frac{1}{2}$ months before the experiment was equal to 1 AE.

For comparison, in some experiments immune as well as non-immune rabbits which were administered the same amount of toxin (75, 100, 150 mouse MLD each) were investigated simultaneously.

The administration of equal doses of toxin into a muscle had a different effect on immune and non-immune rabbits. While no signs of illness appeared in the immune animals, the non-immune rabbits fell ill either with localized or generalized tetanus; in the latter case death ensued in 5-6 days.

The difference was evident not only in the animal's condition but also in the changes in the indicators of chronaxy. The administration of a fatal dose of toxin into the muscle of an immune rabbit caused a slight change in the subordinate chronaxy of the muscle and nerve on the side of toxin administration. These changes disappeared on the 3-4th day; the chronaxy changes of the control non-immune animal were more sharply evident. It should be noted that the lengthening of the chronaxy of the muscle of a non-immune animal was not connected with its rigidity and consequently, with the loss of its contractility since: 1) the lengthening of the chronaxy occurred before the clinical signs of tetanus were found); 2) the tetany of the muscles of the affected extremity of the rabbits which survived the administration of tetanus toxin remained for a prolonged period, while the chronaxy of the muscles gradually returned to the original level (Fig. 1).

Experiments on rabbits, not immune and immune to botulism. In this group of experiments, rabbits were used which had been immunized one time by the subcutaneous administration of 1 ml of precipitated botulinus anatoxin type "A" and revaccinated by the subcutaneous administration of the same amount of anatoxin 8-9 months later. 5 immune and 4 non-immune rabbits were taken for the experiment. The level of the normal fluctuations of the chronaxy was determined in all the rabbits during 3-4 days prior to toxin administration.

After this, botulinus toxin series "A" IEM-2 (Institute of Experimental Medicine) was administered in the anterior tibial muscle in lethal doses (5 mouse MLD per 1 kg of the animal's body weight; 1 mouse MLD = 0.00025 mg). The toxin was administered to the immunized rabbits 1-2 months after revaccination.

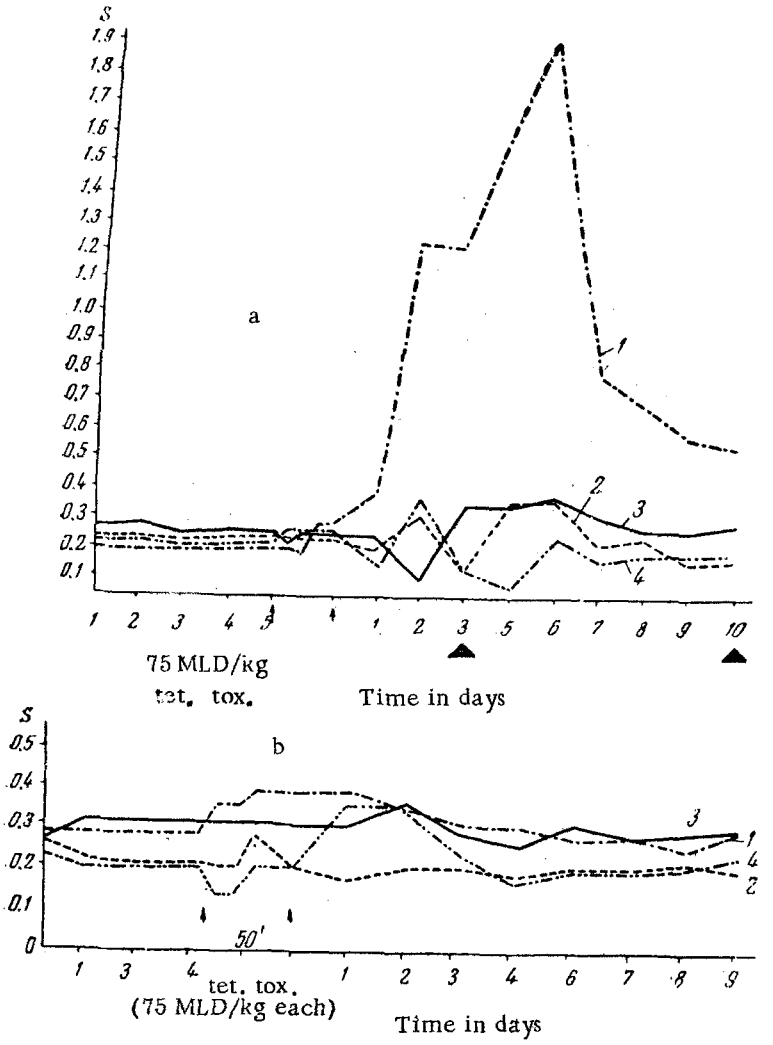

Fig. 1. Changes in the chronaxy after administration of tetanus toxin. (75 mouse MLD/kg) to non-immune (a) and immune (b) rabbits. Arrows mark: the administration of toxin. ▲) local tetanus.

1) tibial muscle, 2) spinal column, 3) brain, 4) sciatic nerve.

In Fig. 2 are shown the results of an experiment in which equal doses of botulinus toxin were administered on the same day to immune and non-immune rabbits.

The administration of such doses of toxin to non-immune animals caused illness within 24 hours, the clinical signs of which were evident as a fall of the muscle tone of the extremities and neck, increased frequency of respiration and the development of rales. As these phenomena grew, the animal's death occurred 3-5 days after toxin administration. On autopsy, hemorrhagic destruction of the upper lobes of the lungs, hyperemia of the tracheal mucosa, and distention of the intestines were found.

No signs of illness were found in immune rabbits after the administration of the same doses of toxin.

As is seen in Fig. 2, the chronaxy changes in immune and non-immune rabbits after toxin administration were different. While it was possible to note a fleeting and weak deviation of the subordinate chronaxy of the muscle and nerve in the immune rabbits, in the non-immune rabbits considerable disruption of the chronaximetric

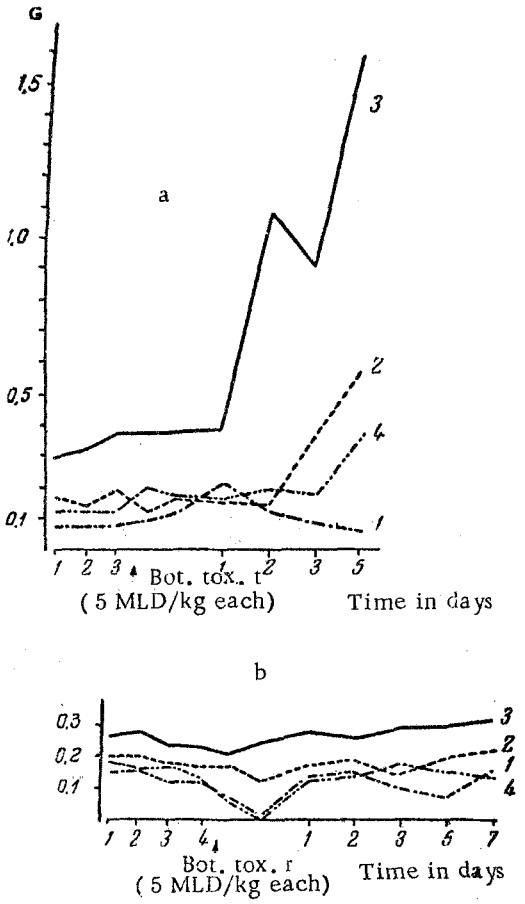

Fig. 2. Changes in the chronaxy of non-immune (a) and immune (b) rabbits after administration of botulinus toxin (5 MLD/kg). Symbols are the same as in Fig. 1.

indicators at all the determined points was observed. The sharpest changes, consisting of a considerable lengthening of the chronaxy, were observed in the motor zone of the cerebral cortex. Sometimes the shortening phase preceded the lengthening phase of the chronaxy of the motor zone of the cerebral cortex.

In the control experiments the following was done: 1) prolonged determination of the chronaxy of normal rabbits; 2) determination of the chronaxy after the administration of physiological solution in the muscle. In order to establish the limit of the normal fluctuations of the chronaxy, in 4 rabbits the rheobase and chronaxy were determined for 6 days at intervals of 1-2 days. Then an interval of 38 days elapsed. At the end of this time, the rheobase and chronaxy were again determined for 7 days. The experimental results indicate that the numerical indicators of the chronaxy are not constant. However, the chronaxy fluctuations of healthy rabbits over a prolonged period of time are insignificant. After the preliminary determination of the chronaxy, 0.25 - 0.3 ml of physiological solution was administered into the tibial muscle of three rabbits, which also did not cause noticeable changes in the chronaxy.

On comparing the changes in chronaxy developing under the influence of tetanus and botulinus toxins in non-immune rabbits, distinct differences were noted. Thus, after the administration of tetanus toxin into the muscle, the most significant changes were observed in the muscle and the least significant ones in the nerve and in the spinal column. Changes in the chronaxy of the motor zone of the cerebral cortex in response to the administration of toxin were noted only in the last days before the animal's death.

In contrast to this, the administration of botulinus toxin into the muscle of non-immune animals caused a significant lengthening of the chronaxy of the motor zone of the cerebral cortex of early onset, while the chronaxy of the muscle, nerve and spinal column changed less significantly.

N. V. Golikov [2] observed the change in the subordinate chronaxy of the muscles and nerves on administration of tetanus toxin to non-immune rabbits. He also noted that the change in chronaxy occurs before the development of the clinical picture of the disease and is characterized by an interchange of the lengthening and shortening phases of the chronaxy.

The administration of toxins to immune animals does not cause significant changes in the chronaxy. Similar differences in the reactions of the tissues of immune and non-immune animals were observed also by A. T. Kravchenko and N. V. Galanova [5], A. D. Ado [1], A. N. Gordienko [3, 4] and others with the action of various endotoxins (brucellosis, typhus, dysentery, diphtheria, anthrax).

The immune state of the system is characterized by a lowering of the sensitivity and by the absence of significant disturbances in the excitability of the tissues and systems of the organism to pathogenic agents. While in non-immune rabbits after the administration of toxins, a considerable rise in the normal chronaxy level of early onset is observed, only a weak and fleeting change in the chronaxy could be observed in immune animals.

SUMMARY

Chronaximetric reactions of immune and nonimmune rabbits after injection of lethal doses of toxin differ . Immune animals reveal no essential changes in chronaxy of muscles, nerve, spinal cord and brain when toxin is injected.

Nonimmune rabbits reveal a considerable change of chronaxy. Most pronounced changes arise in muscle when tetanus toxin is injected and in the motor zone of the cerebral cortex when botulinus toxin is injected. Changes in chronaxy appear earlier than clinical symptoms of the disease.

LITERATURE CITED

[1] A. D. Ado, Antigens as Extreme Stimulants of the Nervous System,* Moscow, 1952.

[2] N. V. Golikov, On the Functional Changes of the Nervous System in Tetanus Intoxication (Pathogenesis of Tetanus). Uchenye Zapiski Leningrad, Gosudarst. Univ. A. A. Zhdanova. Ser. Biol., issue 16, pp. 6-45, 1949.

[3] A. N. Gordienko, The Nervous System and Immunity,* Krasnodar, 1949.

[4] A. N. Gordienko, The Neuro-Reflex Mechanism for Producing Antibodies and Regulating Phagocytosis,*Moscow, 1954.

[5] A. T. Kravchenko and N. V. Galanova, The Third Factor of Acquired Immunity, Immunity and Cellular Allergy,*Moscow, 1948.

* In Russian.