THE INCUBATION PERIOD IN PASSIVE IMMUNITY TO TETANUS TOXIN

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From numerous experimental investigations and clinical observations we know that so-called passive immunity reaches its maximum degree of intensity not immediately after injection of immune serum but after some time. The existence of this characteristic incubation period became known soon after the discovery of the preventive properties of antitoxic serum [19], and different workers accounted for this phenomenon in different ways, depending on their ideas of the mode of action of antitoxin [2, 8, 21, 22]. The existence of this period in the development of passive immunity was regarded by some workers as an indication that passive immunity arises as a result of the action of antibodies on the body of the recipient. Despite the fact that it is now 70 years since this discovery was made, many aspects of the conditions of production of passive immunity and of the action of antitoxin in vivo have received insufficient study. Recently a number of works have appeared in which the significance of the action of tetanus antitoxin on the animal in creating specific resistance is once again examined [7, 11-14, 18].

Considering the practical and theoretical importance of these problems, we undertook the present investigation with the aim of explaining the features of development of passive immunity to tetanus depending on the conditions of administration of tetanus antiserum.

EXPERIMENTAL METHODS

The experiments were based on the production of experimental tetanus. This plan was selected not only because of the frequent interest in the study of passive immunity to tetanus but also because since the work of E. Bering and his co-workers [2] experimental tetanus has become one of the main experimental devices for solving a number of general immunological problems. This has not lost its importance even today [16].

In the experiments we used tetanus antiserum purified and concentrated by the method Diaferm-3 IEM (series 1881), and dry tetanus toxin (series No. 580 IEM AMN SSSR). This latter was first dissolved in different volumes of physiological saline containing glycerin (pH = 6.9). The required dilutions of toxin were made just before each experiment. The investigations were carried out on white rats weighing 125-140 g. The severity of the disease was rated by mortality, survival time of animals who later died, and clinical picture. In order to describe the signs and symptoms of local tetanus we used special symbols; the degree of resistance of the animals to the toxin was judged by the clinical course of the disease. The resistance was conventionally expressed in points (Table 1).

A lethal dose of toxin was chosen as the dose which on injection subcutaneously in a volume of 0.2 ml in the outer surface of the left thigh, caused death of all the animals on the 4th and 5th day. In the first 3 series of experiments tetanus antiserum was given in a dose of 5 units; this dose corresponds roughly to that used in practice for prophylaxis of tetanus (calculated per 1 kg body weight).

TABLE 1

Symbols Used in Describing the Clinical Course of Local Tetanus and the Degree of Resistance of the Animals

Clinical picture	Symbols	Degree of resistance in points
Absence of any signs of disease.	–	10
Barely perceptible increase of extensor tonus (more readily observed rats are lifted up by their tails).	±	8
Clearly expressed increase of extensor tonus. This is mainly observed when the animal moves about.	±	6
Considerable increase of extensor tonus. The paw is semiflexed, the springing joint does not lie on the surface of the ground.	+	4
Sharp increase of extensor tonus. The paw is drawn backwards, and on walking only the phalanges touch the ground, and movement in the joints, especially the springing joint, is restricted.	++	2
Total local tetanus. The paw is extended backwards and takes part in no movements; muscular rigidity; the sole and phalanges are extended; movement in the springing joint is absent, while occasionally a trace of movement may be preserved in the other joints.	+++	0

Estimation of antitoxin in the blood of the rats was made by the usual method of titration in mice. In control tests normal rat serum was used.

The experimental results were treated by statistical variance analysis.

EXPERIMENTAL RESULTS

The purpose of the first series of experiments was to discover how quickly the maximum degree of resistance of animals arises after intramuscular injection of tetanus antiserum.

In these experiments tetanus antiserum was injected (5 units in a volume of 0.2 ml) into the posterior group of muscles of the right thigh, and tetanus toxin (1 lethal dose) – into the posterior group of muscles of the left thigh.

As seen from Table 2 after injection of serum immediately before injection of toxin, none of the animals showed any protection against the disease: in all the rats total local tetanus developed, with characteristic muscular rigidity and restriction of movements at the joints. Three hours after injection of the serum the resistance of the animals was increased (local tetanus did not reach its maximum development), after 6 hours further increase could be observed, and after 12 hours the resistance of the animals was at its highest. Differences in the degree of resistance of the animals of each successive group compared with the resistance of the animals of the preceding groups were significant in every case. The resistance of the animals to tetanus 24 hours after injection of serum was practically the same as that after 12 hours (variations in the degree of resistance are statistically not significant).

Thus by intramuscular injection of tetanus antiserum and tetanus toxin in different places, passive immunity reaches its maximum development not at once after injection of the serum, but after a definite period of time. This result is in accordance with the reports in the literature on the subject.

The next series of experiments were intended to explain the rate of accumulation of antitoxin in the blood of the animals under the conditions of the experiment.

The rats were exsanguinated by section of the cervical vessels. From the blood, serum was prepared

and tested for its antitoxic properties on the day after it was drawn. Each test was carried out with a mixture of equal quantities of sera from 4 rats (0.4 ml of each serum). The entire experiment was repeated 3 times. In the whole experiment 60 rats were used.

As seen from Table 3, as the interval after injection of serum intramuscularly increases, so also does the amount of antitoxin in the blood, and it reaches its maximum in 12 to 24 hours (variations in the antitoxin content after 3, 6 and 12 hours from the injection of serum are statistically significant). After 24 hours the content of antitoxin in the blood was roughly the same as after 12 hours.

TABLE 2

Prophylactic Effect of Tetanus Antiserum Injected at Various Intervals Before Injection of Toxin

Time between injections of serum and toxin (in hours)	Number of animals in the experiment	Clinical course of the local tetanus	Resistance in points (M ± m)
0	10	+++	0
3	15	++	2±0.52
6	15	<+	4.6±0.69
12	10	<±	9±0.3
24	10	<±	9.2±0.41

Note: Intermediate degrees of development of the disease are signified by the additional signs > or <.

The results obtained agree with those of other investigators [20, 21]. Any differences consist in the fact that in the opinion of other workers cited, the maximum level of the antitoxin content of the blood may be observed in some cases on the 2nd-3rd day after subcutaneous or intramuscular injection of tetanus antiserum. This difference may be explained by the fact that we used purified serum, the size of the immune globulin of which reduced by enzyme digestion at the time of purification [1]. Thanks to this purification by the Diaferm-3 IEM method the serum is more rapidly absorbed than the native serum [1].

Comparing the results of the two series of experiments a parallel may be observed between the change in the level of the blood antitoxin and the degree of resistance of the animals. As can be seen from the figure, the curve of growth of resistance of the animals corresponds to the curve of growth of antitoxic properties of the blood.

The results obtained enable the conclusion to be drawn that the degree of resistance of the animals to tetanus toxin in passive immunity is determined by the level of the blood antitoxin content, derived from the site of injection. This conclusion was confirmed by the results of an experiment in which tetanus antiserum was injected in the same dose (5 units) directly into the blood stream (into the vein of the right calf). In these conditions, the simultaneous injection of toxin into the limb muscles produced in the majority of animals merely an insignificant rise in the extensor tonus (from − to ±; it reached ± in only 1 rats out of 20). At the same time, all of 20 rats receiving serum into the muscles of the right thigh developed total local tetanus (+++) with characteristic extensor rigidity and restricted movement in the joints.

Thus on injecting tetanus antiserum directly into the blood stream with the object of creating the required concentration of antitoxin in the minimum time, we observed the same degree of resistance in the animals which develops roughly 9 to 12 hours after intramuscular injection of the same dose of serum.

TABLE 3

Content of Antitoxin in the Blood of Rats at Various Intervals After Injection of Tetanus Antiserum into the Limb Muscles

Interval between taking blood and injection of tetanus antiserum (in hours)	Series of experiments			
	I	II	III	mean
	content of antitoxin in units			
0	0	0	0	0
3	0.09	0.09	>0.08	0.09
6	<0.14	0.13	0.15	0.14
12	0.18	0.19	0.17	0.18
24	>0.2	<0.18	0.17	0.18

TABLE 4

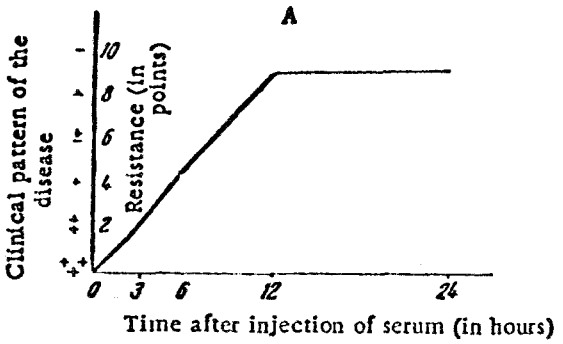
Prophylactic Effect of Tetanus Antiserum, Injected into the Blood as a Mixture with Toxin or Separately at Various Intervals Before Intravenous Injection of the Toxin

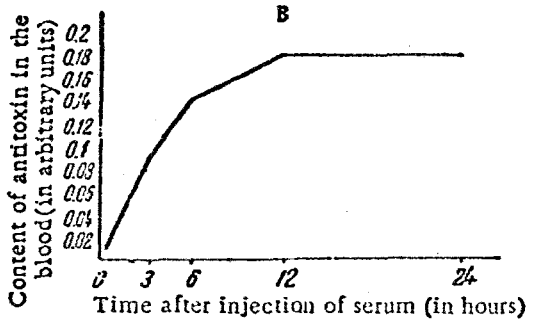
Group No.	Conditions for injection of serum	Dose of serum – 0.02 units				Dose of serum – 0.01 units			
		number of animals in experiment	number developing the disease	number which died	mean survival period, days	number of animals in experiment	number developing the disease	number which died	mean survival period, days
1	As a mixture with toxin	5	0	0	—	5	1	0	—
2	Just before injection of toxin (into another vein)	5	0	0	—	5	4	0	—
3	3 hours before injection of toxin	5	0	0	—	5	5	1	9
4	6 hours before injection of toxin	5	0	0	—	5	5	4*	5.8
5	12 hours before injection of toxin	5	4	0	—	5	5	5	3
6	24 hours before injection of toxin	5	5	1	6	5	5	5	2.8
7	Toxin control (serum not injected	5	5	5	1.2	5	5	5	1.2

* One rat died with the appearance of exhaustion.

The next series of experiments were for the purpose of discovering the form of the passive immunity appearing in response to injection of both toxin and serum into the blood stream.

Doses of 0.02 and 0.01 units of serum in a volume of 0.2 ml were injected into the vein of the right calf; toxin (3.5 lethal doses in a volume of 0.15 ml) was injected into the vein of the left calf at different intervals after injection of the serum. In addition a portion of the animals received a mixture of toxin and serum which had been prepared just before injection into the vein (not kept in the incubator). The results of the experiments are shown in Table 4.





Change in the resistance (A) and blood antitoxin content (B) of rats at various intervals after intramuscular injection of tetanus antiserum.

As seen from Table 4, in this experiment the best prophylactic effect was observed in those cases where the serum was injected either as a mixture with toxin or simultaneously with it. As the length of time between injection of serum and injection of toxin increased, so the prophylactic effect of the tetanus antiserum decreased, and reached its minimum after 12 to 24 hours.

The results of this experiment are thus diametrically opposed to those which were obtained in the experiment in which toxin and serum were injected into the muscles of different limbs. In this present experiment no incubation period could be observed in the development of passive immunity after injection of tetanus antiserum.

The results obtained suggest that the incubation period in passive immunity is the period of resorption of antitoxin and the time for its distribution throughout the body, thereby creating optimal conditions for fixation of the toxin at the site of its injection (or formation) and of its action. Of great importance in this respect is a necessary concentration of antitoxin in the blood, permitting not only blockage of the humoral route of spread of toxin but also access of antitoxin to the appropriate sections of the nervous system on which the toxin acts. The possibility of access of tetanus antitoxin from the blood to the central nervous system may now be considered to be proved [3, 4, 9, 10]. For this reason the duration, presence or absence of the time required to produce the maximum prophylactic effect of antitoxic serum will be determined not only by amount and site of injection but also by conditions of injection and spread of the toxin.

These facts which have been presented suggest that the prophylactic action of tetanus antitoxin is connected primarily with its power to fix toxin. If the prophylactic effect of tetanus antiserum were due to its action on the macroorganism, the existence of an incubation period would be observed in all cases of passive immunity, just as occurs in any reaction of the body to any stimulus. This naturally does not dismiss the importance of the action of the serum proteins, including immune protein, on the body as an additional stimulus modifying the reactive basis of the animal. The importance of this mechanism, like the importance of changes in reactivity due to other stimuli, may vary in different conditions.

From the foregoing it will be understood why in the usual conditions of passive immunity (in the absence of special stimuli modifying the reactivity of the body) a direct relationship is particularly clearly observed between the degree of resistance of animals and their blood antitoxin level. As may be seen from the results presented, even comparatively fine differences in the clinical pattern of local tetanus correspond to changes in the content of antitoxin in the blood. In active immunity such a relationship may not always be present or so clearly expressed [5, 6, 15].

SUMMARY

It was established in experiments on white rats that the time of the maximal immunity against tetanus depends on the method of introduction of antitetanus serum and tetanus toxin. The resistance of the animals to the toxin depends directly on the content of the antitoxin in their blood. The mechanism of action of tetanus antitoxin and conditions of appearance of the passive immunity are discussed.

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