APPEARANCE AND CIRCULATION OF ANTIBODIES AGAINST SERUM PROTEINS IN THE BLOOD STREAM AND THEIR EFFECT ON THE ANTITOXIN LEVEL

V. V. Pushkarev

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The appearance and circulation of antibodies against serum proteins in the blood stream after injection of antitetanus serum under various conditions were studied. The antitoxin level was found to vary inversely with the concentration of antibodies against serum proteins in the blood. The effect of blocking the specific properties of the antitoxin by these antibodies can be overcome by injection of antiserum from another species of animal.

The more successful use of heterogeneic serum preparations is dependent on knowledge of the principles governing the formation of antibodies against serum proteins (ASP) and analysis of their effect on the passive immunity level.

A disturbance of the binding power of tetanus antitoxin by antiserum was observed previously [13, 17], and it was subsequently postulated [16] that the rapid liberation of horse diphtheria antitoxin is dependent on the formation of antibodies against it. The dynamics of the blood antitoxin level was later studied in more detail [2-8, 14,15, 18, 19], and the results showed that repeated injection of the serum is ineffective, for the resulting antibodies against the foreign protein interact with the antitoxin and block its specific determinant.

Since this problem is not only of theoretical interest, but also of considerable practical importance, it was decided to study the dynamics of ASP formation and its relationship with the antitoxin level after single and successive repeated injections of antitetanus serum.

EXPERIMENTAL METHOD

Chinchilla rabbits weighing 2.8-3.5 kg were used. Antitetanus horse serum (D-3) with a titer of 3400 i.u. was injected intramuscularly in a dose of 1 ml/kg body weight. In one of the series of experiments, in which bovine native serum and horse (D-3) antitetanus serum were given in succession the titers of antitoxin were lower (650 and 1,900 i.u., respectively). The bovine serum was accordingly used in twice the volume. The blood antitoxin level of the rabbits was determined daily by titration in albino mice by the usual method, and antibodies against serum proteins were determined by the precipitation test in agar (PTA). Altogether 52 rabbits were used.

EXPERIMENTAL RESULTS

Four series of experiments were performed. The mean values of antitoxin and antibodies against serum proteins in each group of animals are shown in Fig. 1. It is clear from Fig. 1A that the blood antitoxin level in the animals after a single injection of antitetanus serum (experiments of series I) reached a maximum during the first 2 days (M = 20.0 and 22.1 i.u.) after injection of the preparation. On the follow-

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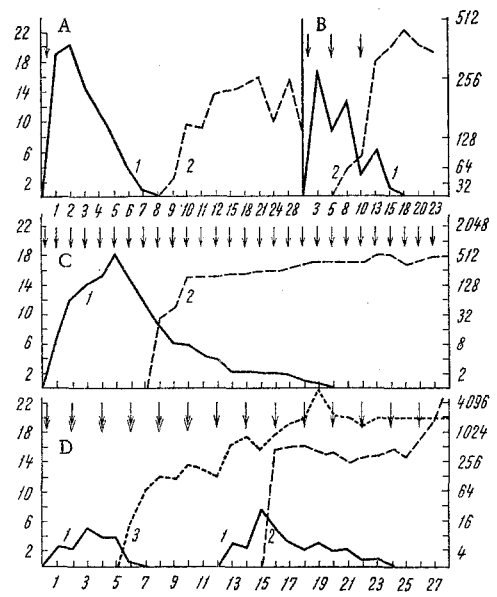

Fig. 1. Dynamics of antitoxin and antibodies against serum proteins in rabbits after single and repeated injections of antitetanus serum. A) After a single injection of D-3 horse antitetanus serum; B) after three injections of D-3 horse antitetanus serum; C) after repeated daily injections of D-3 horse antitetanus serum; D) after repeated successive injections of bovine and horse antitetanus sera. Arrow indicates injection of horse serum; doubled arrow indicates injection of native bovine serum. 1) Titers of antitoxin; 2) titers of antibodies against horse serum proteins; 3) titers of antibodies against bovine serum proteins. Abscissa, days of investigation; ordinate, on the left: titer of antitoxin (in i.u.); on the right: titer of antibodies against serum proteins.

ing days the antitoxin level fell, and the manner of its fall showed certain characteristic features. The greatest decrease in antibody titers (by up to 30%) took place on the 3rd day of the experiment. The antitoxin level subsequently decreased more slowly: on the 4th and 5th days, for instance, the mean decrease was 13.5% daily, and on the 6th and 7th days it was 18.6%. The titers of antitoxin were determined after the 8th day in only a few rabbits, and they varied between 0.1 and 0.5 i.u.

From the 5th to the 9th day of the experiment ASP appeared in the animal's blood in a titer of 1:16-1:256. Their titer reached its highest level between the 12th and 17th day of observation (M = 1:274 and 1:347). Subsequently the ASP titers fell to 1:212-1:256, at which level they remained until the 26th day of observation. This was followed by a sharp decrease in the ASP concentration, and after the 32nd day of the experiment they could no longer be detected in any of the rabbits.

In the experiments of series II (Fig. 1B) the same indices were studied after three injections of horse antitetanus serum at intervals of 5 days. The blood antitoxin level reached a maximum during the first 3 days of the experiment, when its value was 8-30 i.u. (M = 16.5). On the 5th day the antitoxin level fell to 6-14 i.u. (M = 8.9), i.e., by half. The second injection of the serum was accompanied by a less marked increase in the blood antitoxin level of the animals – to 10-20 i.u. (M = 12.4) – while in two rabbits of this group the antitoxin content actually fell. In these animals the ASP titers were higher. The antitoxin titers 5 days after the second injection fell to 0.1-8.0 i.u. (M = 3.2). After the third injection of serum, a further, smaller increase in the antitoxin titer was observed (2-15 i.u.; M = 6.7) than after the first two injections; by the 5th day the antibody titers in half of the animals had fallen to 0.1 i.u., while in the rest they did not exceed 3-4 i.u. (M = 1.6).

The highest mean values of the ASP titer occurred on the 20th-26th days of the experiment, after which they fell gradually. However, these times varied widely among individual animals.

The dynamics of antitoxin and ASP after repeated daily injections of horse antitetanus serum (series III) is illustrated in Fig. 1C. During the first days of the experiment there was a regular increase in the antitoxin titer, to reach a maximum on the 5th day of the investigation (20 i.u.). Later, despite continuing injections of the preparation, the antitoxin level fell sharply, and by the 12th day it had fallen by 80%. In the following 10 days a further but slower disappearance of antitoxin from the blood was observed, and by the 24th day of the experiment its titer was down to 0.01-0.1 i.u., despite the fact that administration of the preparation to the animals continued in the previous dose.

From the 5th-8th day, ASP appeared in the rabbit's blood, and by the 10th day it was detected in dilutions of 1:512 or higher, at which level it remained until the end of the period of observation (5 months). The appearance of ASP coincided with the phase of more rapid disappearance of antitoxin from the blood stream.

The experiments of series IV were undertaken to determine the effectiveness of using sera of different species at the time when further use of the homogeneic serum was ineffective. The results (Fig. 1D)

show that after injection of antitetanus serum from another species of animal, antitoxin appeared in the rabbit's blood, and the dynamics of its level was the same as that found in the previous series of experiments. However, further injections of the heterogeneic serum 7 days after its primary injection likewise were ineffective. This coincided with the time of an accumulation of large quantities of ASP against the proteins of that serum in the blood stream.

The results thus indicate that in response to injection of heterogeneic immunoglobulins the body produces ASP, and their formation is subject to the general rules of the immunological response. These antibodies can be detected by the PTA, the passive hemagglutination test, and the complement fixation test [11, 12]. The rapid fall in the blood antitoxin level coincides with the appearance of ASP.

Investigations [3, 4] have shown that ASP are formed against the heterogeneic determinant of the protein molecule and not against its specific group. However, despite this fact, once they appeared in the blood stream and interacted with the antiserum they blocked its specific determinant, as a result of which subsequent injections of the preparation were almost or completely ineffective. This was also confirmed by the fact that, as a rule, the ASP began to be detectable in high titers, while small amounts of ASP could not be detected for they were evidently completely bound by the protein molecules of the related serum.

The duration of circulation of ASP in the blood depends on the number of injections of the preparation.

This effect of blocking the specific activity of serum preparations by ASP can be overcome by successive administration of sera of different species. The serum of each species possesses optimum effectiveness for only 5-7 days.

Considering the results of the writer's previous investigations with antivirus preparations [9-12] and results obtained with cytotoxic sera [1], it can be concluded that the phenomenon of blocking specific activity of serum preparations by ASP is not a special case, but a general biological rule.

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