The appearance and circulation of antibodies against serum proteins in the bloodstream 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
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) Titors of antitoxin; 2) titors of antibodies against horse serum proteins; 3) titors 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.

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.

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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.

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)