CATABOLISM OF HETEROLOGOUS "DIAFERM" ANTITOXIN

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The disappearance of heterologous antibodies from the blood is connected with the formation of antibodies against the heterologous immune protein, and this applies to antitoxins, especially tetanus antitoxin injected for the purpose of passive immunization or treatment [3, 4, 5, 7, 8, 10]. This process has been observed both in man [5] and in animals; it begins most intensively on the 6th-7th day [3, 5, 10], when the discharge of the newly formed antibodies into the blood stream becomes considerable in scale and is accelerated with each fresh injection of antitoxin if it is given repeatedly [3, 7, 10]. The mechanism of disappearance of antitoxin from the blood is largely unexplained. It is assumed that this process is accompanied by excretion of the antitoxin from the body. However, the process of excretion of antitoxin or of its breakdown products during passive immunization has not been studied in direct experiments, although it is known that during active immunization in man and animals (rabbits) fragments of antibody molecules with a sedimentation coefficient of 0.92-1.2 Svedberg units are excreted in the urine [6, 9, 11].

In the present investigation an attempt was made to discover whether the excretion of antibody fragments of low molecular weight takes place during passive immunization with heterologous antitoxin, how this process is modified during repeated injections of antitoxin, and whether it is correlated with the decrease in the antitoxin titer in the blood and with antibody formation against it.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits weighing 2.5-3 kg. Each series of experiments was conducted on four animals. The rabbits were injected subcutaneously with 9000 antitoxin units of "Diaferm" tetanus antiserum (three injections at intervals of 12 days). Immediately before each injection and 48 h thereafter serum was taken for determination of the antitoxin content (biological testing on mice) and of the titer of antibodies formed against it (ring precipitation test). A mixture of equal volumes of serum from all the experimental animals was used for titration.

On the days after injection of the antitoxin the animals were kept in specially equipped cages similar to those used for studying basal metabolism, and the urine was collected for 4 days. After collection of each sample it was clarified by centrifugation and kept in the frozen state. Proteins were isolated from a mixture of equal volumes of the rabbits' urine and fractionated by the method described above [2]. The principal stages of the procedure were as follows. The urinary proteins were precipitated by a 3.3 M solution of ammonium sulfate. After dialysis against distilled water a saturated solution of ammonium sulfate was added to the protein solution to 50% saturation, the intensely pigmented residue was discarded, and the supernatant was dialyzed against distilled water, concentrated, and fractionated on a Sephadex G-50 column in distilled water. The antitoxic activity of the fractions thus obtained was determined by biological tests on mice. Immunoelectrophoresis and double diffusion in agar were carried out in a micromodification [1], using a rabbit antihorse serum as developer.

EXPERIMENTAL RESULTS

During chromatograph through a Sephadex G-50 column in distilled water the "Diaferm" tetanus antitoxin was eluted in one fraction (one symmetrical peak). The urinary proteins of the intact rabbits not precipitated by ammonium sulfate in 50% saturation were separated into two fractions by filtration through the same column. One of them (peak A) was eluted by the same volume of solvent as the original "Diaferm"

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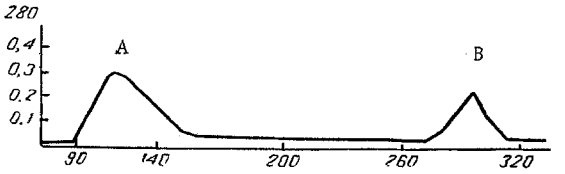

Fig. 1. Chromatography of urinary proteins of rabbits receiving one injection of "Diaferm" tetanus antiserum on a Sephadex G-50 column in distilled water. A and B — peaks of fractions 1 and 2 of urinary proteins.

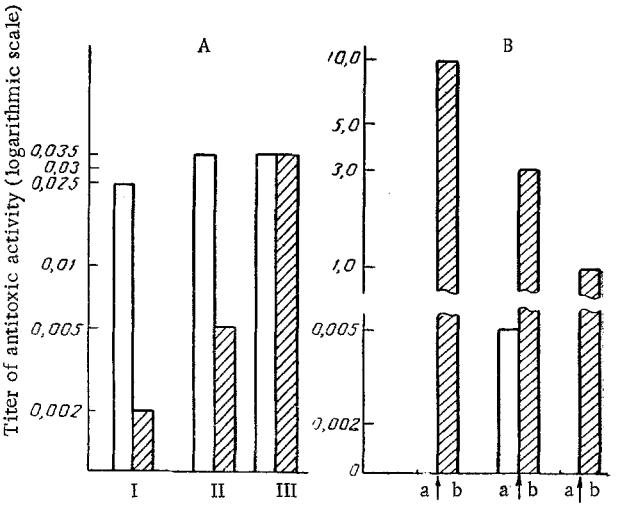

Fig. 2. Antitoxic activity of fractions 1 and 2 of urine (A) and antitoxin content in blood (B) of rabbits during triple passive immunization. A: Unshaded columns — antitoxic activity of fraction 1 (peak A), shaded columns — antitoxic activity of fraction 2 (peak B). I-III) Injections of antitoxin; B: antitoxin content in blood before (a) and 2 days after (b) injection of antitoxin; antitoxin titers in the urine and blood are given on a logarithmic scale.

antitoxin, whereas the other (peak B) was considerable retarded in its passage through the column (Fig. 1). As shown previously [6], the proteins of this fraction were precipitated during ultrafiltration at the rate of 1.2 Sverdberg units.

Fractions with similar physicochemical properties were also obtained during chromatography of the urinary proteins of animals of the experimental groups, receiving 1, 2, and 3 injections of antitoxin, on Sephadex (Fig. 1).

In every case antitoxic activity was found in the fractions A and B isolated from the urine of the passively immunized animals. Comparison between the specific activities per liter of urine showed that the ratio between the antitoxic activity of these fractions in the animals receiving 1, 2, and 3 injections of antitoxin was not identical (Fig. 2A). Whereas the specific antitoxic activity in fraction A did not change significantly, the activity in fraction B rose. After the third injection of antitoxin the specific activity of fraction B was more than 15 times greater than the specific activity of the same fraction in the urine of the same animals after a single injection of antitoxin. At this moment a high content of precipitatins was

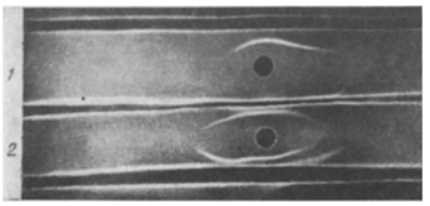

Fig. 3. Immunoelectrophoresis of "Diaferm" horse tetanus antiserum and of the peak A fraction. In the wells: 1) "Diaferm" serum, 2) peak A fraction; in the gutters: rabbit antihorse serum.

found in the blood (titer over 1 : 1000) of the rabbits. The increase in the specific activity in fraction B correlated with the degree of elimination of this fraction from the blood (Fig. 2B).

Experiments with immunoelectrophoresis showed (Fig. 3) that fraction A of the passively immunized animals contained a protein detectable by antiserum against horse globulins and with the same electrophoretic mobility as "Diaferm" antitoxin. Because of the limited quantity of material it was impossible to carry out a similar analysis of the proteins of peak B.

The results described above show that in rabbits immunized passively with heterologous antitoxin excretion of antitoxic protein in the urine takes place in two fractions: the antitoxic activity of the fraction A protein belongs to the "Diaferm" antitoxin, for it is identical in its physicochemical properties to this antitoxin; the antitoxic activity of fraction B is evidently due to a fragment of the "Diaferm" antitoxin molecule, for the proteins of this fraction are precipitated in the ultracentriguge at the speed of 1.2 unit [6], whereas the sedimentation constant of pepsin-treated "diaferm" horse antitoxin is 5 units.

The excretion of globulins (like the "Diaferm" antitoxin), proteolyzed by pepsin, in the urine has also been demonstrated in other investigations [11]. This phenomenon can be explained by assuming that in these globulins the part of the molecule responsible for their reabsorption is destroyed. It is a striking fact that when repeated injections of antitoxin were given in the present experiments the total amount of antitoxin excreted with this fraction did not increase. Hence, the excretion of antitoxin with the urine in fraction A is not connected with the immunologic reaction. It may accordingly be considered that this process is physiological in nature.

Meanwhile, the amount of antitoxin of low molecular weight excreted with fraction B increased with each successive injection of the heterologous antitoxin. This process correlated, on the one hand, with a decrease in the antitoxin content in the blood, and on the other, with the increase in the titer of antibodies against it. These results, coupled with the fact that proteins with analogous physicochemical properties are not present in the composition of "Diaferm" antitoxin, suggests that the antitoxin excreted with fraction B is a product of catabolism of the injected heterologous antitoxin.

Elimination of the "Diaferm" heterologous antitoxin thus takes place in two ways: by excretion of the antitoxin itself in its original form and by excretion of low-molecular weight products of its catabolism. As the content of antibodies against heterologous antitoxin in the blood rises, the importance of the second mechanism (immunologic catabolism) increases. It may be supposed that cells of the reticulo-endothelial system participate in the processes of catabolism of the injected antitoxin. In connection with the observations described above it would be interesting to study the process of elimination of native antitoxin, both heterologous and homologous.

LITERATURE CITED

1. G. I. Abelev and V. S. Tsvetkov, Vorp. Onkol., No. 6, 62 (1960).
2. M. M. Bartova, A. Ya. Kul'berg, Yu. B. Volgin, et al., Vorp. Med. Khimii, No. 3, 12 (1965).
3. A. T. Kravchenko and F. F. Rezepov, Voen.-Med. Zh., No. 1, 48 (1960).
4. G. N. Kryzhanovskii, L. N. Fontalin, and L. A. Pevnitskii, Vestn. Akad. Med. Nauk SSSR, No. 10, 18 (1960).
5. G. N. Kryzhanovskii, K. M. Loban, M. V. D'yakonova, et al., Klin. Med., No. 3, 68 (1962).
6. A. Ya. Kul'berg and L. M. Bartova, Vorp. Med. Khimii, No. 5, 514 (1963).
7. E. Falchetti, C. R. Soc. Biol., 120, 592 (1935).
8. E. Falchetti, C. R. Soc. Biol., 124, 96 (1937).
9. E. Merler, J. Remington, M. Finland, et al., J. Clin. Invest., 42, 1340 (1963).
10. G. Ramon and E. Falchetti, C. R. Soc. Biol., 119, 6 (1935).
11. J. Remington, E. Merler, M. Lerner, et al., Nature, 194, 407 (1962).
12. H. L. Spiegelberg and W. O. Weigle, J. Exp. Med., 121, 323 (1965).