# DISTRIBUTION OF ANTIGEN AND ANTIBODIES IN SMALL GOPHERS IMMUNIZED WITH DEPOSITED ANTIGEN

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In recent years, the prominent role of the lymphoid system in antibody production was demonstrated [2, 5]. With the help of cytological methods it was established that in the lymph nodes and spleen from the effect of antigenic stimulation shifts occur in the cellular composition characterized at first by an accumulation of large reticular and transitory cells, and then plasmatic cells, while the "plasmatization" of the lymphoid organs coincides with the appearance of the antibodies. As a rule, the appearance of antibodies in the blood precedes their development in the regional lymph node [1, 2].

We decided to examine the distribution of antigen and antibodies in small gophers (Citellus pygmaeus Pall.) who were injected with deposited antigen.

## EXPERIMENTAL METHOD

Fraction I of a virulent strain of bubonic plague 1213 which was a relatively homogeneous substance giving a single line in the gel precipitation reaction with antiplague serum was used as the antigen. The minimum neutralizing dose of antigen in the antibody neutralization reaction was 0.004 μg. The antigen was kept in dry form before the experiment it was dissolved in physiological solution, mixed with an aluminum hydroxide suspension and 0.1 ml injected into the skin of one or two of the animal's rear paws.

After various periods of time the animals were anesthetized with chloroform, their chest cavity opened, blood removed from the heart and incisions made in the femoral and axillary veins. Then the animals were perfused through the heart with a volume of warm physiological solution equal to their weight. After perfusion the popliteal and axillary lymph nodes were removed from both sides, the rear paws were cut off at the level of the mid shank, as were the front paws. In some experiments the heart, lungs, liver, kidney, spleen and bone marrow were removed.

The plasmocytic reaction [1] in the regional lymph nodes was studied by counting the cellular elements in 50 visual fields of impression smears stained with azure blue-eosin [4].

The organs and tissues were weighed, ground with a motar and pestle, and the paste removed with physiological solution. The suspension was heated for 30 min. at 56°, centrifuged and the supernatant liquid tested for serological reactions. Because of the small size of the femoral lymph nodes, before preparation of the suspension two or three from animals of the same group killed in the same period were combined. The amount of antigen in the minimum neutralizing doses (MND) and antibodies in serum units (SU) was calculated (geometric mean) for all tissues or organs (for the whole volume of blood in the body, for the whole paw suspension, etc.).

Antibodies were determined by the passive hemagglutination reaction, antigen by the antibody neutralization reaction. Both reactions are extremely sensitive [3]. The inhibition of passive hemagglutination reaction was also used to examine the specificity of the positive results of the passive hemagglutination reaction.

## EXPERIMENTAL RESULTS

After introduction of the deposited antigen (up to 25 g) into the right and left rear paws and precipitin into the front paws of 55 gophers, over the course of two weeks fraction I was found at the site of injection. At the end

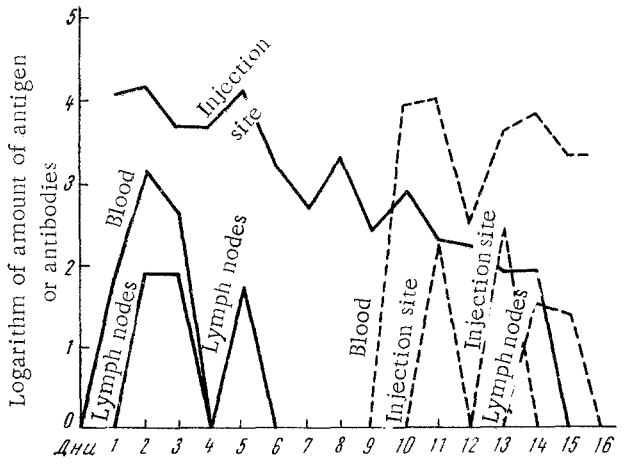


Fig. 1. Distribution of antigen (in MND) and antibodies (in SU) in the body of the small gopher. Solid line—antigen; dotted line—antibody.

of the period of antigen detection it was possible to find antibodies in this placed in some animals. Then a period followed in which it was not possible to detect either antigen or antibodies. Probably the period of predominance of antibodies over antigen is so small that sometimes it was not possible to catch it.

During the whole period we did not find antigen or antibodies either in the front paws or in the parenchymatous organs.

Soon after the injection of antigen fraction I was found in the blood and urine; later, antibodies circulated in the blood serum. The over-all pattern of the distribution of antigen and antibodies is seen in Fig. 1.

The plasmocytic reaction in the popliteal lymph nodes was characterized mainly by maximal accumulation of plasmoblasts on the 8th day.

We attempted to divide the injection site into the actual place of the injection and the surrounding tissue. For this, 49 gophers were immunized and cut open, as described above, but before examination the pulp of the foot (approximately 15 mg) was removed and placed in a small amount of physiological solution. The remaining part of the foot (about 500 mg) was also placed in a small amount of physiological solution for washing. Both wash liquids were combined before investigation. Suspensions were prepared from the pulp and foot which were examined separately.

The antigen concentration in the pulp was always much higher than in the remaining part of the foot, while in the latter the elimination of antigen occurred somewhat more quickly than in the pulp. In one gopher killed on the 11th day antigen was found in the pulp, and antibodies in the surrounding tissue. Antigen in the wash liquid ("free" antigen in soluble form) was found up to the 11th day, that is, even when there was a high titer of antibodies in the animals' blood.

In another series of experiments in a group of small gophers (19) antigen was injected into the right rear paw, and then repeated on the 9th day in the left rear paw (Fig. 2). In a second group of animals (18) antigen was injected both times in both paws with the same interval. The time antigen, could be detected as show in Fig. 2, in the right rear paw was prolonged in comparison with animals who did not receive a repeat injection and was the same as the time of antigen detection in the left paw and in the paws of animals of the group in which antigen was injected both times in both paws.

Thus, an unusual equalization occurred in the time of elimination of antigen from the places in which it was injected. This is also indicated by the fact that antibodies in the right and left popliteal lymph nodes in animals of the first group appeared at the same time and somewhat later than in animals of the second group.

More than that, the impression is created that after injection of the antigen in the left rear paw in animals of the first group the amount of antigen in the right rear paw not only does not decrease but even increases. In all

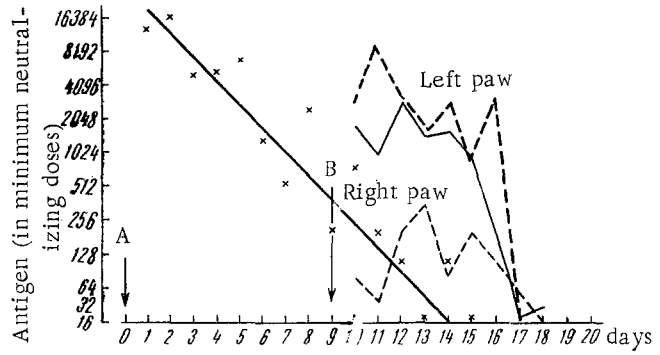


Fig. 2. Dynamics of antigen in injection site in small gophers. Solid, heavy line—antigen injected in both rear paws once; dotted heavy line—antigen in both rear paws after second injection; solid thin line—antigen in left paw after second injection; dotted thin line—antigen in right paw after second injection. A) first injection of antigen in right paw; B) second injection of antigen in left paw.

probability this increase is only apparent, connected with the removal of antibody producing cells or antibodies from the original place of injection to the site of the repeat injection of antigen. Therefore the reaction of the whole organism in response to repeated injection of deposited antigen in different paws depends to a greater degree on the concentration of antigen than on the remoteness of its injection.

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