POSSIBLE PASSAGE OF SPECIFIC ANTIGENS THROUGH THE BLOOD-BRAIN AND BLOOD-EYE BARRIERS IN PLAGUE TOXICOSIS

K. M. Mokhin, R. A. Beloborodov, V. I. Veinblat, and T. A. Kotkina

UDC 616.981.452-07:16.832.9+617.7]-008.6-07

In albino rats with plague toxicosis no specific antigens against Pasteurella pestis can be found in the brain or fluid from the anterior chamber of the eye, although they can be detected in high concentration in the blood and internal organs.

* * *

In plague and plague toxicosis marked disturbances of nervous activity are observed [5, 6, 9]. A decisive role in the genesis of these disturbances is ascribed to the effect of toxic products of Pasteurella pestis on the body [2, 3].

The object of the present investigation was to study the possibility of passage of breakdown products of P. pestis in plague toxicosis through the bloodbrain (BBB) and blood-eye (BEB) barriers.

EXPERIMENTAL METHOD

Experiments were carried out on 124 albino rats, animals sensitive to plague toxin. Toxicosis was produced by intraperitoneal injection of 4 LD_{50} of plague autolysate or cells of P. pestis (strain EV) grown at 28 and 37° and killed with acetone. The animals were killed by decapitation 30 min later or in the agonal period of toxicosis. Blood was removed from the brain by washing out the vessels with physiological saline through a needle inserted into the common carotid artery. To detect breakdown products of P. pestis the following methods were used: 1) gel diffusion [8], 2) immunofluorescence staining of tissue sections [1 7], and 3) the antibody neutralization reaction (ANR) [4].

Agglutinating plague antiserum and antiserum containing antibodies against fraction I, prepared at the "Mikrob" Institute, and also an antitoxic plague antiserum kindly presented by V. N. Metlin, were used in the gel diffusion reaction. Formalinized sheep's erythrocytes, sensitized with fraction I of P. pestis, were used as reacting system in the ANR. Tissue sections were stained with luminescent antisera against capsulo-somatic fraction and fraction I of P. pestis.

EXPERIMENTAL RESULTS

The results of the experiments of series I showed that no antigens of P. pestis could be detected in the brain tissue 30 min after injection of the toxin by the gel diffusion method, whereas fractions I and II (toxic) and other unidentified fractions of P. pestis were readily detected at this time in the blood serum. In the experiments with brain tissue of rats in an agonal state, no breakdown products of P. pestis likewise could be detected by the gel diffusion reaction, but when the reaction was performed with blood serum from animals in an agonal state, clearly defined precipitation lines were observed (Fig. 1).

Investigation of the permeability of the BEB by the gel diffusion method showed that neither during the initial period nor during the period of marked toxicosis could antigens of P. pestis be detected in fluid from the anterior chamber.

By means of the gel diffusion reaction, specific antigens were thus found in the blood serum of albino rats with plague toxicosis, but none were found in the brain or fluid from the eye.

"Mikrob" All-Union Plague Research Institute, Saratov. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 6, pp. 45-47, June, 1969. Original article submitted September 20, 1967.

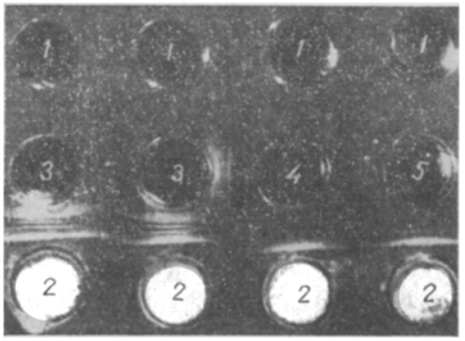

Fig. 1. Gel diffusion reaction.
1) Brain supernatant; 2) blood serum. Plague antisera: 3) agglutinating; 4) against fraction I; 5) antitoxic.

To determine whether a local concentration of plague antigens can form in the brain, a series of experiments was carried out in which brain sections were treated with specific fluorescent antibodies. This method was used also to investigate tissues from the liver, spleen, and lung. As a control, sections of the brain and organs of normal albino rats stained with fluorescent serum, and also tissues from experimental animals stained with luminescent cholera antiserum, were studied.

Examination of brain sections of albino rats sacrificed in both the initial and final periods of toxicosis as a rule revealed no specific luminescence. Different results were obtained in the investigation of spleen, liver, and lung sections. Large formations with bright specific luminescence of the cytoplasm and a negative shadow of the nucleus were observed among the cells and in the intercellular spaces of these organs. No specific luminescence was found in sections of organs of normal animals treated by the same method. The control with luminescent cholera antiserum likewise gave negative results.

The results of this series of experiments indicate that no plague antigen is present in the brain of albino rats in an amount detectable by the immunofluorescence method.

In the next series of experiments the ANR, an immunologic method capable of detecting thousandths of a microgram of plague antigen [4], was used to detect minimal quantities of plague antigens in the brain. However, this method also failed to reveal specific antigen in the brain tissue of rats sacrificed in the initial and final periods of poisoning. A high concentration of plague antigen (titers 1:4000-1:8000) was found in the blood serum 30 min after injection of the toxin, while in animals in an agonal state the antigen titers in the serum were higher still (1:6400-1:12,800). In isolated cases, plague antigen was found in low titers (1:20-1:40) in the brain of both experimental and healthy control animals, as a result of nonspecific factors.

In fluid taken from the eye of animals in various stages of toxicosis, no plague antigens likewise were found by the ANR.

From the agreement between the results of experiments using three different immunologic techniques, it can be concluded that the blood and internal organs of albino rats with plague toxicosis contain large quantities of components of P. pestis, but these substances do not pass through the BBB and BEB. Disturbances of nervous activity observed in plague toxicosis cannot therefore be connected with the direct action of breakdown products of P. pestis on the nerve cells of the brain.

LITERATURE CITED

1. R. A. Beloborodov, in: Problems in Microbiology and Laboratory Diagnosis of Particularly Dangerous Infections [in Russian], Saratov (1965), p. 221.
2. I. V. Domaradskii, Outlines of the Pathogenesis of Plague [in Russian], Moscow (1966).
3. A. G. Kratinov, Abstracts of Proceedings of an Inter-Institute Scientific Conference on Microbiology, Immunology, Laboratory Diagnosis, and Treatment of Particularly Dangerous Infections [in Russian], No. 1, Saratov (1955), p. 28.
4. M. I. Levi and A. G. Momot, in: Collected Scientific Transactions of Élista Plague Station [in Russian], No. 2, Élista (1961), p. 207.
5. K. M. Mokhin, Transactions of Rostov-on-Don Plague Research Institute [in Russian], Vol. 18, Rostov-on-Don (1961), p. 130.
6. G. P. Rudnev, Clinical Features of Plague [in Russian], Rostov-on-Don (1938).
7. A. H. Coons and M S. Kaplan, J. Exp. Med., 91, 1 (1950).
8. O. Ouchterlony, Acta Path. Microbiol. Scand., 25, 186 (1948).
9. R. Pollitzer, La Peste, Genéve (1954).