THE PROBLEM OF ANTHRAX AND IMMUNITY

By Ye. P. Stefanova

-USSR-

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FOREWORD

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Following is the translation of an article by Ye. P. Stefanova in Trudy Vsesoyuznogo Institut-a Eksperimental'nov Veterinarii, No 22, Moscow, 1959, pages 177-181.

As early as 1888, N. F. Gamaleya, in experiments with vaccination of sheep against anthrax, established that in order to produce lasting immunity it was necessary for the organism to react to the introduction of antigen with a slight fever. Consequently, at the very dawn of the development of immunology the Russian scholar foresaw the significance of the organism's reactivity in the process of development of immunity to anthrax. Since then, numerous investigators have confirmed by their own research the conclusions drawn by N. F. Gamaleya.

I. P. Pavlov has proved that the extent of the rise in temperature in fever, as well as the character of the rise and fall of the temperature, depend upon the condition of the nervous system, and that the temperature reaction is a reflex process.

Considering that fever in infectious diseases is the organism's means of self-defense, Pavlov wrote: "The basis for this consists in that the life of many disease-causing micro-organisms is confined to specific degrees of temperature. The temperature need only be changed up or down by two or three degrees to make life difficult for them."

This may explain why, in L. Pasteur's experiments, the chickens, ordinarily not susceptible to anthrax, contracted it when the body temperature was lowered.

Later, I. I. Mechnikov demonstrated that artificial chilling weakens the organism and the microbes are enabled to multiply more rapidly.

There are also numerous evidences of the protective role of inflammatory reaction in immunity. Mechnikov regarded inflammatory reaction as a curative force of the organism. According to him, the protective role of inflammation is due to phagocytosis, which he regarded as the main defense mechanism, peculiar to living creatures on all rungs of the zoologic ladder; as a fitting protective reaction of the organism, inherent in every living thing, that is, a general biologic phenomenon.

Very many works, both of our own and of foreign authors, are devoted to the role of inflammatory reaction in immunity to anthrax. Among them must be classed the works demonstrating the favorable
influence of the so-called stimulating or, as it is often customary to call them, the "depositing" substances upon the development of immunity to anthrax vaccines.

All this indicates that inflammatory reaction and vaccinal fever play a substantial role in the formation of immunity in anthrax inoculation.

Working since 1939 on the study of the nature of anthrax infection and immunity, we have proven experimentally that the nervous system plays an exceptional role in the process of the development of immunity. Let us demonstrate this by the following example:

As is known, Tsenkovskii's first vaccine, from the time of its introduction (1863), has so lowered its immunogenic properties that a second Tsenkovskii vaccine has begun to be used in the last few decades in the practice of anthrax control.

We have repeatedly confirmed by histologic investigations the absence of a reaction by the organism to the introduction of the first vaccine, it being established that the spores of the first vaccine remain for about 5-8 days at the place of introduction without germinating.

Proceeding from I. P. Pavlov's doctrine that no reaction on the periphery takes place without the participation of the central nervous system and that a reflex process underlies the protective reaction, it could be assumed that the first Tsenkovskii vaccine is too weak as an irritant and does not cause the proper irritation of the receptor devices at the place of introduction, so that there is no transmission of the antigen stimulus to the central nervous system.

Our experiments with the introduction of the first Tsenkovskii vaccine into rabbits by the method of suboccipital puncture directly into the spinal fluid have shown that in this case immunity sets in even in a span of time measured by minutes. It has been established that this immunity is of a strictly specific character and in no case can be interpreted as a factor of unspecific retardation. The control rabbits, into which hog erysipelas vaccine or a physiological solution was introduced simultaneously, died of anthrax infection as a result of the inoculation.

Studying the fate of the first vaccine, introduced into the spinal fluid, we established that the bacilli of the first vaccine are very quickly destroyed. In the smear prepared 20 minutes after the introduction of the vaccine, granular accumulations of disintegrated bacilli and lymphocytes were visible in the field of vision, but after three hours only 2-3 leucocytes could be noted. The presence of lymphocytes already in the first minutes after the introduction of the vaccine indicates, as is known, the irritation of the center of the vegetative nervous system.

These investigations have led us to think that, in vaccinating with avirulent cultures, such as the first Tsenkovskii vaccine is, it is necessary to create, at the place of injection, conditions favoring the transmission of an antigen stimulus to the central nervous system. This can be accomplished if conditions for an
intensive, heightened metabolism of the vaccine are created at the place of injection, and if its metabolism is in direct and close connection with the metabolism in the tissue into which the vaccine is injected. That is, in order to heighten and intensify the exchange of matter by the vaccine, the metabolism in the injected tissue must be heightened. This may be done most simply by causing an inflammation at the place of introduction of the vaccine, since the exchange in an inflammation focus is always heightened. In order to cause an inflammatory reaction, we added a small quantity of saponin — 0.1 mg per dose — to the first Tsenkovskiy vaccine. Besides this, we added to the vaccine Na ions, which promote the transmission of nervous stimulation from the periphery to the central nervous system, and we brought the pH of the vaccine up to 8.0-8.5, since it has been known ever since the work of P. N. Andreyev (1898) that an alkaline medium promotes the intensive propagation of vaccine.

The experiments with the vaccination of rabbits by the above-described method have demonstrated the presence of lasting immunity even with a single injection of the first Tsenkovskiy vaccine.

It is known that nobody has thus far succeeded in producing immunity in white mice with the first Tsenkovskiy vaccine, since it is pathogenic for these animals. In the organism of the white mouse, the bacilli multiply without impediment, and phagocytosis, as a rule, is extremely weak or even absent.

We set ourselves the task of studying the question of the possibility of immunizing white mice with the first Tsenkovskiy vaccine. For this purpose, a so-called split vaccine was prepared. The vegetative form of the first Tsenkovskiy vaccine was split in a 1% and a 2% solution of NaHCO₃ in a thermostat. After three days, the culture of the first vaccine represented very fine granular formations settled on the bottom of the flask. Vaccinating the white mice with this split vaccine, we obtained lasting immunity in this species of animal.

The white mice were inoculated intramuscularly with a 0.2 ml dose. After 14-21 days these mice were infected with a 10-fold lethal dose of the ordinary first Tsenkovskiy spore vaccine.

The results of the experiments are shown in the table.

(see next page).
Experiment in the Immunization of White Mice with a Split Culture of the First Tsenkovskiy Vaccine

<table>
<thead>
<tr>
<th>Number of animals inoculated</th>
<th>Time of infection after vaccination</th>
<th>Control infection</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>14 days</td>
<td>10</td>
<td>9 alive, 1 dead</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>3</td>
<td>All dead</td>
</tr>
<tr>
<td>20</td>
<td>21 days</td>
<td>19</td>
<td>14 alive, 5 dead</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>5</td>
<td>All dead</td>
</tr>
<tr>
<td>25</td>
<td>21 days</td>
<td>22</td>
<td>16 alive, 6 dead</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>5</td>
<td>All dead</td>
</tr>
</tbody>
</table>

From the data given above it may be seen that the first split vaccine imparts lasting immunity to white mice.

Later, it was established that the spore form of the first Tsenkovskiy vaccine used for control infection is subjected to destruction and phagocytosis in the organism of the white mice previously immunized with the split vaccine (Fig. 1). In these experiments it was also noted that the bacilli are destroyed outside the leucocytes as well; the latter seize only the already destroyed bacillus cells.

All the foregoing has led us to think that the immunity, as well as the infection, are closely connected with metabolism.

It is known that soda possesses the property of intensifying metabolism in the organism, but it apparently also intensifies metabolism in the bacterial cell itself. In smears taken 20 minutes after the action of the soda, the capsules and swollen cells of the vaccine were well pronounced when the bacilli were colored with methyl red, and after one hour the protoplasm of the bacillary cells broke up into granules colored an intensive red by the methyl red. These data fully agree with the numerous indications in literature that capsule formation in anthrax bacilli has always been noted in media having an alkaline pH.

It was important to ascertain just what happens to the anthrax bacilli in the organism and how the organism itself reacts to the introduction of the vaccine.

For this purpose, we made experiments on rabbits that had been vaccinated with the second Tsenkovskiy vaccine and had remained alive after a control infection with a lethal dose of virus B. anthracis.

A section about 15 cm²/120. Millimeters in diameter was snipped off the lower wall of the abdomen of these rabbits. Around the circumference of this section, a 1% solution of novocain in a
dose of 0.1-0.2 ml was introduced twice at intervals of 30-40 minutes at four points, and the anthrax spore virus was introduced into the center of the snipped-off surface. The purpose of the introduction of novocain was to eliminate nerve influence temporarily and check leucocytosis. Very soon (in 3-6 hours) an edema was formed at the place of injection of the virus and in 18 hours had attained the size of 7 x 10 cm and had the form of a sharply limited, projecting tumor. At different times after inoculation, spinal fluid, edematose fluid and blood from an ear vein were taken from these rabbits to prepare smears.

In the smears of edematose fluid, vegetative forms of B. anthracis were found in place of the spores, and there were anthrax bacilli of unusually large dimensions already 30 minutes after inoculation alongside of the oval forms colored according to Gram (germinating spores); some were surrounded by a capsule, often of a twisted form (Fig. 2).

In the spinal fluid, lymphocytosis was noted in 20 minutes. In 12 hours, there were leucocytes and fibrin reticula in addition to lymphocytes.

In the smears of spinal fluid taken 20-30 minutes and one hour before the death of the animal were observed formations which retained the contours of anthrax bacilli, sometimes of unusually large dimensions, 5-6 times above normal, in the swelling stage and twisted, with parts of the capsule still present (Fig. 3).

It should be noted that when bacteriemia was developing in the blood and organs, the anthrax bacilli in the spinal fluid were also in the destruction stage. This picture of anthrax bacilli destruction was observed 20 minutes after death (Figs. 4-5).

In the smears of blood taken not long (5-10 minutes) before the animal's death, local thickening of the membranes of the erythrocytes was noted; one got the impression that there are inclusions of some kind in the erythrocyte membranes. Sometimes the thickening of the membranes was more considerable on some one side. Powdery accumulations were observed around the erythrocytes. An analogous picture was observed in the fluid of the inflammatory edema. We have made and examined these preparations many times -- without fixation and employing different methods of fixation -- uncolored and colored by various methods (see Fig. 2), and are convinced that the above-described changes are regular in character.

Analyzing the above data, we have arrived at the conviction that infection and immunity in the case of anthrax are closely connected with metabolism in the organism.

In anthrax infection, the action of a very high body temperature results in profound disturbances of the metabolism, which are also promoted by the toxic products of the vital activity of the anthrax bacilli. When the disturbance of the metabolism becomes catastrophic, there is a destruction of the albumins of the organism proper, as we see from the picture of the erythrocytes: when even the albumins of the erythrocyte membranes are dispersed, hemolysis and death set in.
CAPTIONS TO PHOTOGRAPHS

Fig. 1. Destruction of the first vaccine in the organism of a white mouse vaccinated with "split vaccine."

Fig. 2. Rabbit No. 2312. Edema. Thickening of the membrane of erythrocytes and inflated forms of bacteria of large sizes.

Fig. 3. Rabbit No. 2702. Spinal fluid. Destruction of bacil-
lar cells and granular accumulations 20 minutes before death.

Fig. 4. Rabbit No. 2702, the same 20 minutes after death.

Fig. 5. Rabbit No. 2702. Spleen. Destruction of bacil-
cells and phagocytosis.

LITERATURE

Gamaleya, N. F., Uchenye ob infektsii (The Doctrine of Infection), Medgiz, 1931.


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