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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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The mutation and transformation problem in microbes has an extremely great theoretical and practical meaning. In the work "New in Science and in the Biological Outlook" T. D. LUSENKO adduced a number of situations of transformations in vegetable organisms according to the influence of the changed habitat.

There is a great number of works in the field of microbiology on the transformation of the one kind of variant into another of the present time. Thus V. M. TIMANSKII, E. M. KOROBKOVA, K. P. POKROOSKAIA, G. N. LENSKAIA and others announced the obtaining of the microbe of the plague pathogen which is able to produce tuberculosis in rodents. These pathogens are independent types possessing various cultural and fermentative properties. The transformation of the intestinal rods into the paratyphoid BRESLAU culture by growing the intestinal rods in the medium containing killed microbic cells of the BRESLAU paratyphoid culture, is described in N. P. GRACHEVA'S works which were conducted under the Leadership of N. F. GANALEIA. The obtained new microbe possessed cultural, fermentative and immunological properties, inherent to the BRESLAU culture.

V. D. TIMAKOV has informed us about the transformation results of the intestinal rod into the paratyphoid BRESLAU culture, the paratyphoid B and the dysentery microbe; these results were obtained by cultivating the intestinal rod separately under conditions when the dissociation products of other related bacteria (paratyphoid B, Breslau, dysentery) became the nitrogen source of its nutrition.

G. P. Kallna obtained microbes similar to the paratyphoid A by influencing FLEKSHER'S microbial culture with bacteriophagia followed by cultivation on special media.

The mentioned works are of a great practical and theoretical interest as they are giving a new direction toward the obtaining of new variants of micro-organisms with useful characteristics. The persuasive part of these works consists in the transformation of one microbial type into another; this was proved by the fact that the newly obtained micro-organism possesses all biochemical and serological properties of the directed culture.
It must be said that it is extremely difficult to transform one kind of microbial variant into another. Therefore this is, possibly, the cause, why the researchers did not succeed until the present time in obtaining new vaccinal strains by the transformation of one kind of microbe into another.

The recently mentioned situation concerning the absence of specific intraconcurrence in micro-organisms and the presence of such among the variants of different types of microbes was elaborated by researchers and explained in the press.

N. A. KRASIL'MIKOV, by working with a great number of microbial variants determined the possibility of defining the nature of micro-organisms by the help of antagonism phenomena. N. A. KRASIL'MIKOV, while laboring the question of the specific inter and intraconcurrence has proved on a large scale of material that no intraconcurrence exists among microbes. The researcher, applying the method of sowing the cultures on hard nutritive media followed by a layer of microbes-antagonists, had never noticed a pressure some on the growth of cultures of the same name. On the contrary, he always observed one, when experimenting with microbes of different variants.

N. A. KRASIL'MIKOV tried to expose the antagonistic properties in the experimentally obtained variants of actinomyces. The results of these observations showed, that the variants, which possessed antagonistic properties toward remotely related organisms never displayed antagonism toward their own types.

This situation confirms the observation of medical and veterinary microbiology that those strains which were obtained by directed transformation belong to that type of microbes from which they were obtained.

N. A. POKSHISHEVSKII, while studying the changeability of the anthrax pathogen isolated from a killed swine, came to the conclusion that there are conditions when the pseudonarthracic bacilli became pathogenic and can act as pathogens producing anthrax in man and animals. However, it must be said that this pronounced opinion has not been proved in practice. It is possible that the researcher has experimented with an anthrax strain which has been drastically changed in the organism of the swine. There are numerous data on the changeability of anthrax microbes in the swine organism. For instance L. S. NEVSKII indicates that he isolated virulent and mobile microbes, also fully apathogenic ones, from the organism of sick swine.

The biological belonging of the isolated microbes to the anthrax cultures was proved by the author by seeding into MFB, MPA, by microscopy, and also serologically in the precipitation reaction of the anthrax precipitant serum.

D. M. TETERMIK determined an unusually great changeability of anthrax microbes. He isolated from swine, sick with anthrax or from those which has already had anthrax, the greatly changed cultures of a low virulence or, some times, even entirely avirulent ones.

The author came to the conclusion that these cultures are variants of the anthrax pathogen.

Thus, the organism of the swine appears to be an unfavorable medium for the virulent anthrax microbes; there they undergo a drastic change, even losing entirely of partially, their cultural - morphological and virulent properties.

We consider this situation to be convincing as it is based on I. V. NICHURIN'S teachings, which deal with the effect of the habitat.
on the properties of the organism.

S. N. MURCHTSEV has clarified in his published works the changeability and transformation problems of microbes.

The author shows new forms of transmutation (or transition) of one species into another, he also admits possibilities of transition from saprophytes to parasites. For instance, he said, the transition from saprophytes of soil microbes to anthrax pathogen is possible, also the transmutation of anaerobic micro-organisms into new variants of anaerobic microbes.

S. N. MURCHTSEV discusses also the specific nature of living microbial vaccines. For instance, he claims that the micro-organisms of anthrax vaccines used in veterinary practice, are new, even although they are closely related species to the anthrax microbe. These are interesting statements, but the author has not confirmed them yet by experimental data.

In order to specify the question, we found it necessary to publish some data which we have obtained during the study of the specific properties of the anthrax vaccine.

We conducted experimental tests in the anthrax laboratory according to the order of the Veterinary Administration of the USSR Agricultural Ministry and checked the specific particularities of the first and second TSENKOFSKII'S anthrax vaccine and also of the STI vaccine in order to define if these vaccines do possess antagonistic properties toward the virulent strains of the anthrax microbes.

The study of the antagonistic properties of the vaccines was conducted in two different ways. In the first experimental test the vaccine cultures were laid into bacteriological dishes in the form of blocks of the one day agar culture on the filmy colony of the freshly seeded virulent anthrax culture on the surface of the agar.

The results of numerous testing experiments showed that the THENKOVSKII vaccine and the vaccine STI do not possess antagonistic properties toward the virulent strains of the anthrax culture because no enlightenment none (some suppressing the growth) was observed.

Analogical studies were conducted on the strains of low-virulent anthrax cultures, obtained in the laboratory by means of directed influence (Sh-15, 316-1, 94 and 1260-31). The obtained results affirmed the absence of antagonism in the tested cultures.

The second experimental method (S. G. KOLESOV and IU. P. BORISOVICH) for testing the antagonism of TSENKOFSKII'S anthrax vaccine and of the vaccine STI of the strains 94, 916-a was conducted the following way: the vaccine were needed into MPB, into test tubes, followed by adding the cultures of the standard spore virus of anthrax unequal doses and concentration. The vaccines and the anthrax virus were needed in the form of agar culture. After one day of growing they were reseeded for the purpose to be passaged. Such a reseeding process was repeated 7 times.

It was noticed during the reseedings that the cultures, starting with the 3rd-4th generation, began to grow turbid in MPB, although the microscopical test confirmed the presence of clean and typical anthrax cultures in the needing. These cultures were tested for virulence by infecting white mice, guinea pigs and rabbits.
The culture of the STI vaccine caused the death of the rabbits and white mice during a period of 24-36 hrs. The culture of the first TENKOVSKII vaccine produced death in guinea pigs and white mice during 35 hrs. The culture of the strain 24 caused the death of the rabbits and white mice after 36-48 hrs, and the culture of the strain 916-1-a produced death in white mice after 18-24 hrs. The bacteriological examination (autopsy and seeding) showed that the animals died of anthrax.

Thus, after having conducted these experiments, we are not able to state the presence of antagonism of anthrax vaccines and of the new obtained strains 94-916-1-a toward the standard anthrax virus.

These experiments showed that the anthrax virus culture multiplied normally and was not affected by the antagonistic influence of the vaccines and of the strains of low virulence. The rabbits, guinea pigs and white mice together infected with the grown cultures, died a periods which are characteristic for the virulent anthrax culture.

Of considerable interest is the question of the study of the anthrax vaccine STI and of the second TENKOVSKII'S vaccine by taking into consideration the factors of pathogenesis produced by these vaccines in rabbits, and also the examination by the precipitation reaction of the pathologically changed organs of rabbits which died of these vaccines. We chose the application of this method for testing the nature of anthrax vaccines, since the precipitation reaction - as it is well known is considered to be the most valuable reaction, because it is chemically exact.

It is customary in veterinary and medical practice to uncover the natural properties of the micro-organisms and other complicated albumin substances by means of the precipitant anthrax serum and other precipitating serum. The properties of those precipitogens (antigens) which are composed of complicated polysaccharide and lipoidal substances are defined in the precipitation reaction.

It is known in practice that - for instance - the test of the decayed pathological material of cadavers of animals, which died of anthrax, permits to diagnose the disease correctly, even if this is no possible by other methods.

We conducted special experiments in 1952-1953 on the study of the pathogenesis of experimental anthrax in rabbits which have died as a result of the vaccination with the second TENKOVSKII vaccine and with the STI. For this purpose the exudative part of the edema of the dead rabbit was collected and filtered through asbestos cotton until entirely transparent. After this, the edema extracts from different rabbits were examined in the precipitation reaction with the precipitant anthrax serum, which was prepared in two different ways. The serum of the series No. 25 was obtained by the Tobol'sk biofactory by means of hyperimmunization of the horses-producers of the virulent anthrax culture of vegetative form and treated with formalin; the serum of the series No 75 was also obtained by the same biofactory by means of hyperimmunization of the horses-producers of the low virulent serum.

The results of the examination by the precipitation reaction of the properties of the edema extract of rabbits which have died of the STI vaccine and of the TENKOVSKII vaccine, are evident in Table I.
Examination results obtained by precipitation reaction of extract of anthracis edema of rabbits which died of the STI vaccine and of the second Tsnirovskii vaccine

### Table I

<table>
<thead>
<tr>
<th>Serial No. of the precipitation serum</th>
<th>Name of the antigen</th>
<th>Edema of the rabbit which died of the STI vaccine</th>
<th>Edema of the rabbit which died of the STI vaccine</th>
<th>Edema of the rabbit which died of the STI vaccine</th>
<th>Edema of the rabbit which died of the STI vaccine</th>
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</thead>
<tbody>
<tr>
<td>1:4</td>
<td>c:1:10</td>
<td>1:100</td>
<td>1:4</td>
<td>1:50</td>
<td>1:100</td>
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<tr>
<td>25</td>
<td>30&quot;+</td>
<td>35&quot;+</td>
<td>1'20&quot;+</td>
<td>40&quot;+</td>
<td>1'40&quot;+</td>
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<td>40&quot;+</td>
<td>40&quot;+</td>
<td>1'40&quot;+</td>
<td>55&quot;+</td>
<td>1'30&quot;+</td>
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<tr>
<td>Normal serum</td>
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</tbody>
</table>

Examination results obtained by precipitation reaction of extract of anthracis edema of parenchymal organs of rabbits which died of the STI vaccine and of the second Tsnirovskii vaccine

### Table II

<table>
<thead>
<tr>
<th>Standard antigen</th>
<th>Name of the antigen</th>
<th>Rabbit which died of the STI vaccine</th>
<th>Rabbit which died of the STI vaccine</th>
<th>Rabbit which died of the second Tsnirovskii vaccine</th>
<th>Rabbit which died of the second Tsnirovskii vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
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<td>1:10</td>
<td>1:10</td>
<td>1:10</td>
<td>1:10</td>
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<tr>
<td>Liver</td>
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<td>1:10</td>
<td>1:10</td>
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<tr>
<td>Lungs</td>
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<td>1:10</td>
<td>1:10</td>
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<tr>
<td>Edema</td>
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<td>1:10</td>
<td>1:10</td>
<td>1:10</td>
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<tr>
<td>Muscles</td>
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<td>1:10</td>
<td>1:10</td>
<td>1:10</td>
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</tr>
<tr>
<td>Spleen</td>
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<tr>
<td>Liver</td>
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<tr>
<td>Lungs</td>
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<tr>
<td>Edema</td>
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<tr>
<td>Normal serum</td>
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</tbody>
</table>
It is evident, from table I, that the edema extract of rabbits which died of the STI vaccine and of the TSENKOVSKII vaccine form a specific precipitation in rings with the anthrax precipitant serum, obtained by means of the virulent, anthrax culture treated with formalin, as well as with the precipitant serum obtained by the hyperimmunization of the vaccinal strains of low virulence.

Later on, a similar experiment was repeated; the results of it were the following: the edema extracts of the other 4 rabbit, which had died because of the STI vaccine, showed also a specific precipitation in rings with the mentioned series of the precipitant anthrax serum in a dilution 1f 1:10 and 1:100. The precipitant ring appeared in the period of 30 sec. to one minute 30 sec. and in 2 min. 40 seconds.

Besides these tests, there were also conducted experimental examinations by the precipitation reaction of parenchymal organs and muscles affected by the TSENKOVSKII vaccine and STI. It was possible to assume that the anthrax antigen (precipitinogen) can be contained not only in the edema of a dead rabbit, but also in parenchymal organs and in the muscle tissue of organs affected by the vaccine.

The reason for this assumption was the clear pathologoanatonical picture obtained at the autopsy of laboratory animals by the injection of the STI vaccine. At the autopsy, an exudative edema was usually uncovered in the abdominal region.

The muscle tissue was usually much affected at and around the injection spot, its color was red, sometimes, dark rod. The liver was of flabby consistency. The spleen did not appear to be changed, the lungs - hemorrhagic.

The examination of the blood and parenchymal organs for the presence of microbes showed that the microbe of the STI vaccine isolates at 50-75% from the blood, livef, lungs and spleen; - from the edema - at 80-100%. The muscle tissues of rabbits which died from the second TSENKOVSKII vaccine, are analogically affected at the spots of the vaccinal injection. The vaccine culture isolates from all organs.

The results from the parenchymal organs of rabbits (which died of the STI vaccine, and of the second TSENKOVSKII vaccine) are shown in the precipitation reaction on table 2 (page 18). It is evident from table 2 that not only the anthrax edema, but also the parenchymal organs of the rabbits which died of the STI vaccine and of the second TSENKOVSKII vaccine, form ring precipitation with the precipitant anthrax serum.

It must be said, that the precipitant serum used in the present experiment has been obtained by the hyperimmunization of the producers of the virulent anthrax culture of vegetative form, treated with formalin; the serum with the serial No. 86 - by the hyperimmunization method of the producers of the strains of anthrax vaccines of low virulence. The serum of both series was prepared by the Tobol'sk biofactory.

Thus, our experiments prove that, the anthrax vaccines according to their specific properties and to the immunobiological precipitation reaction, are indentical anthrax cultures.

Two precipitation serums - as we have already said - are in preparation at the present time. On of them is obtained by the
hyperimmunization of horses with the virulence anthrax culture of vegetative form inactivated by formalin in the precipitation reaction with antigens from vaccinal microbes. The other vaccine is obtained from vaccinal strains of low virulence, also including the second TSENKOVSKII vaccine. This serum develops a well expressed precipitation not only with antigens of those strains by which the horses were hyperimmunized, but also with antigens of virulent anthrax strains. This situation confirms the presence of an immunobiological identity of the anthrax vaccine represent anthrax cultures, which were purposely changed by special direction and do possess a residual low virulence. This characterizes their high immunogenesis for the prophylactic vaccinations of agricultural animals.

It is necessary to mention that the TSENKOVSKII anthrax vaccine and the vaccin STI are stable and possess stable biological properties. The mentioned vaccines are not able to produce anthrax in big animals. The fact that the vaccines have been used in practice for many years, confirms it.

It is also the be mentioned that we have confirmed the direct interrelation of the residual virulence and the immunogenesis; this is based on test with numerous anthrax strains which underwent a directed change. The more the virulent properties of the strains are decreased so much less immunogenic become the properties they possess when tested on rabbits and sheep.

Thus, according to the experimental data presented on the study of the nature of anthrax vaccines, it is obvious that they differ from the virulent anthrax strains only in one of the basic properties, namely: in the absence of pathogenicity, since the vaccines are not able to cause anthrax infections in animals. It is also notorious that in nature there are cultures of different virulence - more virulent strains and less virulent strains, though all of them are called strains of the anthrax culture. Besides, it is considered that animals, which had anthrax in enzootic or epizootic conditions, caused by a strain, in a natural course of the infection, do acquire a stable immunity against anthrax. However, this situation had not led the researchers to think until the present time that the strains, which produced immunity against anthrax in animals after the latter had been sick with anthrax in natural conditions, do possess other specific properties.

The anthrax cultures, from which the vaccines were obtained, underwent - as well known - a directed (?) effectuation (?) (vosdelstvie) in order to obtain from the vaccinal strains of low virulence, which would be able to produce immunity and also would possess stable properties.

Therefore, the mentioned vaccines should be considered as a variety, or, perhaps, to be more exact - as a new species of anthrax microbes which have lost their pathogenicity toward big animals but which still retain a low virulence and, therefore, possess immunogenic properties. Not to consider these vaccines being of the anthracis microvial type would not be fair, because they possess - as well known - cultural - morphological, biochemical and serological properties which are characteristic for the strains of anthrax. They also possess an expressed development of spores.

The factor of the ability of capsular formation capsulogenesis serves
as indicator for the less of virulence in anthrax vaccines. For instance the second TSINKOVSKII vaccine possesses a more expressed virulence toward laboratory animals than does the STI vaccine, it also possesses a low expressed ability of capsule formation (capsulogenecity).

We have a considerable number of anthrax strains, which were weakened by different means, and which have lost their ability of capsular formation (capsulogenecity).

S. N. MURONTESEV writes in his works: "Vaccinal strains used in practice for the vaccination against anthrax microbe - species of microorganisms, created by man."

It seems to us that the proof based on only one indicator, which shows the absence of pathogenecity toward big animals, meaning the unablness to produce mass infection - is not sufficient for considering the anthrax vaccinal strains as a new species of the anthrasic microbe. This new situation ought to be thoroughly proved by experimental tests, as required in practice and theory.


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