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[illegible] UPRAVLIAEMAIA IZMENCHIVOST MIKROBOV SIBIRSKOI IAZVY S TSEL'IU POLUCHENIIA VAKTSINY

(CONTROLLED VARIABILITY OF ANTHRAX MICROBES FOR THE PURPOSES OF OBTAINING THE VACCINE)

Trudy Gosudarstvennogo Nauchno-Kontrol'nogo Instituta Veterinarnykh Preparatov, Vol. 4. p.193-208. Moscow, 1953.

(In Russian)

CONTROLLED VARIABILITY OF ANTHRAX MICROBES FOR THE PURPOSES OF OBTAINING THE VACCINE

The problems of variability of microorganisms have long been an object of numerous investigations both in the field of practical observations and in the field of theoretical pronouncements. However, only the teaching of I.V. Michurin, which disclosed the mechanism of the phenomena of variability and heredity of organisms, has shown the correct approach to the understanding of the factors of variability of microorganisms and the ways to control this variability to the ends necessary for the national economy.

The great Russian scientist I.V. Michurin was the first to prove by his numerous experiments that living organisms can easily be modified through the action on them of environment and that there is nothing unchanged in the nature of living organisms. T.D. Lysenko, developing Michurin's teaching, assumes that the cause of change of the nature of a living body is the change in environment and in the type of metabolism. Thus, the teaching of Michurin and Lysenko constitutes the corroboration of the fact that the nature of biological properties of living beings, be they complex organisms or simplest microorganisms, can be modified on the desire of the experimenter.

This teaching is the most progressive, arming the researchers with the materialistic theory of the understanding of the most complex laws of nature of living organisms. The teaching of Michurin and Lysenko enables the scientists and specialists in socialist agriculture to successfully solve the problems of remaking the nature of plant and animal organisms.

In the practice of veterinary and medical microbiology the problems of variability of pathogenic microorganisms have been posed repeatedly. But in only rare cases were they resolved correctly.

Thus, for example, by the method of controlled modification of pathogenic microbes a number of investigators (L. Pasteur, L.S. Tsenkovskii, D.V. Konev) succeeded in obtaining microorganisms of attenuated virulence, possessing immunogenic properties. But these were the elementary materialistic approaches, because they were isolated cases in the mass of problems in microbiology and immunology.

The theory of the monomorphists Kon [transliterated] and Koch continued to exist for a long time; certain modified theories of dissociation and cyclogeny supported monomorphism greatly.

The theory of dissociation, which was supported by a number of scientists, including Shtutser, Gartokh [transliterated], and others, came, for example, to the fact that the phenomenon of microbial dissociation permits the placing all occurrences of variability into the frames of fission of the culture into two basic types differing from each other by a number of morphological, biological, cultural and serological properties. Thanks to this, it comes to the fact that a species of microbe forms a circle the limits of which it does not overstep.

The theory of cyclogeny also reduces the variability of microorganisms to the development in a closed circle. Thus, the theory of Enderlein, Lenis, Smit (all transliterated) and others, though by different courses, come to one and the same conclusion, namely, that every microorganism runs through strictly its own cycle of development, including also the sexual process. Under the cycle development is understood the fact that at different stages of the cycle the form of the microbe changes, consequently the considerable multiformity of morphological changes again forms a closed circle the limits of which the microbe does not overstep.

Thus, both the theory of dissociation and the theory of cyclogeny constituted the life-saving factors for monomorphists, and held back for a long time the solution of the problem of variability. However, in the course of the whole history of microbiology and, especially, on the dawn of its emergence and development there were advocates of the variability of microorganisms, because microbiological practice was forever finding the phenomena of variability of microbes. In addition, the facts were universally known when, on the desire of the experimenter, the microbial cultures, subjected to a special, i.e., controlled, action, modified their nature. Hence, in the course of the whole history of microbiology a fierce struggle was going on between the two opposing schools - between the doctrine of variability and the doctrine of the constancy of species.

Dwelling briefly upon the problems of monomorphism, dissociation and cyclogeny, it is necessary to note the facts of penetration of formal genetics into microbiology. Under the influence of the accumulated materials on the variability of microorganisms and the impossibility of a full negation of the factors of variability, the advocates of the immutability of species began to interpret these phenomena from the point of view of formal genetics. The view of De Fris (transliterated) on "mutation" as causelessly and suddenly appearing changes found its acceptance also in microbiology, constituting an explanation in all those cases when the direct action of environment on microorganism was not great. But in those cases when the effect of environment was apparent the changes obtained were interpreted as temporary modifications which sooner or later turn back to the initial state. Even if the factors of partial effect of environment on the modification of the nature of microorganisms were conceded by individual authors, they were declared to be secondary. The basic was considered to be the peculiar property of the microbial cell of transmitting its characteristics to posterity independently of the action of environment.

The ideas of Weismannism were most persistently propagandized in the works of Ravich-Birger, which granted the presence of "genes" in microorganisms and therefore also the impossibility of profound changes of bacterial cells, since "genes", according to Weismann, are eternally immutable.

There were also other authors (Krivisskii, Troitskii, Fisher, Filipchenko) who were the advocates of similar ideas in the matters of variability of microorganisms.

M.N. Ginsburg, too, is entertaining incorrect conceptions in his works, thinking that the external action alone on the microbial cell is not enough for the emergence of mutants. It is necessary that this cell be historically ready to turn into the "adaptively transformed" cell, suitable for the use as a vaccine. Professor Ginsburg thus endows the microbial cell with some sort of peculiar properties of historical readiness of its conversion into the "adaptively transformed cell", i.e., into a mutant. The materialistic principles and the methods of controlled modification of microorganisms for the obtaining of vaccinal strains he considers to be too primitive.

However, it would be erroneous to consider that in the practice of voterinary and medical microbiology there would not be such works which would not reflect the materialistic principles and methods in the obtaining of vaccinal strains of microorganisms and viruses. With the methods of controlled modification of microorganisms were, for example, obtained by L. Pasteur and L.S. Tsenkovskii, anthrax vaccines (1881-1883) and, by D.F. Konev, vaccines against swine erysipelas (1899). Tsenkovskii's and Konev's vaccines are constant biopreparations and have been successfully employed in veterinary practice for many decades.

In addition, there are a number of works on the obtaining, by means of controlled modification, of microbial cultures suitable for the vaccinoprophylaxis of several infectious diseases of man. To their number must be attributed the work of Kal'mett and Coren [transliterated] on the obtaining of the tuberculosis strain BTsZh, and the works of Soviet scientists N.A. Gaiskii and I.N. Khaiskii on the obtaining of vaccinal strains of tularemia by means of controlled modification, and the works of Pokrovskii and Korobkova on the obtaining of vaccinal strains of plague. N.N. Ginsburg obtained the anthrax vaccine strain STI by the method of selection.

The method of controlled variability of microbes is a creative method because it arms the experimenter in the fight against the incorrect ideas in the matters of variability, and it secures the possibility of obtaining more rapidly the microbial cultures with the characteristics and properties which are useful for the national economy. The method of controlled modification delivers the experimenter from passive contemplation and waiting when nature "will bring forth" an organism with the ready useful characteristics and properties.

The work has recently appeared of the head of biochemistry department, All-Union Institute of Experimental Veterinary Medicine, professor G.M. Bosh'ian, "On the nature of viruses and microbes", as well as the book of professor G.P. Kalina, "Variability of pathogenic microorganisms". The authors of these works are most boldly posing the problems of variability of microorganisms and viruses in relation to the action of environment on them.

Thus, for example, G.M. Bosh'ian writes that the concession of constancy of bacterial species did not permit the understanding and correct explanation of the essence of origin of microbial forms which are usually found in virus diseases.

Professor G.P. Kalina, too, poses in a new way the problem concerning the variability and phasic development of microbes. The author believes that, through controlled modification, it is possible to obtain microorganisms possessing new properties. He succeeded in obtaining a complete reorganization of the character of metabolism in microbes, which was accompanied not only by the modification of biochemical properties but also of the antigenic structure.

A huge hindrance in the problems of controlled variability of microorganisms for the purposes of obtaining vaccines were the deeply rooted views of Weismannists-Morganists concerning the impossibility of changing in artificial way the natural properties of the organism. This pseudoscientific theory stated that even if it is possible in some cases to obtain changes in the characteristics of the organism through the action with various means, these changes are temporary since the "genes" - the carriers of hereditary properties in the organism - are found in eternally immutable state.

The whole essence of the reactionary teaching of Weismannists-Morganists about the existence of "eternally immutable genes" in the organism of living beings has been revealed and unmasked by the teaching of I.V. Michurin and T.D. Lysenko.

The materialistic teaching of Michurin and Lysenko has concretely shown the entire insolvency of the reactionary teaching of Weismannists-Morganists. On the basis of his theory of active intervention of the experimenter in the problems of remaking the nature of living organisms, I.V. Michurin evolved over 300 new sorts of higher plants.

T.D. Lysenko, developing Michurin's teaching, has demonstrated in experiment and in practice the correctness of the doctrine concerning the possibility of controlled modification of plant organisms. On the examples of conversion of the summer grain crops into winter and the winter into summer, he and his students have demonstrated the possibility of reorganization of the natural properties of the indicated plants; moreover, the natural properties of the changed plants are stably preserved. These observations and many other similar phenomena in the controlled variability enabled T.D. Lysenko to advance a series of theoretical theses pertaining to the problems of variability and heredity. Thus, for example, T.D. Lysenko reckons that in modification of environment the organism begins to adjust itself and to reorganize its properties; moreover, the chief role is allotted to nutrition. When environment is modified so, too, the type of nutrition of the organism changes, and this in turn leads to the modification of the nature of cells and of the organism as a whole. T.D. Lysenko says that "the cause of modification of the nature of a living body is the change of the type of assimilation, of the type of metabolism" (Agrobiologiia, 1948, p.347).

The cited examples of the controlled variability extend not only to the range of problems in horticulture and plant science but also to the world of animal organisms and plant microorganisms.

In the past 2 years (1949-1950) have appeared a number of works devoted to the problems of controlled variability of pathogenic microorganisms and viruses

(Muroutsev, Vygodchikov, Grinbaum, Krestovnikova, Kravchenko, Fal'kovich, Maiskii and others). However, the majority of authors deal only with the theoretical approaches and generalisations in the solution of the problems in controlled variability of pathogenic microorganisms. Only in a negligible number of works an indication is made to the solution of the problems of variability through the controlled action on microbial cells and viruses.

Thus, I.N. Maiskii reported in 1949 about the obtaining of weakly virulent cultures of tularemia microorganisms from the virulent strains. The author employed the Gaiskii method which consists in modification of the conditions of growth and nutrition of the microbes. These methods made it possible to reduce, in 5-7 months, the virulence in 9 out of 12 strains to the degree of the vaccinal strains. The testing by the author of the immunogenic properties of a number of strains on white mice showed that certain weakly virulent tularemia strains possessed strongly marked immunogenic properties.

G.M. Bosh'ian (1949) advances the thesis that all microorganisms have their filtrable stages of existence. The microbial cell breaks up into invisible (filtrable) particles owing to the adverse effects of environment, since, in the author's opinion, the filtrable forms of microorganisms are the most stable, and in this specifically are perceived the evolutionally formed properties of microbes which are directed to the preservation of life.

The problems raised by G.M. Bosh'ian concerning the nature of viruses and microbes are up-to-date, since they affect the extremely important problems of variability of microorganisms and the stability of filtrable forms of microbes.

On the question of species formation G.M. Bosh'ian stands on the viewpoint that there is a biological law of transmutation of microorganisms from one f[illegible] of their existence into another; that the virus forms and the forms of microbes are of one and the same species, which, by reciprocal transmutation, can evolutionize into also other species of microbes and viruses. Possible also is the transition of microbes-saprophytes into microbes-parasites. The author believes that there is not and there cannot be a sharp border between parasitism and saprophytism, since these forms in nature are continuously crossing over one into the other.

In point of development and variability of viruses and microbes in the sick organism and in experiment, approximately the same point of view is advanced by V.A. Krestovnikova who believes that the filtrable viruses, found in a sick organism, go through the appropriate stages of development, going from the filtrable to the microbial form. In proof of this the author cites the data of experiments with typhus, in which, through the use of special methods of culturing from the blood and the material containing Rickettsia, it is possible to obtain the microorganisms approaching by their properties the Rickettsia, but which do grow on synthetic culture media. They are close also to the microorganisms of Proteus-X. Krestovnikova views these microorganisms as forms intermediate between Rickettsia and Proteus.

Similar viewpoint on the development of microbes and viruses in the organism of the sick is maintained also by a number of other authors (Fal'kovich, Voronkova and Krasil'shchik in the study of the etiology of scarlet fever; Fal'kovich in the study of the interconnection of the virus of grippe and Pfeiffer's bacillus; Krestovnikova, Zhurbina and Izmailova in the study of the problem concerning the nature of bacteriophage of Proteus X_{19} and the bacteriophage of scarlet fever streptococcus).

G.P. Kalina (1949) believes that microbes have the filtrable form of their development and that it is necessary only to know how to detect them in this phase of existence. Microbial cultures can be turned into filtrable forms by means of a special action; moreover, as the author points out, their biological characteristics are strongly modified. Through the action it is possible to obtain a complete change of the character of metabolism of microbes, which conditions a modification of their natural properties. In the case in point the author thinks that such population oversteps the bounds of its species, i.e., it turns into another species. G.P. Kalina reports that he and his coworkers have succeeded, by means of a special action, i.e., going through the filtrable stage, in converting dysentery bacillus into paratyphoid A and into the bacillus of mouse typhus. A check showed that the newly acquired biological properties of the culture were preserved for 7 and 9 months.

It remains to be regretted that the author does not cite data pertaining to the further status of the modified cultures, since the indications that the cultures had stably preserved the newly acquired properties for 7-9 months do not explain what subsequently happened with these cultures, whether they returned to the original state or whether they continued to preserve the acquired properties.

In dealing with the problems of variability of microorganisms, the researchers inevitably touch upon also the problems of species formation, for with the modification of the nature of microbes their specific peculiarities can also be changed. In a number of experiments on the controlled variability the investigators have succeeded in transforming microorganisms from one species into another. However, many investigators still negate the transformation of one species into another, believing that here takes place only the modification of properties of individual characteristics of microbes within the species itself.

Willy-nilly, these investigators maintain the antievolutional theory concerning the constancy of species of microorganisms, for, by rejecting the possibility of transmutation of one species into another by means of a special action, the possibility of remaking the nature on the desire of the experimenter is rejected in principle.

In his work, "New in the science on biological species", T.D. Lysenko points out that the possibility of transmutation of one species into another under natural conditions and in experiment does exist. This thesis was corroborated in a number of scientific research establishments of VASKhNIL in 1948-1950 on the examples of obtaining rye from wheat, wheat from rye and oats, barley and summer rye from ramose wheat.

In explaining the problems of species formation in the plant world, academician Lysenko points out that "Modification of environmental conditions, which is essential for the species specificity of these organisms, sooner or later compels the modification of also the species specificity - one species generate others. Under the influence of the changed conditions, which have become unfavorable for the nature (heredity) of the organisms of the species of plants growing here, in the body of organisms of these species are conceived, formed the embryos of the body of other species which are more adequate to the changed environmental conditions". And further, in developing the theory of species formation, T.D. Lysenko points out that "species - it is a peculiar qualitatively determined state of living forms of matter. An intrinsic characteristic feature of the species of plants, animals and microorganisms are the fixed intraspecific in-

terrelations between the individuals".
These intraspecific interrelations are qualitatively different from the interrelations between the individuals of different species.

S.N. Muromtsev (1950) also points to the transmutation of one species of microbe into another.

The problems of variability of anthrax microbes have been illuminated by a number of investigators (V.M. Kirsanov, F.A. Terent'ev, D.M. Teternik, A.P. Zotov and others). However, these works bore the character of the study of culturo-morphological peculiarities of microbes in action upon them of various biological and chemical substances (immune organism of guinea pig, bacteriophage, culture medium containing lithium chloride). These works established the possibility of appearance of involutional forms of anthrax bacilli (Kirsanov, Terent'ev) and dissociants in form of the formation of colonies of S-form (F.A. Terent'ev, A.P. Zotov, D.M. Teternik and others) after a special action on the culture. It was thus demonstrated that anthrax microbes, in the appropriate influence, can greatly change their morphological properties.

The attempts of some investigators to obtain weakly virulent but immunogenic microbial strains of anthrax culture by the method of roentgen exposure (Batiuk), or by means of action with ultrashort waves and ultraviolet rays (Arkhangel'skii) were not in the meantime crowned with success, since the authors did not propose any anthrax vaccines. The obtaining of weakly virulent strains, possessing the features requisite for anthrax microbes and chiefly the immunogenic properties, is therefore a very difficult task. That precisely is why, alongside the extant works on the variability of anthrax microbes, which establish the possibility of obtaining involutional forms, there are virtually no works on the problems of controlled variability and on the obtaining of anthrax microbes with the consolidated heredity and suitable for vaccino-prophylaxis. However, this does not exhaust the possibilities of continuing the exploration of the methods of obtaining anthrax vaccines.

For this, it seems to us, it was necessary to employ the methods of controlled modification of anthrax microbes, where the acting agent would correspond to the influences to which the microbe is subjected under natural conditions of existence. Other investigators also point to this. G.P. Kalina, for example, thinks that the modifications of microbial cultures develop sooner and easier the less keen and the longer the action on the microbial cell. As concerns the so-called mutagenous factors (roentgen, radium and others), they do not produce profound and stable changes in the microbes.

From the existing works on controlled modification of microbial cultures, it is seen that the greatest effect is obtained in the action on the microbial cell with temperature factors, in introduction into the organism of an unsusceptible or immune organism and in action of immune serum, bacteriophage, bile, etc. Here pertain the works on the obtaining of modified forms of anthrax, erysipelas, dysentery and tularemia microorganisms, of plague and tuberculosis bacilli. Thus, for example, the use of increased temperature in culturing anthrax microbes enabled L. Pasteur and L.S. Tsenkovskii to obtain effective vaccines. For the obtaining of anti-erysipelas vaccines, D.F. Konev employed the method of passing the cultures of swine erysipelas through the organism of young rabbits.

It was therefore important in carrying out the experiments in controlled modification of anthrax microbes to choose such methods of action on the culture which would condition a profound reorganization of the nature of microbes. Naturally, this required the factors not of temporary but of prolonged action, i.e., suc[illegible] vironment as would condition the reorganization of nutrition of microbes.

Organisms grow and develop, as is known, within the limits of fixed norms (temperature, composition of culture medium, its pH, etc.), and the changes taking place in the organism do not affect its hereditary characteristics so long as the environment meets the needs of the organism, its nature. But if the environment changes, ceasing to be the customary medium suitable for the development of the organism, the latter either does not develop and dies, or, in the process of forced development and adaptation to the new environment, inevitably changes its heredity. Thus, the cause of modification of hereditary properties of microorganisms is the change of the conditions of life, the change of the type of nutrition, of the type of metabolism.

In 1950, in the laboratory for control of anthracic preparations, S.G. Kolesov and IU.F. Borisovich carried out the first part of the work on the exploration of the methods for obtaining a safe anthrax vaccine for inoculation of sheep and goats against anthrax.

The posing of the question concerning the obtaining of a safer anthrax vaccine was conditioned by the fact that the Tsenkovskii and STI [Sanitary Technical Institute of the Red Army] vaccines, being immunogenic preparations, possess the property of causing complications in animals.

We in our experiments employed the methods of controlled action on the microbial culture of anthrax with increased temperature, immune serum, immune rabbit organism, and ultraviolet rays. The basic part of the experiments was carried out with the use of the first two methods, for it was assumed that in the action with increased temperature and immune serum on the virulent microbial anthrax culture it would be possible to obtain more positive results. As will be seen below, this assumption was in the main affirmed, since in carrying out many experiments we succeeded in a number of cases in obtaining anthrax strains with sharply modified biological properties.

Without dwelling in detail upon the culture-morphological and precipitinogenic properties which the newly obtained anthrax strains possess, since a report on this is given in IU.F. Borisovich's article, we consider it necessary to point briefly to the problems of morphological status of microbial cultures in the process of their modification.

As was expected, with the modification of environmental conditions, conditions in which the anthrax strains were grown, with the modification of the type of nutrition, the type of metabolism, changes occurred also in the properties of microbes. In the first generations appeared morphological and cultural changes in the majority of the strains, which were characterized by the formation of granular forms of the bacilli with their subsequent breakup into minute particles. Those morphological changes occurred faster and were more marked in the action on the microbial cultures with temperature factors. It should be noted that the appearance of such type of changes in microorganisms alternated periodically in the course of a number of generations, and then developed the constant phase of development of the cultures without the formation of granular forms of microbes. In the process of appearance of the granular forms of rods and threads and their disintegration into minute particles, other sharp morphological changes, for example, the appearance of spheroid or bulb-like forms, were not observed. It must be noted that the anthrax bacilli, subjected to the action of increased temperature, become shorter and in the majority of cases are distributed individually or in pairs, but not in the form of threads. Hence, when cultured in meat peptone broth such strains usually produce a diffuse growth, and on agar, side by side with the colonies of R-form, the intermediate RS- and SR-colonies appear initially in a definite amount, and subsequently - the colonies of S-form.

The use of other methods of action on anthrax microbes (organism of immune rabbits, ultraviolet rays) did not give positive results in the sense of modification of the culturo-morphological and virulent properties.

In the study of virulent properties of anthrax strains subjected to modification, we found that under the influence of the immune serum only a negligible number of strains had the property of losing the virulence for rabbits, guinea pigs and white mice. Moreover, the modification of virulent properties proceeded in one case considerably faster, and in another case more slowly.

Thus, for example, strain No.1260 after 25 generations became avirulent for rabbits; after 28 generations - for guinea pigs; and after 31 generations it was practically safe also for white mice. The strain of the standard sporal virus lost the virulence for rabbits after 41 generations, and for guinea pigs - only after 94 generations.

The modification of virulent properties of anthrax strains to the side of their reduction is achieved faster and in the greater number of cases under the action of increased temperature. Thus, for the 14 strains which were subjected to temperature action, four strains became avirulent for rabbits and two of them lost the virulence also for guinea pigs. In the check for virulence the culture of the strains did not always produce death of all guinea pigs; the doses employed were usually large (0.5 - 1 cc for guinea pigs, and 3-3 cc for rabbits), in concentration of 3 billion microbial bodies.

It is characteristic that the weakly virulent, edema-causing strains STI, 916-1 and Sh-15 became fully avirulent for guinea pigs and white mice after 4-5 generations under increased temperature, while these same strains, under the influence of immune serum, preserved their virulent properties for guinea pigs and white mice for many generations.

Thus, the culturing of anthrax microbes under increased temperature permits the achievement of the attenuation of their virulent properties within shorter periods. It must be noted that the method of action with increased temperature which we employed differed considerably from the methods described by Pasteur and Tsenkovskii. What induced us to this was the circumstance that already before the start of the experiments, it was established in the laboratory of anthrax preparations that in the prolonged action of 42.5 - 43° temperature a decrease in virulent properties of the cultures did not develop. And this is understandable, for it was at the same time established that, despite the increased temperature, a portion of anthrax bacilli were converted into the sporal state in which the modification of the natural properties of anthrax cultures is evidently extremely difficult. That is why, in carrying out experiments in controlled modification of anthrax culture we employed the method of action on the young microbial cells with increased temperature. The same principle was employed also in the action on anthrax cultures with the immune serum. In addition, we in this case laid at the foundation the doctrine of Michurin which states that the reorganization of the organism proceeds considerably more effectively if the action of environment is exerted on the young organism.

Having obtained weakly virulent cultures of anthrax, it was necessary to test them for immunogenic properties on laboratory animals. In this case the tests were arranged on rabbits. The following results were obtained.

It is seen from Table I that the strains obtained possess marked immunogenic properties. It should be noted that according as the number of generations increased the virulence of the strains decreased, and together with it their immunogenic properties also decreased. Thus, for example, strains No.42

and 43 possess more marked immunogenic properties than the strain No.46. Strains No.61 and 64 possess stronger immunogenic properties than strains No.65 and 68.

This circumstance must point to the fact that the ability of anthrax strains to develop in animals an immunity against anthrax is evidently associated with the residual virulence.

True, this circumstance is not observed in all cases. It is known, for example, that the first Tsenkovskii vaccine does not develop immunity in rabbits, although it has a strong virulence for white mice. But the strain No.45 is practically safe for white mice, but it has the ability to develop immunity in rabbits against anthrax.

Our laboratory has a number of strains avirulent for white mice, which were obtained under the action of increased temperature from the strains STI, 916-1 and Sh-15, as well as the Nevskii strain, but they do not possess immunogenic properties. It means, therefore, that the residual virulence in the vaccinal anthrax strains must be associated not only with their virulence for this or the other species of laboratory animals, for example, white mice and guinea pigs, but also with the reactivity, i.e., with the residual virulence of such strains as are capable of producing the corresponding changes in the organism of a vaccinated animal.

The preliminary data of our experiments ~~in~~ testing the immunogenic properties of modified anthrax strains ~~constitute~~ the first stage of works in the ~~field of~~ exploration of a safer anthrax vaccine. The second, ~~not less important~~ stage ~~of works~~ will ~~be~~ the ~~problems of~~ study of the stable properties of the strains obtained. These problems are ~~very~~ important because only the preservation of the constancy of biological properties of microbes insures their practical value as vaccinal strains.

Under the action of increased temperature were obtained four weakly virulent strains which were tested for immunogenic properties on rabbits. The results of this test are presented in Table II.

It is seen from the data in Table II that all the strains, except strain No.88, in single inoculation possessed high immunogenic properties in the test on rabbits. It must be noted, moreover, that all these strains had virulence for guinea pigs and white mice, but were safe for rabbits. In point of their virulent properties they can be put on the same footing with the second Tsenkovskii vaccine, while some of them - with the first Tsenkovskii vaccine.

Strain No.Sh-15, too, has marked immunogenic properties. It must be noted that this strain, as well as the strain No.916-1 were obtained earlier by means of controlled action and selection and, in point of biological properties, they differ considerably from other weakly virulent cultures by the fact that they do not cause a marked sepsis in guinea pigs but develop a reaction at the place of introduction in the injection of large doses.

The question may arise whether this means that we have anthrax strains with consolidated, hereditarily modified biological properties. Or are these the strains which still do not have the constancy of biological properties? An affirmative answer to the question pertaining the constancy of the newly acquired biological properties by the anthrax strains cannot yet be given because the period of observation is brief. However, proceeding from the materialistic positions of I.V. Michurin and T.D. Lysenko that the organism in a new medium, in new experimental conditions reorganizes its nature (heredity) in the process of nutrition, in the process of development, it is necessary to consider that the obtained strains, which have lost the virulence to a certain degree, must not have the property of restoring it.

In the process of modification of the natural properties of microorganisms, in the period of the loosened state there may be certain negligible fluctuations in the virulent and other properties of the microbes, but their return to the initial state is apparently impossible. We had the occasion to observe the phenomenon when, in testing, soon after the completion of the action of the appropriate factor, one or the other strain of anthrax culture lost the virulence for guinea pigs. But after a certain number of transplants on the ordinary culture media these strains partially restored their virulence, causing death of guinea pigs in infection with large doses and after longer periods of time (8-10-15-20 days). We did not observe an intensification of the virulent properties of the strains to the degree of pathogenicity for rabbits. In such cases, i.e., in obtaining modified cultures which had to a certain measure lost their virulent properties, we can, it seems to us, most probably expect not an intensification of virulence, but its decrease, providing the special methods of stabilisation of the cultures in the process of keeping are not employed.

Out of the fact that the inheritance of newly acquired biological properties of microbial cultures of anthrax is fully possible - it cannot be claimed that the new characteristics and properties can be easily effected and, mainly, easily consolidated in posterity. For this it is first and foremost necessary to create the appropriate conditions of development of microbes and the conditions for the consolidation of their newly acquired characteristics and properties. Only after this can the question concerning the reconstruction and consolidation of the new natural, i.e., hereditary properties of microbial cultures, be resolved.

In examining the problems of variability of microorganisms, we stand on the point of view that the prospect of immunoprophylaxis of infectious diseases of animals must in the majority of cases belong to the living microbial vaccines.

True, the ways and methods of obtaining living microbial vaccines are more difficult. However, this does not mean that it is impossible to obtain such vaccines; the mass experiments of Michurinites in the area of controlled modification of the nature of living organisms assure us in this. Absolutely incorrect are the investigators who believe that by the method of controlled action it is far from always possible to obtain effective vaccines, citing the irrepeatability of the methods of Jenner with the variola vaccine and of Tsenkovskii with the anthrax vaccines.

In conclusion, it must be observed that the experiments which we have carried out in controlled modification of microbial cultures of anthrax attest to the possibility of obtaining microbes with the strongly modified properties. Moreover, it was established that a number of the modified strains possess marked immunogenic properties.

Our experiments have demonstrated also the possibility of conversion of anthrax microbes from parasites into saprophytes by means of a special action of the medium.

In principle, the question relating to the development of new methods of controlled modification of anthrax microorganisms may be considered as having been solved. However, the work carried out in this direction may be viewed merely as the first stage on the road of exploration of a new anthrax vaccine, inasmuch as the anthrax strains which have been obtained must be subjected to a further study for immunogenic properties on large animals, and for the constancy of the acquired characteristics.

LL/rbp

LITERATURE

1. G.M. Bosh'ian. On the nature of viruses and microbes. Medgiz, 1949.

2. I.F. Batiuk. Attenuation of B. anthracis under the influence of roentgen rays in order to obtain antianthrax vaccines. ZhMEI, Vol.18, No.1, 1937.

3. G.V. Vygodchikov and F.T. Grinbaum. Concerning the problem of variability of microbes in the works of Soviet microbiologists.

4. N.N. Ginzburg. Anthrax vaccine STI (on the revision of the problem concerning the origin and essence of vaccinal strains). Sbornik rabot NIIEG of the Red Army, No.1.Medgiz, Moscow, 1946.

5. G.P. Kalina. Variability of pathogenic microorganisms. Gosmedizdat, Ukraine SSR, 1949.

6. V.M. Kirsanov. On the question of pleomorphism of the anthrax microbe. Trudy GIZV, Vol.2, No.1. 1924.

7. A.T. Kravchenko. Influence of external factors on the variability of viruses.

8. L.A. Kalinichenko. Michurinite doctrine - the foundation of scientific biology. Academy of Medical Sciences, USSR. Moscow, 1949.

9. V.A. Krestovnikova. On the doctrine of the stages of development of microorganisms. Medgiz, Moscow, 1950.

10. T.D. Lysenko. Agrobiologiia. Sel'khozgiz, Moscow, 1948.

11. T.D. Lysenko. New in the science concerning the biological species. Doklady VASKhNIL, No.2. 1950.

12. I.V. Michurin. Works. Vol.IV. Sel'khozgiz, Moscow, 1948.

13. I.N. Maiskii. Variability of the tularemia microbe under the influence of external factors.

14. S.N. Muromtsev. Problems of modern microbiology in the light of the Michurinite doctrine. "Pravda". Moscow, 1950.

15. F.A. Terent'ev. Morphological variability of B. anthracis "Anthrax". Sel'khozgiz, Moscow, 1940.

16. M.O. Streshinskii. Controlled variability of bacteria under the influence of the products of vital action of other forms of microorganisms. "Zhurnal obshchei biologii", Vol.XI, No.2, 1950.

TABLE I

Test of immunogenic properties of anthrax strains subjected to the action of immune sera

Strain No.	Number of tests	Number of inoculated rabbits	Multiple of inoculation of rabbits	Dose of the 1st inoculation of rabbits (in ml)	Dose of the 2nd inoculation of rabbits (in ml)	Control infection		Died	Survived	Percent
						Days after the 1st inoculation	Dose of virus (in ml)			
42	1	5	Double	2	3	28	1	-	5	100.0
43	4	17	"	1	3	15-28	1	3	14	82.4
43	2	8	Single	3	-	25	1	2	6	75.0
46	1	5	"	3	-	17	1	3	2	40.0
46	1	3	Double	2	3	17	1	1	2	66.6
61	1	6	"	1	3	19	1	-	6	100.0
64	2	11	"	1	3	19	1	-	11	100.0
65	1	5	"	2	2.5	24	1	2	3	60.0
65	3	9	Single	3	-	23-24	1	3	6	66.6
68	1	5	"	3	-	25	1	1	4	80.0
	Control	25	-	-	-	-	1	18	7	

TABLE II

Strain No.	Number of tests	Number of inoculated rabbits	Multiple of inoculation of rabbits	Dose of the 1st inoculation (in ml)	Dose of the 2nd inoculation (in ml)	Control infection		Died	Survived	Percent
						Days after the 1st inoculation	Dose of virus (in ml)			
83	3	13	Single	3	-	14-29	1	2	11	84.7
83	1	4	Double	2	3	19	1	3	1	25.0
80	2	9	Single	3	-	14-29	1	2	7	77.8
80	1	3	Double	2	3	19	1	1	2	66.6
82	2	10	Single	3	-	14	1	2	8	80.0
85	2	10	"	3	-	34	1	2	8	80.0
85	1	5	Double	2	3	24	1	1	4	80.0
87	1	5	"	2	2.5	24	1	1	4	80.0
87	1	5	Single	3	-	34	1	1	4	80.0
Sh-15	1	5	"	2	-	23	1	1	4	80.0
88	2	9	"	2	-	23	1	4	5	55.6
88	1	4	Double	3	5	15	1	-	4	100.0
	Control	10	-	-	-	-	1	8	2	