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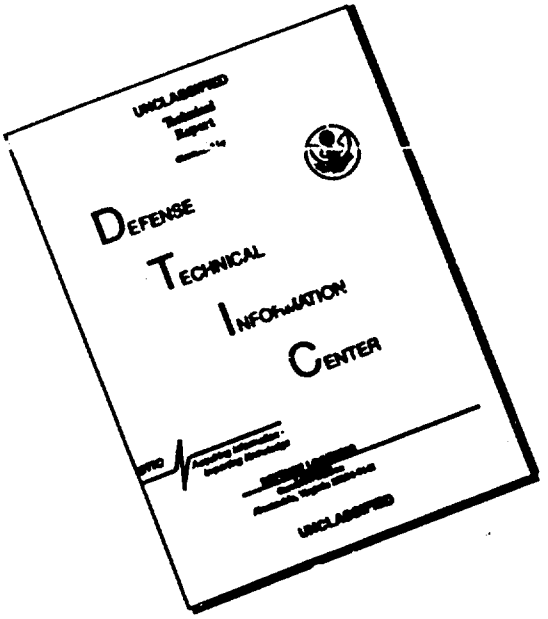

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Vaktsiny protiva sibirskoi iazvy

Vaccines against anthrax

In Biologicheskie i khimioterapevticheskie veterinarnye preparaty /Biological and chemotherapeutical veterinary preparations/, Moscow, 1948. 63-78 cl Um314

(In Russian)

VACCINES AGAINST ANTHRAX

The outstanding results of preventive inoculations against anthrax, obtained by Pasteur, soon attracted general attention and the vaccination of agricultural animals (especially sheep) with Pasteur vaccines began to be carried out not only in France but in other countries as well.

In 1883 in Russia Professor L. S. TSENKOVSKII prepared, according to the Pasteur principle, his anthrax vaccines. To obtain these vaccines Tsenkovskii took a virulent anthrax culture isolated from a rabbit that just died, planted it on chicken broth and placed it in incubator at 42.5° for growing. The asporogenic cultures obtained under such temperature gradually became weaker in their virulence under the action of oxygen in the air. Testing daily the virulent properties of the grown cultures on experimental animals, Tsenkovskii selected cultures of 2 degrees of weakness. The First vaccine he obtained as a result of a 12 day cultivation in incubator at 42.5° C; it killed all the white mice used, 1/3 of the gophers in 3 - 6 days, and caused fever - intense in rabbits and mild in sheep. The Second vaccine was obtained following a 6 - 7 day growth in incubator - 42.5° C; it killed all inoculated mice, 3/4 of gophers used.

1/3 of rabbits, and 1 - 2 of the 10 inoculated sheep. Tsenkovskii converted his vaccines from the bacillary form into the sporal form by growing and keeping them in incubator at 35° C for 5 - 6 days.

In the initial years the practical work of Pasteur and Tsenkovskii established that the broth sporal vaccines of anthrax lose their immunogenic properties at prolonged storage; in this connection not few failures occurred in control testing of vaccinated animals. The maintenance of constant properties by the Chamberland method - by means of periodic regeneration on culture media - proved unsatisfactory. Tsenkovskii therefore suggested an original method of maintaining matrices of the vaccines in 30% aqueous solution of chemically pure neutral glycerin. Having determined the suitability of his system of preservation of the vaccines, Tsenkovskii announced that "the greatest obstacle in practical application of Pasteur inoculations - inconstancy of the properties of the vaccines - is removed by preserving them in glycerin. Subsequently the method of preserving the matrices of anthrax vaccines in glycerin solution proved correct and for 60 years it has brilliantly justified itself.

Tsenkovskii also established the possibility to clean the vaccines from alien microflora by passing them through the animal organism. In order to test the constant properties of his vaccines, Tsenkovskii submitted them to multiple passages through the organism of gophers. The First vaccine was passed 19 times and the Second 10 times. The vaccines of each passage were tested on sheep for harmlessness and immunogenic properties. As a result the following was established:

1) in multiple passages the vaccines preserve their original virulent and immunogenic properties; and, (2) their periodic regeneration by means of passages through the organism of laboratory animals contributes to the consolidation of their constant properties. And this, as we shall see below, has been confirmed by practice because, thanks only to the systematic regeneration of matrices of the vaccines through the organism of white mice and gophers, can their constant properties be preserved.

TSENKOVSKII arranged his first experiment of vaccination of sheep against anthrax on May 10, 1883. Eleven sheep, initially vaccinated with the First and then with the Second vaccine, were subjected, 16 days after the injection of the Second vaccine, to controlled infection with the anthrax virus simultaneously with 2 un-vaccinated sheep. The unvaccinated sheep died of anthrax in 30 hours, while all those vaccinated remained alive. Later the experiments on vaccination of sheep with the TSENKOVSKII vaccines and on controlled infection were considerably expanded both by Tsenkovskii himself and by his nearest associates - CHALASHNIKOV and SKADOVSKII, and always with favorable results. The investigation on the length of immunity conducted in 1887, established that 13 months after vaccination the sheep possessed strained immunity (out of 20 vaccinated sheep 2 died; out of 10 controls - 9 died).

The excellent immunogenic properties of the Tsenkovskii vaccines were subsequently confirmed by numerous experiments and by their extensive utilization.

In connection with the favorable references about Tsenkovskii

vaccines there arose a necessity, chiefly in Southern Russia, of organising several bacteriological stations for manufacture of these vaccines. At first the vaccines were prepared by cultivation in broth for 3 days. A planting in broth was made from sporal cultures (matrices) which are kept in glycerin solution. Utilisation of the 3 - day broth vaccines of vegetative form[1] made it too difficult and restricted their use for inoculations because the period of fitness of such vaccines was 3 - 4 days. And only since 1896-1897 GORDZIALKOVSKII introduced into practice the manufacture and utilisation of Tsenkovskii vaccines in form of sporal culture grown on broth and contained in glycerin solution. These broth growths contain considerable amount of spores.

In the first years of utilisation of the Tsenkovskii vaccines, and also of the French (Pasteur) vaccines, together with numerous positive results there have been cases of failures. Such, for example, was the case of a mass murrain, after a vaccination by French vaccines, on the estate of PANKEEV, known under the name of "PANKEEV history" which remained essentially unexplained. There, after injection of the First vaccine out of 4,414 inoculated sheep 3,549 died, i. e., 80.3%. According to some hypotheses this was the result of a fatal mistake (instead of the First vaccine, virus was used) or it was atavism of the First vaccine to the degree of weakened anthrax virus.

A special commission was organized in 1890 to test the harmlessness and fitness of the Tsenkovskii vaccines. This commission conducted extensive experiments on vaccination of sheep, cattle, horses and pigs.

Footnote: In the 3-day broth growth there is always up to 25% of useful spores.

In Beloterka 6,055 sheep (young and grown) were inoculated; the mortality after the inoculation was 0.1%. The test of strength and the longevity of immunity established that the sheep infected with anthax remained alive a month and 8 months later, while 90-100% of the controls died. The commission therfore came to the conclusion that the Tsenkovskii vaccines possess the requisite virulent and immunogenic properties which they possessed at the time that TSENKOVSKII obtained them. After this the TSENKOVSKII vaccines began to be extensively utilized throughout Russia.

An important modification in the manufacture of TSENKOVSKII vaccines was introduced in 1910 by professor S. N. VYSHELESSKII. He proposed to prepare them not on broth but on poptonoless agar. This modification improved considerably the quality of the vaccines and made their manufacture cheaper and simpler.

In 1890-1891 professor LANGE (Kazan) obtained the First and Second Tsenkovskii vaccines, according to the Pastour principle. The author undertook the task to obtain vaccines which would produce a more moderate reaction, in comparison with TSENKOVSKII vaccines, in inoculated animals.

In order to obtain his vaccines LANGE took anthrax culture which had been isolated from the carcass of a dead bull, and grew it in incubator at 42.5° C: to obtain the First vaccine - 21 days; to obtain the Second - 14 days. The prepared vaccines possessed the following virulence: the First vaccine caused death of all mice and 50% of young guinea pigs; while the Second vaccine caused loss of 50-60% of grown guinea

pigs and 10 - 15% of rabbits. The sheep, inoculated with the 2nd vaccine, reacted severely; sometimes fatally but not over 1% of them.

The LANGE vaccines were for a time utilized for inoculations of horses chiefly in provinces along the Volga. A number of experimenters believed that the LANGE vaccines were more practical in vaccination of horses (less complications were noted) since the 1st LANGE vaccine is more virulent while the 2nd is less virulent in comparison with corresponding TSENKOVSKII vaccines. Later, however, in extensive commissioned experiments (SARATOV and DON Commissions) with the TSENKOVSKII, LANGE and French vaccines, it was established that, in point of vaccinal qualities, the advantage belonged to the TSENKOVSKII vaccines.

Of the anthrax vaccines employed abroad (besides Pastour's) ZOBERNHEIM, MATSUOKI, and KITCHENER (glucocidic) vaccines must be mentioned.

In 1934 Professor TERENT'EV proposed a saponin anthrax vaccine. This vaccine represents the spores of anthrax of TSENKOVSKII 2nd vaccine, matrix No. 71, contained in 3% glucocide - saponin solution. The animals are inoculated once with this saponin - vaccine, without previous vaccination with the 1st vaccine. This makes the inoculation of domestic animals against anthrax considerably cheaper and simpler. Immunogenic properties of saponin - vaccine are high.

In 1933 - 34 STAMATIN, studying the morphology and biology of virulent cultures of anthrax grown on coagulated, defibrinated or citrated horse blood, noted that in this process there occur individual colonies which have a dried and coarse appearance and that the bacilli from these colonies do not have capsules.

In injection of this variant into white mice and guinea pigs an intense swelling is produced at the site of inoculation, in which is contained a considerable amount of acapsular anthrax bacilli. In cases of death of the guinea pigs the acapsular agent of anthrax is isolated only from parenchymatous organs and not from cardiac blood. The sheep inoculated with the STANATIN acapsular, swelling causing variety of the agent, developed strong immunity.

In 1962 in the USSR Professor N. N. GINSBURG proposed a new anthrax vaccine - STI -1 (Sanitary - Technical Institute of Soviet Army) consisting of the acapsular, swelling - producing variant which he obtained from the virulent species of anthrax. The acapsular variety STI - 1 possesses the capacity to cause edema of a considerable size in guinea pigs and white mice; the acapsular pthogen is isolated from the edema and the parenchymatous organs of the dead mice and guinea pigs in great masses; it is more rarely isolated from the blood of the heart of the guinea pigs and white mice. The strain of the vaccine STI does not possess a stable ability to cause death in the infected mice and guinea pigs, however, it has some peculiar acapsular properties and the ability of causing death in white mice and guinea pigs. These specific properties are taken as the basis for the control of the vaccine STI.

The results of a four-year practice in the production of STI vaccine in bioplants are summarised in the following resolutions:

1. The strain possesses stable edema-causing properties.

2. There are cases when the culture of the STI vaccine is isolated from the blood of the heart of the dead guinea pigs.

3. Other series of the STI vaccine might possess an increased virulence toward rabbits.

4. The infection of white mice and guinea pigs by vaccination does not always cause death in white mice and guinea pigs, since there are cases when a part of the white mice does survive and when all of the guinea pigs perish or when all of them survive.

5. The STI vaccine of an increased virulence toward rabbits, does not cause any complications when tested on big animals (sheep, cattle, horses) if administered at ordinary or even at increased doses.

A commission has established, by testing the immunogenic properties of the anthrax STI vaccine on sheep, that the vaccine causes a "tension" immunity for a period of 15 months (after that period the immunity has not been checked on the sheep).

The vaccine STI has advantages over the TSENKOVSKII's vaccine, since the animals have to be vaccinated just once with this STI vaccine and there is no need to free them from work (horses and work oxen).

However, it must be added, that there were cases with complications in horses, cattle, and, especially, in sheep and goats, when the vaccine STI was used in mass vaccination. These complications often resulted in the loss of young animals, especially in lambs and kids. Therefore, the conclusion drawn from this experiment proves that the STI vaccine is not completely harmless to agricultural animals.

However, in regard to the stability of the properties of the vaccine, we must abstain from any categorical decisions, since the Vaccinal strain STI possesses entirely new cultural-biological properties.

The question of the stable properties of the vaccinal strain STI will be answered after a certain period of time, when more experimental data will have been obtained.

/begin. p.63/

1. PRESERVATION, REGENERATION AND EXAMINATION OF MATRICES OF ANTHRAX

The importance and significance of the regeneration of matrices of Tsenkovskii's vaccine for the preservation of their stable properties has been mentioned already before. However, it must be said that in the time when no refocillation of the vaccines by passaging them through the organism of white mice was done (from 1920 to 1932), the vaccines have lost their biological properties and changed them considerably. Thus, for instance, the 1st Tsenkovskii's vaccine did not kill white mice anymore and the 2nd one - had an increased virulence which caused death in rabbits. In connection with this, the Commission on Matrices has conducted work in 1932 on the investigation and separation of the matrices in accordance with the requirements for their utilization. The results were: the matrices of the first vaccine no. 20 and of the 2nd vaccine no. 71 were separated from the others. Furthermore, the matrix of the first vaccine no. 20 was replaced by the analogical matrix no. 18 which was in better accordance with the instructions.

Since 1932, the preservation and regeneration of the matrices of Tsenkovskii's vaccines and their distribution to bioplants was conducted by the State Scientific-Control Institute of the USSR Ministry of Agriculture. Thanks to the yearly regeneration of the matrices by passaging them through the organism of white mice, the matrices have preserved their cultural and virulent properties.

The method of regeneration of the matrices consists in the following: Three white mice are infected with the matrices at 0.1 cm^3 (first passage). The second passage is done by a subcutaneous injection of the following three mice by injecting into them blood of the heart of the mice which died of the first passaging and so-forth up to three or four passages. The blood obtained from the heart of the last mouse which died of the last passage must be separated from the blood serum of mice by seeding it twice on fowl flesh bouillon prepared according to Tsenkovskii's method.

The culture, primarily examined for its purity and typicality of growth in regard to anthrax (Tsenkovskii's 1st vaccine grows on bouillon by forming a cotton-like film on the bottom of the flask; Tsenkovskii's 2nd vaccine grows diffusibly; both vaccines form on agar typical colonies of the R - form), is to be seeded on fowl flesh bouillon contained in flat-bottomed and two-necked bottles of the Khar'kov type; the bouillon layer should be not thicker than 2 - 3 mm. The seeded bottles have to be placed into the thermostat for growing and for the development of spores; they must be kept there at a temperature of 32 - 34° for 6 - 7 days. After a perfect spore formation is reached, (80 - 90°/$_o$ spores on the field of vision through the microscope) the spores are enclosed into a 30°/$_o$ solution of chemically pure glycerin at the following rates: 20°/$_o$ spore culture of the matrices of the 1st vaccine — 80°/$_o$ of glycerin solution and 10°/$_o$ of the matrix culture of the 2nd vaccine — 90°/$_o$ of glycerin solution. After 30 days the regenerated matrices must undergo an investigational test for their virulent properties and the results should be: the first vaccine should cause death in all mice at rates of 0.01 to 0.0001 cm^3 and should

/begin. p. 62/ not cause death in guinea pigs at a dose of 0.2 cm^3; the second vaccine should cause death in all guinea pigs at doses of 0.1 to 0.001 cm^3 and should not cause death in rabbits at doses of 0.5 cm^3. Furthermore, prior to the delivery to bioplants, the matrices must be checked by an analogical test for virulence after each distribution into bottles.

In 1936 - 1938, the Commission on Matrices (P. A. TERENT'EV, S. G. KOLESOV and V. N. DENISOV) conducted experiments at large on the investigation of the immunogenic properties of the Tsenkovskii's - vaccines and on sheep saponin vaccines. The results of the control-tests have established that the sheep had strong immunity infection with anthrax. For instance, 7 months after the infection of 15 sheep, all of them remained alive, whereas the 4 control sheep died of anthrax 36 - 48 hours after the vaccination. Thirteen months after vaccination, one out of the 14 vaccinated sheep died; two out of the four control sheep died of anthrax within 36-53 hours; the other two suffered a severe form of it. After the infection of five sheep seven months after vaccination with saponin vaccine and 4 sheep 13 months after the vaccination with the same vaccine, all vaccinated sheep remained alive (one control for both, for the matrices and saponin vaccines).

2. METHOD OF PREPARATION OF THE TSENKOVSKII's VACCINE

The Tsenkovskii's vaccines are prepared from the matrices of the 1st vaccine no. 18 and the second vaccine no. 71, which are distributed by the State Scientific-Control Institute. The matrixes are delivered in sealed Pasteur pipets equipped with labels showing the name of the matrix,

the date of its regeneration and distribution. A description (passport) with the indication of the cultural and virulent properties of the matrices is also added.

For the multiplication of the matrices, apeptonic fowl flesh bouillon is used, which is prepared in accordance with Tsenkovskii's method: 1 part defatted ground fowl flesh + 4 parts of water boiled on a low flame for 30 minutes; to one liter of meat water - 13 g chemically pure common salt and 0.7 cm^3 12.5% phosphoric acid added. Thereafter, the meat water is boiled for another 30 minutes and filtrated; distilled water is added as much as is needed in order to reach the primary volume; pH 7.2 is established and boiled again for 15 minutes; then filtrated into bottles and sterilized in the autoclave for 30 minutes at one atmosphere.

The seeding of matrices for multiplication is done by Pasteur's pipet which must be shook thoroughly and carefully checked through the microscope for the absence of mold. For the elimination of microbes, the outside of the pipet must be treated with alcohol - ether. The end of the pipet must be detached by breaking with fired (proflambirovanye) pincers; thereafter the matrices are taken and seeded at 10 - 15 drops per flask or bottle of the fowl (hen's) [begin. p.70] bouillon. After the inscription of the date of the seeding, the name of the vaccine and the number of its series, the flasks are placed for 14 - 16 hours into the thermostat at a temperature of 34° for growing.

The matrix obtained from one pipet is to be used just once, that means that from the content of one pipet, only one vaccine series can be obtained.

Tsenkovskii's vaccines are grown on apeptonic agar prepared from meat bouillon (1 part of ground meat per 4 parts of distilled water) or of Hottinger's broth by adding 2% of Odessa agar-agar. Simultaneously, the vaccines were grown on a hydrolytic medium. The nutritive agar is to be distributed into flat bottles (matrass) of transparent glass and even sidewalls; it must be sterilized at 1 atmosphere for 30 minutes. Prior to seeding, the bottles have to be checked for sterility by keeping them in the thermostat at a temperature of 36 - 37°.

For typicality and purity of growth, prior to the seeding into the agar of the matrasses, the bouillon seedings of the matrix vaccine must be examined on a smashed drop or on a stained smear macroscopically and microscopically. The macroscopical matrix of the 1st vaccine, usually looks like a transparent bouillon with a sediment (cotton-like film) on the bottom of the flask; whereas the matrix of the 2nd vaccine grows diffusively. In the dab, the first one is composed of immobile bacilli; long and short threads; the second one - of separate immobile bacilli and short threads. In the smears, as a rule, are present straight rods which adopt the color of the rod or thread easily. Those cultures of matrices, which contain involutionary forms (rods with a pear-like bulging and threads of short and long limbs) are considered as not suitable. After purity and typicality of the matrix seedlings are established, they are seeded in a sterile room on agar by means of a siphon (a shut method) into the matrass bottles over a flame; moreover, only as much of the quantity of the matrices together with the condensed liquid should be poured into the matrasses, as might be used for the even wetting of the agar surface. After the seeding

and the inscription of the name, date and number of series, the matrasses must be placed for 6 - 7 days into the thermostat at a temperature of 32 - 34° (the matrasses have to be placed with the agar upwards) for multiplication and for the formation of spores. Twenty four hours thereafter, the matrices, together with the seedings, have to be examined carefully for growth, purity and typicality; a magnifying glass must be used for this purpose and the surface of the agar should not be wetted; those matrices in which colonies of foreign microbes or slimy growth or partial lysis is observed, must be considered as not suitable. It is well known that the matrices of the Tsenkovskii's vaccine do form typical uneven colonies of the R - form; however, it is also possible that in some rare cases, atypical, even S-colonies might be observed. Matrasses which contain such atypic S-form colonies, must be rejected by all means. For the investigation of the spore formation of the seeded /begin. p.17 vaccine, after 4 days 10 - 15 % of the flasks must be examined microscopically, that means by the smashed drop method. On some bioplants, the preliminary examination of the vaccine for the formation of spores is not conducted; it is considered being sufficient if the testing of the vaccine in the matrass bottles takes place after the vaccine had been kept in the thermostat for 6 - 7 days.

In order to wash off from the agar the spore culture of the vaccine, a sterile physiological solution at a rate of 70-80 cm^3 must be poured through a siphon into the bottles. Thereafter, in order to wet the agar surface with the culture, the matrasses with the agar downwards, have to be left for 20 - 30 minutes. At the indicated time, the matrasses must be

shaken as mentioned before and also as long in a circulating movement, until the culture is washed off from the agar. After the washing the vaccine must be tested microscopically for purity and value of the spore formation; this must be done on a squashed drop. Those matrasses which were proved being pure and having in the microscopic field of vision the necessary amount (not less than 80°/₀) of the full value spores, might be filled into the bottles: the 1st vaccine together with 40°/₀ of the aquatic solution of glycerin ("conservant"), approximately 350 - 400 cm^2 of the agar surface per 1 liter of glycerin solution; the 2nd vaccine is to be filled first into a (bottle) - mixer and then distributed into flasks with the 40°/₀ aquatic solution of glycerin, approximately with 60-70 cm^2 of the agar surface per 1 liter glycerin solution. The emulsion must be filled from the matrasses into the flasks with the "conservant" /aquatic glycerin solution/ through a sterile siphon with a hose under normal pressure. A 40°/₀ chemically pure glycerin solution on distilled water must be prepared in bottles of 18 - 20 liter capacity /begin. p.72/ 12 - 13 liters each. The bottles with the glycerin solution must be mounted with two siphons: with a short one equipped with a little bag (made either of silk or linen) on the end for the filtration of the emulsion of the vaccine which will be poured into the bottles, and with a long one - for the distribution of the vaccine. The solution in the bottles must be sterilized in the autoclave at $1^1/_2$ atmospheres during two hours.

The preparation of the Tsenkovskii's vaccines is permitted not only on glycerin but also on the physiological solution. The period of suitability is two years - for the vaccine on glycerin and one year - for the vaccine on the physiological solution. That amount of vaccine which is

seeded from the same seedlings on the same day is considered being one series.

3. PREPARATION METHOD OF THE SAPONIN VACCINE

The saponin vaccine is prepared from the matrix of Tsenkovskii's 2nd vaccine no. 71. The preparation of the seedlings and the growing of the culture on an apeptonic agar, also the testing for purity and spore formation is done by the same method which was prescribed for the matrix of Tsenkovskii's 2nd vaccine. The emulsion of the 2nd vaccine, which had been washed by the physiological solution from the matrasses, must be poured into the 3°/₀ solution of saponin. The saponin solution must be prepared the following way: Saponin which had been checked, is diluted in a bottle of a preliminarily sterilized physiological solution under $1\frac{1}{2}$ atmospheres pressure during 2 hours at a rate of obtaining 3°/₀ of saponin. After a total dilution of the saponin, the bottles with the solution are sterilized during 30 minutes at 115°. In case, after sterilization, the pH of the saponin solution is lower than 6.0, a phosphate mixture in the amount of 5°/₀ must be added. The phosphate mixture must be sterilized separately and added to the sterile solution of saponin prior to the addition of the wash out of the culture. The phosphate mixture is prepared on the preliminarily dried mono-derivative potassium phosphate KH_2PO_4 and di-derivative sodium phosphate No. 2 HPO which had been dried preliminarily; they must be diluted in distilled water (9.078 g KH_2PO_4 per 1 liter and 11.874 g Na_2HPO_4 per 1 liter) and mixed in the proportion of 19.2 cm^3 KH_2PO_4 and 80.8 cm^3 Na_2HPO_4.

Prior to utilization in bioplants, saponin must be checked for reactiveness and toxicity, and a test must be conducted for its reaction

to sugar and for the hemolytic index in the form of a 3°/. solution. For the reactiveness test, saponin was injected subcutaneously to two horses at a dose of 0.3 cm^3 and to six rabbits at a dose of 0.1 cm^3; 10 - 12 hours after the injection, the temperature in horses started to rise and was 0.5 - 1.5° higher after 18 - 24 hours. Such temperature lasts usually 1 - 1 $^1/_2$ days and returns thereafter to normal. On the injection spot of saponin, after 6 - 12 hours emerges a spreading swelling in the size of 5 x 5 - 5 x 8 cm. After 1 - 2 days, the swelling decreases and hardens. In vaccinated rabbits on the spot of the saponin injection, necrosis of the skin should appear (not more than in 50°/.). For /Begin. p. 73/ toxicity, saponin must be tested on 12 white mice in doses of 0.05 - 0.1 - 0.15 cm^3; four mice to each dose; saponin corresponds to the standard; if one part of the white mice dies from a dose of 0.05 cm^3, consequently the dose of 0.1 and 0.15 cm^3 causes the death of all white mice. The Heinz reaction might be used for the saponin test, if there is no hydrolysis on hand; the test of the hemolytic properties should be conducted according to the instructions which are described in the preparation of the saponin vaccine.

4. PREPARATION METHOD OF THE ANTHRAX VACCINE STI

The STI vaccine is prepared from the standard vaccinal strain which consists of a sporular acapsular anthrax variant STI-1. The standard strain is preserved in a 40°/. solution of chemically pure glycerin on distilled water. To the bioplants, the standard strain is distributed in sealed Pasteur's pipets by the State Scientific-Control Institute. The pipets are all labeled and have the inscription of the name of the strain, the date of ... preparation and the date of the distribution. To the strain

is also added a description (passport) of the cultural-biological properties.

For obtaining a culture of the standard strain STI, nutritive bouillon is used; the bouillon must be prepared of the Hottinger broth. The meat broth, obtained according to Hottinger's classical prescription, must be diluted 1:5 - 1:6 in distilled water. Per one liter of this broth 2.5 g NaCl, 0.2 g KCl and 1 g Na_2HPO_4 must be added. By rendering the solution more caustic in adding a 4% solution of NaOH, the pH 7.2 - 7.3 is established; thereafter, the nutritive bouillon is to be filtered and sterilized at 1 atmosphere for 30 minutes. The seeding of the standard strain on the nutritive bouillon is done in the amount of 0.5 - 1 cm^3 by following the rules for sterility. After the seeding, the matrasses should not be shaken but placed for growing into the thermostat at a temperature of 33 - 34° for 18 - 24 hours. After being kept in the thermostat, the bouillon on the bottom of the flask starts to grow in the form of cotton or in the form of a tiny net; the upper layer of the bouillon is transparent. Within the mass of the broth, flakes of the growing culture are suspended in a considerable small amount. In case, diffusive growth and a coating appears on the top, the matrasses must be considered as not acceptable. The culture contains in the divided drop immobile bacilli and long threads, whereas in the smear - stained rods and threads.

The vaccine STI must be grown on a nutritive agar, prepared from the Hottinger broth by the above mentioned method and by adding to it 2.5 - 3% of the Arkhangel'sk agar-agar or hydrolytic medium. The nutritive medium must be filled into matrasses and sterilized at 1 atmosphere

during 30 minutes. Thereafter, the medium is to be checked for sterility in the thermostat during two days [48 hours] and kept in the plant for ripening at ordinary temperature for 0 - 5 days.
[Begin. p.7]

After the purity and typicality of the cultures are established by examination in a sterile room by following all regulations for sterility, the matrices are seeded on the nutritive agar into the matrasses at an amount of 3 - 5 cm^3 into each. After the name of the vaccine, the date of its seeding and the number of the series is inscribed, the surface of the agar must be thoroughly wetted, and thereafter, the matrasses placed with the agar downwards for 2 - 3 hours. The growing of the vaccine is done in the thermostat at an upward position of the agar and at a temperature of 33 - 34° during 48 - 72 hours. After 48 hours, the matrasses have to be checked carefully macroscopically for typicality and purity and microscopically for purityand spore formation. The colonies of the cultures are usually dry and of a greyish color; in a dab or stained smear the spore of the whole value are contained in the amount of 70-80°/. in the field of vision of the microscope in proportion to the bacillar forms. If the spore formation is not sufficient, the matrasses have to be kept in the thermostat for up to 72 hours. Thereafter, the culture in the matrasses must be treated with turpentine, by introducing 2 cm^3 of turpentine with pincers into the cork of the matrasses; thereafter, the matrasses have to be placed back into the thermostat for 24 hours for the acceleration of the process of the spore of formation. After the effect of the vapor of turpentine during the 24 hours and after a selective microscopy, which must show in the dab or in the smear not less than 90 - 100°/. spores; the

washing off of the culture is carried out by using distilled water at 60 - 70 cm^3 per each matrasse; the use of pebble or beads might be helpful, but not necessary. The sucking of the culture from the matrasses is done into a sterile measured bottle filled with beads and inside containing a filter in the form of a bag of gauze. The washed off culture is then subjected to shaking for 2 - 3 hours, thereafter a sample of it is taken for the determination of the optical concentration of the initial (mother) washout and for the examination of the growth for purity. To the initial (mother washout, at 1 $^1/_2$ atmospheres for 2 hours, 30% of the sterilized glycerin is added; thereafter, the washout is placed into the refrigerator at a temperature of — 10° for 5 days [60 hours]. At the indicated period, after having obtained positive results, the initial washed agar is added and filled into prepared bottles with a 30% solution of glycerin on distilled water, which was sterilized at 1 $^1/_2$ atmospheres for 2 hours by calculating of obtaining a vaccine of a 100 million spore concentration per 1 cm^3 as to the optical specific standard of the STI vaccine. The optical specific standard of the STI vaccine corresponds like this: 200 million spore - to 1 milliard according to the standard of the Central State Scientific-Control Institute of the USSR Ministry of Health (Protection).

5. PACKAGING OF VACCINES

The vaccines must be packaged the same day when they were bottled. The packaging into flasks is done by using a sterile siphon with a rubber hose under normal pressure [begin. p. 75] by placing the bottle with the vaccine one meter higher than the working table; the first portion of the

vaccine sucked by pulling the rubber backwards. For an even distribution of the spores, the bottle with the vaccine must be shaken periodically before and during the packaging. The first portion of the vaccine - at the amount of 150 - 200 cm^3, must be rejected.

The packaging must be done over a flame of a burner, under a bell glass, in a sterile room and by adhering to the regulations for sterility. The dishes used in the packaging of the vaccines, and the rubber corks must be sterilized under 1 $^1/_2$ atmospheres for 2 hours.

After the packaging and corking of the bottles, the latter must be sealed by resin of prime quality (gum mastic), stamped with the stamp of the bioplant and labeled. On the labels must be indicated: the name of the vaccine, the name of the bioplant which had prepared the vaccine, the number of the series, the date of suitability, the number of the State Control and the doses for all types of agricultural animals. For the first vaccine - must be used a navy blue label, for the second vaccine - a red one, for the saponin vaccine and the vaccine STI - white ones. The ready packaged vaccines must be kept in a dark and dry room at the temperature of + 2 - to + 15°.

6. CONTROL OF THE VACCINES

Prior to distribution, each series of the vaccines must be examined by the State Control of the bioplant. The vaccines are checked for purity of the growth, for MPB, MPA and MPPB. The seedings are observed during seven days, whereas those vaccines which were proved to be soiled must be rejected. For the biological control, healthy animals and those in a normal state must be employed; they should not have been used for experiment before

and should have the following weight: mice - 17 - 20 g, guinea pigs - 350 - 450 g and rabbits - 1.5 - 2.5 kg; for the control of the STI vaccine, animals of a maximal weight must be employed.

The determination of the concentration of the spores in the Tsenkovskii's vaccines must be done according to BLAGOVA's method, by seeding on agar into Petri dishes. For this purpose into the test tubes with fused agar 1 cm^3 of the initial suspension (1:1,000,000 of the first vaccine and 1:100,000 of the second vaccine) is at a temperature of 55° with a graded pipet, on which the graduation is marked up to the end.

Into three tubes it is introduced at - 0.33 cm^3 and into the 4th, the pipet is flushed with the agar itself. Thereafter, the agar with the vaccine must be shaken briskly, poured into dishes, cooled for growing and placed into the thermostat together with the four tubes of the agar. After 2 days /24 hours/ the colonies which have grown in the dishes and tubes must be counted.

The examination of the Tsenkovskii's vaccines for virulence must be done the following way: three white mice must be infected with the 1st vaccine at a dose of 0.01 cm^3 each, also 2 guinea pigs at a dose of 0.2 cm^3 each. The white mice will die of anthrax not later than within 100 hours, /begin. p. 76/ the guinea pigs will survive. With the 2nd vaccine - two guinea pigs must be infected at a dose of 0.2 cm^3 and also 4 rabbits - at a dose of 0.5 cm^3. The guinea pigs die of anthrax after 3 - 4 days, all rabbits survive, or nearly all; that means that out of the four, one rabbit may die. In case the results turn out to be different and it is obvious that the first vaccine is of lower virulence toward the

white mice and the second vaccine toward the guinea pigs - or the virulence of the first vaccine is increased toward guinea pigs and that of the second vaccine - toward rabbits, the control test must be repeated and, if analogical results are obtained, the vaccine must be rejected.

On experimental animals the testing of the saponin vaccine is done the following way: For testing the local reaction to saponin, three rabbits are infected subcutaneously at 0.2 cm^3 and three guinea pigs also subcutaneously at doses of 0.1 cm^3. In these animals, at the spot of the injection, a swelling appears on the second day (a reaction to saponin), the size of the swelling in guinea pigs is 2 x 3 cm and in rabbits - 3 x 5 cm. After 2 - 3 days, in all animals necrosis of the skin emerges and little ulcers start to form. The rabbits do survive, but all guinea pigs die of anthrax or, sometimes, two out of the three infected. In order to test the matrices, the 24 hour old bouillon culture of the saponin vaccine must be injected subcutaneously to 2 rabbits at a dose of 0.5 cm^3 and to 2 guinea pigs at 0.1 cm^3. The rabbits survive, but the guinea pigs die. In case one or both rabbits die and the guinea pigs survive, the test must be repeated. If analogical results are obtained - the vaccine must be rejected.

The test of the concentration of the spores of the STI vaccine is done according to the specific optical standardized method, indicated before; the test of the quantity of the living germs - by seeding on the agar into six Petri dishes at 0.2 cm^3 of a suspension of 10,000, 1,000 and 100 spores according to the specific optical standard. The amount of the living spores in the vaccine fluctuates in the limits of 25 - 35%, in proportion to

the concentration which equals 100 millions in 1 cm^3 of the vaccine. Determining the quantity of the living spores on the agar, the typicality of the colonies must also be taken into consideration, the colonies must correspond to the type of the P-form. For controlling and testing the vaccine for the absence of capsules in bacilli, the vaccine must be seeded onto the coagulated equine serum in order to become separate colonies. The colonies which have grown on the coagulated serum must be dry and not slimy; the microbes of these colonies should not have capsules, specially when stained by the ordinary methods.

When five rabbits are subcutaneously inoculated with the STI vaccine at a dose of 5 cm^3 each, all of them must survive. In some of the rabbits not very big edemas, in the size of a pigeons egg, might emerge. In case, at the first or second control one part of the rabbits dies and shows anthracic discharge from the edema or from the parenchymatous organs, the vaccine must be tested again for its harmlessness on five sheep at a 4 - fold dose and the material must be sent to the State Scientific-Control Institute for approval.

The vaccine is acceptable if just 2 - 4 out of the five guinea pigs which were vaccinated subcutaneously with 1 cm^3 of the vaccine died /Begin. p. 77/ within 4 - 7 days and also when the culture of the vaccine is isolated in large masses from the parenchymatous organs only, whereas the blood must remain sterile. After 24 - 48 hours, at the inoculation spot (the interior part of the surface of the femur) nearly in all vaccinated guinea pigs edema emerges in the size of a pigeon's egg which might expand and reach both inguinal folds and cover the stomach. The culture of the vaccine

which is isolated from the dead white mice and guinea pigs, should not have capsules.

7. PRACTICAL UTILIZATION OF THE ANTHRAX VACCINE

The anthrax vaccines (Tsenkovskii's, the saponin vaccine and the vaccine STI) prior to their application, must be thoroughly investigated by a veterinary specialist; only these vaccines can be used for the vaccination of agricultural animals which correspond to the established standard. The anthrax vaccines must have labels on the flasks showing the number of the series, the date of the preparation, the suitability date, the number of the State Control and the doses. The flasks must be thoroughly closed with rubber corks, sealed with gum mastic and have the stamp of the bioplant which had prepared the vaccine. Those flasks which show broken seals and a growth of mold and flakes within the mass, must be rejected. The suitability period of the 1st and 2nd Tsenkovskii's vaccine on glycerin is two years; the same - on the physiological solution - one year; the saponin vaccine - six months, and that of the vaccine STI on glycerin - tow years and on the physiological solution - one year.

Vaccination with the vaccines against anthrax are not permitted on those farms infected with acute infectious diseases and also in the following cases when: a) the animal's temperature is higher than normal, b) the animal is under 2 months of age, c) animals are in the last two months of pregnancy (with the Tsenkovskii's vaccine and the saponin vaccine) and in the second half of the last month of pregnancy (with the vaccine STI) and d) animals are exhausted and weak.

The vaccination must be carried out by the veterinary surgeon or by a veterinary fel'dsher under the supervision of a veterinary surgeon. The syringes and needles must be sterilized by boiling for 30 minutes prior to the vaccination and during the vaccination. The injection spot must be shaved and disinfected with a 3% solution of carbolic acid and denatured spirit.

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Doses of the vaccines to be used for agricultural animals (in cm^3)

Kind of animal	Animal's Age	Name of Vaccines			
		Tsenkovskii's 1st vaccine	Tsenkovskii's 2nd vaccine	Saponin vaccine	STI vaccine
Horses	Up to $1\frac{1}{2}$ yrs.	0.3-0.5	0.1	0.1-0.2	0.5
	Over $1\frac{1}{2}$ yrs.	1	0.3	0.3	1
Camels	Up to 2 yrs.	0.5	0.2	0.1-0.2	1
	Over 2 yrs.	1	0.3	0.3	1.5
Cattle	Up to $1\frac{1}{2}$ yrs.	0.5	0.2	0.1-0.2	0.75
	Over $1\frac{1}{2}$ yrs.	1	0.5	0.3	1.5
Sheep and goats	Over 2 months	0.3	0.1	0.1	0.25
Swine	Up to 1 year	0.2-0.3	0.1	0.1-0.2	0.25
	Over 1 year	0.5	0.2	0.3	0.5

In case complications arise, the vaccination must be stopped. The sick animals are then subjected to treatments with the anti-anthrax serum and also to medicamental treatments. The veterinary surgeon of the Raion must be consulted in each case. Concerning the time of conducting vaccinations against anthrax and also in regard to the application of other vaccines, indications will be found in the instructions of the applications of those vaccines.

10/30/56