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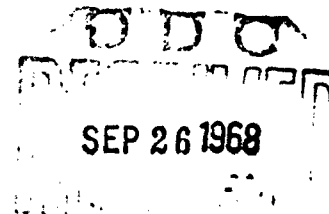
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Poluchenio sirbirolazvennykh shtamov dlia
tselei vaktsinoprofilaktiki.

Obtainance of anthrax strains for the purpose
of prophylactic vaccination

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(In Russian)

Obtainance of anthrax strains for the purpose
of prophylactic vaccination

The finding of methods of obtaining anthrax strains with
a decreased virulence and with strongly pronounced immunogenic qualities
is of considerable theoretical and practical importance.

The method of "directed effectuation" of the micro-organisms
is one of the basic methods used in the practice of microbiology for
obtaining vaccine strains.

The great Russian scientist I.V. NICHURIN proved by his
numerous experiments that living organisms can easily be changed by
the experimenter by effectuating⁴² their external habitat and also, that
there is nothing unchangeable in the world of living organisms.

T.D. LYSENKO, in developing NICHURIN'S teachings, considers
that the change of the nature of a living body is the change of the
external habitat and of the type of the metabolism. Thus, NICHURIN'S
teachings confirm that the nature of living beings - either the most
complicated organisms or the lowest [protozoa] micro-organisms - can
be changed at the will of the experimenter.

Therefore the method of "directed effectuation" upon the
virulent microbes must be used in order to obtain new vaccine strains
which possess stable, greatly reduced virulence and immunogenic
properties useful for vaccinoprophylaxis.

These two methods of obtaining anthrax vaccines are known
in literature. The method of L. PASTEUR and L.S. TSENKOVSKII - by
effectuating [subjecting] the virulent anthrax strains to increased
temperature - and the method of isolating the noncapsular edemic
population on the blood serum (STANATIN, MAC STERNE, and N.N. GINSBURG).

Both methods have enabled the researchers to present effective
anthrax vaccines for practical use.

The TSENKOSKII and STI anthrax vaccines are used in practice
at the present time for prophylactic inoculations against anthrax.
These vaccines have obvious immunogenic qualities and are effective
preparations. However, these vaccine can also produce postvaccinal

complications.

The experiments of some researchers to improve the TSENKOVSKII vaccine (F.A. TEREENT'EV) and to obtain new vaccine (I.F. BATOK, I.I. ARKHANGEL'SKII, D.R. ROMANOV and others), proved to be unsuccessful. Further experiments were needed for the obtainance of effective and less harmful anthrax vaccines. We chose the method of "directed effectuation" on micro-organisms in order to obtain weak virulent strains good for prophylactic vaccination of agricultural animals against anthrax.

Different methods have been used in our laboratory, such as: the effect of increased temperature, the immune anthrax serum and the method of isolating the population on the conglutated serum.

Fifteen strains of virulent anthrax culture were used. This is the history of these strains which succumbed to mutation: strain No. 916 was obtained from the aborted fetus of a cow in 1949; strain Sh-2 was isolated from the cadaver of a swine in 1935; strain No. 1260 was separated from the cadaver of a cow in 1949; the standard sporiforous virus was isolated from a cadaver of a horse in 1950; the strains K-5 - was isolated from the cadaver of a cow in 1934 and strain K-12 - from the cadaver of a swine in 1935.

The cultivation on prepared [agar] serums resulted in obtaining the strain 916-1 (KOLESOV, GUTMAN) and the strain Sh-15 (KOLESOV) in 1949. The strain 916-1 possessed the ability to cause edemas in white mice and guinea pigs, seldom in rabbits; it did not cause capsulas. It caused death nearly in all mice and guinea pigs; it did not cause capsulas. The strain caused death in the majority of white mice and - not over 10-20% in guinea pigs. Comparing the obtained and mentioned strains with the vaccine STI - it must be said - that the strain 916-1 has a greater virulence and the strain Sh-15 a less virulence than the vaccine STI. The strains do correspond in their cultural and morphological properties with the anthrax micro-organisms; they formed R-form colonies on MPA and grow like cotton on the bottom of the test tube. The strain 916-1 is able to produce more spores than the strain Sh-15.

The strains 1260-31 and 94 were obtained in 1950-1951 by the method of effectuating the culture of the antianthrax serum. Strain 94 was effectuated a standard sporiforous virus. The strains correspond in their cultural properties with the typical anthrax micro-organism. The strains do not form capsulas. The strain 1260-1 is fully apathogenic to white mice; the strain 94 possesses a weak virulence toward white mice and young guinea pigs; 20-25% of the guinea pigs die. Several strains were obtained by increased temperature; they were of weak virulence. Two of them, namely, strain K-5-39 and strain K-12-46 were studied because of their practical interest. The strains were able to grow on an agar culture medium; by forming RS-forms, they grow diffusively and formed moired weaves when shaken. They have a distinctive spore formation, they do not form capsulas. The strains are virulent to white mice and guinea pigs.

The study of the strains which were obtained by the method of "directed effectuation" on the immunogenity on rabbits showed that they do possess distinctive immunogenic properties if administered in great doses.

TABLE 1

Control of the immunogenic properties of anthrax cultures
which underwent a directed mutation.

No of the strain	Quantity of tests	Quantity of inoculated rabbits	Quantity of conducted vaccinations of rabbits	Dosis of vaccination in ml	Control infection		Died	Survived	%
					How many days after Vaccination	Dosis of the virus			
1260-31	2	8	Once	3	28	1	2	6	75
94	1	5	"	3	25	1	1	4	80
R-5-39	2	10	"	3	14	1	2	8	80
R-12-46	2	10	"	3	34	1	2	8	80
Sh-15	1	5	"	2	23	1	1	4	30
Control	2	10	-	-	-	1	8	2	-

It has been determined that the strain Sh-15 has a more expressed immunogenicity than the strains No. 94, 1260-31 and K-5-39, this was obvious after the study of the four strains on the immunogenic properties of sheep in 1951.

Only one sheep from the 8 which were inoculated with the vaccine prepared from the strain Sh-15, died four months before control infection. The other 7 sheep showed only a low and short thermal reaction.

Three sheep which were inoculated 15 days before the control infection, survived; they did not show any thermal or general reaction to the fatal doses of the anthrax virus. Four of 5 control sheep which were not vaccinated, died of anthrax.

A careful study in 1951 and 1952 of the vaccine from strain Sh-15 concerning its cultural, morphological and virulent properties showed that the vaccine still retained the properties which it had possessed in 1949 when it was obtained, and also those which it had at the primary test - in 1950.

In regard to the cultural morphological properties - the vaccine possesses all characteristics of an anthrax vaccine.

This vaccine - because of its weak virulence - caused death in white mice only when it was administered in massive doses. However guinea pigs remained alive to the greatest extent. The vaccine is harmless to rabbits.

A vaccine, for testing its virulence and immunogenesis, was prepared from the strain Sh-15 in the form of a spore culture fixed in a 20% or 30% (per cent) solution of glycerin at a concentration of 40 to 60 million spores for one ml.

TABLE No. 2

Data obtained from tests of the vaccine for harmlessness in -
1951-1952

Kind of animals	Quantity	Doses (in ml)	Number of test	Local reaction		Died	Survived %				
				Any reaction	Size of the reaction						
				+	++	+++					
Rabbits	17	2	3	3	6	3	1	16	94.2		
	8	3	2	-	4	4	-	8	100.0		
	8	5	1	4	1	-	-	1	8		
	33	0.3	6	7	6	10	9	1	32	97.0	
Guinea pigs	4	1	1	-	1	1	2	-	4	100.0	
	38		7	4	15	3	11	3	8	30	79.0
	42		8	4	16	6	13	3	8	34	81.0

The vaccine from strain Sh-15 has been tested for its immunogenesis on rabbits many times in the period of 1951-1952. It was proved every time that the administration optimal doses (1-2ml) guarantees the formation of evident immunity in rabbits. Even small doses (0.3-0.5 ml) produce insusceptibility to anthrax of over 50% in rabbits, at the death of all control animals.

A number of tests were recently scheduled for the study of the increase of immunogenic properties of the vaccine prepared from the Sh-15 strain by adding aluminum hydroxide as nonspecific stimulator. It was proved by a number of tests that the aluminum hydroxide increases the immunogenesis of the vaccine.

TABLE No. 3

Data obtained from tests of the vaccine for immunogenetic properties
in rabbits in 1951 - 1952

Name of the vaccine	Number of tests	Number of rabbits	Dosis in ml	Died	Survived	%
Glycerinated vaccine	2	9	2	1	8	88.8
	3	16	1	7	9	56.2
	1	3	0.5	1	4	80.0
	2	15	0.3	7	8	53.3
Total	1	45		16	29	64.4
Glycerinated vaccine	2	5	2	-	5	100
with	1	11	1	2	9	81.8
Ao(OH)s	2	3	0.5	-	5	100.0
added		14	0.3	6	8	57.1
Virus control	2	35	-	8	27	77.1
		8		8	-	-

Positive results have been obtained from the vaccination of agricultural animals in 1951 and 1952 testing the vaccine from the strain Sh-15 for harmlessness.

Thus, there were vaccinated in the second half of November 1951 eleven horses with 1 ml and one foal with a dose of 0.3 ml, nine cows with a dose of 1 ml and three calves with - 0.3 ml, twenty sheep with - 0.2 ml and ten lambs with a dose of 0.1 ml.

The local reaction to the inoculated vaccine was limited and not strong, the temperature of the body increased 0.5 - 1 only and lasted just for a short time (1-2 days).

The harmlessness of the vaccine also has been tested and proved on young goats and sheep in October 1952.

The vaccine used in the vaccination of goats was either glycerinated vaccine ~~was administered~~ vaccine with aluminum hydroxide added. The vaccine was administered in the usual dose (0.2 ml) or in doses which were 2-3 times or even 4 times greater. There were 33 goats vaccinated. It should be mentioned that the vaccination took place in October, at an unfavorable, rainy season. However, notwithstanding these circumstances, the vaccination was a success, since the local reaction in the animals was insignificant and the temperature, which had risen 0.4 - 1.4 only, lasted just 1-2 days. From all the vaccinated goats just one little goat had a high temperature. Thirty six sheep were vaccinated with the same vaccine, at the same time, among them ten 2-3 months old lambs.

The sheep were vaccinated with a 0.2 ml dose. These vaccinations did not have any complications either; the local reaction was insignificant, the temperature rise was of short duration.

Thus, the preliminary tests have proved that the vaccine is harmless if inoculated to young goats and sheep.

Conclusion

1. The new anthrax vaccine obtained by "directed effectuation" and selection proved to be harmless and immunogenic, when tested on rabbits.
2. The same vaccine, tested for harmlessness on sheep and goats in the conditions of the Institute, showed that it is harmless and stimulates just a minor local and thermal reaction.
3. It was determined that the vaccine produces stable immunity at a control after 15 days and after 4 months subsequent inoculation; this was proved by testing the vaccine for stability and length of the immunity period on sheep.
4. Further studies of the vaccine should be conducted in testing its harmlessness and effectiveness on agricultural animals at large and in rigorous experiments for the length and stability of its produced immunity.

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