

AN EXPERIMENTAL STUDY OF THE VACCINE PROPERTIES OF A BR. ABORTUS VARIANT
OBTAINED UNDER THE EFFECT OF PHAGE TB BACTERIOPHAGE

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AN EXPERIMENTAL STUDY OF THE VACCINE PROPERTIES OF A BR. ABORTUS VARIANT,
OBTAINED UNDER THE EFFECT OF BRUCELLA TB BACTERIOPHAGE

[Following is the translation of an article by N. N. Ostrovskaya, Gameleya Institute of Epidemiology and Microbiology, AMN, USSR, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) Vol. 2, No 7, 1965, pages 36--41. It was submitted on 24 Mar 1964. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

As was already reported (Ostrovskaya, 1957, 1960, 1961), under the influence of brucella TB phage on the virulent No 146 culture of Br. abortus, a number of variants were obtained with a diversely changed morphology of colonies, cells and other properties of the initial culture, especially virulence.

Here our attention was drawn to the cultures of Br. abortus No. 75-P, 74-C and 84-C. They were isolated from various organs of guinea pigs following several passages through the organism of these animals of one of the phage variants of Br. abortus No. 146 (4th variant) and based on virulence turned out to be close to the vaccine strain of Br. abortus No 19-BA. It is interesting to study the vaccine properties of these cultures.

At the present time the problem of obtaining new vaccine strains of brucella is very urgent, since for the approved prophylactic vaccination of persons vaccines are used which are prepared only from the strains of Br. abortus No 19 and 19-BA. Searches for new vaccine strains of brucella and their study have been the concern of many investigators for a number of years (Kotlyarova, 1950; Chernysheva and Orlov, 1951; Herzberg and Elberg, 1953; Elberg et al., 1953). Up until now the vaccines proposed by the authors have still not been used for the vaccination of humans.

The cultures studied by us were dissociated. They caused a positive test with tryptaflavine and a weakly positive thermoprecipitation reaction. This distinguished them from the majority of vaccine strains of brucella. The problem concerning the possibility of utilizing the dissociated cultures of brucella as vaccines is disputable. There are reports concerning the weak immunogenicity of the R-forms of brucella (Jacotot et al., 1960), however positive data has also been obtained concerning the immunogenicity of nonagglutinogenic R-cultures (Orlov and Borisovich, 1956; McEwen, 1942; Sicro and Rosenbusch, 1952; Prochaszka, 1960; Schaaf and Jartsveld, 1961).

In all probability the suitability of dissociated cultures of brucella as vaccines depends on the nature and depth of dissociation, on the degree of change of the antigenic structure of the microbial cells. Therefore the problem concerning their utilization should be resolved each time concerning the specific culture.

The present report presents the results of a study of the above named cultures of the 4th variant of Br. abortus No 146. As already pointed out, all the cultures were dissociated, their populations turned out to be persistently homogeneous and consisted entirely of R-forms (following staining according to White and Wilson, 1951). Based on reducing capability and other differential tests the cultures were typical brucella of the abortus species. They differed

from the initial culture by a lowered virulence, resistance to the TB phage, and lowered agglutinogenicity. These properties of the culture were strongly preserved for more than 5 years.

As is known, the vaccine strains of brucella, in addition to a lowered virulence and pathogenicity, should satisfy a number of requirements, and in particular they should possess a good ability to take root and be able to remain for a sufficiently long time in the organism of animals (Vershilova, 1961). Namely these properties were studied by us in the above mentioned cultures in an experiment on guinea pigs.

Subcutaneously these animals received 1 ml of a suspension of the culture (in a physiological solution) which contained $2 \cdot 10^9$ microbial cells (this dose is usually used in the tests on the vaccination of guinea pigs with a culture of Br. abortus No 19-BA). The animals were subjected to investigation in various periods (after 1, 2, 3, 4, and 6 months) following the administration of the culture. The results obtained testified to the similar dynamics of the process for all the cultures. Thus, brucella in the stated dose takes root well, conditioning a significant index of inoculability (65-50%) in the first periods of investigation (after 1 and even 2 months). After 3 months following the administration of the culture the index of inoculability is noticeably lowered (down to 23%), after 4 months brucella are sown from individual lymph nodes, and after 6 months all the seedings turn out to be sterile. Approximately the same indices are observed with the administration of this dose of brucella of the vaccine strain No 19-BA (Vershilova, 1950 and 1954).

Studies were made of the intensity and duration of immunity, dynamics of accumulation of agglutinins, level of sensitization of animals in various periods following vaccination, and also of the pathomorphological changes in the organs and lymph nodes.* The tests were conducted on 255 guinea pigs. Since the preliminary tests showed that the greatest immunogenicity is possessed by the Br. abortus culture No 84-C, we conducted all the subsequent tests just with this strain. [*Pathomorphological investigations conducted by N. A. Grekova.]

For studying the intensity of immunity, various doses of the virulent culture No 565 of Br. melitensis -- 2, 5, 25 and 250 ID*-- were administered to the guinea pigs after 45 days following vaccination (the period of the maximum intensity of immunity). [ID*-- one infecting dose of this culture equals 10--15 brucella.] For purposes of control the culture in the same doses was administered simultaneously to nonvaccinated animals. In order to establish the nature of the vaccine process, during the period of infection of the test animals and also in periods following infection when they were investigated*, a control investigation was made of a specific amount (no less than 3) of vaccinated but not infected animals. [*The animals were investigated after 30 days following infection.]

With infection, after 45 days following vaccination during the phase of nonsterile immunity, a 100% nonsusceptibility to 2 and 5 infectious doses of a virulent culture (see the table) was observed in the guinea pigs. Increasing the infection dose up to 25 and 250 infectious doses led to a break in immunity

in half of the animals. Here the nature of the infection in the infected animals was diverse: In some a culture was isolated only from the regional or the remote lymph nodes (mainly following the administration of 25ID), in others a generalized infection was observed (in 4 out of 12 guinea pigs, infected with 250 ID). Upon comparing the index of inoculability of a virulent culture in the control (nonvaccinated) and test animals, it was established that in the first it equaled 80--83%, and in the second -- all told 17.4--26.7%. The latter indicated the partial protection of the organism of vaccinated animals, expressed in the limitation of multiplication of virulent brucella.

Since even in the period of the maximum intensity of immunity the infection of some of the animals was observed following the administration of a large dose of a virulent culture, in the experiments for testing the duration of immunity we infected the vaccinated animals with only a small amount of Br. melitensis No 565 -- 30 to 45 microbial cells, which corresponded to 2--3 infectious doses. In this test the animals were infected after 2, 3, 4, 5 and 6 months following vaccination. The results obtained (see table) showed that even after 4--6 months following vaccination, when in all the animals a complete purification of the organism from the vaccine culture set in (phase of sterile immunity), it was possible to establish 100% nonsusceptibility to the stated dose of a virulent culture.

In an additional test which was set up it was established that an increase of the dose of the culture during infection up to 20 and 200 ID in that period (6 months following vaccination) led to the infection of all the vaccinated animals, but the index of infection in them, just as in some of the guinea pigs which were infected during the period of nonsterile immunity, was considerably lower than in the control animals. It should be noted that based on the intensity of immunity which was conditioned in guinea pigs with the No 84-C culture, it was very close to the immunity caused by the vaccine strains of Br. abortus No 19-BA, 104-M and Rev-1 (Vershilova and Kurdina, 1963).

The titer of agglutinins was checked in all the animals during all the periods of the bacteriological investigation. The Wright reaction as a rule was set up with two antigens at the same time -- with the Wright antigen and with the antigen from the homologous culture (Br. abortus No 84-C). In all cases it was possible to observe a difference in the results of this reaction depending on the antigen used. Thus, with the standard antigen the titer of antibodies in all the periods of investigation was low (1:20--1:40), while with the homologous culture it reached considerably higher indices -- 1:280--1:400. In the control animals, infected only with the culture of Br. melitensis No 565, the titers of antibodies with the Wright antigen and the homologous antigen were practically the same (see drawing). The results obtained may be explained by a certain change in the composition of the antigen complex of the No-84-C culture in comparison with the S-cultures of this species of brucella.

The comparatively greater accumulation of agglutinins in the serum of vaccinated animals which were specific for the vaccine culture opens the possibility for the serological differentiation of vaccinated from infected and brucellosis stricken animals and man.

It is known that as a rule brucellosis infection is accompanied by a significant sensitization of the organism which complicates the course of the disease. Vaccination is also accompanied by sensitization, especially the repeated administration of the vaccine. To a certain degree this circumstance limits the carrying out of revaccination (Vershilova, Grekova, 1959; Balandin and Sazykin, 1964; Taran et al., 1964). Therefore one of the main missions in the research for finding new vaccine strains is obtaining cultures with a minimum sensitizing effect on a sensitive organism.

When studying the No 84-C culture it was established that it exerted a relatively weak sensitizing action on the organism of guinea pigs. When comparing the degree of sensitization of pigs which was caused by the vaccine strains of brucella No 19-BA, 104-M and the culture of No 84-C, it was established that the greatest sensitizing action was caused by a culture of 104-M (87.1% of the pigs reacted positively), second place was occupied by the culture of No 19-BA (58.3% reacted positively), and third -- the culture of No 84-C (a positive Burnet test was noted in only 18.6% of the pigs). In all cases the investigation was conducted in 45--60 days following vaccination. The vaccination doses were as follows: $2 \cdot 10^9$ microbial cells for the cultures of No 19-BA and 84-C and $2 \cdot 10^5$ microbial cells for the culture of No 104-M. There were 90--116 animals in each group.

During the pathomorphological investigation of the organs and lymph nodes of guinea pigs in various periods following their intake of $2 \cdot 10^9$ microbial cells of a culture of No 84-C a picture of a typical vaccinal process was observed. It was expressed somewhat more weakly than during the administration of the vaccine strain of Br. abortus No 19-BA.

The data presented testifies to the fact that a culture of Br. abortus No 84-C, when it is administered to guinea pigs in a quantity of $2 \cdot 10^9$ microbial cells, is capable of stimulating the formation of an immunity of moderate intensity in the guinea pigs. We propose that the study of this culture should continue, since with a weak pathogenicity and a not too great sensitizing capability it can be used as a vaccine. Besides this, a certain peculiarity in the antigenic structure of the culture studied opens the possibility of the serological differentiation of inoculated animals and man from infected and brucellosis stricken animals and man.

Conclusions

1. A culture of Br. abortus No 84-C, obtained as a result of the influence of TB phage on the fully virulent culture of Br. abortus No 146, was a persistent R-variant with a weakened virulence and pathogenicity.

2. Following administration to guinea pigs in a dose of 10^9 microbial cells the No 84-C culture of Br. abortus took root easily in the organism of the animals and caused benign pathomorphological changes, which indicated a weakly expressed vaccine process and which was preserved for 2 months.

3. Following administration to guinea pigs of $2 \cdot 10^9$ microbial cells of

this culture a 100% nonsusceptibility was observed to 2 and 5 infecting doses of a virulent culture of the melitensis species and this was preserved for a period of 6 months (period of observation); increasing the dose of infection during the period of the nonsterile immunity phase led to a break in it in 50% of the guinea pigs, however the index of infection in the vaccinated animals was significantly lower than in the control, which indicated the presence of a partial immunity in them.

4. The culture of Br. abortus No 84-C sensitized guinea pigs comparatively weakly. As a result of the peculiarity in the antigenic structure of this culture the titers of the reaction with the Wright antigen in the vaccinated guinea pigs were significantly lower than with a homologous culture.

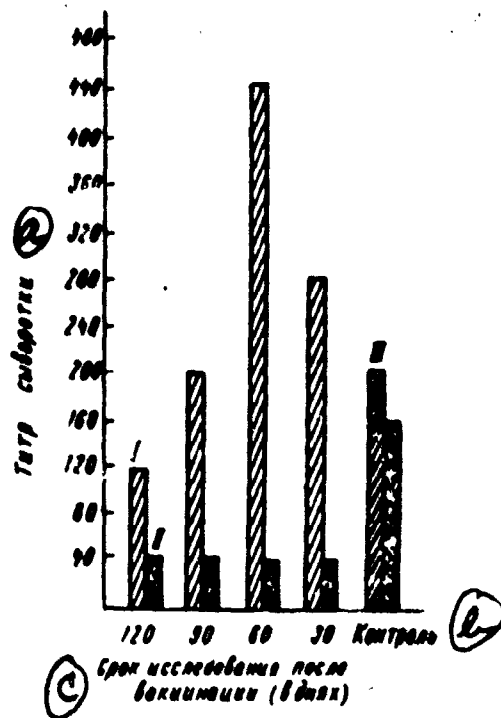
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Immunity in guinea pigs, vaccinated with a culture of Br. abortus No 84-C, following infection in various periods following vaccination.

Period of infection following vaccination (in months)	Dose of infection with a virulent culture of Br. melitensis No 565 (in ID)	Number of guinea pigs in the test (numerator) and number infected (denominator)		Index of inoculability (in %) for the guinea pigs			
		Vaccinated	Nonvaccinated (control)	Vaccinated		Nonvaccinated (control)	
				with vaccine culture	with virulent culture	with vaccine culture	with virulent culture
1	2	12/0	10/10	58.3	0	60.5	
1½	2	12/1	4/4	28.3	0.6	80.0	
	5	12/0	4/4	21.4	0	83.0	
2	25	9/5	-	25.2	17.4	---	
	250	12/7	-	15.4	26.7	---	
2	2	6/0	5/5	20.4	0	71.0	
3	2	5/0	4/4	0	0	76.0	
4	2	9/1	12/12	0.9	1.9	51.1	
6	2	9/0	9/9	0	0	57.5	



The Wright reaction in guinea pigs, vaccinated with the culture of Br. abortus No 84-C (10^9 microbial cells).

I - Culture of Br. abortus No 84-C; II - Wright antigen; III - Culture of Br. melitensis No 565 (10^1 microbial cells).

a - Titer of serum; b - Control; c - Period of investigation following vaccination (in days)