AD 678902

9 (1)

TRANSLATION NO. 1090

DATE: July 1968

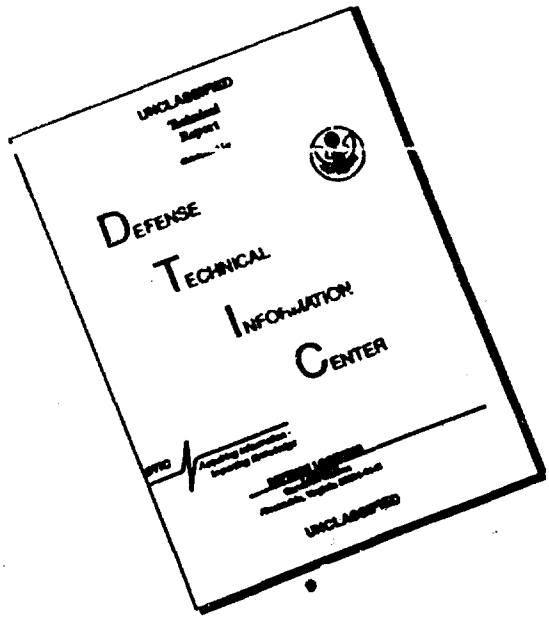
DDC AVAILABILITY NOTICE

This document has been approved for public release and sale; its distribution is unlimited.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

13

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

AF, 0 98
9-1

THE BIOLOGICAL CHARACTERISTICS OF INAGGLUTINABLE CULTURES OF BRUCELLA MELITENSIS

/Following is a translation of an article by N.N. Ostrovskaya of the Institute of Epidemiology and Microbiology imeni Gamaleya of the Academy of Medical Sciences of the USSR in the Russian-language periodical Zhurnal mikrobiologii, epidemiologii, i immunobiologii (Journal of Microbiology, Epidemiology, and Immunobiology), No 7, 1963, pages 125-131. The article was submitted on 11 November 1961./

In the last several years Brucella cultures isolated from the blood of patients and from animals which were not agglutinated by standard brucellosis serum but were agglutinated by homologous sera and also by the serum of the patients from which they were isolated began to attract attention. Drozhevkina (1954-1956), as a result of a study of similar cultures of the melitensis type, proposed that like the salmonella the inagglutinable cultures be considered as being V-cultures having a surface Vi-antigen. According to her data such strains are more virulent and highly antigenic and consequently more full valued. Drozhevkina considers that agglutinable cultures are R-cultures minus the Vi-antigen and with decreased immunogenous properties in comparison to the V-cultures. Also, Wolff, Dinger (1951), Renoux, Mahaffey (1955), and Antonov (1958) consider that such cultures are disassociated R-forms with a changed antigenic structure.

Thus there are two opposite concepts in evaluating

inagglutinable Brucella cultures which are icentical in their properties. Taking into account the practical and theoretical importance of this question, we decided to make the fullest possible study of the inagglutinable cultures available to us and to make an appropriate evaluation of them.

In this report we give the results of a biological study of inagglutinable cultures of Br. melitensis in comparison with agglutinable test cultures /See Note/. In this work we studied the morphology of colonies and cells, the state of dissociation of cultures using the method of Wright and Wilson (1951), and the agglutinability of the cultures with various sera (standard brucellosis agglutinating serum, Drozhevkina's so-called Vi-serum, and sera obtained by us for inagglutinable Brucella cultures); we also studied the populations of the cultures and determined the virulence of the strains by infecting guinea pigs with various doses of Brucella organisms (10^1, 10^2, 10^3, 10^4, and 10^5 microbial cells) and by studying the cultures isolated from various organs of the animals. Altogether we studied 10 strains of Br. melitensis, of which 7 were inagglutinable strains which were isolated from the blood of patients (various times of isolation), one was an agglutinable culture isolated from a hare, and the others were test culture No 565 and reference strain 16-M. The last three cultures were used for control purposes.

/Note/: Some reports contain the results of study of the pathomorphological changes in the organs of guinea pigs infected with these cultures (Grekova) and of study of the chemical composition of antigenic complexes (Dubrovskaya).

The general characteristics of the cultures are given in Table 1.

The cultures which were studied did not differ from the control cultures with respect to the morphology of the cells and tinctural aspects. With respect to their reducing capacity all the inagglutinable cultures behaved like typical Br. melitensis strains. A characteristic of these cultures was the fact that they were not agglutinated by standard brucellosis serum but gave a positive test with tripaflavine and a thermal precipitation reaction. Thus, with respect to their properties they were all completely identical to the cultures described by

Drozhevkina as being V-cultures and they differed in this respect from the control cultures. The latter were agglutinated to a titer by standard brucellosis serum but were not agglutinated in tripaflavine, i.e., they were typical undissociated Brucella cultures.

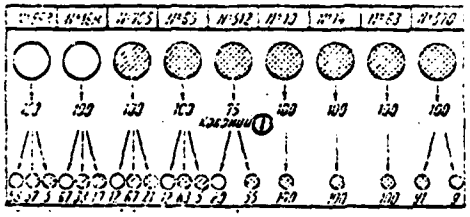
In studying the virulence a large difference was also found in the biological characteristics of agglutinable and inagglutinable Brucella cultures. Thus, control strains (No 565, 16-M, 998) upon being administered to guinea pigs in amounts of 10 and 100 microbial cells (2 and 20 lethal doses) caused generalized infection in all the animals with a high semination index. The virulence of all the inagglutinable cultures was considerably lower than that of the control cultures; doses of 10^1 and 10^2 microbial cells in most cases did not cause infection of the animals. With large doses it was possible to establish a different degree of weakening of the virulence for various inagglutinable cultures. Thus, relatively great virulence was found for strains No 705 and 65. The administration of these cultures to animals in doses of 10^3 microbial cells led to generalized infection with a high semination index. Other cultures for the same dose gave a small index of secretion (cultures No 512 and 14) or did not cause any infection at all (cultures No 10, 63, 570). Upon the administration of even large doses of the latter cultures (10^5, 10^6, 10^9 microbial cells) only very weak development of the infection was observed. For example, upon administering strain No 570 to the animals in an amount of 10^9 microbial cells only one culture was isolated from the inguinal lymph node of one of three infected guinea pigs.

We assumed that the different degree of lowering of the virulence in the strains which were studied depended on the quantitative relation of the agglutinable and inagglutinable species which comprised the population of the cultures. In order to confirm this assumption all cultures were placed in a dish with agar and from the secretion of each culture 100 colonies were isolated. The agglutinability and the presence of dissociation were determined for the cultures of isolated colonies; some colonies of each culture were studied in greater detail; their virulence, agglutinogenic properties, etc. were determined. The population of most cultures of not only the inagglutinable but also of the control types were heterogeneous and consisted of undissociated agglutinable species and others of varying dissociation (Figure 1).

Table 1

General characteristics of the cultures which were studied

Number of the culture	Conditions, time, and place of the isolation of the culture	Characteristics of the population according to the strain of the colony	Dissociated in testing with trypaflavine	Reducing ability	Agglutination with sera: Standard serum	Agglutination with sera: V-sera with respect to the dissociated cultures	Infectivity by a phage	Index of infectivity (in %) in infection with various doses (for each dose for three guinea pigs): 10^1	10^2	10^3	10^4	10^5	10^6	10^7
565	Standard	Heterogeneous	–	Lowered	+	–	–	80	80	86				
16-M	Reference	30% dissociated	–	"	+	–	+	11.1	66.6	61.1	66.6	70.3		
998	From a hare (1958, Kazakhstan)	40% dissociated	–	"	+	–	+	56	66	66.6	70.4	80		
705	From the fetus of a sheep (1955, Rostov-on-the-Don)	80% dissociated	++++	"	Lowered (1:200++)	+	+	0	0	48	77.7	55.5	66.6	
512	Hemoculture (1952, Rostov-on-the-Don)	50% dissociated	++++	"	–	+	+	3.7	22.2	22.2	22.2	26		
65	Hemoculture (1959, Central Asia)	70% dissociated	++++	"	–	+	+	0	29.6	85	70.3	85		
10		100% dissociated	++++	"	–	+	+	0	0	0	0	14.8		
14		Typical	++++	"	–	+	+	0	0	7.4	14.8	7.1		
63		" "	++++	"	–	+	+	0	0	0	3.7	7.4		
570	Hemoculture (1952, Stavropol')	" "	+++	"	–	+	+	0	0	0	7.4	3.6	11.1	3.6



(1) Colonies

O undissociated, agglutinable
⊜ dissociated, inagglutinable in standard serum
⊕ dissociated, agglutinable
⊖ dissociated and inagglutinable in standard serum with respect to dissociated cultures

Figure 1. Characteristics of the population of cultures upon cultering in a dish with agar.

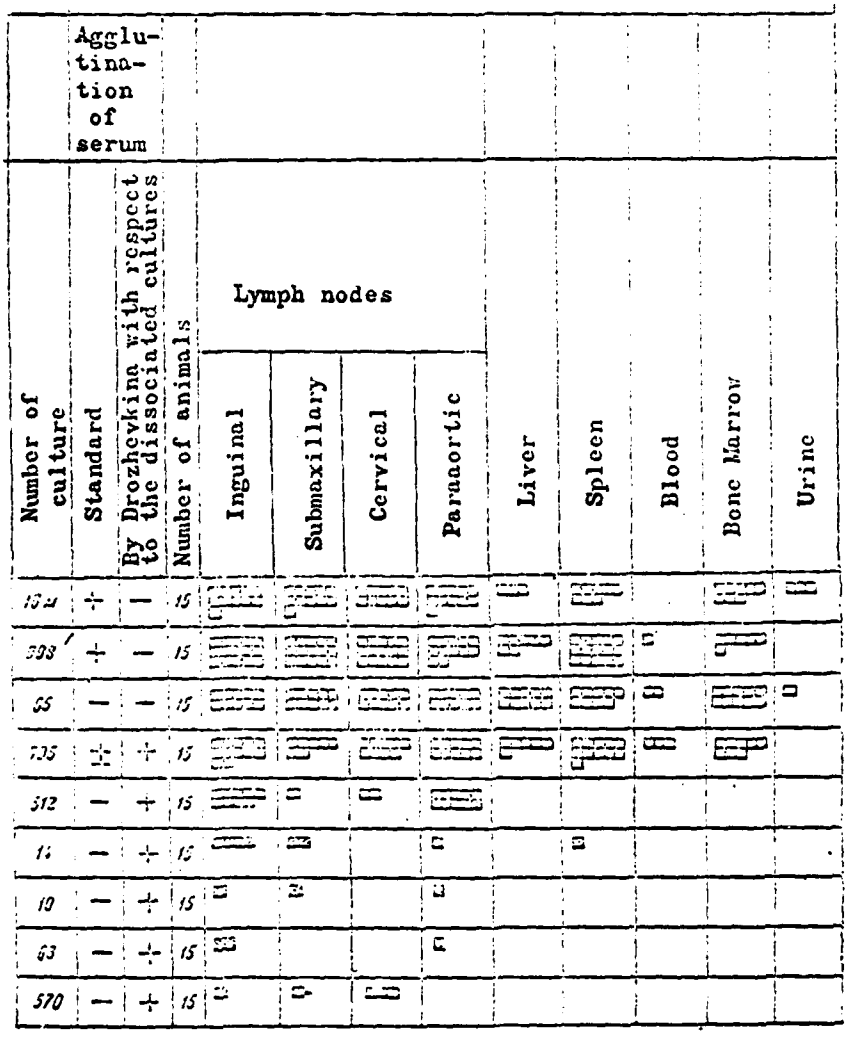
It was established that the quantity of agglutinable undissociated species in different cultures varied. Thus, in a population of undissociated cultures (No 16-M and 998) there was a predominance of undissociated cells; in cultures which were inagglutinable but with greater residual virulence (No 512, 705, 65) it was noted that there was only a small amount of undissociated species; cultures with very low virulence (No 63, 14, 10) had a homogeneous population consisting only of inagglutinable species with positive tests for dissociation. Thus, the study of inagglutinable Brucella cultures showed that their virulence is based on and is determined by the quantitative relation of the normal cells (agglutinable, undissociated) which enter into the composition of the population.

As a result of the study of the population of cultures it was also found that along with the inagglutinable dissociated species the population includes agglutinable

cells which give positive tests for dissociation. It is possible that both they and the others were in various degrees of dissociation.

Then we studied the development of infection in animals upon administering inagglutinable brucella strains to them, especially strains of a heterogeneous population; we also studied the nature of cultures isolated from various organs of the animals. The results of these investigations showed that in most cases agglutinable and undissociated cultures were isolated. This circumstance showed that if the infecting is done with a culture wherein the population contains both inagglutinable dissociated species and even a small percentage of normal cells, the development of the infection will be based on the multiplication of these very cells (Figure 2). Thus, in our tests wherein we infected guinea pigs with inagglutinable cultures No 65, 705, and 512 in which the population was heterogeneous, in most cases agglutinable undissociated cultures were isolated from the organs of the animals (157 of 159 cultures which were isolated). With two cultures a positive tripaflavine test was obtained.. In no case was the presence of agglutinable cultures found in the organs of this group of animals. At the same time, upon infecting guinea pigs with inagglutinable brucella cultures wherein the population consisted wholely of dissociated inagglutinable species, the small number of cultures secreted from the organs of the animals was identical with that of the initial strain. A consequence of the weak settling of inagglutinable cultures in the organism of the animals and, as the results of a pathomorphological investigation (Grekova) showed, of the slight stimulation of the reticuloendthelial system was their rather weakly expressed serological reactions.

This applied in particular to animals which were infected with inagglutinable cultures having a homogeneous population (No 63, 10, 14). In this a relation was observed between the level of the titer of the antibodies and the antigens which were taken into the reaction (the ordinary Wright's antigen or culture corresponded to the culture with which the infection was derived). In all cases with Wright's antigen a positive agglutination reaction was observed with a considerably lesser dilution of the serum than with the corresponding culture (with Wright's antigen -- 1:10 to 1:40, with the culture -- 1:80 to 1:120).



1 square -- culture isolated from the indicated
□ -- culture not dissociated, agglutinable
▨ -- culture dissociated, agglutinable
▩ -- culture dissociated, inagglutionable

Figure 2. Distribution of cultures with respect to the organs of animals infected with various strains of Brucella melitensis

For guinea pigs infected with agglutinable cultures, Wright's reaction, as a rule, reached much higher titers and did not depend on the antigen taken into the reaction.

This circumstance indicates some difference in the antigenic structure of agglutinable and inagglutinable Brucella cultures and consequently points to a qualitative difference in the antibodies which have been formed.

Thus, the results which were obtained indicate that inagglutinable strains are dissociated Brucella cultures with weakened virulence and changed antigenic structure.

In order to obtain fuller proof and support for this evaluation of inagglutinable cultures a study was made along the same line to examine agglutinable and inagglutinable cultures obtained from individual colonies of each strain of a heterogeneous population. With respect to their morphological and cultural properties these cultures were typical Brucella organisms while at the same time they differed sharply from each other with respect to signs of dissociation, virulence, and antigenicity. Table 2 contains their characteristics which give evidence of the difference in degree of virulence of agglutinable and inagglutinable cultures obtained from various colonies of the same initial strains. All the agglutinable cultures (the 11th colony of strain No 512, the 4th colony of strain No 705, etc.) set a much higher secretion index for all doses of infection than was the case with inagglutinable colonies of these strains (the 22nd colony of strain 512, the 1st colony of strain 705, the 2nd colony of strain No 65, etc.).

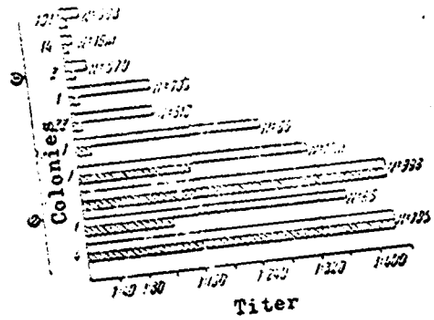
The titers of Wright's reaction for guinea pigs infected with inagglutinable cultures from separate colonies were very low and, like the initial strains depended on the antigens which were taken into the reaction -- Wright's antigen or the infecting culture (Figure 3).

In order to determine the antigenic capacity of the strains which were studied we conducted tests involving the immunization of rabbits. The results which were obtained showed the considerably lesser antigenic capacity of inagglutinable Brucella cultures in comparison with agglutinable cultures.

Table 2

Virulence of agglutionable and inagglutinable cultures obtained from separate colonies

Number of Culture	Colory	Dissociation	Agglutination		Index of secretion (in %)[1] upon infection with various doses (in microbial cells)						
			Standard serum	V-serum and with respect to in-agglutinable cultures	10^1	10^2	10^3	10^1	10^5	10^4	10^9
512	22-Я	Positive	Negative	Positive	0	0	3.7	3.7	11.1		
705	1-Я	"	"	"	7.4	3.7	3.7	14.8	7.4	7.4	
705	50-Я	"	"	"	0	0	0	0	0		11.1
570	2-Я	"	"	"	0	0	0	5.5	5.5	3.7	
65	2-Я	"	"	"	0	0	3.7	7.4	14.8		
16-M	14-Я	"	"	"	0	0	3.7	3.7	7.4		
998	101-Я	"	"	"	0	0	0	0	11.1		
512	11-Я	Negative	Positive	Negative	18.5	29	7.4	22.2	31.1		
705	4-Я	"	"	"	70.3	74	70.3	80	66.6	80	
65	1-Я	"	"	"	48.1	78	78.2	77.7	85		



— Wright's reaction with Wright's antigen
— Wright's reaction with the culture taken for infection
— Inagglutinable cultures
— Agglutinable cultures

Figure 3. Wright's reaction in guinea pigs infected with agglutinable and inagglutinable Brucella cultures obtained from separate colonies of strains which were studied (infecting dose of 10^5 microbial cells).

Using Castellani's test for the absorption of antibodies, it was also found that there is some difference in their antigenic structure.

The inagglutinable Brucella cultures which we studied which were similar in their properties to Drozhevkina's V-cultures can, on the basis of the results which we obtained, be considered only as dissociated cultures. All the strains had sharply weakened virulence; they were all weakly antigenic and caused slight stimulation of the reticuloendothelial system in the organism of guinea pigs even in the case of infection with large doses.

Conclusions

1. The inagglutinable cultures of Br. melitensis which we studied were typical with respect to their morphological and cultural aspects and also with respect to their ability to reduce aniline dyes. A distinctive feature was the presence of positive signs of dissociation

and the loss of the ability to be agglutinated by a specific antibrucellosis serum. With respect to these aspects they were identical to the cultures described by Drozhevkina as V-cultures.

2. The inagglutinable Brucella cultures had lowered virulence in various degrees (in comparison with the agglutinable cultures). The somewhat large residual virulence of some of the inagglutinable strains (No 512, 705, 65) could be explained by the presence in the population of a small percentage of agglutinable undissociated species, the predominant settlement of which in the organism of the guinea pigs was the basis for the development of infection.

3. In contrast to Drozhevkina's opinion, we consider inagglutinable Brucella cultures only as being at a certain stage of dissociation with lowered virulence, a changed antigenic structure, and weakly expressed antigenic capacity.

4. For the identification of such freshly isolated Brucella cultures it is expedient to use the corresponding agglutinating serum.

BIBLIOGRAPHY

1. Antonov, V.K., In the book: Sbornik rabot po brutsellezu (Collection of Works on Brucellosis), Alma-Ata, 1958, No 6, page 3.

2. Ibid., page 14.

3. Drozhevkina, M.S., Zh. mikrobiol. (Journal of Microbiology), 1954, No 5, page 24.

4. Ibid., Trudy Rostovsk.-na-Donu nauchno-issled. protivochumnogo in-ta (Works of the Rostov-on-the-Don Scientific Research Anti-Plague Institute), 1956, Vol 10, page 399.

5. Ibid., page 354.

6. Renoux, G., Mahaffey, L.W., Ann. Inst. Pasteur, 1955, v. 88, page 528.

7. White, P.G., Wilson, J.B., J. Bact., 1951, v. 61, page 239.

8. Wolff, H.I., Dinger, J.E., J. Path. Bact., 1951, v. 63, page 163.

5570
CSO: 10057-D