STUDIES ON BACTERIAL VARIATION AND SELECTIVE ENVIRONMENTS

II. The Effects of Sera from Brucella-infected Animals and from Normal Animals of Different Species upon the Variation of Brucella abortus

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In previous reports (Braun, 1946, 1948) it has been shown that the addition of small amounts of normal serum, or certain of its globulin fractions, to buffered broth suppresses the establishment of nonsmooth variants (mutants) in growing smooth populations of *Brucella abortus*. The sera, or their fractions, which were used in these studies had been obtained from normal cows, guinea pigs, rabbits, or humans, i.e., from susceptible donors that had never been exposed to *Brucella*. However, when the serum was obtained from donors that had been infected with a virulent *Brucella abortus* culture, or had been vaccinated with strain 19 of *Brucella abortus*, results were obtained which differed from those previously presented for normal sera.

It was also found that normal sera from species with relative insusceptibility to *Brucella* infections failed to produce the selective effect previously described for normal sera from *Brucella*-susceptible species. These observations, with sera from infected animals and with normal sera from different species, will be reported in this paper.

EXPERIMENTAL DATA

As in previous studies on the *in vitro* effect of sera, varying amounts of Seitz-filtered serum were added to 5 ml of buffered beef extract broth cultures, usually in concentrations from 2 to 20 per cent; the most significant data were obtained in cultures containing 5 to 10 per cent serum. In the experiments with sera from infected animals, these cultures were inoculated with either a smooth clone isolated from strain 19, yielding approximately 30 per cent nonsmooth types after 10 days of growth in cultures without serum (dissociation index = 30 per cent) or a virulent S culture with a dissociation index of 22 per cent. In the experiments with sera from different species, only the smooth clone isolated from strain 19 was used. All data are based on at least triplicate tests with each concentration of the various serum samples.

The effect of sera from Brucella-infected animals. Sera from 42 cows were tested. Nine of these cows had never been exposed to a virulent strain of *Brucella*

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abortus and had not been vaccinated with strain 19 (a strain of low virulence which is commonly used for vaccination). The addition of serum from any of these normal animals to buffered broth cultures suppressed the establishment of nonsmooth variants during the 10-day growth period of originally smooth populations, both in cultures started with 19S or virulent S (see examples in table 1). However, the addition of serum from any of 20 animals that had been vaccinated

TABLE 1

Examples of the effect of sera from normal, infected, and vaccinated cows upon the establishment of variant types in vitro

STATUS OF COW	AMOUNT OF SERUM ADDED TO 5 ML OF BROTH	DISSOCIATION INDEX (%) OF CULTURES INOCULATED WITH		REMARKS
		S 2583 (From strain 19)	S 6232 (Virulent)	
Normal.....	0.5	0	0	Nonvaccinated and noninfected
Normal.....	0.5	0	0	Nonvaccinated and noninfected
Vaccinated....	0.5	42 R* & M*	98 I*	Vaccinated 17 months before testing
Vaccinated....	0.5	24 R* & M*	70 I*	Vaccinated 24 months before testing
Vaccinated....	0.5	0	0	Vaccinated 37 months before testing
Infected.....	0.5	0	25 R & M	First positive culture obtained 15 months before testing
Infected.....	0.5	0	15 R & M	First positive culture obtained 19 months before testing
Infected and vaccinated...	0.5	21 R & M	36 I, R, & M	Positive culture 12 months, vaccinated 11 months before testing
Infected and vaccinated...	0.5	26 R & M	39 I, R, & M	Positive culture 17 months, last vaccinated 12 months before testing
Controls.....	None	32 R & M	22 R & M	

* Predominant types of variants found.

within 28 months prior to bleeding failed to suppress the establishment of nonsmooth types (table 1). In cultures originally inoculated with 19S, varying percentages of rough and mucoid types were observed after 10 days of growth; in cultures inoculated with the virulent S a high percentage of intermediate types were found. (These intermediate types proved to be avirulent for guinea pigs.) Such results were obtained both with sera containing detectable agglutinins and with sera that had ceased to produce agglutination reactions. Serum obtained from six animals that had been vaccinated 37 months or more prior to

bleeding behaved like normal serum, i.e., suppressed the establishment of nonsmooth types. Serum obtained from six animals that had been naturally infected but had never been vaccinated[2] produced different results with the two test cultures; when added to cultures of virulent S these sera failed to suppress the establishment of nonsmooth variants, but when added to cultures of 19S no nonsmooth types established themselves, i.e., "infected" serum had the same effect as "normal" serum upon organisms of strain 19 (table 1). Serum samples obtained from seven animals that had been vaccinated with strain 19 and had also been naturally infected failed to suppress the establishment of nonsmooth variants in originally virulent S or avirulent (strain 19) S cultures; however, the types found in virulent cultures after the addition of "infected plus vaccinated" serum contained a large number of intermediate types in addition to R and M, whereas R and M types only were observed after the addition of "infected" serum (table 1).

These effects produced by sera from exposed cows were confirmed with a limited number of human sera, including samples from persons having had contact with strain 19 or virulent cultures (but showing no clinical signs of brucellosis), and with a number of sera from infected guinea pigs and rabbits. The results were identical with those from the examples compiled in table 1, and the general effect of serum from normal donors and donors exposed to strain 19, virulent cultures, or a combination thereof, upon the variation of *Brucella* can now be summarized as shown in table 2. The potential value of these results for diagnostic procedures is evident, especially since the strikingly different effects produced by sera from infected and vaccinated donors is independent of the presence of agglutination titers, as witnessed by the results obtained with sera from vaccinated animals that had ceased to show detectable agglutinins.

The lack of any correlation between the presence of agglutinins and the change in selective serum factors after exposure has been substantiated by observations on serum samples obtained periodically after inoculation of strain 19S into cows and rabbits. It was found that sera from these inoculated animals maintain their selective effect until approximately 4 weeks after the appearance of agglutinins, i.e., sera obtained during the early postexposure period suppress the establishment of nonsmooth types in originally smooth broth cultures exactly as has been described for sera from normal, nonexposed animals. Serum obtained more than 4 weeks after the appearance of agglutinins, however, will permit the establishment of nonsmooth types, as shown in tables 1 and 2; and, as already mentioned, this altered selective effect can be observed for at least 2 years after the disappearance of agglutinins.

It should be added that the percentage of nonsmooth types which establish themselves in cultures to which "exposed" serum has been added can actually be higher than the percentage of nonsmooth types found in control cultures without serum inoculated with the same S clone. This has been observed especially

[2] Blood cultures from these animals had been positive approximately 10 months prior to bleeding; no attempts were made to culture blood samples at the time blood was obtained for the studies here reported.

when serum obtained from animals within the early months after exposure was used. However, the data are insufficient to determine whether the percentage of nonsmooths establishing themselves within "exposed serum broth cultures" may serve as an indicator of the period that has elapsed since exposure. In any

TABLE 2

Summary of the effects of sera from donors exposed in different ways

SERUM DONOR'S STATUS	EFFECT OF ADDITION OF SERUM UPON THE ESTABLISHMENT OF VARIANT TYPES IN BROTH CULTURES OF	
	S 2583 (from 19)	S 6232 (virulent)
Normal	−*	−
Vaccinated (exposure to strain 19S)	+† R & M types	+ I types
Infected (exposure to virulent S)	−	+ R & M types
Infected and vaccinated	+ R & M types	+ I, R, & M types

* − = no nonsmooth types after 10 days of growth.
† + = nonsmooth types present after 10 days of growth.

TABLE 3

The effect of the addition of sera from various species upon the establishment of nonsmooth types ("dissociation") in broth cultures inoculated with a smooth clone

NORMAL SERA FROM THE FOLLOWING SPECIES	
Suppress dissociation *in vitro*	Fail to suppress dissociation *in vitro*
Man	Hamster
Cow	Rat
Rabbit	Mouse
Guinea pig	Chicken
Hog	Quail
Cat	Duck
	Pheasant

Example

TYPE OF SERUM ADDED	AMOUNT	D.I.(%)
Normal human	4%	0
Normal bovine	4%	0
Normal rat	4%	22
None	—	22

event, these last-mentioned observations indicate that not only is there a post-exposure disappearance of the selective serum effect, but that during certain periods after exposure, at least, the establishment of nonsmooth types may be enhanced.

The effect of sera from different species. Table 3 summarizes the results obtained from over 300 cultures. It appears unnecessary to go into detailed descriptions

of the individual observations. It can be seen that normal sera from 6 of the 13 species tested suppressed the establishment of nonsmooth types, whereas sera from the other 7 species failed to do so, i.e., in the presence of the latter sera the establishment of nonsmooth types proceeded exactly as in control cultures without serum.[3] It is obvious that there is a remarkable agreement between the known susceptibility to *Brucella* infections of all the species from which "dissociation-suppressing" serum is obtained and the known relative insusceptibility to *Brucella* infections of those species that lack the selective serum factor.

DISCUSSION

These results demonstrate that in *Brucella*-susceptible species the selective serum factor, suppressing the establishment of nonsmooth types *in vitro*, is temporarily inactive after infection, and that in species with relative insusceptibility to *Brucella* infections the selective serum factor is entirely lacking. This means that after infection or vaccination the serum activity of susceptible species becomes temporarily like that of nonsusceptible species.

If these observations *in vitro* reflected similar effects *in vivo*, one would be tempted to suggest a possible causal relationship between susceptibility and serum selectivity. Actually, it has been possible to demonstrate that the difference in selectivity *in vitro* of normal sera from different species is directly correlated with differences of selective effects *in vivo* of the same species (Braun and Hauge, 1948): nonsmooth types inoculated into mice (serum nonselective *in vitro*) can be recovered from the spleen after several weeks whereas the same nonsmooth types cannot be recovered after inoculation into guinea pigs or rabbits (whose normal serum suppresses the establishment of nonsmooth types *in vitro*). These observations thus support the suggestion that susceptibility is correlated with the presence of a normal serum factor suppressing the establishment of nonsmooth, usually avirulent types, whereas a lack of this factor will be associated with increased resistance.

One might speculate that after exposure to *Brucella* the establishment of the virulent smooth type would be initially forced in all those species listed on the left side of table 3 (susceptible species), whereas after the infection of, for example, a mouse or duck with any type (S, R, or M, etc.) the establishment of the avirulent, nonsmooth types would be favored in the absence of any selective factor. Furthermore, the disappearance of the selective suppression *in vitro* when serum from infected, susceptible animals is used suggests that after exposure *in vivo* selectivity may similarly change and permit the establishment of avirulent, nonsmooth variants. This would coincide with the known increased resistance to subsequent infections of infected or vaccinated animals and is supported by the reports on the isolation of variant types of various pathogens from carriers or convalescent hosts.

An additional instance of change in selective serum activity, presumably associated with changes in resistance, was observed during late pregnancy in a cow.

[3] In cultures containing 10 to 20 per cent of mouse serum the establishment of nonsmooth types was actually enhanced.

Serum from this animal, which had served as donor for normal serum for over a year, suddenly failed to suppress dissociation *in vitro*. This change occurred during the sixth month of pregnancy and was found to persist until calving, after which the former selective activity returned immediately. During a subsequent pregnancy of the same animal the same type of change in serum activity after the sixth month of gestation was observed. The clinical literature contains many references to increased resistance to various infections during late pregnancy (Perla and Marmorston, 1941), and it becomes a challenging question whether this altered resistance could be associated with changes in selective serum activity as observed in this one animal.

The mechanism responsible for this temporary loss of selective *in vitro* activity of serum from susceptible animals after infection is still obscure. After some early and preliminary observations on the effect of sera from infected animals (Braun, 1946), it was suggested that the suppression of S types due to the presence of S antibodies may be stronger than the suppression of rough and mucoid types by factors normally present in serum, and so-called dissociation may thereafter occur in the presence of antiserum. However, the more recent observations on the lack of correlation between the presence of agglutinins and alteration of the serum effect make such an explanation unlikely. Some other mechanism must be looked for. In this connection it may be of interest to mention some preliminary observations on absorption experiments which indicate the complexity of changes occurring after infection. It was observed that the selective activity of normal serum from *Brucella*-susceptible species cannot be absorbed by exposure of the serum to R and M types, the types that are suppressed by normal sera. However, if serum is obtained from such animals during that brief period after infection when the serum still retains its selective effect in the presence of agglutinins, the non-S-suppressing activity can be absorbed by exposure to R and M for 3 hours at 37 C.

In conclusion it may be pointed out that the different results obtained when sera from *Brucella*-susceptible animals exposed to strain 19S or a virulent S were used in cultures inoculated with a 19S clone or a virulent S clone not only provide an effective tool for differential diagnosis, but appear to indicate a lack of close relationship between strain 19 of *Brucella abortus* and a virulent culture of the same species.

SUMMARY

The previously described selective activity of normal sera from *Brucella*-susceptible species suppressing the establishment of nonsmooth *Brucella abortus* types in smooth broth cultures was found to be lacking in sera from infected animals of these susceptible species, and was also found to be absent in sera from normal animals of nonsusceptible species.

The change in activity of sera from infected animals belonging to susceptible species occurred approximately 4 weeks after agglutinins were first detected and persisted for at least 2 years after their disappearance.

Different effects were produced by sera from infected or vaccinated animals

upon the variation of a virulent culture or a strain with low virulence (strain 19), respectively. This suggests that the effect of an animal's serum upon *in vitro* variation may be utilized for diagnostic tests, indicating the status of an animal in regard to *Brucella* infection or past vaccination independently of agglutination titers.

The possibility of a causal relationship between serum selectivity and susceptibility has been discussed.

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