a predisposing factor in humans who develop SLE and antibodies to nucleoproteins (*5*). Our preliminary data indicate that there may be similar sex differences in the occurrence of this autoantibody in A/J mice. We have not found histological changes in these mice resembling those of SLE in humans, but these studies are still incomplete.

Further serological studies have not yet revealed serum γ-globulin that will combine with heterologous DNA such as that found in patients who are acutely ill with systemic lupus erythematosus (*4*).

We have described here the occurrence of a spontaneous autoimmune-like process in A/J mice. The origin of this phenomenon is unknown. Since the A/J mouse is a widely known, commercially produced strain, not noted for any tendency toward short survival, it is evident that early mortality from a lupus-like disease does not occur in a large proportion of the population. If multiple genes are involved in susceptibility to early development of clinically manifest disease, it is possible that lethal genes have been removed from the population by selection of healthy breeding stock. This impression is supported by our preliminary histopathologic studies. It may be possible that further immunologic stimulation with appropriate antigenic material, or hybridization with the NZB strain of Bielschowsky (*6*) or other mouse strains may produce a more fully manifest disease (*7*).

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7. We have recently found antinuclear γ-globulin in serum from some 6- to 12-month-old mice of the C57BL/6J strain. Our studies do not completely exclude the occurrence of small amounts of antibody to nucleoprotein in individual mice of the negative strains listed in this report, since only pooled serum has so far been tested.
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Brucella-Agglutinating Antibodies: Relation of Mercaptoethanol Stability to Complement Fixation

Abstract. Brucella-*agglutinating antibodies from selected bovine blood serums and milk samples did not fix complement, and after treatment with 2-mercaptoethanol they lost agglutinating power. After infection of calves with* Brucella abortus, *strain 19, agglutinins for* Brucella *that were inactivated by mercaptoethanol appeared earlier than those stable to mercaptoethanol. Under the conditions of these experiments, the appearance and development of complement-fixing capacity coincided closely with the mercaptoethanol stability of the agglutinins for* Brucella.

Rapidly sedimenting 12*S* to 19*S* antibodies which agglutinate *Brucella abortus* antigen have been reported in the serum (*1*) and milk (*2*) of certain individual cattle. These have been differentiated from the common 7*S* agglutinins on the basis of ultracentrifugation, isoelectric point, heat lability, and loss of activity upon treatment with 2-mercaptoethanol (ME).

After parenteral injection of cattle with living *Brucella abortus*, strain 19, a class of agglutinating antibodies that is inactivated by mercaptoethanol (ME-susceptible) and by heat has been observed to appear earlier and persist longer than a class that is resistant to these treatments (*3*). Complement-fixing antibodies for *Brucella* have been reported to appear later and recede earlier than agglutinating antibodies after inoculation of cattle with *Brucella abortus,* strain 19, vaccine (*4*).

This report describes experiments concerning the relation between complement fixation and the susceptibility of *Brucella*-agglutinating antibodies in bovine serum and milk to treatment with mercaptoethanol.

Bovine serums were tested by the USDA 48-hour standard tube agglutination test (*5*) accompanied by a duplicate tube test containing 0.05*M* mercaptoethanol in each serum-antigen dilution from 1:25 to at least one tube dilution greater than the end titer of the tube agglutination test. Bovine milk samples, with and without prior treatment with 0.1*M* mercaptoethanol for 18 hours, were tested by the USDA serial dilution *Brucella* ring test (*6*).

In the complement-fixation test, 0.2-ml portions of appropriately standardized reagents were used. The fixation period was 60 minutes at 37°C. Three units of complement were used in the test proper, but to avoid possible compounding of anticomplementary effects, one-half amounts were used in both serum and antigen controls. The procedure was further modified by adding 0.01 ml of fresh normal bovine serum to each test and to the antigen control (*7*).

For the first experiment antibody preparations were isolated from bovine serums and wheys in which *Brucella* agglutinins were susceptible to inactivation by mercaptoethanol and also by heat (*3*). The preparations were isolated by adsorption on *Brucella* antigen followed by elution with water acidified to *p*H 3.5 with acetic acid and, after further purification (*2*), added to serums and milk without antibodies to *Brucella* to obtain adjusted titers of 1:100 for the two tests. In contrast, these preparations had complement-fixation titers of less than 1:4. Exposure of the adjusted preparations to mercaptoethanol decreased their serum agglutination test and milk ring test titers to less than 1:25.

In the second experiment, *Brucella*-agglutinating serums from five cows and milk from three cows were examined with the results presented in Table 1. Agglutination titers varying from 1:3200 to 1:50 were observed for these serums and milk samples. Similar titers were observed for serums 1 and 2 and milk 8 by the complement-fixation test and also after exposure to mercaptoethanol in the tube agglutination test. In contrast, for serums 3, 4, and 5 and milk samples 6 and 7, reactions at 1:4 and greater were not observed with the complement-fixation test and, after exposure to mercaptoethanol, agglutination titers decreased to less than 1:25 for the tube agglutination and ring tests.

In the third experiment a 5-month-old calf was injected subcutaneously with vaccine containing approximately 80×10^8 living organisms of *Brucella abortus*, strain 19. Serum, obtained from blood collected at intervals as indicated in Table 2, was examined by the tube agglutination test, with and without exposure to mercaptoethanol, and also by the complement-fixation test. *Brucella*-agglutinating antibodies were first observed with the tube agglutination test in serum collected on day 3 after injection, at a dilution of 1:25 or greater. Antibodies with agglutination activity in the presence of

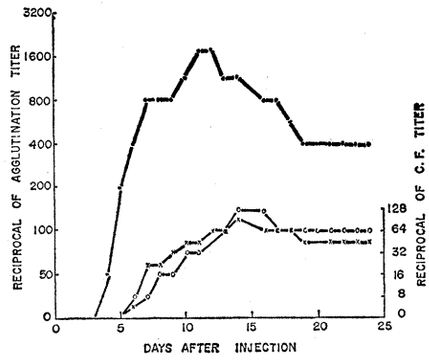

Fig. 1. Arithmetic mean of agglutination and complement-fixation results with serums of four calves following injection with viable cells of *Brucella abortus*, strain 19. Closed circles, tube agglutination without mercaptoethanol treatment; open circles, tube agglutination with mercaptoethanol treatment; *X*, complement fixation.

Table 1. Comparison of serologic tests of selected serums and milk samples for *Brucella* antibodies, with and without mercaptoethanol treatment. The serum and milk samples were selected on the basis of resistance or susceptibility of the *Brucella* antibodies to treatment with 0.05*M* 2-mercaptoethanol. STT, standard tube agglutination test; BRT, *Brucella* ring test; CF, complement-fixation test; (a), not treated with mercaptoethanol; (b), treated with mercaptoethanol. Titers are expressed as reciprocals.

Specimen	Titers				
	STT		BRT		CF
	(a)	(b)	(a)	(b)	(a)
		Serum			
1	3200	3200			2048
2	50	50			32
3	200	<25			<4
4	200	<25			<4
5	100	<25			<4
		Milk			
6			200	<25	<4
7			400	<25	<4
8			1600	800	256

Table 2. Comparison of complement-fixation and tube agglutination tests of serums collected at intervals from a calf injected with viable *Brucella abortus*, strain 19, vaccine. The titers are expressed as the reciprocals of the complement-fixation or agglutination titers. STT, standard tube agglutination test; CF, complement-fixation test; (a) not treated with mercaptoethanol; (b) treated with mercaptoethanol.

Days after injection	Titers		
	STT		C.F.
	(a)	(b)	(a)
0	<25	<25	<4
1	NC*	NC*	NC*
2	NC*	NC*	NC*
3	37	<25	<4
4	200	<25	<4
5	800	50	4
6	1600	75	12
7	2400	100	48
8	3200	100	48
9	3200	100	48
11	3200	100	96
21	2400	100	48

* NC = Serum not collected.

mercaptoethanol were first detectable in serum on the 5th day after infection, and this coincided with the first detectable appearance of complement-fixing antibodies at a dilution of serum of 1:4 or greater on day 5. Agglutination titers of the serum treated with mercaptoethanol were comparable to those of the complement-fixation test, while the serum titers obtained with the tube agglutination test in the absence of this compound rose to a high of 1:3200 by the 8th day after infection, indicating that a large proportion of the antibody was inactivated by mercaptoethanol. These data are generally comparable to differences observed between the tube agglutination test and the heat inactivation test after vaccination of calves with vaccine prepared from strain 19 (*3, 4*).

The exeperiment with *Br. abortus*, strain 19, was repeated with four additional calves. Arithmetic means of the results, presented in Fig. 1, indicate a pattern very similar to that exhibited by the single calf (Table 2). Correlation of the appearance, development, and persistence of antibodies which fix complement and are resistant to mercaptoethanol suggests that this and other differences in physicochemical properties offers an explanation for the generally observed appearance of *Brucella*-agglutinating reactions prior to complement-fixing reactions (*4*).

The data are consistent in indicating that ME-susceptible *Brucella*-agglutinating antibodies occuring in bovine serum and milk do not fix complement. In this respect they differ from the ME-resistant agglutinins examined. Preliminary data indicate that these bovine ME-susceptible antibodies are macroglobulin types sedimenting more rapidly than the 7*S* type, ME-resistant antibodies (*1, 2*). They appear to resemble certain rapidly sedimenting antibodies which have been reported in serums of humans (*8*), guinea pigs (*9*), rabbits (*10*), and chickens (*11*) in that they appear early after infection and are inactivated by mercaptoethanol. Their failure to fix complement in the agglutination reactions implies that they may differ in composition or configuration from the ME-stable and complement-fixing antibodies for *Brucella*.

The difference might be due to the absence in the ME-susceptible antibodies of a fragment essential for complement fixation. Thus Heremans *et al.* (*12*) suggested that the failure of γ_{1A}-agglutinins for *Brucella* to fix complement may be due to the lack of fragment III which is present in γ_{ss}- (7*S*) globulins. However, it is not known which fragment of the 7*S* globulins is actually responsible for the fixation of complement (*13, 14*).

Heremans *et al.* (*15*) have recently reported that of three immunoelectrophoretically distinguishable agglutinins for *Brucella* isolated from human serum, the γ_{ss}- (7*S*γ) fixed complement intensely, the γ_{1m}- (19*S*γ) moderately, and the γ_{1A}- not at all. It will be interesting to ascertain if the agglutinins to *Brucella* of bovine serum and milk which do not fix complement can be similarly classified.

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