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On the Characteristics of *Anti-Br. abortus* Strain 19 Agents in Albumin and Globulin Fractions of Bovine Serum

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Summary

There are two agents in bovine serum which are bactericidal for *Brucella abortus* strain 19 but not for strain 544. One is contained in an albumin fraction of the serum and another is in a globulin fraction. They act cooperatively with each other on strain 19 under the existence of Mg^{2+} but neither of them acts on the cells by oneself.

In this paper, some characteristics of the agents in the two fractions were studied. From the results, it became clear that the agent in the globulin fraction was resistant to heat-treatment at 56°C, inactivated by acid-treatment at pH 3 to 4 and specifically removed by absorption with *Br. abortus*, while another agent in the albumin fraction was heat-labile, acid-stable and not removable by the absorption and that the anti-strain 19 activity of the mixture of these agents was temperature-dependent and the optimal temperature was about 37°C. These facts show that the former agent seems to be a kind of antibody and the other seems to be some components of complement.

In our preliminary report (1), it was shown that bovine sera were bactericidal for avirulent *Br. abortus*, strain 19, but not for virulent one, strain 544, and that this anti-strain 19 activity disappeared by heat- or acid-treatment and by absorption with brucella organisms and was temperature-dependent. Next, it was shown that a permeable factor and a non-permeable factor in the serum were necessary to the bactericidal action on strain 19 and that the former factor was Mg^{2+} (2). It was also shown that the later factor was divided into two fractions, one of which was a globulin fraction which was salted out at between 0 and 33 per cent saturation of ammonium sulfate and the other was an albumin fraction which was obtained at between 50 and 100 per cent saturation. It was also observed that neither of them acted alone on strain 19 but that they cooperated with each other to affect the organisms (3).

In this paper, some characteristics of the anti-strain 19 agents in the two fractions were observed.

Materials and Methods

Bacterial Cells

For detection of bactericidal activity and absorption test, brucella organisms were prepared according to the method described in the previous paper (1, 3). Cells of *Salmonella abortus equi* (strain Shurei) were prepared in the same way from cultures on Trypticase Soy agar (BBL) plate at 37°C for 18 hours.

Fractionation of Serum

Globulin and albumin fractions of bovine serum were obtained by the method described in the previous paper (3). The protein concentration of these fractions was adjusted to 10 mg per ml with Mg-Tris buffer.

Estimation of Anti-strain 19 Activity

Each 0.2 ml of the two fractions, treated or untreated according to the purpose of experiments, were mixed and 0.1 ml of the bacterial suspension containing 10^2 viable cells of *Br. abortus* strain 19 were added. After incubation for 3 hours at 37°C, the bactericidal rate which represents the anti-st. 19 activity was determined.

Absorption Test

The absorption of anti-st. 19 agents with brucella organisms was carried out according to the method described in the previous paper (1). The method was correspondingly applied for the absorption with salmonella organisms.

Results

Heat-stability of Globulin and Albumin Fractions

As shown in Table 1, the bactericidal rates for *Br. abortus* st. 19 of the mixtures, which consisted of the gloublin fraction heated at 56°C for varying times from 5 to 30 minutes and the intact albumin fraction, were more than about 80 per cent of the value of the control. From the result, it is known that the anti-st. 19 agent in the globulin fraction is relatively heat-stable. On the contrary, remarkable loss of the anti-st. 19 activity of the mixtures, consisted of the intact globulin fraction and the heat-treated albumin fraction, occured within 10 minutes of the heat-treatment so that the albumin fraction is extreamly heat-labile.

Acid-stability of Globulin and Albumin Fractions

The globulin fraction was adjusted to pH 3 or 4 with 0.1 N HCl and kept for an hour at room temperature. It was then readjusted to pH 7.2 with 0.1 N NaOH. These acid-treated fractions were mixed with the untreated albumin fraction to examine their anti-st. 19 activity. On the other hand, mixtures consisting of the untreated globulin fraction and the acid-treated albumin fraction were treated in

TABLE 1. *Effects of Heat-treatment on the Anti-st. 19 Activity of Globulin and Albumin Fractions*

Fraction		Period of treatment (56°C)	Anti-st. 19 activity[2]	Relative activity
Untreated	Treated[1]			
Albumin	Globulin	5 min	80%	93
		10	78	91
		20	73	85
		30	68	79
Globulin	Albumin	5	36	42
		10	14	16
		20	0	0
		30	0	0
Control[3]			86	100

1) Globulin or albumin fraction was previously treated by heat at 56°C for varying periods, then untreated fraction was added.
2) Bactericidal rate for *Br. abortus* st. 19.
3) Untreated globulin and albumin fractions were used.

TABLE 2. *Effects of Acid-treatment on the Anti-st. 19 Activity of Globulin and Albumin Fractions*

Fraction		pH of Treatment	Anti-st. 19 activity[2]	Relative activity
Untreated	Treated[1]			
Albumin	Globulin	4.0	11%	12
		3.0	0	0
Globulin	Albumin	4.0	92	101
		3.0	83	91
Control[3]		7.2	91	100

1) Globulin or albumin fraction was previously kept at corresponding pH for an hour and adjusted to pH 7.2, then untreated fraction was added.
2) Bactericidal rate for *Br. abortus* st. 19.
3) Untreated globulin and albumin fractions were used.

the same way to test for their activity.

The results are shown in Table 2, in which it is shown that the former mixtures lost their anti-st. 19 activity but the latter were not affected. So, the globulin fraction is unstable in acid and the albumin fraction is acid-resistant.

Optimal Temperature of Anti-strain 19 Activity

Effects of incubation temperatures on the anti-st. 19 activity of the mixture consisting of the intact globulin and albumin fractions was examined for 4°C to 37°C. As shown in Table 3, the activity increased with the rise of temperature. The highest value was given at 37°C. Therefore the anti-st. 19 activity is temperature-dependent and the optimal temperature may be 37°C or higher.

TABLE 3. *Effects of Incubation Temperature on the Anti-st. 19 Activity of Mixture Consisting Globulin and Albumin Fractions*

Incubation temperature	Anti-st. 19 activity[1]	Relative activity
4°C	18%	19
15	12	13
25	71	77
30	83	90
37	92	100

1) Bactericidal rate for *Br. abortus* st. 19.

Effects of Absorption with Brucella and Salmonella Organisms on Anti-strain 19 Activity

The anti-st. 19 activity of the mixtures consisted of two fractions unabsorbed and absorbed with *Br. abortus* st. 19/st. 544 or *Salmonella abortus equi* was detected. The results are shown in Table 4. The activity of the mixtures which consisted of the unabsorbed albumin fraction and the globulin fraction absorbed with brucella organisms disappeared completely. On the contrary, the mixtures consisting of the unabsorbed globulin fraction and the albumin fraction absorbed with the organisms kept the activity just like the control. While, the activity of the two fractions was not affected by the absorption with salmonella organisms. These facts indicate that the anti-st. 19 agent in the globulin fraction combines with the two strains of *Br. abortus* but not with *Sal. abortus equi*, and that the agent in the albumin fraction does not combine directly with the cells.

TABLE 4. *Effects of Absorption of Globulin or Albumin Fraction with Heat-killed Brucella and Salmonella on the Anti-st. 19 Activity.*

Fraction		Absorbed with	Anti-st. 19 activity[2]	Relative activity
Unabsorbed	Absorbed[1]			
Albumin	Globulin	*Br. abortus* st. 19	—	—
		Br. abortus st. 544	—	—
		Sal. abortus equi	91	99
Globulin	Albumin	*Br. abortus* st. 19	92	100
		Br. abortus st. 544	88	96
		Sal. abortus equi	91	99
Control[3]		None	92	100

1) Absorption was carried out at 4°C.
2) Bactericidal rate for *Br. abortus* st. 19.
3) Unabsorbed albumin and globulin fractions were used.

Discussion

It was reported that there was a bactericidal factor in bovine serum acting on *Br. abortus* strain 19 cooported with Mg^{2+} (2) and that the factor in the serum was

heat-labile, acid-labile, temperature-dependent and removable by absorption with heat-killed *Br. abortus* (1) and that the factor was divided into two agents belonging to albumin and globulin fractions respectively. However this activity appeared only when the two fractions were coexistent (3).

From the results obtained in the present paper, it has become clear that the acid-lability and the removal by the absorption of the factor in bovine serum is due to the agent in the globulin fraction and that the heat-lability of the serum depends on the agent in the albumin fraction. The facts that the absorption of the agent in the globulin fraction with bacterial cell was specific to *Br. abortus* and the agent was resistant to heating at 56°C seem to indicate that the agent in this fraction is a kind of antibody. Also, the facts that the agent in the albumin fraction was not removed by the absorption and that its activity was easily lost at 56°C seem to indicate that the agent is some components of complement. The facts that Mg^{2+} was a requirement in the anti-st. 19 action (2) and that this action was temperature-dependent should support the above assumption.

References

1) Nakamura, M., and Katsuno, M., *Tohoku J. Agr. Res.* **25**, 77 (1974)
2) Katsuno, M., and Nakamura, M., *Tohoku J. Agr. Res.* **26**, 71 (1975)
3) Nakamura, M., and Katsuno, M., *Tohoku J. Agr. Res.* **27**, 40 (1976)