Ethylenediaminetetraacetic Acid (Disodium Salt)-Labile Bovine Immunoglobulin M Fc Binding to *Brucella abortus*: a Cause of Nonspecific Agglutination

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It was demonstrated by a radioimmunoassay procedure that *Brucella abortus* agglutinins from noninfected cattle sera, absorbed to *B. abortus* antigen and eluted with ethylenediaminetetraacetic acid (EDTA), was immunoglobulin M that bound to that bacterium by its Fc portion. The EDTA-eluted immunoglobulin M agglutinated intact *B. abortus* cells but not erythrocytes treated with *B. abortus* lipopolysaccharide. The specificity of the EDTA-eluted immunoglobulin was for *B. abortus*, although a small titer to *Yersinia enterocolitica* serotype O:9 was observed. In contrast, immunoglobulin M purified from the serum of a cow injected 7 days previously with heat-killed *B. abortus* bound to the antigen by its Fab portion, was not labile to EDTA treatment, cross-reacted extensively with *Y. enterocolitica* serotype O:9, and agglutinated various other bacterial antigens and normal erythrocytes.

Nonspecific reaction of cattle sera with *Brucella abortus* antigens is a problem in the routine serological diagnosis of brucellosis. These nonspecific reactions are not confined to agglutination (13–15, 32) and complement fixation tests (24) but occur with primary binding assays, such as the enzyme-linked immunosorbent assay (19; B. Stemshorn, Ph.D. thesis, University of Guelph, 1979). Two serum proteins have been implicated in agglutination tests, a macroglobulin, probably immunoglobulin (IgM), and a slower-sedimenting serum component (13–15, 32).

Since agglutination tests are most commonly used for screening, a number of serological procedures have been devised to eliminate nonspecific agglutinins. These include heat treatment of the sera (1, 14, 29), the addition of 2-mercaptoethanol (31) or rivanol (22), and the use of acidified antigen preparations (30). These methods destroy or inactivate IgM and IgM-like molecules indiscriminately and could therefore fail to detect cattle infected with *B. abortus*, which might be producing only IgM antibody. Another technique to reduce nonspecific agglutination relies on divalent cation chelators, such as ethylenediaminetetraacetic acid (EDTA) (24, 34). An understanding of the mechanism by which chelating agents reduce agglutination may provide useful information on the cause of nonspe-

cific serological reactions. The mechanism of binding and the specificity of EDTA-labile IgM isolated from sera of nonspecific *B. abortus* reactor cattle were studied.

MATERIALS AND METHODS

Cattle sera. Sera were collected from 10 cows with EDTA-labile agglutination titers to *B. abortus* ranging from 1:64 to 1:1,024 and from 1 cow seven days after intramuscular injection of 1.0 ml of 8% heat-killed *B. abortus* strain 413 (the *B. abortus* preparations were supplied by J. A. J. Carrière, Animal Diseases Research Institute Nepean, Nepean, Ontario, Canada). Agglutination titers were determined as previously described (24).

Preparation of EDTA eluates. *B. abortus* cells (strain 413, heat killed) were washed three times in 0.1 M phosphate buffer containing 0.15 M NaCl (PBS), three times in PBS containing 10 mM EDTA (PBS-EDTA), and then three times in PBS by centrifugation at 9,600 × *g* for 15 min at 4°C). A 1-ml amount of packed cells was suspended in 10 ml of serum by stirring at 4°C for 12 h and then pelleted by centrifugation (9,600 × *g* for 15 min at 4°C). The supernatants were tested for residual *B. abortus* agglutination activity, found negative at a 1:4 serum dilution, and pooled to constitute the "absorbed serum." The cell pellets were washed three times in PBS as described above, with the supernatant in each case being discarded, followed by resuspension in PBS-EDTA by stirring at 4°C for 3 h. The supernatant was recovered by centrifugation as described above, and the cell pellets were washed one additional time in PBS-EDTA. The two PBS-EDTA wash supernatants prepared from each serum were pooled, dialyzed against PBS, and

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concentrated to 1.0 ml by dialysis against polyvinyl-pyrrolidone.

The EDTA eluates from 8 of the 10 sera were pooled, and the pool was tested for its agglutination titer to *B. abortus* (24) and for the presence of IgM, IgG1, IgG2, and IgA by standard double gel immuno-diffusion (27) and immunoelectrophoretic techniques (33), using heavy chain-specific antisera (see Table 1). The pooled material was chromatographed on Sephacryl S200 [Pharmacia (Canada) Ltd., Dorval, Quebec, Canada; bed (180 by 5 cm) at a flow rate of 28 ml/h run reverse to gravity by a peristaltic pump and maintained at 4°C] equilibrated in 0.1 M tris-(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.5) and containing 1.0 M NaCl and 10 mM EDTA. The void volume protein pea, monitored at 280 nm, was collected, dialyzed against PBS, and adjusted to a protein content of 10.0 mg/ml, using optical density at 280 nm and $E_{1\%}^{1\ cm} = 13.7$. This IgM, which was shown to contain no measurable contaminants by gel immunodiffusion with a rabbit anti-bovine globulin serum, had an agglutination titer of 1:10,240 to *B. abortus*.

Preparation of IgM. Serum IgM from one cow injected 7 days previously with heat-killed *B. abortus* and from a pool of sera absorbed with *B. abortus* cells were prepared by a combination of euglobulin precipitation and column chromatography (J. C. Cambier and J. E. Butler, Program Abstr. Annu. Meet. Am. Soc. Microbiol. 1973, M27, p. 78). These preparations were tested for purity by gel immunodiffusion and immunoelectrophoresis.

Papain digestion of IgM. The three IgM preparations were reduced and alkylated as previously described (23). The monomeric IgM was separated from nonreduced IgM by gel filtration chromatography (Sephacryl S200, equilibrated in 0.1 M Tris-hydrochloride [pH 7.5] containing 1.0 M NaCl and 10 mM EDTA). The monomeric IgM was digested with papain (type IV, Sigma Chemical Co., St. Louis, Mo.) by the method previously described (9), with two exceptions: first, all protein determinations were based on optical density at 280 nm, using $E_{1\%}^{1\ cm} = 13.7$, and second, 3% papain (protein/protein) was used for digestion. Monomeric IgM was separated from the proteolytic cleavage fragments by gel filtration (Sephadex G-100 equilibrated in 0.2 M Tris–0.5 M glycine, pH 7.6). With anion-exchange chromatography (Whatman DE-52, Mandel Scientific Co., Montreal, Quebec, Canada) equilibrated in 0.01 M Tris-hydrochloride (pH 8.6) containing 1 mM EDTA, a fragment was eluted with the starting buffer. This fragment was similar to the IgM Fab described previously (26) on the basis of charge, and the presence of L-chains was confirmed by gel immunodiffusion. Application of a buffer gradient (0.01 M Tris-hydrochloride to 0.01 M Tris-hydrochloride with 0.5 M NaCl [pH 8.6] containing 1 mM EDTA) resulted in the elution of a second fragment similar to IgM Fc (26) and containing class-specific antigenic determinants of IgM. Immunoelectrophoretic analysis of the two fragments, referred to as IgM Fab and IgM Fc, is presented in Fig. 1. Antisera prepared in guinea pigs to the Fc fragments of the three IgM preparations revealed complete identity in gel immunodiffusion
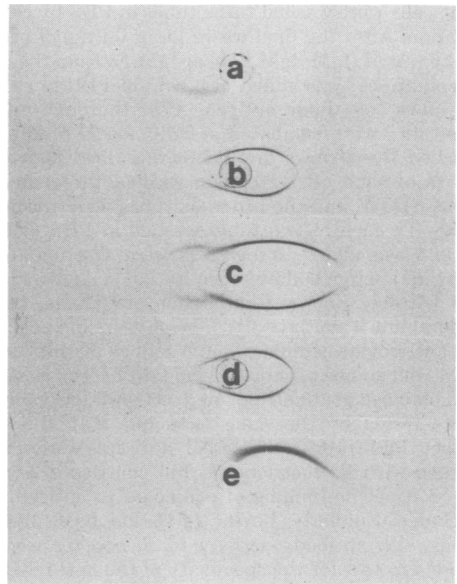

FIG. 1. *Immunoelectrophoresis of IgM and its papain fragments. Anode is on the left. (a) IgM F_c; (b and d) IgM; (c) IgM after digestion with papain; (e) IgM Fab. All troughs contained rabbit anti-bovine gamma globulin.*

with IgM and EDTA-eluted IgM as the antigens and failed to react with other serum constituents.

IgM and fragment preparations were dialyzed and stored lyophilized.

Preparation of antigens for agglutination tests. Fourteen species of bacteria (see Table 2) isolated from cattle or obtained from the Animal Diseases Research Institute culture collection were propagated in tryptic soy broth (Difco Laboratories, Detroit, Mich.) at 37°C for 48 h. The live bacterial cells were then harvested and washed three times in 0.15 M NaCl by centrifugation (4,000 × *g* for 15 min at 4°C) and resuspended in 0.15 M NaCl containing 0.5% phenol to an optical density of 0.5 at 420 nm. These suspensions were used as antigens with equal volumes of doubling IgM dilutions as for *B. abortus*. Titers represent the final IgM dilutions to agglutinate approximately 50% of the cells, as determined by visual inspection.

Similarly, erythrocytes from seven mammalian species (Table 2) were washed three times in 0.15 M NaCl by centrifugation (1,000 × *g* for 10 min at 4°C). Cells were made up to a final concentration of 1% (vol/vol) and used as antigens in titrating the three IgM preparations.

Radioimmunoassay for binding of IgM and its papain fragments to *B. abortus* cells. Basically, an indirect radioimmunoassay, such as that described previously (28) was used. Amounts of 0.1 ml of *B. abortus* previously washed three times in PBS-EDTA (strain 413, heat killed, 8% [vol/vol]) were dispensed into polystyrene tubes (10 by 75 mm; Falcon Plastics, Fisher Scientific Co. Ltd., Ottawa, Ontario, Canada).

The cells were washed three times in PBS by centrifugation. After the final wash, 1.0 or 0.1 mg of protein to be tested (IgM, IgM Fab, or IgM Fc from the three preparations) was added in 0.1 ml of PBS to each of 12 tubes containing antigen. After thorough mixing, the tubes were incubated at 22°C for 20 h and then washed three times in PBS as described above. Six tubes of each set were then washed three times in PBS-EDTA, and the other six tubes were washed in PBS. To each of two tubes washed in PBS or PBS-EDTA was added 10 mg of antisera, fractionated by 33% ammonium sulfate precipitation and labeled with ^{125}I (Atomic Energy Commission of Canada) by the chloramine T method (21). The antisera included rabbit anti-bovine gamma globulin, guinea pig anti-bovine IgM, and guinea pig anti-bovine IgM Fc. For a control, radiolabeled goat anti-bovine IgG1 and -IgG2 antisera were tested by the same technique with the three intact IgM preparations. All antisera were preabsorbed with *B. abortus* cells, but controls to account for nonspecific binding of conjugate to antigen were included. Similarly, bovine IgG1 and IgG2 preparations with antibody activity to *B. abortus* were included to test for the specificity of the anti-IgM antisera in the radioimmunoassay. All tubes were enumerated for radioactivity retained in the cell pellets after three washes in PBS. Counts per minute were averaged and are presented as radioactivity bound as a percentage of the total counts per minute added (see Table 4).

Zymosan, cobra venom factor, ammonium hydroxide, and immune complex treatment of sera. The 10 sera originally used in the preparation of EDTA-labile IgM were treated with zymosan, cobra venom factor, unrelated immune complexes, or ammonium hydroxide or heated to 56°C for 30 min. Zymosan (Sigma Chemical Co.) was used to prepare bovine sera free from C3 (18). Absence of C3 from the absorbed sera was ascertained by gel immunodiffusion against a rabbit anti-bovine C3 antiserum prepared in our laboratory. No hemolytic activity remained in the absorbed sera (4).

Cobra venom factor (Sigma Chemical Co.) was prepared by the method outlined previously (5). Bovine sera were treated with 25 U of cobra venom factor per ml, incubated for 30 min at 37°C, and then tested for hemolytic activity (4) and found negative.

Immune complexes consisting of formalinized washed sheep erythrocytes treated with a bovine anti-sheep erythrocyte antiserum in the presence of 10 mM EDTA were washed a total of six times in PBS by centrifugation (1,500 × *g* for 10 min at 4°C). Bovine sera (1.5 ml) were treated with 0.5 ml of packed antibody-treated sheep erythrocytes for 1 h at 22°C with constant stirring. The sera were separated from the erythrocytes by centrifugation (1,500 × *g* for 10 min at 4°C).

The bovine sera were treated with ammonium hydroxide (18) for 45 min at 37°C. The sera were then restored to a pH of 7.2 with hydrochloric acid. No hemolytic activity remained in these sera.

All sera, untreated or treated with zymosan, cobra venom factor, immune complexes, or ammonium hydroxide or heated to 56°C for 30 min were titrated for

their agglutinin activity against *B. abortus* in the presence or absence of 10 mM EDTA.

Indirect hemagglutination. *B. abortus* (strain 413, heat killed) was dialyzed against distilled water, lyophilized, and then used to prepare crude lipopolysaccharide by Redfearn's modified phenol-water method as described previously (3). Lyophilized lipopolysaccharide was suspended (5 mg/ml) in 0.25 N NaOH, heated to 56°C for 1 h, neutralized with acetic acid, and then precipitated with 3 volumes of ethanol (7). The precipitate was collected by centrifugation, dissolved in and dialyzed against distilled water, and made up to a concentration of 2 mg of lipopolysaccharide per ml (based on the weight of starting material) and stored at −20°C.

A 1-ml amount of lipopolysaccharide was diluted 1:4 with PBS, and 1.0 ml of 5% sheep erythrocytes was added while swirling. The mixture was incubated at 37°C for 30 min. The sheep erythrocytes were washed three times with 10 ml of PBS and finally suspended to 10.0 ml with PBS (0.5% sensitized cells).

Sera were absorbed with 0.05 ml of packed sheep erythrocytes per 0.5 ml of serum. The EDTA eluate was tested without absorption with sheep erythrocytes. An initial 1:5 dilution of test sample was made in PBS, and further twofold dilutions were made in U-bottom microtiter plates, using 0.1 ml of PBS containing 0.01% NaN$_3$ and 2.5% of the heat-inactivated normal rabbit serum per well. A 25-μl drop of sensitized or control erythrocytes was added to each well containing 100 μl of each test dilution. The plates were shaken to mix the contents of the wells and then incubated for 18 h at 22°C, after which sedimentation patterns were observed.

EDTA-eluted IgM was absorbed with an equal volume of packed sensitized sheep erythrocytes. The absorbed IgM was then titrated for *B. abortus* agglutinins.

RESULTS

The serological properties of the three IgM preparations (EDTA-eluted, induced, and *B. abortus*-absorbed IgM) are described in Table 1. From this data, it is clear that the EDTA-eluted and induced IgM preparations have very high agglutination titers to *B. abortus*, the EDTA-eluted IgM being completely labile in the presence of the chelating agent (titer below 1:4). The *B. abortus*-induced IgM was not affected by EDTA treatment, and no antibody activity could be demonstrated in the *B. abortus*-absorbed IgM. However, both the *B. abortus*-induced and -absorbed IgM preparations contained antibody of various titers to 14 common gram-negative and gram-positive bacteria as well as to erythrocytes from seven mammals (Table 2). The EDTA-eluted IgM, on the other hand, only contained agglutinins to *B. abortus* and had a low titer to *Yersinia enterocolitica* serotype O:9 (Table 2). Although this "specificity" appeared to be limited to *B. abortus*, sheep erythrocytes coated with *B. abortus* lipopolysac-

TABLE 1. *Serological properties of purified IgM preparations*

IgM	Agglutination titer[a]		Protein concn (mg/ml)[b]	IgM[c]	IgG1	IgG2	IgA
	0.15 M NaCl	10 mM EDTA					
EDTA eluted	10,240	<4	10.0	+	−	−	−
Induced[d]	5,120	5,120	10.2	+	−	−	−
Absorbed[e]	<4	<4	10.0	+	−	−	−

[a] Reciprocal of the last dilution to agglutinate 50% of the antigen (*B. abortus*).

[b] Measured at 280 nm, using $E_1^{\frac{1\%}{cm}} = 13.7$.

[c] Based on gel immunodiffusion and immunoelectrophoresis.

[d] Serum IgM from a cow injected 7 days previously with *B. abortus* (heat killed).

[e] Serum IgM absorbed free of antibody activity to *B. abortus*.

TABLE 2. *Serological activities of IgM preparations with various antigens*

Antigen	Agglutination titer (reciprocal)		
	EDTA-eluted IgM[a]	Induced IgM[b]	Absorbed IgM[c]
Bacterial species			
B. abortus (heat killed)	1,024	512	
Salmonella pullorum		64	32
E. coli		512	1,024
Pasteurella hemolytica		64	64
Alcaligenes faecalis		8	16
Proteus mirabilis		32	128
Y. enterocolitica O:3		32	16
Y. enterocolitica O:9	8	512	32
Yersinia pseudotuberculosis		16	16
Pseudomonas aeruginosa		64	128
Enterobacter sp.		128	256
Bacillus sp.		64	32
Staphylococcus aureus		32	64
Streptococcus sp.		64	64
Erythrocytes			
Guinea pig		8	8
Rabbit		8	16
Sheep		8	4
Goat		16	4
Horse		16	8
Pig		32	32
Human		64	32

[a] The three IgM preparations were used at a concentration of 1 mg/ml.

[b] IgM prepared from serum of a cow injected 7 days previously with heat-killed *B. abortus*.

[c] IgM absorbed with *B. abortus*.

TABLE 3. *Indirect hemagglutination test titers obtained with the EDTA eluate and control sera*

Sample	Titer
EDTA eluate	1:10
Negative serum pool	1:20
Positive serum A/8	1:2,560
Pool of sera from strain 19 vaccinated calves	1:2,560

charide were not agglutinated by the EDTA-eluted IgM (Table 3). Similarly, no reduction in the agglutination titer of the EDTA-eluted IgM

to *B. abortus* was noted after absorption with sensitized erythrocytes. The indirect hemagglutinating activity of the isolated, induced IgM was not evaluated, but the loss of direct agglutinating activity for *B. abortus* in comparison to the original serum was only in the order of 1 log.

To ascertain the portion of the EDTA-eluted IgM molecule which bound to *B. abortus* cells, the papain digestion fragments of this IgM (Fig. 1) and those of *B. abortus*-induced and -absorbed IgM as well as the intact IgM molecules were reacted with *B. abortus* cells. This binding was in turn detected by the addition of ^{125}I-anti-bovine gamma globulin, ^{125}I-anti-bovine IgM, and ^{125}I-anti-bovine IgM Fc antisera. The results show that EDTA-eluted IgM and its Fc fragment bound equally well to *B. abortus* and that most of this binding was EDTA labile (Table 4). EDTA-eluted IgM Fab binding was much more resistant to chelation. The reason for the initial EDTA lability of the Fab binding IgM is not understood but may be a result of weak binding by IgM molecules perhaps attached to the antigen by only a fraction of their antigen binding sites and therefore removed by ion exchange by the EDTA. This IgM may account for the small *Y. enterocolitica* titer observed (Table 2). The *B. abortus*-induced IgM and its Fab fragment bound to *B. abortus*, whereas the Fc portion did not. This binding was also relatively resistant to chelation. No binding was observed with the *B. abortus*-absorbed IgM or its papain digestion fragments. Control results for the radioimmunoassay procedure are compiled in Table 5.

Treatment of the 10 sera which contained EDTA-labile agglutinins with cobra venom factor, ammonium hydroxide, zymosan, or immune complexes or heating at 56°C for 30 min did not alter their agglutinin titers in the presence or absence of EDTA from untreated sera.

DISCUSSION

A population of IgM molecules which nonspecifically agglutinate *B. abortus* cells via interaction by their Fc portion is described. This Fc-

TABLE 4. *Percent binding of EDTA-eluted, B. abortus-absorbed and -induced bovine IgM, IgM Fab, and IgM Fc fragments to B. abortus cells as indirectly detected with ^{125}I antisera*

IgM sample	Amt added (mg)	RaBGG[a] (1,069,000 cpm)[b]		GPaIgM (748,000 cpm)		GPaIgM Fc (636,000 cpm)	
		Saline (0.11%)[c]	EDTA (0.10%)	Saline (0.09%)	EDTA (0.13%)	Saline (0.16%)	EDTA (0.18%)
EDTA eluate							
19S IgM	1.0	3.05[d]	1.01	5.87	1.55	1.79	1.03
	0.1	0.41	0.12	0.75	0.18	0.28	0.20
IgM Fab	1.0	0.72	0.61	1.38	1.09	0.18	0.21
	0.1	0.13	0.13	0.23	0.19	0.17	0.23
IgM Fc	1.0	3.07	0.41	5.94	0.55	5.49	0.76
	0.1	1.34	0.11	2.18	0.19	2.44	0.24
IgM (induced)							
19S IgM	1.0	1.71	1.44	2.11	1.90	1.78	1.45
	0.1	0.21	0.17	0.29	0.17	0.28	0.22
IgM Fab	1.0	2.18	1.83	3.29	2.69	0.19	0.20
	0.1	0.26	0.20	0.35	0.30	0.17	0.23
IgM Fc	1.0	0.18	0.16	0.18	0.24	0.27	0.22
	0.1	0.16	0.13	0.17	0.18	0.22	0.23
IgM (absorbed)							
19S IgM	1.0	0.18	0.17	0.18	0.21	0.21	0.27
	0.1	0.16	0.12	0.15	0.21	0.19	0.20
IgM Fab	1.0	0.16	0.16	0.23	0.23	0.19	0.26
	0.1	0.15	0.14	0.16	0.20	0.19	0.21
IgM Fc	1.0	0.12	0.14	0.22	0.18	0.21	0.22
	0.1	0.13	0.15	0.17	0.17	0.20	0.22

[a] RaBGG, Rabbit anti-bovine gamma globulin; GPaIgM, guinea pig anti-bovine IgM; GPaIgM Fc, guinea pig anti-bovine IgM Fc.

[b] Total radioactivity added in counts per minute per 10 mg of antiserum.

[c] Percent binding of antiserum conjugate to *B. abortus* washed in saline or EDTA.

[d] Percent binding with regards to the total amount of conjugate (10 mg) added is indicated.

mediated agglutination process was shown to be largely labile in the presence of divalent cation chelators, such as EDTA. A small portion of the IgM bound to *B. abortus* by the Fab part, and this binding was not affected by EDTA treatment. By destruction or removal of serum complement components C3 (with cobra venom factor and zymosan) and C3 and C4 (by ammonium hydroxide treatment) or by absorption of the sera with unrelated immune complexes made with bovine antibody, the involvement of complement components and conglutinin may reasonably be discounted.

Studies of the binding site on *B. abortus* for the EDTA-labile IgM remain inconclusive. The protein was only capable of agglutinating intact bacterial cells but not erythrocytes coated with crude lipopolysaccharide, the major cell surface component. Interestingly, the EDTA-eluted IgM did not agglutinate other bacterial species tested except for *Y. enterocolitica* serotype O:9, to a small extent (Table 2). This suggests a specific interaction between this IgM and *B. abortus*, as *Y. enterocolitica* serotype O:9 and *B. abortus* have common antigenic determinants (16). This interaction is perhaps caused by the EDTA-resistant Fab portion of IgM.

Binding of immunoglobulin molecules by their Fc portion to bacterial cells has been described previously in the cases of streptococci (17), *Di-*

TABLE 5. *Radioimmunoassay controls for specificity*[a]

Sample	RaBGG[b] (1,069,000 cpm)[c]	GPaIgM (780,000 cpm)	GPaIgM Fc (636,000 cpm)	GaIgG1 (810,000 cpm)	GaIgG2 (922,000 cpm)
IgM					
EDTA eluted[d]	3.05[e]	5.87	1.79	0.20	0.17
Induced	1.71	2.11	1.78	0.21	0.17
Absorbed	0.18	0.18	0.21	0.19	0.15
IgG1	1.43	0.15	0.19	2.99	0.21
IgG2	1.56	0.16	0.19	0.26	3.38
Antigen (*B. abortus*)	0.11	0.09	0.16	0.18	0.14

[a] Data represent the percent bound of each antibody.

[b] RaBGG, Rabbit anti-bovine gamma globulin; GPaIgM, guinea pig anti-bovine IgM; GPaIgM Fc, guinea pig anti-bovine IgM Fc; GaIgG1, goat anti-bovine IgG1; GaIgG2, goat anti-bovine IgG2.

[c] Total radioactivity added in counts per minute per 10 mg of antiserum.

[d] A 1.0-mg amount of each purified protein was added to *B. abortus* cells. All preparations had antibody activity to *B. abortus*, except for the absorbed IgM.

[e] Percent of the total amount of radioactivity added (10 mg) which bound to the antigen-antibody complex.

plococcus pneumoniae (35), *Escherichia coli* (36), *Staphylococcus aureus* Cowan 1 strain, and IgG from a variety of mammals (10, 11). This binding of *S. aureus* is mediated by protein A of the bacteria. Although the chemical nature of the protein A-Fc interaction is not fully understood, an ion-exchange phenomenon may be involved as different subclasses of IgG may be eluted from protein A at different hydrogen ion concentrations (6, 8). Similarly, ion exchange may be responsible for the elution of IgM from *B. abortus* by EDTA. By using ethylenediamine diacetic acid or diaminopropane tetraacetic acid, the former containing two negative charges and the latter having four negative charges but a slightly different molecular configuration, agglutination of *B. abortus* cells was not affected. Calcium or other divalent cations added in a molar excess can overcome the EDTA lability of the agglutinin, whereas hydroxyquinolone, also a chelating agent, has no effect. Therefore, the reaction appears not to be divalent cation-dependent, but rather, divalent cations neutralize the effects of the EDTA. The effect of EDTA on anion-exchange chromatography has previously been described (K. Nielsen and J. R. Duncan, submitted for publication). It would appear that interference by EDTA with agglutination depends on its four negatively charged groups in a certain configuration interacting with either the *B. abortus* cell or with the IgM molecule. It is unlikely that the EDTA acts on the bacterial cell, as no effects were noted with IgM obtained from a cow infected with *B. abortus* (Table 4). Thus, the IgM molecules involved in this EDTA-labile interaction may be, in some fashion, physiochemically altered. One hypothesis is that such a change could be the result of normal

catabolic processes in which case the level of this IgM should be fairly similar in most cattle. However, of 2,000 cattle tested, 12 were found to have elevated agglutinin levels considered non-specific (K. Nielsen, J. R. Duncan, B. Stem-shorn, and G. Ruckerbauer, in J. E. Butler, ed., *Proceedings of the International Symposium of Ruminant Immune Systems*, in press). The normal catabolic processes may be responsible for the low levels of EDTA-labile IgM agglutinins observed in most cattle (Nielsen et al., in press); however, other factors must be involved in cases of elevation. Therefore, elevated levels may be the result of accelerated aging of molecules or conditions that interfere with their elimination. Natural aging and the signal for excretion of serum glycoproteins have been suggested to be the result of desialation of carbohydrate residues (2). It is conceivable that bacterial or serum glycosidases desialate IgM, exposing chemical structures capable of reacting with *B. abortus*. This has clearly been shown in the case of other glycoproteins and hepatic cells (2). Desialation has also been demonstrated to increase cryoinsolubility (12, 20), and the *B. abortus* agglutinins may be altered, or cryoprecipitable proteins found normally in low levels may be found in increased levels in various disease states. The liver may be responsible for the excretion of these molecules; however, if the liver transport mechanism becomes overloaded or damaged, such molecules would remain in circulation. Therefore, any bacterial infection could cause an increase in nonspecific *B. abortus* agglutinins.

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