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BACTERIAL-AGGLUTINATING IMMUNOGLOBULINS IN BOVINE SERUM: A POSSIBLE ROLE IN FEEDLOT BLOAT CONTROL

by

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INTRODUCTION

Bloat often occurs in ruminants fed low-roughage, high-concentrate, feedlot rations. Although bloated feedlot cattle may not die, the economic losses due to poor feed efficiency or low milk production are significant. Complex interactions of animal, feed and microbial factors are associated with the occurrence of feedlot bloat (Meyer, 1969; Hungate, 1966).

To control feedlot bloat, it is necessary to prevent the retention of fermentation gases. This can only be done by controlling the many interacting factors involved in froth production (Meyer, 1969). Studies of bloat prevention are numerous, involving natural and artificial roughages, antibiotics, and surface active agents. The use of a quaternary ammonium compound has shown promise in the recent work of Meyer (1972). As for many drugs used in food animals, clearance and assay problems for this bloat controlling agent exist. An approach to the bloat problem, which has received only limited attention, is the role of the bovine secretory immune system in the control of bloat provoking rumen microorganisms (Gettings, 1970). This study investigates the properties and reactivity of agglutinating agents in bovine serum, and discusses a possible role of the secretory immune system in bloat control.

REVIEW OF LITERATURE

Feedlot Bloat

Microbial digestion in the ruminoreticulum of cattle provides nutrients for the host which are not available to monogastric animals on the same ration. However, the microbial fermentation process results in the production of methane and carbon dioxide. These gases cannot be used by the host and must be eliminated. This is usually done by eructation. If eructation does not occur, or if the cardia or esophagus is blocked so that fermentation gases cannot be freely passed, distention of the ruminoreticulum will result from the increase in gas pressure. This condition is called gastric tympany, or more commonly bloat (Meyer, 1969).

Microflora of Feedlot Cattle

The microflora of ruminant animals varies with the ration being fed. Feedlot rations, composed chiefly of starch, cause amylolytic bacteria to predominate. The prevalent amylolytic bacteria of feedlot ruminants are: Streptococcus bovis, Butryvibrio sp., Bacteroides ruminicola, Lactobacillus sp., Succinimonas sp., Selenomonas sp., and Peptostreptococcus elsdenii (Hungate, 1966).

In short term studies, Streptococcus bovis was shown to predominate in the rumen of feedlot cattle (Gall and Huhtanen, 1951; Phillipson, 1951; Bauman and Foster, 1956; Gutierrez et al., 1959; Mishra, 1964; Meyer, 1969). When animals are maintained on feedlot rations for several weeks, the rumen streptococcal concentration is not considerably different from hay fed ruminants (Perry et al., 1955; Hungate, 1957; Bryant

et al., 1961; Meyer, 1969). Hartman et al. (1962) found the number of rumen Streptococci did not increase until feedlot bloat symptoms had appeared. Smith et al. (1953) reported the appearance of iodophilic Streptococcus concurrent with frothing. An abundance of encapsulated rumen bacteria has been found in animals fed feedlot rations (Lindahl et al., 1957; Gutierrez et al., 1959; Bryant et al., 1961). These workers postulated that bacterial capsules may entrap fermentation gases and be a causative agent of bloat. The incidence and severity of feedlot bloat correlates with increased rumen fluid viscosity and increased numbers of encapsulated rumen bacteria (Jacobson et al., 1957; Gutierrez et al., 1961). Bacterial capsular material may strengthen or increase viscosity of rumen fluid (Meyer and Bartley, 1971). Most encapsulated bacteria associated with feedlot bloat are thought to be type D streptococci, as described by Hobson and Mann (1955). The capsule of group D streptococci and a cell-free polysaccharide in the culture media contained mostly rhamnose, galactose and galacturonic acid (Hobson and MacPherson, 1954). Ruminant levels of rhamnose-containing cell-free polysaccharides have been correlated with rumen viscosity and bloat severity (Meyer and Bartley, 1971). Streptococcus bovis, a group D streptococci, produces a polysaccharide capsule (Hobson and MacPherson, 1954; Bailey and Oxford, 1958; Kane and Karakawa, 1969). A water soluble dextran is produced by S. bovis when sufficient sucrose is present (Niven et al., 1941, 1946; Oxford, 1951, Barnes et al., 1961). Dextran does not accumulate in the rumen; presumably, it is digested by other organisms (Bailey, 1959).

Hartman et al. (1962) found that when rumen contents from a bloat susceptible and a nonsusceptible animal were exchanged the microflora and physical characteristics soon reverted to the former type. This indicates

that the symbiotic relations among rumen microbes can be influenced by animal factors as well as rations and can affect the animal susceptibility to feedlot bloat.

Salivation in Feedlot Cattle

In the ruminant, saliva not only aids in the swallowing of feed, but also plays an important role in microbial digestion. Saliva serves as a buffering agent which controls the pH of the rumen during microbial production of volatile fatty acids (VFA). In turn, VFA can stimulate salivation (Meyer, 1969).

In relation to bloat, the mucin of saliva has been shown to inhibit froth formation (Van Horn and Bartley, 1961; Bartley and Yadava, 1961; Meyer, 1969). However, the rumen mucinolytic bacteria can destroy the antifrothing ability of mucin (Fina et al., 1961; Mishra et al., 1967, 1968). Some rumen strains of Butrivibrio fibrisolvens, Selenomonas ruminatium, Streptococcus bovis and Peptostreptococcus elsdenii present in feedlot cattle have been found to be actively mucinolytic (Mishra et al., 1968).

The capacity for saliva production appears to be a heritable trait. The rate and amount of saliva secreted was found to be similar within a set of identical twin cows, but distinctly different among twin sets (Lyttleton, 1960; Mendel and Boda, 1961; Meyer et al., 1964; Meyer, 1969). Bloat susceptible cows appear to secrete less saliva than nonsusceptible cows (Mendel and Boda, 1961; Meyer et al., 1964; Meyer, 1969). A decreased rate of salivary secretion has been reported for low roughage rations (Bailey and Balch, 1961; Wilson, 1963; Wilson and Tribe, 1963; Oltjen et al., 1965; Putnam et al., 1966; Meyer, 1969). Thus, the

natural antifrothing effect of saliva is diminished on feedlot rations because of reduced production of saliva and excessive concentration of mucinolytic flora.

Bovine Immunoglobulins

Three antigenically distinct classes of immunoglobulins, IgG, IgA, and IgM have been identified in cattle (Butler, 1971). Two subclasses of IgG immunoglobulins are currently recognized: IgG_1 and IgG_2. IgG_1 is normally the most abundant immunoglobulin in the serum and lactial secretions of the cow (Butler, 1971; Mach and Pahud, 1971a). IgG_2 makes up about 26% of the total serum immunoglobulins and carries A_1 and A_2 allotypic heavy chain markers (Butler, 1971; Mach and Pahud, 1971a; Blakeslee et al., 1971). A B_1 allotype marker has been found on IgG, IgM, and IgA light chains (Blakeslee et al., 1971; Mach and Pahud, 1971a). Bovine IgM is present in significant amounts in serum and colostrum and at low levels in saliva (Mach and Pahud, 1971a; Butler, 1971). Small amounts of IgA, in both the monomeric and secretory form are present in serum (Porter and Noakes, 1970; Mach and Pahud, 1971a). Secretory IgA (SIgA) is the major immunoglobulin in saliva, lacrimal, and gastrointestional secretions. SIgA is a minor component of colostrum, compared with the high concentration of IgG_1. The cow seems to have a secretory immune system similar to humans, with the exception of the mammary gland, which has acquired a special function of transporting IgG from serum to the lacteal secretions (Mach and Pahud, 1971, 1971a). Cells, thought to produce IgA, have been identified in the ileum, duodenum, colon, lungs, lower nasal mucosa, oral pharyngeal mucosa, thymus, salivary and lacrimal glands of the cow (Butler et al., 1972; Yurchak et al., 1971).

Control of Feedlot Bloat

Studies of feedlot bloat prevention have involved antibiotics, and surface active agents. Neither of these has adequately controlled bloat (Bartley and Meyer, 1967; Van Horn et al., 1963). A recently developed quaternary ammonium salt has shown definite bloat controlling ability (Meyer, 1972). Natural and artificial forms of roughage control bloat by effecting rumen motility, salivation, feed and microbial factors (Meyer, 1969). However natural roughage is not used in feedlot rations because of its low energy content. Artificial roughages have run into durability and regurgitation problems (Bartley, 1971). Antibodies which agglutinate rumen bacteria have been detected in bovine serum. Bovine sera studied by Sharpe et al. (1969) contained relatively high titers of agglutinating antibodies against strains of anaerobic bacteria isolated from the bovine rumen. Gettings (1970) considered a similar agent in bovine serum capable of agglutinating S. bovis. It was thought that the agent might be an animal factor affecting bloat susceptibility. He found no appreciable variation in the S. bovis agglutinating ability of serum from bloaters versus non-bloaters. This agent was referred to as an immune factor. However, tests were not performed to indicate that it was an immunoglobulin. The specificity of the agglutinating agent was not thoroughly studied.

MATERIALS AND METHODS

Sources of Bacterial Cultures

Streptococcus bovis, strain 124 was provided by Dr. Marvin D. Bryant, University of Illinois, Urbana. S. bovis, strains 18M2 and 2B were provided by Dr. Peter N. Hobson and Mr. S. O. Mann, Rowett Research Institute, Aberdeen, Scotland. All other bacteria cultures were obtained from Dr. L. R. Fina, Division of Biology.

Collection of Bovine Serum

A Jersey cow, X-10, supplied by Dr. E. E. Bartley, Department of Dairy and Poultry Science, was used for blood collection throughout this project. Jugular blood was collected in 50 ml sterile jars by Dr. Ronel Meyer, Department of Dairy and Poultry Science, and the author. The clot was loosened with a wood applicator stick, jars were incubated at 37 C for one hour to complete the clot, and allowed to stand overnight at 4 C. The clot was removed, serum centrifuged at 2000 g for 20 minutes, then decanted into sterile tubes and stored at -4 C.

Growth and Preparation of Bacterial Antigens

The bacteria were grown in 50 ml of trypticase soy broth for 12-18 hours at 37 C, harvested by centrifugation at 3500 g for 5 minutes, washed with three 20 ml volumes of phenolyzed buffered saline (0.5% phenol, 0.85% NaCl, 0.05M sodium phosphate), pH 7.0, and resuspended in a small volume of phenolyzed buffered saline. The concentrations of the bacterial suspensions were adjusted to 150 Klett units (Klett-Summerson) which was comparable to McFarland tube 6 (Kabat and Mayer, 1964;

McFarland, 1907; Appendix, p. 39). The bacterial antigens were then ready for use in the tube agglutination test.

Tube Agglutination Test

Whole bovine serum was diluted 1:25, 1:50, 1:100, 1:200, 1:400, 1:800 or further when necessary, as described by Kabat and Mayer (1964, Appendix, p. 40). A 0.5 ml volume of the bacterial suspension was added to each tube. The serum, bacteria suspensions were mixed then incubated overnight at 37 C. The agglutination titer was obtained by recording the highest dilution at which agglutination occurred.

DEAE Cellulose Preparation

Diethylaminoethyl (DEAE) cellulose (fine mesh, exchange capacity 0.89 meg/g, Sigma Chemical Co., St. Louis, Mo.) was suspended in 30 times its weight of distilled water, stirred vigorously, and allowed to settle 2 hours. The supernatant fluid containing the fine particles was decanted, and the washing was continued until the supernatant fluid was clear after 30 min of settling. The DEAE cellulose was then washed on a coarse sintered glass funnel with 0.125N NaOH, washed to neutrality with water, washed in a solution of 1% HCl, and finally equilibrated by washing in the starting buffer (Young, 1966).

Purification of Bovine Gamma Globulin

Bovine gamma globulin (BGG), Cohn fraction II was purchased from Calbiochem, La Jolla, California. Further purification of the BGG was necessary, since Cohn fraction II contains non-immunoglobin proteins (Rodkey, 1973). The additional purification was accomplished by DEAE

column chromatography. A slurry of regenerated DEAE cellulose in the starting buffer, 0.0175M sodium phosphate, pH 6.9, was packed into a 2.2 x 10 cm column and equilibrated with an additional 50 ml of starting buffer. The buffer level was lowered to the column surface and 150 mg of impure BGG (50 mg/ml) was deposited at the head of the column. The column was then charged with 150 ml of 0.0175M sodium phosphate buffer, pH 6.9, and 10 ml fractions were collected. The optical density of each fraction was read at 280 nm (Gilford spectrophotometer, model 240, Oberlin, Ohio). Fractions with optical densities greater than 0.1 were pooled for concentration. The amount of purified gamma globulin eluted was calculated from the extinction coefficient $E^{1\%}_{1\ cm} = 15$ (Rodkey, 1973). The above procedure was repeated a sufficient number of times to obtain the quantity of purified BGG needed for rabbit injection, antiserum assay, and immunoadsorbant preparation.

Preparation of Rabbit Anti-Bovine Gamma Globulin

Three New Zealand White rabbits supplied by Mrs. Harry Whitney, RFD #4, Manhattan, Kansas were used for antiserum production. Each rabbit was injected subcutaneously at four locations over the back with an emulsion of 5 mg purified BGG in 1.5 ml of phosphate buffered saline and 1.5 ml of complete Freund's adjuvant. Each rabbit received an identical dosage in a second injection given three weeks after the first. Ten days after the second injection 50 ml blood was collected from the marginal ear vein of each rabbit, at weekly intervals. The antiserum was removed from the clot as previously described in obtaining bovine serum.

Quantitative Precipitin Test

The quantitative precipitin test (Kabat and Mayer, 1964, Appendix, p. 41) was used to measure the amount of precipitating antibody in the rabbit anti-BGG antiserum prepared for this study. Constant amounts of rabbit antiserum (0.5 ml) were added to dilutions of purified bovine gamma globulin and incubated at 37 C for 30 minutes. The precipitates were removed by centrifugation, washed with borate buffered saline (0.05M, pH 8.0, 0.85% NaCl), then solubilized by the addition of 1.0 ml of 0.5N NaOH (Kabat and Mayer, 1964). The optical density of each tube was read at 280 nm and total protein calculated from $E^{1\%}_{1\ cm} = 15$. Since the amount of antigen present in each tube was known, the amount of antibody which precipitated at the equivalence point could be calculated. This gave the amount of precipitating antibody in the rabbit anti-BGG antiserum. The antibody levels of the antisera were checked at 14, 21, 31, and 59 days after the first injection.

Preparation of Immunoadsorbant Column

The immunoadsorbant column was prepared with sepharose (Sigma Chemical Co., St. Louis, Mo.) by the method of Axen et al. (1967) and others (Porath et al., 1967; Cutrecasas et al., 1968; Wolfsy et al., 1969; Appendix, p. 43). A slurry of 100 g sepharose and 100 ml 0.1M sodium bicarbonate buffer, pH 9.0, was made and cooled in an ice bath. The sepharose was activated by: (1) adjusting the pH to 11.0 - 11.5, (2) adding a solution of 13.3 g CNBr (Aldrich Chemical Co., Cedar Knolls, N. J.) in 117 ml of 1.88M dimethylformamide, and (3) holding the pH at 11.0 - 11.5 for 9 minutes. The activated sepharose was washed on a

sintered glass filter under suction with 2 liters water at 4 C, followed by 3 liters 0.1M bicarbonate, pH 9.0, then resuspended in 100 ml bicarbonate buffer and returned to the ice bath. While maintaining the pH of the activated sepharose at 9.0, 270 mg DEAE-purified bovine gamma globulin, 15 mg/ml in bicarbonate buffer, was added dropwise with stirring. The pH was held at 9.0 for one hour, then the sepharose-BGG slurry was stirred overnight at 4 C. The sepharose-BGG matrix was next washed under suction on a coarse sintered glass filter with 1 liter of 0.1M NH_4OH, pH 9.0, followed by a 2 liter wash with borate buffered saline (BSB). The washed matrix, resuspended in 100 ml of BSB, was poured into a 4.4 x 10 cm column and charged with an additional 500 ml of BSB. The immunoadsorbant column was ready for use.

Preparation of Specifically Purified Rabbit Anti-Bovine Gamma Globulin

The immunoadsorbant column was used to prepare specifically purified anti-bovine gamma globulin. The sepharose column was charged with 225 ml of pooled rabbit anti-BGG antiserum which had been dialyzed against five 4 liter volumes of borate buffered saline. The adsorbed serum eluted from the column at the rate of 0.5 ml per minute. The column was then flushed with 750 ml of BSB. The antibodies were eluted from the column by denaturation with 0.2M glycine-GCl buffer, pH 2.4. Fractions of 5 ml each were collected and the optical densities read at 280 nm. The pooled fractions containing the eluted antibodies were concentrated from 250 ml to 7.7 ml by vacuum dialysis and dialyzed against two 4 liter volumes of BSB at 4 C. The final concentration of the anti-BGG solution was 26.0 mg/ml, resulting in a yield of 200 mg of specific purified antibody.

This antibody solution was used to adsorb the immunoglobulin fraction from whole bovine serum.

Adsorption of Immunoglobulins from Whole Bovine Serum

One tenth milliliter of monospecific anti-BGG (26.0 mg/ml) was added to 0.1 ml of whole bovine serum (WBS). After incubation for 5 minutes at 37 C, the precipitate was pelleted by centrifugation at 3000 g for 5 minutes. Two additional 0.1 ml volumes of anti-BGG were added to the WBS with the same incubation and centrifugation time as above. The addition of a fourth 0.1 ml volume of anti-BGG resulted in only slight turbidity and was followed by incubation for 10 minutes at 37 C, and centrifugation for 5 minutes at 3000 g. To insure antibody excess, and thus complete antigen inactivation, a fifth 0.1 ml volume of anti-BGG was added, incubated for 30 minutes at 37 C and stored overnight at 4 C. The precipitate from the addition of 0.5 ml of anti-BGG was pelleted by centrifugation at 3000 g for 5 minutes. The whole bovine serum, anti-BGG mixture in the presence of the precipitate was diluted to 2.4 ml by addition of 1.9 ml BSB. The precipitate was resuspended in the diluted supernatant and repelleted by centrifugation at 3000 g for 5 minutes. The supernatant was removed. For the control, 0.1 ml bovine serum was adsorbed with normal rabbit gamma globulin, 26.0 mg/ml (supplied by Dr. L. Scott Rodkey, Division of Biology), and incubated sequentially with the bovine serum, anti-BGG mixture. Since the addition of 1.9 ml BSB resulted in a 1:25 dilution of WBS, the adsorbed sera were properly diluted for tube agglutination test.

Immunoelectrophoresis Procedures

The purity of the bovine gamma globulin and the specificity of the prepared anti-serum were checked by immunoelectrophoresis (Appendix, p. 44). Purified agar (Difco Laboratories, Detroit, Michigan) 1.25% in 0.025M sodium barbital buffer, pH 8.2, was used as the support medium (Appendix, p. 46). The protein samples were subjected to 30 V for 80 minutes in an ice cooled chamber (National Instrument Laboratories, Rockville, Maryland). The developing antisera were added and allowed to diffuse overnight in 100% humidity chamber, room temperature. The soluble protein was removed from the developed slides by washing at room temperature for 24 hours in BSB. The agar support medium was dried to a thin film under a wet paper towel and circulating air. The protein precipitin bands were stained with 0.5% light green stain in 7:2:1, 95% MetOH:H_2O: glacial acetic acid, followed by three washes with the same solvent.

RESULTS

Bovine Gamma Globulin Purification

The elution pattern of purified BGG from DEAE cellulose chromatography appears in Figure I. The purity of the BGG was checked by immunoelectrophoresis. Rabbit anti-bovine serum (Immunologic Research Supply Co., West Lafayette, Indiana) was used as the developing antiserum. Only immunoglobulins IgG_1 and IgG_2 were detected in the purified BGG used for rabbit injection and sepharose attachment (Figure II).

Rabbit Anti-BGG Preparation

The amount of precipitating antibodies present in the rabbit antiserum was checked by the quantitative precipitin test. Antibody levels at several periods after the first injection are shown in Table I. The amount of precipitating anti-BGG antibodies ranged from the pre-injection level of 0.0 mg/ml to 2.97 mg/ml at 59 days. The level of anti-BGG antibodies in pooled serum prior to immunoadsorption was 2.77 mg/ml.

Specifically Purified Anti-BGG Preparation

The specificity of antibodies eluted from the immunoadsorbant column was checked by immunoelectrophoresis (Figure III). The antibodies were specific for only the immunoglobulins of bovine serum.

Adsorption of Bovine Serum by Monospecific Rabbit Anti-BGG

Immunoelectrophoresis was used to check for complete adsorption of the immunoglobulins from bovine serum. Fetal calf serum, shown to be very

low in immunoglobulins was used for comparison (Pierce and Feinstein, 1965, Figure IV). The immunoelectrophoresis results (Figure IV) indicated complete adsorption of the immunoglobulins from bovine serum.

Tube Agglutination Test of Adsorbed Bovine Serum

The agglutination titers of bovine serum adsorbed with monospecific anti-BGG and normal rabbit gamma globulin were measured. Streptococcus bovis 124 and Staphylococcus aureus were used as bacterial antigens. Results appear in Table II. The agglutinating ability of the bovine serum was completely removed by adsorption with specifically purified anti-BGG. Adsorption with normal rabbit gamma globulin did not affect the titer.

Agglutination Titers of Bovine Serum Against a Variety of Bacterial Antigens

To examine the reactivity of the agglutinins of bovine serum, several strains of S. bovis, various other streptococcal species and bacteria genera were grown as previously described. The agglutination titer of bovine serum to each organism was measured by the tube agglutination test. The agglutination titer was near 1:200 for all organisms, except S. aureus, which was agglutinated to a dilution of 1:6400. Table III summarizes the results.

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FIGURE I. Elution Pattern of Purified BGG from DEAE Cellulose with 0.0175M Sodium Phosphate Buffer, pH 6.9.

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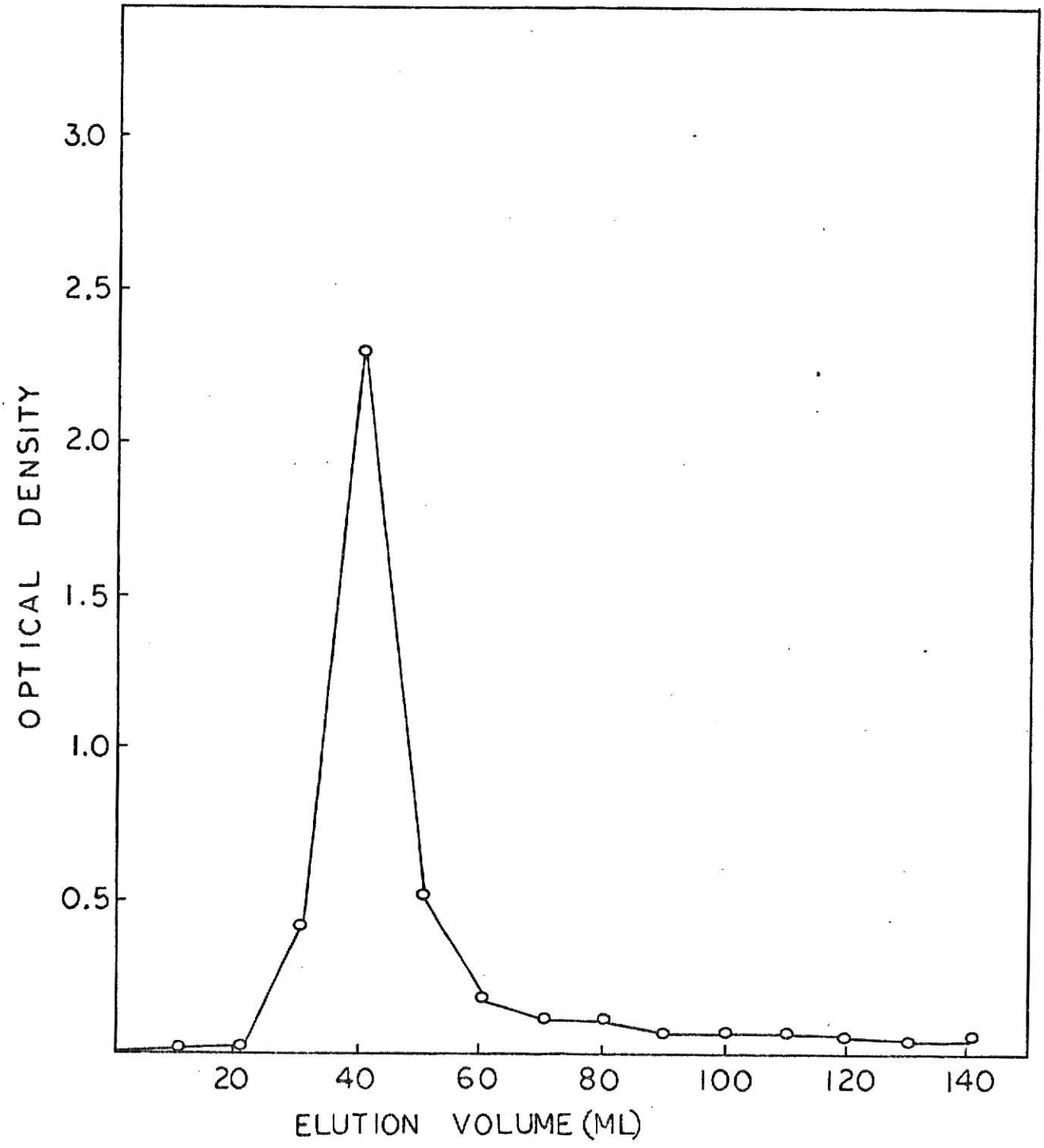
OPTICAL DENSITY
3.0
2.5
2.0
1.5
1.0
0.5
20
40
60
80
100
120
140
ELUTION VOLUME (ML)

FIGURE II. Purity of BGG
(F) BGG for sepharose attachment,
(G) Rabbit anti-whole bovine serum,
(H) Whole bovine serum, (I) BGG for
rabbit injection

FIGURE III. Specificity of Anti-BGG Antibodies
(J) Rabbit anti-BGG antibodies from
immunoadsorbant column, (K) Whole
bovine serum (WBS), (L) Rabbit
anti-WBS, (M) Rabbit anti-BGG serum

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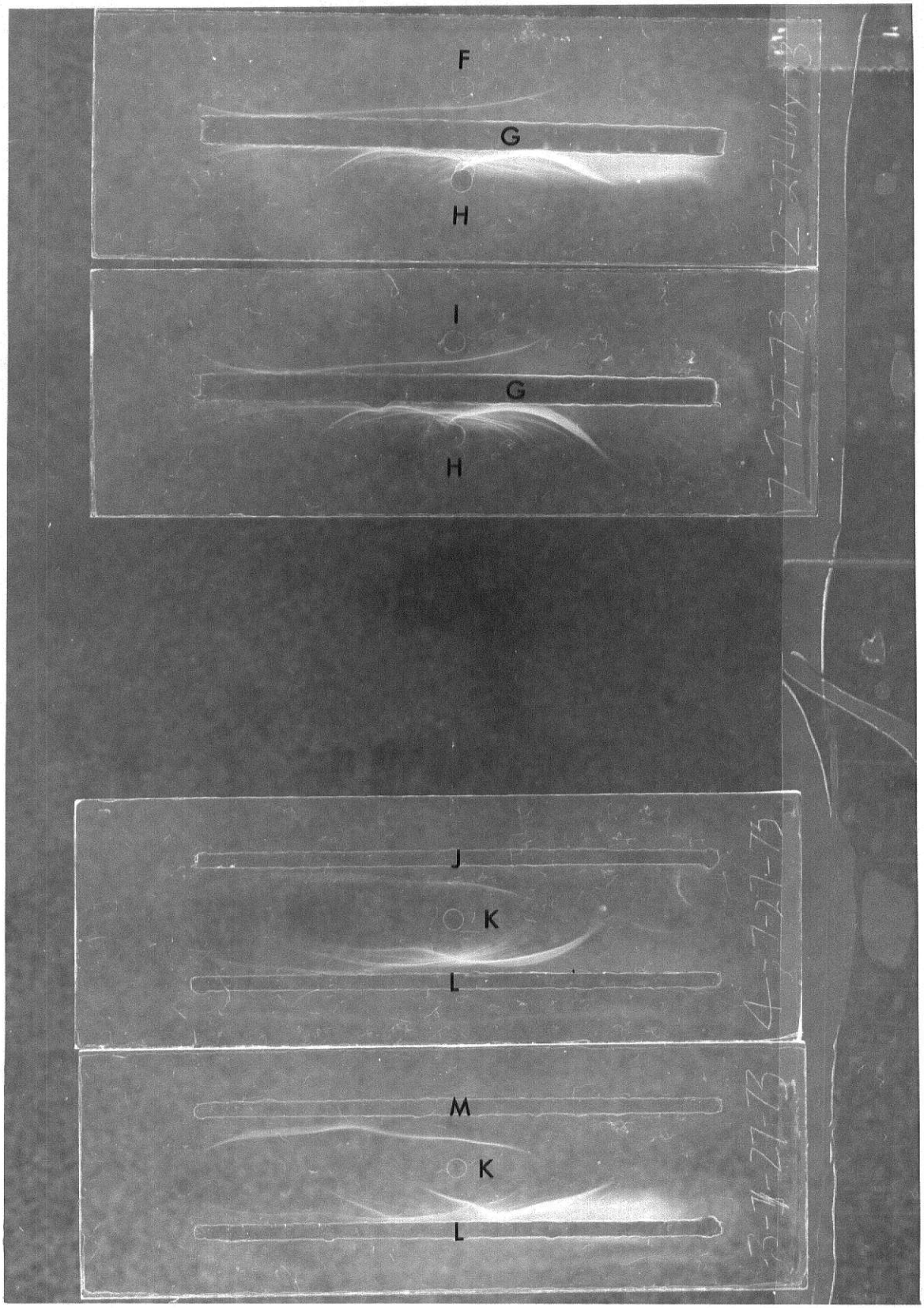
F
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FIGURE IV. Adsorption of Bovine Serum (A) Whole bovine serum (WBS), (B) Rabbit anti-WBS, (C) Fetal calf serum, (D) Purified BGG, (E) Adsorbed bovine serum

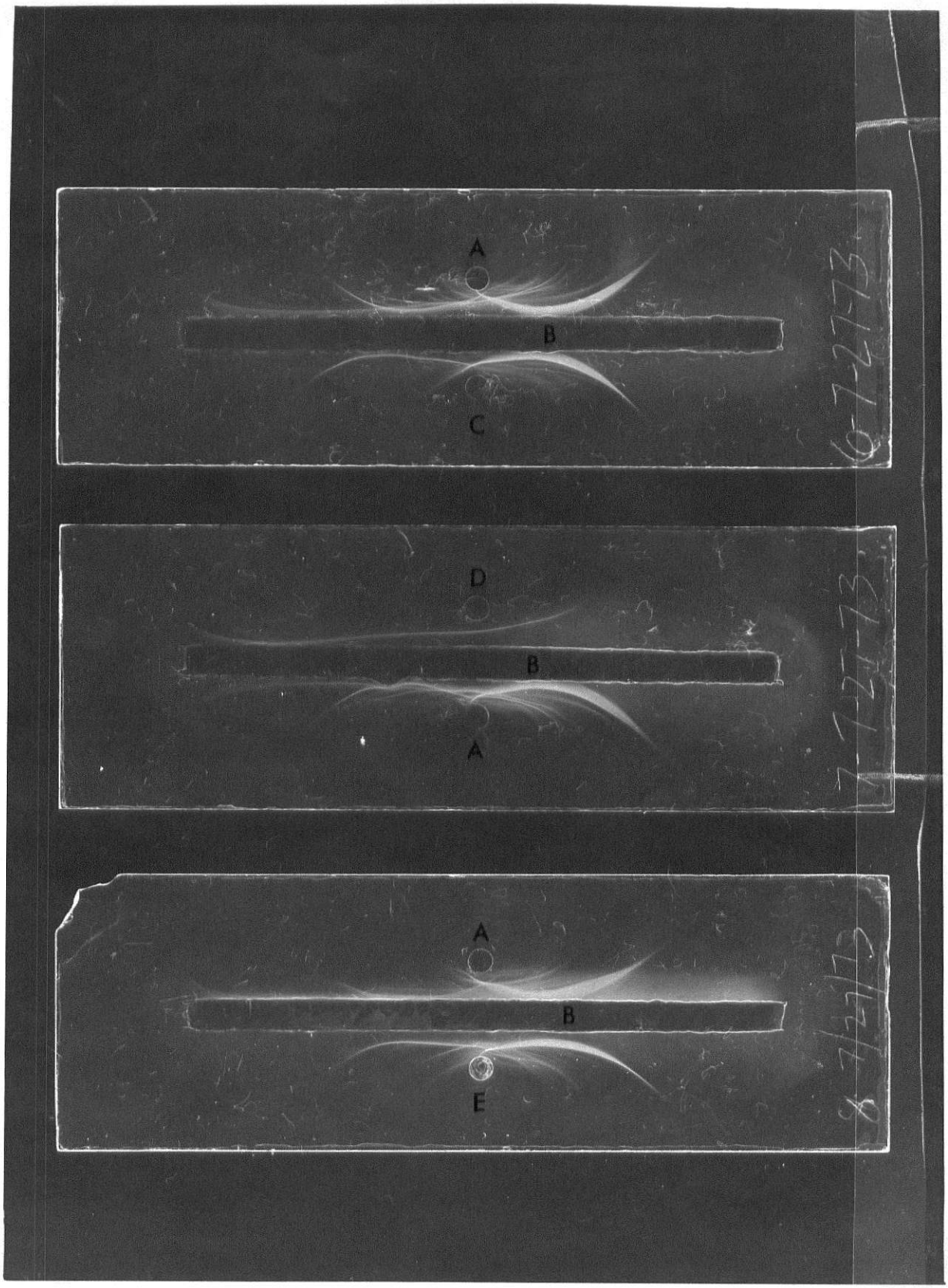
A
B
C
D
B
A
A
B
E

TABLE I

Quantitative Precipitin Test

Days after injection 1	mg/ml anti-BGG
0	0
14	0.39
21	0.37
31	1.68
59	2.97
Pooled Antisera	2.77

TABLE II

Reactions of Adsorbed Bovine Serum

Treatment of Bovine Serum	Agglutination Titer	
	S. bovis	S. aureus
Adsorbed with Anti-BGG	0	0
Adsorbed with normal BGG	1:200	1:6400

TABLE III

Agglutination Titer of Whole Bovine Serum to a Variety of Bacterial Antigens

Organism	Titer
Streptococcus bovis 124	1:200
Streptococcus bovis 18M2	1:200
Streptococcus bovis 2B	1:200
Streptococcus faecalis	1:200
Streptococcus faecium, var. *durans*	1:100
Streptococcus salivaris	1:100
Streptococcus lactis	1:100
Streptococcus sp, Group A	1:100
Streptococcus sp, Group B	1:100
Streptococcus sp, Group C	1:100
Streptococcus sp, Group D	1:200
Staphylococcus aureus	1:6400
Staphylococcus epidermidis	1:50
Escherichia coli	0
Proteus vulgaris	0
Bacillus subtilis	0
Serratia marcescens	0
Leuconostoc mesenteroides	0
Leuconostoc dextranicum	0

DISCUSSION AND CONCLUSION

A bacterial agglutinating factor is present in bovine serum. The factor was shown to be an immunoglobulin by first, preparing rabbit anti-bovine gamma globulin antiserum. Next, specifically purified anti-BGG antibodies were recovered from a sepharose immunoadsorbant column. Bovine serum was adsorbed with the specifically purified anti-BGG antibodies. The adsorbed serum no longer agglutinated Streptococcus bovis or Staphylococcus aureus. This indicated that the agglutinating factor was an immunoglobulin.

The immunoglobulin reacted with antigens on three strains of Streptococcus bovis, Streptococcus faecalis, Streptococcus faecium var. durans, Streptococcus salivaris, Streptococcus lactis, Streptococci sp. Groups A, B, C, D, Staphylococcus aureus, and Staphylococcus epidermidis. The antibodies showed no specificity for surface antigens of Escherichia coli, Proteus vulgaris, Bacillus subtilis, Serratia marcescens, Leuconostoc mesenteroids, and Leuconostoc dextranicum. The precision factor of two, inherent in the tube agglutination test, makes the slight titer variations among organisms insignificant, with the exception of S. aureus (Kabat and Mayer, 1964).

The surface structures on the reacting bacterial antigens are quite similar. S. bovis strain specificity involves structural differences in the cell wall and capsular carbohydrates (Medrek and Barnes, 1962; Deibel, 1964; Elliot, 1960). Purified cell wall and capsular polysaccharides of several S. bovis strains have been shown to cross react with strain specific antiserum (Kane and Karakawa, 1971). Streptococcus bovis, S. faecalis, and S. faecium var. durans belong to Lancefield Group D

(Deibel, 1964). The agglutination of Streptococcus lactis, a member of Group N and the streptococci of Groups A, B, C, makes it unlikely that the Group D antigen is a common antigen. The intracellular location of the Group D antigen a glycerol techoic acid, makes it quite inaccessible to agglutinating antibodies (Wicken et al., 1963, 1963a; Elliot, 1960). The cell wall carbohydrates of S. bovis S19, and Group A streptococci, and the ribitol techoic acid of Staphylococcus aureus share a β-N-acetylglucosamine determinant (Kane and Karakawa, 1969; Torii et al., 1964; Barkulis, 1966; Morse, 1962). The cell wall antigens of Groups A-H and K-S are quite similar in chemical composition (Slade and Slamp, 1962). Many gram-positive bacteria, including the hemolytic streptococci Groups A-G, K, L, N, Streptococcus salivaris, Staphylococcus aureus, several Bacillus species, and Leuconostoc mesenteroides, contain a common polyglycero-phosphate (techoic acid) antigen (McCarty, 1959).

A common antigen could be shared by all the reacting bacteria. A β-N-acetylglucosamine is a prevalent moiety in many of the cell wall carbohydrates. The techoic acids of gram-positive organisms cannot be excluded from the list of antigens possibly shared (Juergans et al., 1963; McCarty, 1959). The mucopeptides of all gram-positive bacteria are quite similar in chemical and structural composition, differing only in cross-linking peptides (Slade and Slamp, 1962; Krause and McCarty, 1961; Kane et al., 1969; Abdulla, 1965; Karakawa and Krause, 1966; Lehninger, 1970). The mucopeptides, though possibly masked, are a third universal component.

These similarities in the agglutinated bacteria suggest that one population of antibodies in bovine serum could be agglutinating all these bacteria. If this is the case, the agglutinating immunoglobulins could

be termed isoagglutinins (Weiner, 1951; Springer et al., 1959; Kabat and Mayer, 1964; Abdulla and Schwab, 1965; Decker et al., 1972). The much higher agglutination titer to S. aureus might suggest that a lower concentration of the common antigen is present on S. aureus. Another possibility is that different populations of antibodies are agglutinating the various bacteria. The specificity of the agglutinating antibodies should be established. This could be accomplished by adsorbing samples of bovine serum with one of the reacting organisms and then checking the agglutination titer of the adsorbed serum against all of the other organisms.

It has been suggested that ruminant conditions are particularly favorable for the formation of antibodies capable of agglutinating rumen organisms (Reiter and Oram, 1967). The agglutinating antibodies, shown to be present in bovine serum, were possibly induced by high concentrations of Streptococcus bovis often present in feedlot cattle (Sharpe et al., 1969; Gutierrez et al., 1959; Gall and Huhtanen, 1951; Phillipson, 1951; Bauman and Foster, 1956; Mishra, 1964; Meyer, 1969).

Salivation rates decrease considerably on low-roughage rations (Bailey and Balch, 1961; Wilson, 1963; Wilson and Tribe, 1963; Oltjen et al., 1965; Putnam et al., 1966; Meyer, 1969). Feedlot bloat studies show that salivation rates are lower in bloaters than non-bloaters (Mendel and Boda, 1961; Meyer et al., 1964; Meyer, 1969). Susceptibility to bloat has been shown to be a hereditary animal trait (Knapp et al., 1943; Bartley, 1958; Meyer, 1969). Mucinolytic bacteria of feedlot animals counteract the anti-frothing action of salivary mucin, therefore increasing the bloat-provoking effects of low salivation rates (Mishra et al., 1968; Meyer, 1969). Mucinolytic activity has been found to be higher in bloat

susceptible than in non-susceptible identical twin cows (Mishra et al., 1967). Gettings (1970) proposed that a saliva factor other than mucin could possibly affect the froth of feedlot bloat. He stated that agglutinating antibodies could have merit as a control of feedlot bloat in cattle. The mechanism suggested was prevention of capsule formation on Streptococcus bovis by secretion of salivary antibodies into the rumen. Ruminant levels of rhamnose-containing cell-free polysaccharides, present in most streptococci, have been correlated with rumen viscosity and bloat severity (Meyer and Bartley, 1971). Limited studies have not detected immunoglobulins in rumen wall secretions; therefore, in this discussion, the rumen wall is not considered as a source for anti-bacterial antibodies (Mach and Pahud, 1971).

The bovine immune system could be exposed to bacterial antigens in many ways. Microbial cells may be translocated across the rumen wall and eventually enter lymph nodes (Wolochow et al., 1966; Grys, 1966; Sharpe et al., 1969). Histological alterations and abscesses on the rumen wall are caused by low rumen pH levels in feedlot cattle (Bartley, 1971). Such abscesses could expose the immune system to rumen microorganisms through the blood vascular system. The plasma cells of the lamina propia known to secrete mostly IgA, may have been induced to produce anti-bacterial antibodies (Vaerman, 1970; Crabbe et al., 1968). Vaerman (1970) has shown that plasma muscosal cells of the dog account for as much as 80% of the IgA in mesenteric lymph. The IgA molecules are then transferred to the blood through the thoracic duct. If a similar transfer of IgA occurs in cattle, this would be a way of getting IgA antibodies specific for rumen microorganisms from the lamina propia to the serum. The nasal and respiratory mucosa are also vulnerable to bacterial antigen exposure. The

crowded conditions and poor waste removal practices which prevail in many feedlots provide an environment for exposure of the respiratory tracts of cattle to rumen bacteria.

If antibodies against rumen microorganisms can aid in the control of bloat, they must enter the rumen. Secretion of antibodies into the rumen through saliva raises questions of transport, salivary gland synthesis, antibody concentration, and rumen survivability of antibodies. These questions must be discussed. IgA has been found to be the predominant immunoglobulin in bovine saliva, a trend consistent with most mammalian exocrine secretions (Vaerman, 1970; Butler, 1971; Mach and Pahud, 1971a). Therefore, if anti-bacterial antibodies exist in bovine saliva, they are most likely from the IgA class. There is strong evidence to support that bovine serum IgA is of secretory origin as described by Vaerman (1970) for the dog (Kiddy et al., 1971). Selective secretion of immunoglobulins from serum to saliva may occur in a manner similar to the transport of IgG_1 from serum to milk (Kiddy et al., 1971). Synthesis of SIgA by bovine salivary glands has been indicated by several studies (Yurchak et al., 1971; Butler et al., 1972). The large amounts of saliva secreted by cattle have prompted workers to rate the bovine as one of the highest known producers of IgA (Mach and Pahud, 1971, 1971a). SIgA is able to resist digestive enzymes of the gastrointestinal tract (Tomasi and Bienenstock, 1968). The survival of bovine SIgA to the perhaps more severe conditions of the rumen is not known.

Streptococcus bovis agglutinating antibodies, shown to be present in bovine serum, may have a possible role in the control of feedlot bloat in cattle. S. bovis and other rumen microorganisms may be affected by antibodies entering the rumen in saliva. Anti-bacterial antibodies in

saliva would quite possibly be IgA and of secretory origin. The two most likely sources of salivary IgA are (1) selective secretion from serum to saliva, or (2) from localized synthesis in the salivary glands. If selective secretion of IgA occurs in the salivary glands, a promising source of the anti-bacterial antibodies would be plasma cells in the lamina propia of the rumen wall. If antibodies are synthesized in the salivary glands, it is more difficult to explain how salivary plasma cells were induced to produce antibodies specific for rumen bacteria. The anti-bacterial salivary antibodies could control bloat in several ways. Since IgA does not fix complement and to explain the presence of complement in the rumen would be difficult, bacterial lysis by complement is probably not a possibility (Tomasi and Bienenstock, 1968). If antibodies were present in sufficient numbers and antigenic sites on each bacterium were plentiful, the antibodies could mask the bacteria in a way which would prevent capsule attachment. The agglutination of the bacteria within the rumen could aid in the removal of the bacteria from the rumen and prevent accumulation of the bacteria in sufficient numbers to cause froth formation.

This speculative discussion has accumulated evidence to suggest that further work on the control of feedlot bloat by anti-bacterial antibodies is warranted. Establishment of the specificity of the agglutinating antibodies and a demonstration of specific antibodies in saliva and rumen fluid would add support to the speculated mechanism of feedlot bloat control presented in this manuscript.

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APPENDIX

MCFARLAND TURBIDITY SCALE

Material:

1. $BaCl_2$, Barium Chloride, 1% (w/w) in H_2O
2. H_2SO_4, Sulfuric Acid, 1% (w/w) in H_2O

Method:

McFarland Scale No.	ml. 1% $BaCl_2$	ml 1% H_2SO_4
1	1	99
2	2	98
3	3	97
4	4	96
5	5	95
6	6	94
7	7	93
8	8	92
9	9	91
10	10	90

TUBE AGGLUTINATION TEST

Material:

1. Suspension of Bacterial Antigen (150 Klett Units)
2. Phenolyzed Phosphate Buffered Saline (0.05M PO_4, 0.5% Phenol, 0.85% NaCl)
3. Antiserum

Method:

1. Label seven tubes (5 x 100 ml), add 0.5 ml of phenolyzed saline to tubes 2-7.
2. Dilute the antiserum 1:25 with phenolyzed saline.
3. Add 0.5 ml of diluted antiserum to tubes 1 and 2.
4. Mix tube 2, remove 0.5 ml and transfer to tube 3.
5. Repeat step 4 for tubes 3, 4, 5, and 6.
6. Discard 0.5 ml from tube 6.
7. Tube 7 contains only phenolyzed saline and is the control.
8. Add 0.5 ml of the bacterial antigen suspension.
9. Mix all tubes well.
10. Incubate tubes overnight at 37 C; observe the presence or absence of agglutination in each tube.

QUANTITATIVE PRECIPITIN TEST

Material:

1. Purified bovine gamma globulin, dialyzed against BSB (1.455 mg/ml).
2. Rabbit anti-BGG antiserum, dialyzed against BSB.
3. Borate buffered saline (0.05M, 0.85% NaCl).

Method:

Rough Quantitation

1. Label tubes 1-10; add 0.5 ml borate buffered saline to tubes 2-10.
2. Add 0.5 ml purified bovine gamma globulin to tubes 1 and 2.
3. Mix tube 2, remove 0.5 ml from tube, add to tube 3.
4. Repeat Step 3 for tubes 3-10, discarding 0.5 ml from tube 10.
5. Add 0.5 ml antiserum to each tube (1-10), and mix.
6. Incubate all tubes for 30 minutes at 37 C.
7. Centrifuge down precipitate and quantitate visually, and record interval of equivalence in terms of amount of antigen added.

Final Quantitation:

1. Label sufficient tubes to span the interval of equivalence in 10 µg increments.
2. Calculate the volume of purified BGG needed in each tube, to decrease the quantity of BGG by 10 µg increments.
3. Add the calculated volume to each tube.
4. Add BSB to each tube to bring the total volume to 0.5 ml.
5. Add 0.5 ml rabbit anti-BGG to each tube, and mix all tubes.
6. Incubate for 30 minutes at 37 C.
7. Centrifuge down precipitate, decant supernatant, wash all precipitates three times with 2 ml BSB.

QUANTITATIVE PRECIPITIN TEST Continued

8. Drain pellets well.
9. Add 1.0 ml 0.5N NaOH to each tube, mix to solubilize precipitates.
10. Read O.D. of each tube at 280 nm.
11. Calculate total protein in precipitates by $E^{1\%}_{1\ cm} = 15$.
12. Calculate antibody protein by subtracting amount of antigen in each tube from total protein.

IMMUNOADSORBANT COLUMN

Materials:

1. 1 liter 0.1M Sodium Bicarbonate buffer, pH 9.0. Make a 0.1M solution of Sodium Bicarbonate and adjust pH to 9.0, 4 C for use.

2. 1 liter distilled water, 4 C for use.

3. Sepharose 4B (Sigma), 15 ml or 15 g of packed beads, suspended in 15 ml of bicarbonate buffer.

4. Protein solution, 3 ml of 10 mg/ml in bicarb buffer.

5. Cyanogen bromide (Aldrich), to 2 g of CNBr add 15 ml of water and 2.5 ml of dimethylformamide (conc.). Prepare this reagent last and use only in well ventilated hood.

6. 200 ml 0.01M NH_4OH, adjust pH to 9.0.

7. 4M NaOH, 50 ml.

8. Borate buffered saline (0.05M borate, 0.85% NaCl, pH 8.0).

9. Increase reagents proportionally for larger volumes of sepharose.

Method:

1. Stir sepharose in ice bath under pH meter. Quickly adjust pH to 11-11.5 with 4M NaOH. Pour in CNBr solution. Maintain pH at 11.5 for 9 minutes.

2. Wash slurry under suction first with cold water, then with bicarb buffer. Resuspend sepharose in original volume by adding 15 ml bicarb buffer. Complete this operation as rapidly as possible.

3. Stir slurry in ice bath and add protein dropwise. Maintain pH at 9.0 for 1 hour then allow to stir overnight at 4 C.

4. Wash slurry with NH_4OH solution, then with borate buffered saline. This step may be done under suction or in a column.

5. Column is ready for use.

IMMUNOELECTROPHORESIS PROCEDURES

Material:

1. Electrophoresis chamber and slide mounting (National Instrument Laboratories, Rockville, Maryland)
2. Voltage source, warm up before use
3. Voltmeter
4. Die for cutting slide pattern (National Instrument Laboratories, Rockville, Maryland)
5. Vacuum pump
6. Pasteur pipettes, micropipettes, trough cutter
7. Barbital buffer (0.05 molar pH 8.2), 4 C
8. Borate buffered saline (0.05M buffer, 0.85% NaCl, pH 8.0)

Procedure:

1. Cut agar coated slides from petri dish with spatula and place on slide mounting.
2. With die, cut desired pattern.
3. Remove agar from circular wells with Pasteur pipette and vacuum pump.
4. Fill wells with protein solution.
5. Place slides in electrophoresis chamber containing cold barbital buffer and ice.
6. Wet and position paper wicks.
7. Cover the chamber, attach leads from voltage source, adjust voltage to 30 V.
8. Electrophorese for 80 min, check voltage periodically.
9. Shut off power, disconnect leads.
10. Remove slides, remove agar from troughs, add developing antiserum.
11. Allow the developing antiserum to diffuse overnight in a 100% humidity chamber at room temperature.

IMMUNOELECTROPHORESIS PROCEDURES Continued

12. Wash slides for 24 hours with several changes of borate buffered saline.

13. Dry slides by covering with wet paper towel and evaporating to dryness with circulating air.

14. Stain slides with 0.5% light green stain in 95% MetOH:H_2O:glacial acetic, 7:2:1, 15 minutes.

15. Rinse 3 times with MetOH:H_2O:glacial acetic, 7:2:1, 15 minutes each rinse.

16. Allow to dry, mount and label.

PREPARATION OF IMMUNOELECTROPHORESIS SLIDES

Material: (For 32 slides, 4/petri dish)

1. Purified agar (Difco), 150 ml, 2.5% in distilled H_2O, sterile.
2. Inoagar (Difco), 300 ml, 1.25% in distilled H_2O, sterile.
3. Barbital buffer, 0.05M, pH 8.2, 150 ml, sterile.
4. 8 sterile petri dishes.
5. 32 sterile, thoroughly clean or new slides, with etched numbers.

Method:

1. Mark the petri dishes and the table top to insure that the petri dishes are in the same position for both agar additions.
2. Add 35 ml of ionagar to each dish, allow to harden.
3. Position 4 slides on the agar of each dish. Leave a small space between slides and keep slides away from edge of dish.
4. Mix 150 ml of barbital buffer with 150 ml of purified agar.
5. Paint slide surfaces with 1:5 dilution of purified agar, buffer solution. Apply 3 coats, with drying between coats (hand hair dryer is useful).
6. Add 35 ml of purified agar-buffer solution to each dish, allow to harden.
7. Store petri dishes at 4 C in plastic bag with wet towels for moisture.

BACTERIAL-AGGLUTINATING IMMUNOGLOBULINS IN BOVINE SERUM: A POSSIBLE ROLE IN FEEDLOT BLOAT CONTROL

by

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A study was made of a factor present in bovine serum which agglutinated Streptococcus bovis. Previous work in this laboratory has shown this factor to be present in 100% of sera examined from twenty-eight animals. The factor was characterized, in the present investigation using immunological procedures. First, rabbit and anti-bovine gamma globulin (anti-BGG) was produced. Next, specifically purified anti-BGG antibodies were recovered from a sepharose immunoadsorbant column. Bovine serum was adsorbed with the specifically purified anti-BGG antibodies. The adsorbed serum no longer agglutinated Streptococcus bovis or Staphylococcus aureus. This showed that the agglutinating factor was an immunoglobulin.

The bovine serum agglutinated three strains of Streptococcus bovis, Streptococcus faecalis, Streptococcus faecium var. durans, Streptococcus salivaris, Streptococcus lactis, Streptococci sp. Groups A, B, C, D, Staphylococcus aureus and Staphylococcus epidermidis. The antibodies showed no specificity for Escherichia coli, Proteus vulgaris, Bacillus subtillis, Serratia marcescens, Leuconostoc mesenteroides and Leuconostoc dextranicum. Common antigens which are possibly shared by the agglutinated organisms are discussed.

It is proposed that the S. bovis-agglutinating antibodies in bovine serum have a role in the control of feedlot bloat in cattle. This hypothesis suggests that S. bovis and other rumen organisms may be affected by salivary IgA antibodies entering the rumen. It is speculated that the antibodies may counteract the froth formation by preventing capsule attachment on S. bovis or by agglutinating the organisms.