

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

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by

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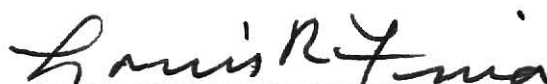
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To my wife

Mary Jane

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INTRODUCTION

The increasing practice of fattening cattle on high-grain rations has brought feedlot bloat, with its associated economic losses, to the fore-front of research. The etiology of feedlot bloat is relatively unknown, and established legume bloat treatments do not work effectively. The complexity of the feedlot bloat syndrome is indicated by factors known to have a bearing on the severity of bloat: heredity, feed mixtures, saliva flow, rumen physiology, and rumen flora (Meyer, 1972). However, recent studies have lead investigators to postulate that an animal factor may influence the severity of bloat (Hartman et al 1962, and Gettings, 1970). The use of a quaternary ammonium compound by Meyer (1972) has shown promise. However, clearance and assay problems must be solved to gain FDA approval for use on food animals.

The role of the bovine secretory immune system controlling bloat-provoking microorganisms has received limited attention. Gettings (1970) suggested that all bovine sera contain a factor which inhibited capsule formation of Streptococcus bovis, a prolific dextran-producing rumen organism implicated in feedlot bloat (Gutierrez, 1959). Investigation was continued into a possible salivary factor against the capsule of Streptococcus bovis, which may in turn affect feedlot bloat.

REVIEW OF LITERATURE

Bloat

Bloat is defined by Hungate (1966) as a build-up of pressure in the rumen. Bloat was then divided into two types: frothy and free-gas bloat, both of which prevent eructation of microbial fermentation gases, followed by distention of the ruminoreticulum (Dougherty and Habel, 1955). Jacobsen and Lindahl (1955) suggested that feedlot bloat might sometimes be of a free-gas type, and Hungate (1966) stated that frothy bloat had been observed in animals fed a high-grain ration. One definite difference between legume bloat and feedlot bloat is a time factor. Legume bloat may occur the first day of consumption of legumes, while feedlot bloat occurs only after prolonged feeding periods of concentrated rations. Feedlot bloat appears to have more confusing factors (listed earlier) involved in its etiology than does legume bloat.

Etiology of Feedlot Bloat

Hungate et al (1955) advanced the possibility that slime production by rumen organisms could be a factor in frothy legume bloat. Jacobsen and Lindahl (1955) further suggested that this slime traps fermentation gases and results in frothy digesta which then causes the feedlot bloat symptoms.

In studies of ruminal micro-flora, Bryant et al (1961) found little, if any, correlation between occurrence or numbers of any predominant groups of bacteria and feedlot bloat. However, Jacobsen et al

(1957) found a high positive correlation between percentages of encapsulated microorganisms and occurrence of bloat. Gutierrez et al (1959) and Yadava (1961) strongly implicated Streptococcus bovis and Peptostreptococcus elsdenii as causative agents. Hartman et al (1962), however, found little correlation between numbers of streptococci and bloat in the rumen of animals on bloat provoking rations. No information was given concerning attempts to study a possible increase in encapsulation of streptococci. He did find that exchange of ruminal contents between clinically bloating cattle and non-susceptible cattle resulted in reversion to their respective bloating conditions. This indicates that an animal factor, as well as a nutritional factor may be involved. Thus the etiology of feedlot bloat remains indefinite.

Saliva and Its Effects on Bloat

Historically, the only known prophylaxis for bloat has been to feed long hay along with the ration. Balch (1958) and Bailey (1959) have shown that fibrous feeds, such as hay, stimulate four to five times as much saliva per unit weight of feed consumed as do less fibrous feeds, e.g., fresh grass. Furthermore, non-bloaters secrete greater quantities of saliva than do bloaters. This trait appears to be heritable, as does bloat susceptibility (Mendel and Boda 1961, Meyer et al 1964, and Meyer, 1969). Van Horn and Bartley (1961) reported that saliva increased the rate of gas released in vitro from the frothy ingesta of legume bloat, and that reduced salivation was a definite factor in legume bloat etiology. Bartley and Yadava (1961) demonstrated that mucin in saliva was responsible for this gas release, and that some other animal mucins were equally effective anti-foaming agents, both

in vitro and in vivo. In studies of the short duration of this effect in vivo, Fina et al (1961) isolated rumen bacteria that destroyed salivary mucin and increased the severity of bloat. Mishra et al (1967) studied these mucinolytic bacteria and found that they were quantitatively greater in bloaters than in non-bloaters. They postulated that eating succulent feeds reduced saliva flow and that mucinolytic bacteria further lowered the amount of mucin able to break foaming. These mucinolytic bacteria included Butrivibro fibrisolvens, Selenomonas ruminatium, Streptococcus bovis, and Peptostreptococcus elsdenii. Reduction of saliva flow previously was considered to affect only rumen buffering capacity and to aid in swallowing and regurgitation (Boda 1961, Boda et al 1965, Hawkins et al 1965, and Olytin et al 1965).

Mucin, however, has not been shown to be effective on feedlot bloat and bloat guard (poloxaline¹) does not work effectively. (Bartley 1972). A recently developed quaternary ammonium salt has shown definite bloat controlling ability (Meyer, 1972). Other means of feedlot bloat control are unknown except for the possible natural bovine immune system as suggested by Gettings (1970) and Tillinghast (1973).

Streptococcus bovis

One of the organisms implicated in feedlot bloat is Streptococcus bovis (Gutierrez et al, 1959, and Yadava, 1961). It was known that carbon dioxide enhanced the growth of this organism (Macpherson 1955,

¹Smith, Kline, and French Laboratories, No. 18667, Phila. Pa.

Prescott et al 1955, Prescott and Stutts 1955), and Deilbel and Niven (1955) demonstrated that tween 80 would substitute for CO₂ as a growth stimulant. Dain et al (1956) showed that CO₂ also greatly enhanced dextran production and that tween 80 would substitute for CO₂ in aerobic slime production, but not anaerobic slime production. Barnes et al (1961) were unable to separate conditions for growth and dextran production in the presence of sucrose. Sensitive immunochemical cross-reaction tests with Type II pneumococcus antiserum were used, and it was postulated that other investigators were not using tests sensitive enough to detect the low amounts of dextran produced.

Bailey and Oxford (1959) found that dextran isolated from Streptococcus bovis grown in a liquid sucrose media was not a true capsule. The true capsule contained glucose, galactose, rhamnose, and uronic acid while the dextran consisted primarily of glucose and did not contain uronic acid or rhamnose. They recommended that growth of Streptococcus bovis for vaccines be carried out repeatedly in media free of glucose, fructose, and sucrose to avoid preparing the vaccine to the dextran rather than the cell itself.

Medrek and Barnes (1962) separated Streptococcus bovis into 12 serological types on the basis of type-specific carbohydrates. While Barnes et al (1961) reported that Streptococcus bovis dextran was not antigenic per se, Kane and Karakawa (1970) have shown that some Streptococcus bovis type-specific reactions are due to the capsular dextrans, which they refer to as glucans. They state that antigenic portions of the intact cell-glucan entity are the alpha 1-6 and alpha 1-4 linkages of the carbohydrate chains of the glucans. Their evidence also indicates

a chemical analogy between the capsular dextran and clinical dextran.

Streptococcus bovis classically is a Lancefield Group D streptococci, on the basis of its group reactions shared with Streptococcus lactis, durans, liquefaciens, faecalis, and faecium (Elliot, 1960). The Group D antigen has been identified as a peculiar type of glycerol-teichoic acid involving D-alanine (Wicken et al, 1963). The type-specific carbohydrates of Medrek and Barnes (1962) and Kane and Karakawa (1971) are not the Group D antigen as identified by Wicken et al (1963) (Deibel et al, 1964).

Bovine Immunoglobins

Three distinct classes of immunoglobulins, IgG, IgA, and IgM, are recognized in cattle (Butler, 1971). Of the two accepted sub-classes of IgG, IgG₁ predominates over IgG₂ until immediately before parturition. IgG₁ is transported from serum to lacteal secretions (Smith, 1946), but the presence of a transport component is debatable (Butler et al, 1971).

Bovine IgM is present in significant amounts in serum and colostrum and at low levels in saliva, milk, tears, and nasal secretions (Mach and Pahud, 1971).

Secretory IgA (SIgA) is the major immunoglobulin in saliva, lacrimal, and gastrointestinal secretions, and a minor component of colostrum. Butler et al (1972) has identified cells thought to produce SIgA in the ileum, duodenum, colon, lungs, thymus, salivary, and lacrimal glands of the cow.

The bovine secretory immune system appears to be similar to the human system, except for the specialized function of IgG transport

from serum to lacteal secretions (Mach and Pahud, 1971). The bovine follows the mammalian trend of IgA dominance in exocrine secretions. Cattle thus rate as one of the highest known producers of IgA due to their phenomenal production of saliva (Mach and Pahud, 1971).

MATERIALS AND METHODS

Saliva

Parotid saliva was collected with the aid and under the direction of Dr. Erle E. Bartley and Dr. Ronel Meyer from fistulated cattle of varying breeds and ages. Volumes of collection varied from 100 to 5000 milliliters and were made during various seasons of the year. The saliva was cleared by centrifugation in a Sorval RC2-B centrifuge at 8000 x g for 15 minutes. The clarified saliva was concentrated by vacuum dialysis, then dialyzed against physiological saline to remove urea and other low molecular weight molecules and ions. A usual procedure was to concentrate 100 to 200 times followed by storage at -4 C if not used immediately.

Blood

Blood samples were provided by Dr. Erle E. Bartley and allowed to clot, first at room temperature, then at 4 C for complete clot retraction. Centrifugation with a Servall angular head centrifuge at 5000 x g for 25 minutes cleared the serum. After holding at 56 C for 30 minutes the serum was stored at -4 C.

Organisms Used

Streptococcus bovis strain 7H4 was obtained from Dr. Marvin P. Bryant (University of Illinois, Urbana). Two strains, 18M2 and 2B, were obtained from Dr. Peter N. Hobson and Mr. S. O. Mann (Rowett Research Institute, Aberdeen, Scotland). Escherichia coli, Staphylococcus aureus and Streptococcus lactis, faecalis, durans, lique-

faciens, faecium, and salivarius, were laboratory stock cultures. Finally, Dr. George L. Marchin (Kansas State University) donated streptococcus strains representing Lancefield Groups A, B and C. All cultures were maintained on trypticase-soy agar slants containing 0.5% soluble starch. Heavily encapsulated streptococcus cultures were prepared by growth in thioglycolate broth or trypticase-soy broth, both with 5-10% added sucrose. Incubation was at 37 C for 12-24 hours in a CO₂ and H₂ atmosphere (Gaspaks²) in a Brewer anaerobic jar. Minimally encapsulated cultures were prepared on plates of nutrient agar incubated aerobically for 12 hours at 37 C.

Antigen Preparations

1) Whole Cell

Encapsulated cells were centrifuged from broth at 5000 x g on a Servall angular head centrifuge for 10 minutes, washed three times and resuspended in physiological saline containing 0.5% formalin. Unencapsulated cells were scraped from the agar plates into a small amount of saline, mixed well, centrifuged, washed and resuspended in formalized saline as above. The cell concentrations were adjusted to 60 Klett units (Klett-Summerson) and used immediately.

2) Capsule

After centrifugation and resuspension as in whole cell preparations, the broth-grown cells were homogenized at medium speed in a Waring blender for 10 minutes. Cells were removed by centrifugation and the super-

²BBL, Division of BioQuest, Cockeysville, Maryland. 21030

nate was concentrated 50 to 100 times by vacuum dialysis. The concentrate was purified using ethanol precipitation methods described by Kabat and Mayer (1961). A modified procedure is given in the appendix.

Fractionation of Serum and Saliva

A diethylaminoethyl cellulose (DEAE-cellulose) column (30 x 400 mm) was prepared using DEAE-cellulose (0.88 meq-gm) (Sigma Chemical Corporation). The cellulose was hydrated in distilled water according to Young (1966) and excess water removed by sinter glass filtration over a partial vacuum. The cellulose was then washed successively with 0.25 M NaOH, distilled water, 0.1 M HCl, distilled water, the starting eluant of the chromatography, and then packed under 0.5 to 1.0 meter water pressure into glass columns. Samples applied were either 30-50 ml of serum or concentrated saliva. In either case, the sample was dialyzed against the starting buffer of the elution prior to the chromatographic procedure. Elutant volumes were 300 ml per step, except where noted below, and each fraction was concentrated 10 to 25 times by volume, then dialyzed against physiological saline plus 0.05 M phosphate buffer, pH 7.0 (PBS). Elutant buffers were:

- 1) 0.005 M Tris-HCl pH 8
- 2) 0.01 M Tris-HCl pH 8
- 3) 0.02 M Tris-HCl pH 8
- 4) 0.04 M Tris-HCl pH 8
- 5) 0.04 M Tris-HCl pH 8 + 0.04 M NaCl
- 6) 0.04 M Tris-HCl pH 8 + 0.08 M NaCl
- 7) 0.04 M Tris-HCl pH 8 + 0.14 M NaCl
- 8) 0.04 M Tris-HCl pH 8 + 0.20 M NaCl
- 9) 0.04 M Tris-HCl pH 8 + 1.00 M NaCl

(From Vaerman, 1970)

At times an elution gradient was prepared using a liter of buffer #8 mixing into a liter of buffer #1, followed by a 500 ml wash of #8. A gradient formed by mixing a liter each of buffer #9 into buffer #8 was used to complete the separation. The two pooled fractions were vacuum concentrated, dialyzed against PBS, and used in the following tests whenever fractions #1-8 or #8-9 are referred to as a sample. These fractions were stored at -4 C until used.

Antigen-Antibody Tests

1) Complement-fixation

In preliminary experiments to determine the presence of salivary antibodies, the standard complement-fixation test was employed, with the exception of extending the first incubation period³ to 12 hours at 4 C rather than 30 minutes at 37 C (Kabat and Mayer, 1961).

Saliva and serum preparations from four heifers of varying breeds were tested unaltered. Preliminary tests showed that 7H4 cells were the best encapsulated cells, and antigen preparations of these cells were used (as described above).

2) Bacterial Tube Agglutination

A standard bacterial tube agglutination test (see appendix) was used. In each case the sample was diluted stepwise as shown, and

³Complement-fixation tests using bacterial antigens require longer incubation periods. (Carpenter, 1956).

antigens prepared as described. Samples used were one of the fractions of serum or saliva prepared as discussed earlier. A titre was established as that dilution at which complete agglutination occurred.

3) Capillary Precipitin

A capillary precipitin test similar to the one described by Swift et al (1943) was employed to test the purified capsular material described against serum, saliva, and pooled fractions of each. No attempt to quantitate was made, since only the presence or absence of a specific reaction was being determined.

Isolated Streptococcus bovis 18M2 capsular material was first purified by Sephadex G-25 filtration (Fig. 1). Serial dilutions were then drawn into 1 x 100 cm capillary tubes followed by serial dilutions of fractions to be tested in order to approximate optimal proportions. Air spaces were left above and below (but not between) the fluids. The ends of the tubes were sealed with modeling clay, and they were placed vertically into a wood block with the sera layer up (Greenblast et al, 1971). A hand lens was used to view the tubes for precipitin formation immediately, after two hours, and after 24 hours, all at 37 C.

4) Ouchterlony Double Diffusion

To avoid problems with equivalence points often encountered in precipitin tests, Ouchterlony double-diffusion plates were prepared according to a method described by Kabat and Mayer (1961). Figure 2 shows how the wells were arranged. The central well was charged with individual fractions of sera or saliva, and the outer wells were

charged with serially diluted capsular preparations from Sephadex G-25 filtration (marked as tube a, Fig. 1) with all fractions previously dialyzed against the borate buffer used to make the diffusion agar (see Fig. 2). The test was repeated with different dilutions of serum and saliva fractions in the central well, with serially diluted capsular material in the circumferential wells.

Capsule Inhibition

Experiments were designed to test whole serum, pooled fractions of serum, concentrated saliva, and pooled fractions of saliva for their ability to prevent or inhibit encapsulation of Streptococcus bovis. The tested fractions were mixed with thioglycolate medium (plus 5% sucrose) in final dilutions ranging from 1:10 to 1:25. Controls were: a) the same medium plus an amount of 0.05 M phosphate buffer, pH 7.0, to equal the dilution in the test tubes; b) sterility controls on the fractions tested; and c) normal rabbit sera plus medium diluted as in the tested fractions. Tubes were inoculated from overnight broth cultures and incubated at 37 C under CO₂ and H₂ as before. Samples were taken every two hours to monitor the process microscopically, and quantitatively via a total hexose test.

Following centrifugation and washing several times in buffer, cells were resuspended in distilled water to equal concentrations as determined by a Bausch and Lomb Spectronic 20 at 420 nm. Serial dilutions of these cells were then tested for total hexose using the phenol-sulfuric method of Dubois et al (1956) (see appendix). Results were used to quantitatively compare microscopic examination with the amount of hexose present by using a glucose standard curve.

Fig. 1: Sephadex G-25 filtration of capsular material from Streptococcus bovis 18M2 prepared according to Kabat and Mayer (1961). The column measured 1.0 x 90 cm, and elution was by distilled water at 4 ml/hr. Volume of eluent is plotted (abscissa) vs. optical density (ordinate) at 220 nm using a Gilford spectrophotometer, model 240.

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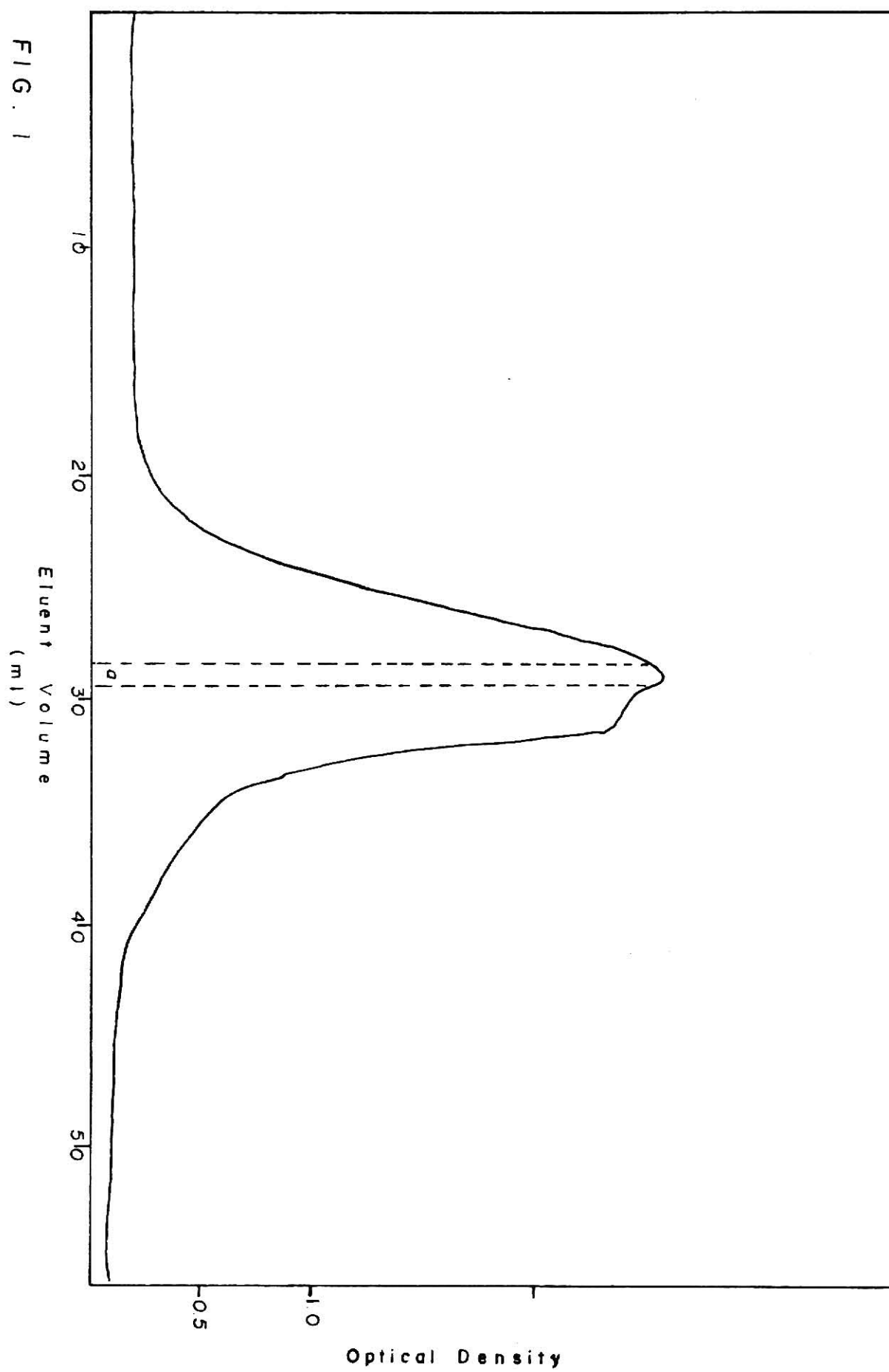


Fig. 2: Ouchterlony plates used as described by Kabat and Mayer (1961). Agar base was 2% Ionagar in Clark and Lub's (1917) borate buffer, pH 8.2, plus physiological saline. The dimensions shown are in millimeters.

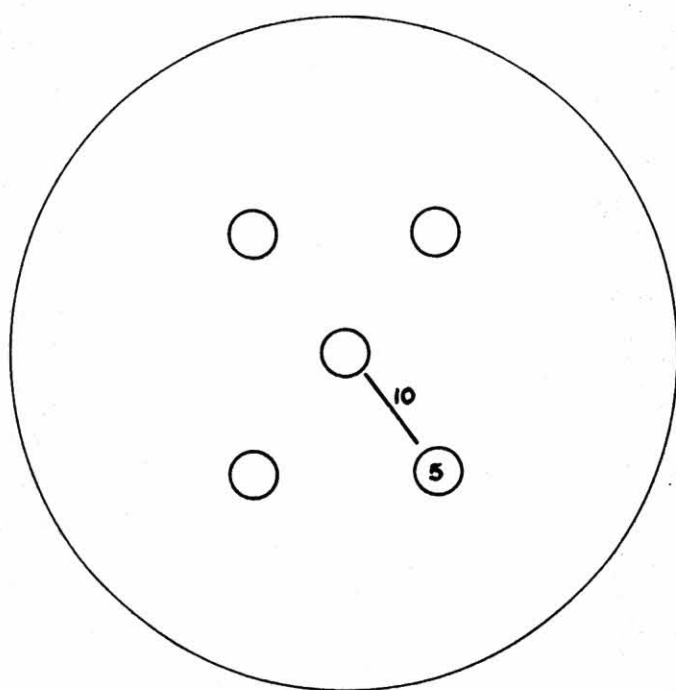


FIG. 2

Capsule Stripping

Experiments were designed to test serum and saliva fractions for their ability to strip capsular material from Streptococcus bovis cells. Encapsulated cells were harvested by centrifugation from broth, washed four times in 0.5% formalized saline, and resuspended in this saline to a concentration of 40 Klett units. Two ml of cells were mixed with 0.1 ml volumes of either serum, serum fractions, saliva, or saliva fractions, and incubated at 37 C for 48 hours. Controls were cells alone plus 0.05 M phosphate buffer, pH 7, fractions alone with the buffer, normal rabbit serum alone with the buffer, and normal rabbit serum incubated with cells. Following incubation, cells were removed from the test tube liquid by membrane filtration (0.45 micron). The filtrate was then tested for total hexose (Dubois et al, 1956).

Purified capsular material was tested in a similar manner. A distilled water suspension of purified Streptococcus bovis 18M2 capsular material (marked as tube a, Fig. 1) was incubated with 0.1 ml of the above fractions, and a blood micro-glucose test (see appendix) was performed for liberated glucose (Park and Johnson, 1949). Controls were capsule alone, fractions alone in buffer, and normal rabbit serum alone in buffer and with the capsular suspension.

Adsorption of Serum and Saliva Agglutinating Factor(s)

Several streptococcus species were tested for their ability to adsorb the tube agglutinating factor present in serum and saliva fractions. Streptococcus species used were: bovis (18M2, 2B, 7H4), lactis, faecalis, and salivarius, all grown in broth as described. Escherichia coli was

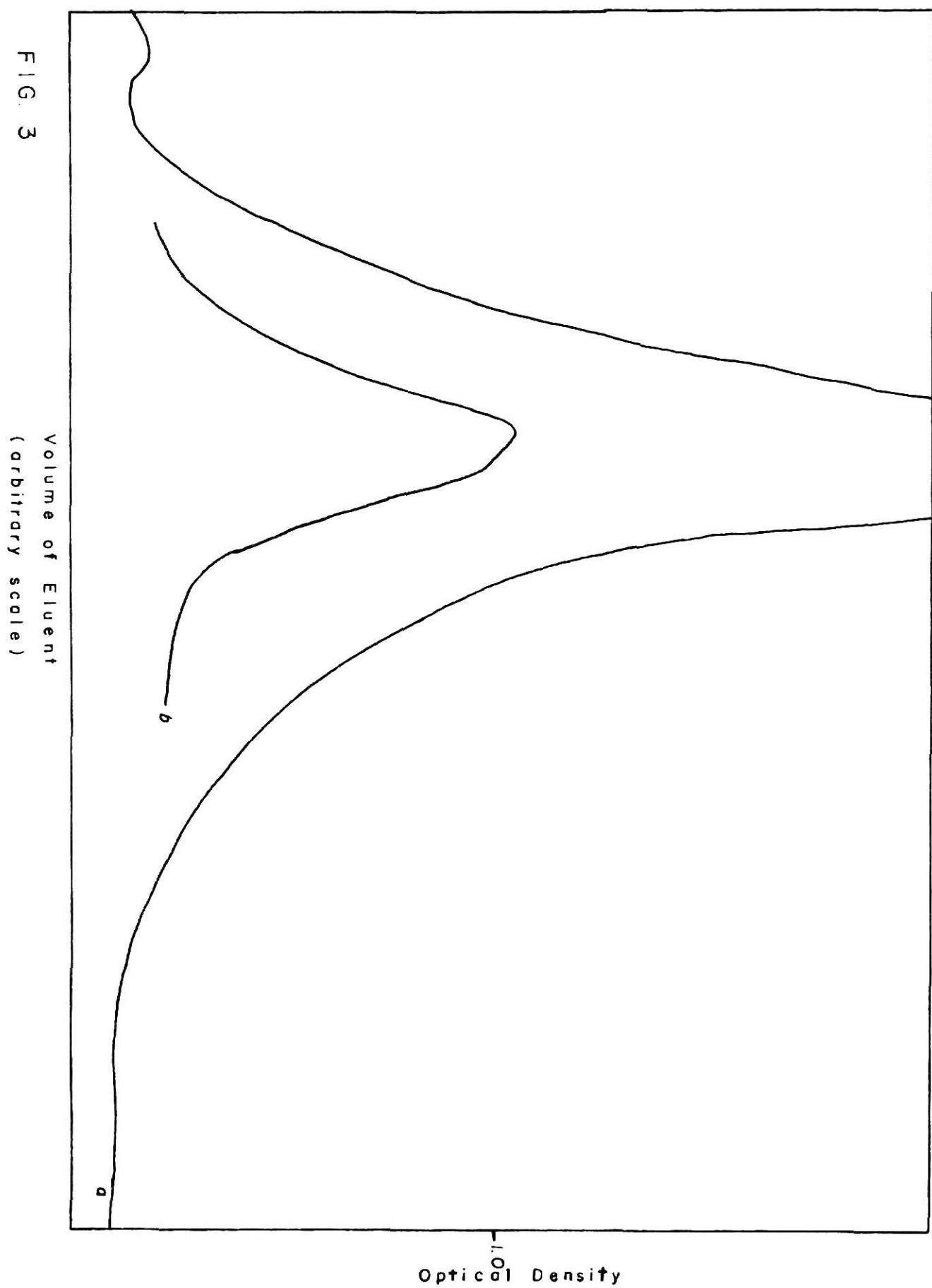
grown in nutrient broth with shaking at 37 C. Cells were washed repeatedly to free them of broth, and then resuspended in 0.5% formalized saline, and incubated at room temperature for 12 hours to kill the cells. Cells were centrifuged at 5000 x g for 20 minutes and 4 ml of serum or saliva fractions were added individually to the packed cells. Following incubation at 4 C for 24 hours and centrifugation to remove the cells, adsorption was repeated with freshly packed formalized cells. This procedure was continued with the entire series of cells and sera until the Streptococcus bovis 18M2 tube agglutination titre in the serum was adsorbed by the 18M2 organism. Following centrifugation, adsorbed sera and saliva fractions were tested, as described earlier, for their tube agglutination titre versus several antigens.

Adsorption of Serum and Saliva Agglutinating Factor(s) by Sephadex G-25

Two ml of serum and/or saliva (5 mg protein/ml) were passed through Sephadex G-25 columns (1.0 x 90 cm) on a recycling basis at 4 C. After the fourth passage at 4 ml/hr, fractions were collected and the A₂₈₀ peak was concentrated to the original volume applied (see Fig. 3). Serum and saliva kept at 4 C served as controls, as did Proteus OX19 antisera⁴ which was passed through an identical column. Serum and saliva were used along with controls in tube agglutination tests, and in capsule inhibition tests, as described.

⁴Difco Laboratories, Detroit, Michigan

Fig. 3: Sephadex G-25 gel filtration of bovine serum and saliva using PBS as elutant. Column measured 10 x 900 mm and elution was at 4 ml/hr. Absorbance was read at 280 nm (Gilford spectrophotometer). Curve a is for serum and curve b is for saliva.



Elution of Factor(s) Removed by Sephadex G-25

Tests were performed to assay for removal of material by Sephadex. Two ml of highly titred serum (titre to Strept. bovis 18M2) was recycled overnight at 4 ml/hr through a 2.0 x 40 cm Sephadex G-25 column. Following exhaustive washing with PBS, 0.1 M glycine buffer pH 2.5 was added to the column and fractions were collected and read at A_{280} (Gilford Spectrophotometer) (Fig. 4). Tubes reading >0.05 were pooled, concentrated to 2 ml by vacuum dialysis, and dialyzed against PBS. The eluent was then used in bacterial tube agglutination tests against Strept. bovis, faecalis, lactis, and salivarius.

After clarification, 600 ml of saliva was vacuum concentrated to 15 ml and added to the Sephadex column for recycling overnight at 4 ml/hr. Following PBS washing and glycine elution as above, fractions were read (A_{280} ; Fig. 4), concentrated to 0.5 ml, and dialyzed against PBS (all steps at 4 C). The dialysate was then used in tube agglutination tests against various antigens listed above.

Anti-BGG Adsorption of Sephadex Isolated Factor(s) in Serum or Saliva

One tenth ml of serum glycine eluent (1.2 mg/ml) was adsorbed by 0.1 ml anti-bovine gamma globulin (0.6 mg/ml) prepared in this laboratory (Tillinghast 1973). Following incubation for 5 minutes at 37 C, the precipitate was pelleted at 3000 x g for 5 minutes. Addition of another 0.1 ml anti-BGG resulted in no turbidity. The pellet was resuspended, incubated for 10 minutes at 37 C, and finally overnight at 4 C.

Fifteen hundred milliliters of cleared saliva was chromatographed on DEAE-cellulose as described. Pooled fraction #1-8 was concentrated

Fig. 4: Glycine elution of Sephadex G-25 adsorption of serum (a) and saliva (b). Column measured 16 x 40 mm and flow rate was 4 ml/hr. Volume eluted is plotted verses A_{280} .

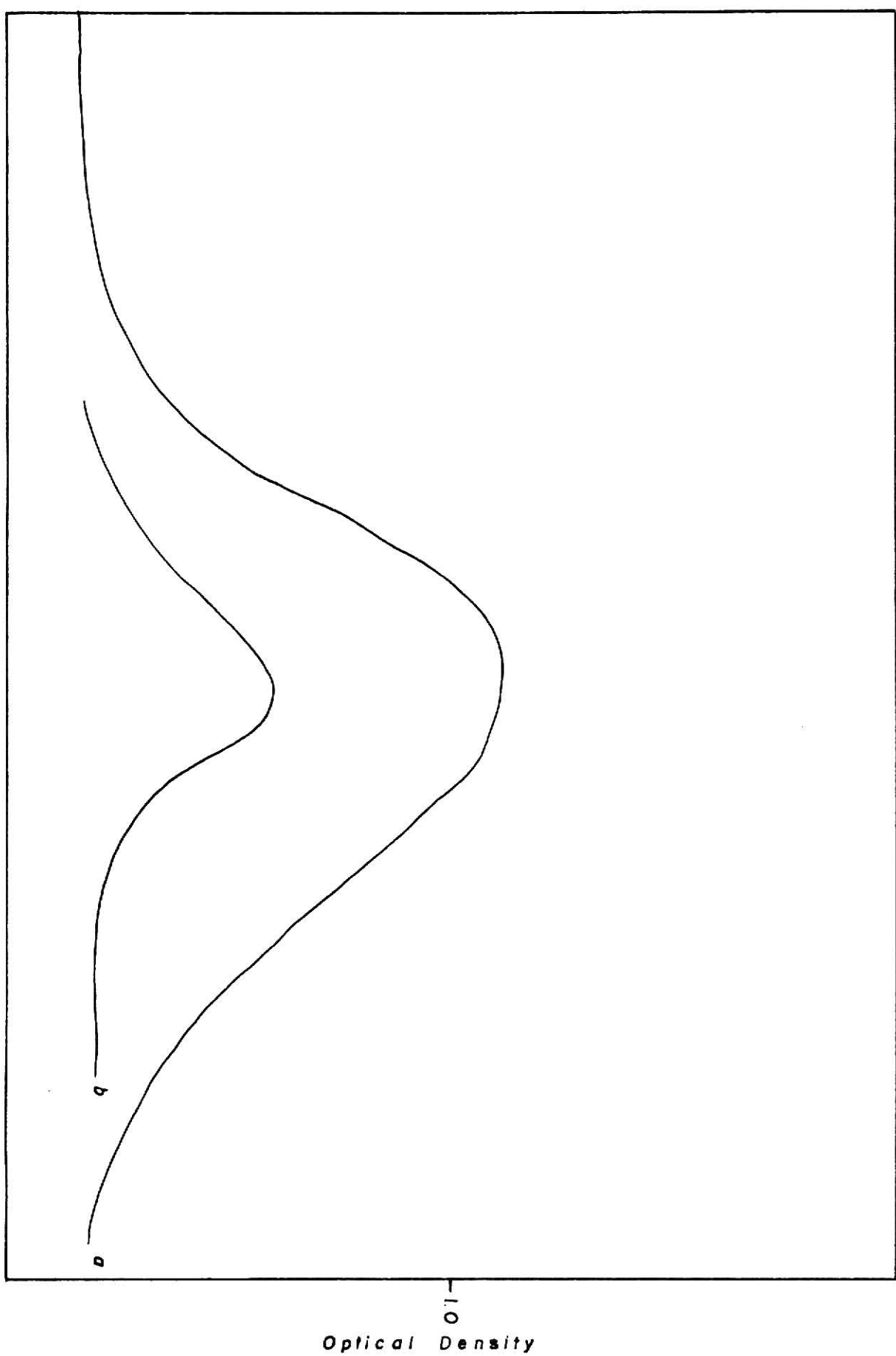


FIG. 4

Volume of Eluent
(arbitrary scale)

Optical Density

to 100 ml by vacuum dialysis, dialyzed against PBS, and added to a Sephadex G-25 column (2.0 x 40 cm) for recycling overnight at 4 ml/hr. All steps were performed at 4 C. Two tenths ml (1.9 mg/ml) from 1 ml of concentrated, dialyzed (PBS) glycine eluent was adsorbed by 0.1 ml of anti-BGG. Addition of another 0.1 ml following incubation and centrifugation as above, showed no increased turbidity. The pellet was resuspended and incubated as above.

Following centrifugation at 3000 x g for 5 minutes, the supernates were used in tube agglutination tests. For controls serum and saliva isolated factors were mixed with normal rabbit gamma globulin (2.6 mg/ml), incubated as above, and used in tube agglutination tests.

RESULTS AND DISCUSSION

Separation of Serum and Saliva into Fractions

The separation of bovine serum into fractions was accomplished as described by Vaerman (1970). In this procedure, buffers 1-7 elute IgG₁; 6-8 elute IgG₂; 7-8 elute IgM; and 7-8 elute IgA. Because of this overlap, gradients of 1-8 (pooled immunoglobulins) and 8-9 (little or no immunoglobulins) were often used. A more detailed description of this procedure as well as its implications and justification is found in Vaerman (1970).

The same procedure was used on bovine saliva, with agglutination tests performed to see where the factor eluted. The factor was found to elute in buffers #1-8 as was the case in serum. Saliva was then routinely separated by the same procedure used for serum. This DEAE-chromatography also served as a clarification system for saliva. Upon concentration, saliva becomes quite dark and viscous with accumulation of feed particles not removed by centrifugation. Use of millipore filtration for clarification failed due to viscosity of the solution.

Complement-Fixation

Table 1 shows saliva's small, but perceptible, titre to the encapsulated test organism 7H4, supporting preliminary findings (Gettings, 1970). Neither serum nor saliva shows any titre to unencapsulated cells of the same strain. From this small sample, it would appear that there is a correlation between higher serum titre and higher saliva titre.

Table 1. Titre of 7H4 against saliva and serum by the complement-fixation test.

Experimental ₁ animal	<u>Serum titre to 7H4</u>		<u>Saliva titre to 7H4</u>	
	capsules		capsules	
	+	-	+	-
34D	1:320	-	1:40	-
35D	1:160	-	1:10	-
36D	1:320	-	1:40	-
37D	1:160	-	1:20	-

¹The number shown is the number given by Dr. E. E. Bartley.

Bacterial Tube Agglutination Tests

Table 2 shows tube agglutination titres of bovine normal sera tested against several organisms. A precision factor of two, inherent in the tube agglutination tests, makes the slight titre variations among these sera insignificant, as is the variation in each individual serum's titre to different organisms.

Table 3 shows a representative serum's titre to various antigens. Also included are representative saliva fractions versus the same antigens. The DEAE-cellulose fractions of serum shown were concentrated back to the original applied volume and dialyzed against PBS. With saliva it was necessary to concentrate the fractions 400-600 times the applied volume, the concentration necessary varying from animal to animal. For this reason saliva is reported as the number of milligrams of protein per milliliter after concentration in the following tests.

Capillary Precipitin Tests

No precipitin reaction was found in any capillary tube of the dilution series of purified capsular material versus the dilution series of the serum and saliva fractions.

Ouchterlony Double Diffusion Tests

There was no evidence of any reaction in the series of plates using purified capsular material versus the tested saliva and serum fractions.

Table 2: Bacterial tube agglutination tests of various bovine sera against several Streptococcus bovis strains used as antigens.

Table 2.

Experimental animal ^a	Antigen ^b		
	1	2	3
94B	1:200	1:100	1:100
115B	1:200	1:200	1:200
130B	1:400	1:200	1:200
169B	1:200	1:100	1:100
25C	1:200	1:200	1:200
46C	1:200	1:100	1:200
98C	1:200	1:200	1:200
285C	1:400	1:200	1:200
295C	1:400	1:400	1:400
11D	1:400	1:400	1:400
34D	1:400	1:400	1:400
35D	1:400	1:400	1:400
36D	1:400	1:400	1:400
37D	1:200	1:200	1:200
47D	1:200	1:200	1:200
48D	1:400	1:200	1:400
68D	1:200	1:400	1:200
69D	1:400	1:400	1:400
161E	1:200	1:400	1:400
169E	1:200	1:200	1:200
173E	1:200	1:200	1:200
174E	1:200	1:200	1:100
190E	1:200	1:100	1:200
194E	1:200	1:200	1:200
109F	1:200	1:100	1:200
110F	1:200	1:100	1:100
112F	1:400	1:200	1:200
113F	1:400	1:200	1:100
116F	1:200	1:100	1:200
118F	1:400	1:200	1:200
fetal calf serum	-	-	-

^aNumbers are the ones used by Dr. E. E. Bartley.

^bAntigens used were:

1. Streptococcus bovis 18M2 grown in trypticase-soy broth plus 5% sucrose.
2. Streptococcus bovis 7H4 grown as above.
3. Streptococcus bovis 2B grown as above.

Table 3. Bacterial tube agglutination titres of various fractions of saliva and serum to several antigens.

Table 3.

Fraction	Antigen ⁴		1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6	7a	7b
normal bovine serum			1:400	-	1:400	-	1:100	-	1:400	-	1:200	-	-	1:400	-
serum #1-8			1:400	-	1:400	-	1:100	-	1:400	-	1:200	-	-	1:400	-
serum #8-9			-	-	-	-	-	-	-	-	-	-	-	-	-
whole saliva ¹			-	-	-	-	-	-	-	-	-	-	-	-	-
saliva #1-8 ²			1:100	-	1:200	-	1:100	-	1:100	-	1:50	-	-	1:200	-
saliva #8-9 ³			-	-	1:50	-	-	-	1:25	-	-	-	-	1:25	-
fetal bovine serum			-	-	-	-	-	-	-	-	-	-	-	-	-

¹0.2 mg/ml protein by biuret method

²7.2 mg/ml protein by biuret method

³33.0 mg/ml protein by biuret method

⁴Antigens used were:

- 1) Streptococcus bovis strain 7H4 grown in broth (a), and on agar plates (b)
- 2) Streptococcus bovis strain 18M2 grown in broth (a), and on agar plates (b)
- 3) Streptococcus bovis strain 2B grown in broth (a), and on agar plates (b)
- 4) Streptococcus lactis grown in broth (a), and on agar plates (b)
- 5) Streptococcus faecalis grown in broth (a), and on agar plates (b)
- 6) Escherichia coli grown in nutrient broth
- 7) Streptococcus salivarius grown in broth (a), and on agar plates (b)

Capsule Inhibition

In viewing cultures for any decrease in encapsulation, no method used showed any significant reduction by serum or saliva. Microscopic detection of microbial capsules is at best haphazard. Techniques used vary from organism to organism, and researcher to researcher, with more established techniques involving India ink, nigrosin, and Congo red, all negative-staining techniques. White (1947) suggests using an alcoholic methylene blue positive stain in addition to Congo red negative staining. None of these methods gave consistent or satisfactory results. Darkfield and phase-contrast microscopy were more convenient and less time consuming, and were on par with staining techniques for results.

Physiologically, addition of these materials to cultures resulted in an increase in cell numbers over non-serum controls--up to five times as many cells with normal sera diluted 1:20 by media.

The quantitative method selected (Bubois, 1956) also failed to show any real reduction in encapsulation by normal bovine serum or by saliva at a sensitivity of 10 $\mu\text{gm/ml}$. The apparent difference found between the non-serum control and the bovine serum test was negated by the normal rabbit serum control (unpublished data).

Capsule Stripping

There was no detectable release of glucose from the purified capsule of Streptococcus bovis 18M2, at a level of sensitivity of 2-10 μgm (Bark and Johnson, 1949), by any fraction of saliva or serum, nor was there any evidence of a spontaneous breakdown.

Furthermore, no fraction shows any ability to strip capsular material from whole organisms (Table 4) at a level of sensitivity of 10 ugm/ml (Dubois, 1956).

Adsorption of Serum and Saliva Agglutinating Fractions

The adsorption of normal serum by Streptococcus bovis cells required approximately 10^{10} cells for complete removal of agglutinating titre. Table 5 shows results of bacterial tube agglutination test on adsorbed sera and saliva in various combinations with several antigens.

The table shows clearly that all the tested streptococci (exceptions noted below) adsorbed the titre to Streptococcus bovis 18M2, and also, Streptococcus bovis 18M2 adsorbed the titres to these other species. Only E. coli did not adsorb any titre. Of interest is the fact that the titre to Staphylococcus aureus was also adsorbed by the streptococci species, but that not all of the Streptococcus salivarius titre was adsorbed, nor was any of the Group C streptococci titre.

Adsorption of Serum and Saliva Agglutinating Factor(s) by Sephadex G-25

The passage of serum and saliva through Sephadex G-25 columns removed the titre to Streptococcus bovis. Serum and saliva controls kept at the same experimental temperature retained their titres, as did antisera to Proteus OX19 that was passed through an identical Sephadex G-25 column. Table 6 illustrates these results.

Table 4: Capsule stripping tests using various serum and saliva fractions against three antigens.

Table 4.

Tube	tested fraction (0.1 ml)	organism (2 ml)	ml PO_4 Buffer pH 7	ugm hexose
1	normal serum	18M2	0.9	-
2	normal serum	7H4	0.9	-
3	normal serum	2B	0.9	-
4	normal serum	-	2.0	-
5	serum #1-8	18M2	0.9	-
6	serum #1-8	7H4	0.9	-
7	serum #1-8	2B	0.9	-
8	serum #1-8	-	2.9	-
9	serum #8-9	18M2	0.9	-
10	serum #8-9	7H4	0.9	-
11	serum #8-9	2B	0.9	-
12	serum #8-9	-	2.9	-
13	saliva #1-8 ¹	18M2	0.9	-
14	saliva #1-8	7H4	0.9	-
15	saliva #1-8	2B	0.9	-
16	saliva #1-8	-	2.9	-
17	saliva #8-9 ²	18M2	0.9	-
18	saliva #8-9	7H4	0.9	-
19	saliva #8-9	2B	0.9	-
20	saliva #8-9	-	2.9	-
21	rabbit serum	18M2	0.9	-
22	rabbit serum	7H4	0.9	-
23	rabbit serum	2B	0.9	-
24	rabbit serum	-	2.9	-
25		18M2	1.0	-
26		7H4	1.0	-
27		2B	1.0	-
28		-	3.0	-

¹Cellulose fractions 1-8 concentrated to 5 mg/ml

²Cellulose fractions 8-9 concentrated to 4.4 mg/ml

Table 5: Bacterial tube agglutination tests of bacterial-adsorbed normal bovine sera and saliva versus several antigens.

Table 5.

Antigen ¹	versus	sera adsorbed by bacterial cells ¹						
		1	2	3	4	5	6	7
1		-	-	-	-	-	-	1:400
2		-	-	-	-	-	-	1:400
3		-	-	-	-	-	-	1:200
4		-	-	-	-	-	-	1:400
5		1:25	1:25	1:25	1:25		1:25	1:200
6		-	-	-	-	-	-	1:200
8		-	-	-	-	-	-	1:100
9		-	-	-	-	-	-	1:50
10		-	-	-	-	-	-	1:50
11		1:100	1:100	1:100	1:100	1:100	1:100	1:100

Antigen ¹	versus	saliva adsorbed by bacterial cells ¹						
1		-	-	-	-	-	-	1:50
2		-	-	-	-	-	-	1:50
3		-	-	-	-	-	-	1:50
4		-	-	-	-	-	-	1:50
5		-	-	-	-	-	-	1:50
6		-	-	-	-	-	-	1:50
8		-	-	-	-	-	-	1:25
9		-	-	-	-	-	-	-
10		-	-	-	-	-	-	-
11		-	-	-	-	-	-	-

¹Antigens and bacterial cells employed were:

1. Streptococcus bovis 18M2
2. Streptococcus bovis 7H4
3. Streptococcus bovis 2B
4. Streptococcus faecalis
5. Streptococcus salivarius
6. Streptococcus lactis
7. Escherichia coli
8. Staphylococcus aureus
9. Group A streptococci (Courtesy Dr. George Marchin)
10. Group B streptococci (Courtesy Dr. George Marchin)
11. Group C streptococci (Courtesy Dr. George Marchin)

Elution of Factor(s) Removed by Sephadex G-25

Following concentration back to the applied volume and dialysis against PBS, the serum eluent showed a titre to Strept. bovis, faecalis, lactis, and salivarius (Table 6). This test differentiates between two possible Sephadex actions: denaturation of the factor responsible for the titre to Streptococcus bovis, or actual removal of the factor.

Figure 4 shows that there was indeed some protein retained by the Sephadex, and that 0.1 M glycine buffer pH 2.5 could elute this material.

Saliva showed similar results except that a comparison of curve a to curve b in Fig. 4 shows a difference in amount of material present. Tube agglutination tests showed that the material adsorbed by Sephadex was responsible for the titre to Strept. bovis, faecalis, lactis and salivarius (Table 6).

In quantitative studies, the glycine eluent from two ml of serum was calculated to be 0.8 mg protein/ml (using $E_{1\text{cm}}^{1\%} = 15$), while the total from 600 ml of saliva was 0.75 mg protein (0.0013 mg/ml).

Adsorption of Sephadex Isolated Factor(s) in Serum and Saliva

Table 7 shows titres of serum and saliva fractions before and after adsorption with anti-BGG. In all cases any titre, that was present after addition of RGG, was removed by anti-BGG.

Table 6: Bacterial tube agglutination tests of several antigens versus serum and saliva, before and after adsorption by Sephadex G-25.

Table 6.

sample tested	Antigen ¹						
	1	2	3	4	5	6	7
Normal bovine serum Before adsorption	1:400	1:400	1:200	1:400	1:500	1:200	1:100
Normal bovine serum After adsorption	-	-	-	-	-	1:50	-
Glycine eluent of the Sephadex column	1:200	1:200	1:200	1:200	1:200	1:25	1:50
Concentrated saliva (5gm protein/ml)	1:50	1:50	-	1:50	1:50	1:50	-
Concentrated saliva After Sephadex (to applied volume)	-	-	-	-	-	-	-
Glycine eluent of Sephadex column (1.1mg protein/ml)	1:100	1:100	1:100	1:100	1:100	1:100	-

¹Antigens used were:

- 1,2,3 Streptococcus bovis 18M2, 7H4, and 2B respectively
 4 Streptococcus faecalis
 5 Streptococcus lactis
 6 Streptococcus salivarius
 7 Staphylococcus aureus

Table 7. Titres of serum and saliva glycine fractions before and after adsorption with anti-BGG.

Table 7.

sample tested	Antigen ¹						
	1	2	3	4	5	6	7
Glycine eluent of G-25 column + RGG	1:200	1:200	1:100	1:200	1:200	-	1:50
Glycine eluent of G-25 column + anti-BGG ³	-	-	-	-	-	-	-
Saliva-glycine eluent of G-25 column + RGG	1:100	1:100	1:50	1:100	1:100	1:50	-
Saliva-glycine eluent of G-25 column + anti-BGG	-	-	-	-	-	-	-

¹Antigens used were:

- 1,2,3 Streptococcus bovis 18M2, 7H4, and 2B respectively
 4 Streptococcus faecalis
 5 Streptococcus lactis
 6 Streptococcus salivarius
 7 Staphylococcus aureus

CONCLUSIONS

These experiments confirm the existence of a serum factor involving Streptococcus bovis, and prove the existence of a salivary factor capable of agglutinating the organism. In both cases these agglutinins show cross-reactions among various streptococci. The chemical basis for these cross-reactions is probably a dextran-like cellular component, as shown by Sephadex adsorption experiments. Contrasting aerobically grown cells with broth-sucrose grown cells leads to the conclusion that the 'extra-cellular slime' is responsible for the reactions.

Of some doubt though is the fact that these reactions should be considered cross-reactions. It is possible that specificity to dextran is very good, and all these cross-reacting streptococci share dextran. Tests indicate that Streptococcus salivarius cross-reacts with these agglutinin factors. Strept. salivarius primarily produces levan, but most strains produce an insoluble dextran as well (Niven et al, 1941a, 1941b). True levan is antigenically dissimilar to dextran, so either the agglutinin is nonspecific or Strept. salivarius dextran is responsible for the agglutination. Of all organisms tested only Group C streptococci demonstrated a serum and saliva titre not associated with this dextran-agglutinating immunoglobulin.

The only serum tested that was without Streptococcus bovis titre was fetal calf serum, which is deficient in gamma globulins. Adsorption of normal bovine serum with anti-bovine gamma globulin (anti-BGG) changes serum to the immunological equivalent of fetal calf serum -- resulting in no titre.

Capillary precipitin and Ouchterlony double diffusion tests gave negative results using both serum and saliva against purified capsular material. Apparently the isolated capsular material was of insufficient size or configuration to be recognized by the immunoglobulins. Sephadex was recognized but is truly massive compared to any isolatable dextran molecule.

Quantitative analysis of the immunoglobulins, as isolated by Sephadex, shows saliva contains a relatively small amount compared to serum. When the 100-300 liters of saliva produced daily (Bailey, 1959 and Balch, 1958) are considered, saliva appears to contribute a significant amount of immunoglobulin. However, Kabat and Mayer (1961) have demonstrated that a likely optimal ratio of antibody to free polysaccharide is 100:1 (weight to weight). Thus the 150-400 mg of parotid immunoglobulin produced daily could only react with 1.5 - 4.0 mg of free polysaccharide.

Extrapolation of bacterial adsorption studies reveal that salivary immunoglobulin could agglutinate 3×10^{11} - 1×10^{12} cells. A bloating bovine may contain 1.5×10^{14} Streptococcus bovis cells (Bartley, 1973b). A bloat affect by parotid saliva alone is doubtful considering cell division rates. Furthermore, in vitro adsorption tends to minimize the amount of antibody attached/cell and in vivo reactions require a large excess of antibody. The amount of antibody necessary to agglutinate an antigen in vitro is quite small compared to the amount necessary to completely react in vivo.

Submaxillary saliva is produced equivalently with parotid saliva during eating periods (Bailey and Balch, 1961). Submaxillary saliva produce 5 times as much protein as parotid saliva (Phillipson and

Mangan, 1959). The relative amount of immunoglobulin produced is unknown.

Secretory IgA (SIgA) is the major immunoglobulin occurring in saliva (Vaerman, 1970 and Butler, 1971). SIgA, like serum IgA, does not fix complement. A very good complement-fixation titre for serum against Strept. bovis, and a much lower (possibly zero) titre for saliva, substantiates the probability of salivary immunoglobulins being SIgA and not transduced IgG from serum.

Tests for capsule inhibition and/or capsule stripping were negative, either due to low sensitivity of the quantitative test, or to the fact that there was no action by saliva or serum. Comparison of agar grown and broth grown cells showed that the latter had 40% more cell glucose (unpublished data). Any significant reduction by the factor would have narrowed this gap. Incubating serum with organisms in thioglycolate medium did result in a drastic increase in final cell numbers. Quantitative glucose tests on turbidometrically matched cell samples showed that these cells had much less glucose. Rabbit serum controls, however, equaled or bettered this reduction, showing a physiological and nutritional growth factor for Strept. bovis rather than any action by immunoglobulins, or other factors, on capsules. Incubation of concentrated saliva with organisms in thioglycolate broth had no such effect, probably because of a lack of nutritional enrichments. Furthermore, use of Sephadex-adsorbed agglutinins of both serum and saliva in these inhibition studies yielded no lowering of total glucose content (unpublished data).

The source of inducement of this factor is a matter of speculation. It may or may not be of microbial inducement. Obviously this has not

been shown. Studies involving complete removal of dextran from the environment of a new born calf would need to be performed to separate inducement and natural origin theories of production. It is noted that natural antibodies tend to be principally IgM (Decker et al, 1971), and little IgM is found in saliva (Vaerman, 1970 and Butler 1971).

Tillinghast (1973) speculated on means of possible exposure for microbial inducement of serum immunoglobulins by rumen bacteria. He considered translocation of microbes across the rumen wall and entry to lymph nodes as a strong possibility. Vaerman (1970) reports that 80% of canine serum IgA is produced by the lamina propria of intestinal mucosa. Tillinghast (1973) suggested a similar action could occur in ruminants. It is a short speculative step from serum IgA to transduction into saliva with the addition of a secretory piece (to make SIgA). Other possible means of inducement include nasal and respiratory mucosa. Butler et al (1972) has shown these areas have the capability to produce immunoglobulin, and feedlot conditions make the possibility of exposure a probability. Direct stimulation of salivary tissue by rumen microorganisms contained in the regurgitate is another possibility.

If immunoglobulins against rumen microorganisms are to aid in bloat control, they must enter, and survive, in the rumen. Entry of SIgA is easily visualized, but entry of serum immunoglobulins is harder to comprehend. Histological alterations and abscesses on the rumen wall could allow exchange of microbes and immunoglobulin between the rumen and the vascular system (Bartley, 1973). This is highly unlikely to occur to any extent. Tomasi and Bienenstock (1968) report that SIgA is relatively resistant to gastrointestinal enzymes. Its survival potential under rigorous rumen conditions is unknown and warrants

study. There are no reports on any attempts to isolate immunoglobulins from the rumen.

Another open question is the mechanism of possible action by immunoglobulins towards control of feedlot bloat. Bacterial lysis is probably not a possibility as IgA does not fix complement which in turn would be hard to explain in the rumen. Tillinghast (1973) suggested that immunoglobulins introduced in the rumen before onset of feedlot bloat, could serve to mask bacteria in such a way as to prevent capsule formation. Results of this study exclude this possibility.

In summary, serum and saliva have an immunoglobulin system that can agglutinate, in vitro, micro-organisms which exude a dextran slime. Many of the reactions to other organisms reported in bovine sera are probably due to cross-reaction with this system. The significance of this factor in vivo is unknown.

Immunoglobulins in saliva are probably SIgA of unknown inducement and place of origin. The immunoglobulin is present in all sera tested. Agglutination of dextran by this immunoglobulin shows that many organisms previously thought not to produce dextran are now known to do so. The means of action of the normal bovine immunogenic system on controlling feedlot bloat is not established, but can only be a source of speculation. Parotid saliva alone probably does not control feedlot bloat. The amount of immunoglobulin produced by submaxillary glands is unknown and warrants study before discarding salivary antibody control of feedlot bloat as a viable hypothesis.

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APPENDIX

Bacterial Tube Agglutination Test

1. For each sample of serum to be tested, label 5 agglutination tubes consecutively from 1 to 5.
2. With B.A.I. pipettes add the following amounts of the serum to be tested to each tube as follows:

Tube	Amount	Dilution
1	0.08 ml	1:25
2	0.04	1:50
3	0.02	1:100
4	0.01	1:200
5	0.005	1:400

3. Pipette 2 ml of antigen into each serum tube and also 2 ml of antigen to an empty tube as a control, or use the antigen with another serum such as normal rabbit serum as was done in these experiments.
4. Mix the tubes well and incubate at 37 C for 42-48 hours.
5. Read and record results as follows:
 - + indicates complete agglutination--no button of cells in the bottom of the tube
 - indicates no agglutination--no appearance of fluffy sediment
 - I indicates incomplete agglutination--some buttoning, but less than control

The highest dilution showing complete agglutination is the titer of the serum.

Weil-Felix Reaction

1. Prepare serial dilutions in 0.5 ml amounts of the suspected serum and Bacto-Proteus Control Serum in Kahn tubes in the following manner:
2. Place eight Kahn tubes in a rack for each serum to be tested.
3. Pipette 0.5 ml of 0.85 per cent sodium chloride into the last seven tubes.
4. Prepare a 1:10 dilution of the serum by adding 0.5 ml of serum to 4.5 ml of 0.85 per cent sodium chloride. Mix thoroughly.
5. Add 0.5 ml of the 1:10 serum dilution to tubes #1 and #2.
6. Mix the contents of tube #2 and transfer 0.5 ml of the mixture to tube #3. Mix thoroughly.
7. Continue carrying the 0.5 ml of the serum dilutions through tube #7. Discard 0.5 ml from tube #7 after mixing thoroughly. Tube #8 is the antigen control tube.
8. Add 0.5 ml of Bacto-Proteus Antigen to each of the eight tubes.
9. Shake the rack to mix the antigen-serum mixture.
10. Place the rack in a water bath at 37 C for two hours followed by storage in the refrigerator overnight.
11. Record agglutination by observing the tube for complete or partial agglutination. Complete agglutination is noted when the supernatant fluid is cleared of bacterial cells and by clumping of the organisms in large blue flakes which settle rapidly to the bottom of the tube when agitated. Partial agglutination is noted by an incomplete clearing of the supernatant with small clumps of organisms. A negative reaction is noted as in tube #8 where no clumping is visible and the organisms remain in suspension.

Quantitative Hexose Test

1. Match bacterial cell concentration at A_{280} on a Bausch and Lomb Spectronic 20.
2. Serially dilute each series of cells by mixing 1 ml into 1 ml of distilled water in the following tube, repeat until discarding 1 ml from the last tube. Use 1 ml of distilled water as a blank.
3. Add 1 ml of 5% phenol to each tube.
4. Add 5 ml of concentrated sulfuric acid with a large opening pipette. Mix gently on a vortex stirrer.
5. Let stand for 10 minutes at room temperature.
6. Cool in water bath to 23 C.
7. Read tubes at A_{280} in a Spectronic 20.
8. Calculate the amount of glucose from a standard curve made by serially diluting a stock solution of glucose.

(modified from Dubois, 1956)

Reducing Sugars Submicrodetermination

General Principle:

The ferrocyanide formed from ferricyanide by the reducing sugar present in the sample is measured colorimetrically by its combination with Fe^{+3} (yielding Prussian blue).

Procedure:

1. Pipet 1 ml of sample into a centrifuge tube and add 2-3 drops of 10% trichloroacetic acid.
2. Mix well and centrifuge for 5 minutes at low speed.
3. Transfer the deproteinized sample into a clean graduated borosilicate test tube and bring the volume to 3 ml.
4. Set up a series of standards by pipetting 3 ml of different concentrations of glucose standards (2,4,8,10 $\mu\text{g}/\text{ml}$) into different clean test tubes.
5. Set up a blank by pipetting 3 ml of water into a tube.
6. Add 1 ml each of carbonate cyanide solution and ferricyanide solution into each tube.
7. Mix well and leave for 15 minutes in a boiling water bath.
8. Cool the tubes well and add 5 ml of ferric iron solution, mix.
9. After 15 minutes, read the color against the reagent blank at 690 m μ .
10. Determine the unknown concentration from a plot of the standards concentration versus adsorbance.

Preparation of Reagents:

1. 10% trichloroacetic acid
Weigh 10 g of trichloroacetic acid into a 100 ml volumetric flask and bring to the mark with distilled water.
2. ferricyanide solution
Weigh 0.5 g of potassium ferricyanide into a one liter volumetric flask and bring to mark with distilled water. Store in a brown bottle.

3. carbonate-cyanide solution
Weigh 5.3 g of sodium carbonate and 0.65 g of potassium cyanide into a one liter volumetric flask and bring to volume with distilled water.
4. ferric iron solution
Weigh 1.5 g of ferric ammonium sulfate and 1 g of Duponol into a one liter volumetric flask and bring to volume with 0.05 N sulfuric acid.
5. 0.05 N sulfuric acid
Dilute 0.14 ml concentrated sulfuric acid to 100 ml with distilled water.

(after Park and Johnson, 1949)

Polysaccharide Capsule Preparations

1. Broth grown cells were washed three times in physiological saline followed by centrifugation at 3000 x g for 10 minutes.
2. The cells were resuspended in enough distilled water to cover the blades of a Waring blender and homogenized at medium speed for 10 minutes.
3. Cells were removed by centrifugation (500 x g for 10 minutes).
4. The supernate was concentrated by vacuum dialysis as necessary.
5. Chloroform and n-butyl alcohol was added to the solution in a 1:5 and 1:25 ratio respectively, and shaken mechanically for 1-2 hours.
6. Following centrifugation at low speed for 30 minutes, the aqueous layer (top) was sucked off and the chloroform-butyl alcohol treatment repeated. This treatment continued until no denatured protein emulsion was formed after 8 hours of shaking. At each step the emulsion layer was washed with distilled water and the washings combined with the main solution.
7. After addition of 20 g of sodium acetate and 2 ml of glacial acetic acid, 95% ethyl alcohol was added to precipitate the polysaccharide (1-1.5 volumes). The solution was allowed to stand overnight, before collection by centrifugation.
8. The precipitate was dissolved in a small quantity of distilled water, and enough alcohol was added to make the solution turbid. After centrifugation at 2000 x g for 10 minutes the precipitate was discarded (polysaccharide does not flocculate in the absence of electrolyte).
9. Flocculation occurred immediately upon addition of 10 g of sodium acetate and 1 ml of glacial acetic acid.
10. After centrifugation at 500 x g for 15 minutes, the collected precipitate was dissolved in a small quantity of distilled water for later use. Storage was at 4 C.

(modified from Kabat and Mayer, 1961)

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

by

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Saliva is thought to have a therapeutic effect on clinical feedlot bloat. The bovine immune system could act on bloat through salivation. Experiments relating the bovine immune system to a suspected causative agent of bloat (Streptococcus bovis) are reported.

All bovine sera tested contain agglutinating immunoglobulins against Strept. bovis. Similar immunoglobulins are shown for parotid saliva. Cells grown with added sucrose produce extra-cellular dextran (capsule) and are agglutinated. Strept. bovis cells grown aerobically without supplemental sucrose are not agglutinated by either serum or saliva. Sephadex adsorption studies show that the serum and saliva immunoglobulin specificity may be the capsular dextran and provide specific means for isolating these immunoglobulins. Isolated capsular dextran shows no precipitin reactions with saliva or serum indicating size or configuration changes in isolation.

No capsule inhibition by serum or saliva immunoglobulins was detected microscopically or by quantitative hexose tests. In vitro enzymatic capsular degradation by either saliva or serum does not occur on whole cells or free polysaccharide.

Preliminary calculations show that parotid saliva alone may not provide sufficient antibody to significantly aid in feedlot bloat prophylaxis. Sub-maxillary saliva contain five times as much protein as parotid, but no information is known about relative immunoglobulin secretion. Inducement of these immunoglobulins is a matter for speculation, no proof of microbial inducement is presented.