

UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Animal Husbandry Research Division
Beltsville, Maryland

February 16, 1960

TO : Participants in Conference on Rumen Function

FROM : N. R. Ellis, Animal Husbandry Research Division,
Agricultural Research Center, Beltsville, Maryland

SUBJECT: Report of Fifth Conference on Rumen Function

Attached is a copy of the abstracts and list of participants for the conference held at the Maryland Hotel, Chicago, Illinois, on December 2 - 3, 1959.

The Conference convened at 9:30 AM on December 2 and was concluded at approximately 1:00 PM on December 3.

As in previous conferences, the Chairmen of the several panels organized the programs for their respective panels. They, along with the people who presented the papers, are commended for the choice and excellence of the material presented and the orderly presentation and discussion within the scheduled time. A total of 35 papers were presented with discussions of a few additional studies not listed on the program.

At the close of the meeting it was agreed to continue the plan of holding these conferences. There was some discussion of possible changes in time and place of meeting and in coverage of subject matter. It was agreed to have the same panel Chairmen continue or to have them arrange for a successor. It appears advisable to take up with the Chairmen any changes for the next conference in 1961, a few months in advance of the projected meeting.

Due to the extended absence of H. W. Marston while on a foreign travel assignment, the immediate arrangements for the Conference and the preparation of this report were handled through the Animal Husbandry Research Division. Credit is due to both H. W. Marston and Ivan Lindahl who made the preliminary arrangements for the program but were unable to attend the Conference.

Attachment

REPORT ON
CONFERENCE ON RUMEN FUNCTION
held at
Maryland Hotel, Chicago, Illinois
December 2-3, 1959

The following persons were in attendance during the two-day meeting:

| <u>NAME</u> | <u>ORGANIZATION</u> |
|--------------------|---|
| Allen, R. S. | Chemistry Dept., Iowa State Univ., Ames |
| Azukas, June | Michigan State Univ., East Lansing |
| Barnes, R. F. | ARS-USDA, Dept. of Agronomy, Purdue University, Lafayette, Indiana |
| Barrentine, B. F. | Dept. of Chemistry, Mississippi State Univ., State College |
| Beinhart, E. G. | ARS-USDA, Botany Dept., Clemson College, Clemson, South Carolina |
| Brown, L. R. | Dept. of Entomology, Iowa State Univ., Ames |
| Brown, R. E. | Dept. of Dairy Science, Univ. of Illinois, Urbana |
| Cole, H. H. | Animal Husb. Dept., Univ. of California, Davis |
| Colvin, H. W., Jr. | Dept. of Animal Industry, University of Arkansas, Fayetteville |
| Conrad, H. R. | Dept. of Dairy Science, Ohio Agr. Exp. Station, Wooster |
| Cunningham, H. M. | Research Branch, Canada Dept. of Agriculture, Ottawa |
| Davis, R. E. | AHRD-USDA, Beltsville, Maryland |
| Dehority, B. A. | Dept. of Animal Science, Ohio Agr. Exp. Sta., Wooster |
| Donefer, E. | Dept. of Nutrition, Macdonald College, Quebec, Canada |
| Donoho, Alvin | Univ. of Missouri, Columbia |
| Dougherty, R. W. | New York State Veterinary College, Cornell Univ., Ithaca, New York |
| Durbin, C. G. | FDA-HEW, Washington 25, D. C. |
| Ellis, N. R. | AHRD-USDA, Beltsville, Maryland |
| Ely, Ray E. | ARS-SESD-USDA, Washington, D. C. |
| Emery, R. S. | Dept. of Dairy Husb., Michigan State Univ., East Lansing |
| Erwin, E. S. | Monsanto Chemical Co., St. Louis, Missouri |
| Essig, H. W. | Mississippi State Univ., State College |
| Foote, L. E. | Dept. of Veterinary Science, Louisiana State University, Baton Rouge |
| Gaddum, J. H. | Inst. of Animal Physiology, Babraham, Cambridge, England |
| Garner, G. B. | Dept. Agr. Chem., Univ. of Missouri, Columbia |
| Gessert, R. A. | The Upjohn Co., Kalamazoo, Michigan |
| Goetsch, G. D. | Dept. of Veterinary Science, Purdue Univ., Lafayette, Indiana |
| Gutierrez, J. | AHRD-USDA, Beltsville, Maryland |

| <u>NAME</u> | <u>ORGANIZATION</u> |
|-------------------|---|
| Hale, W. H. | Chas. Pfizer & Co., Terre Haute, Indiana |
| Hardie, W. B. | Chas. Pfizer & Co., Terre Haute, Indiana |
| Hartman, P. A. | Dept. of Bacteriology, Iowa State University, Ames |
| Hobel, R. E. | New York State Veterinary College, Cornell Univ., Ithaca, New York |
| Hodgson, R. E. | AHRD-USDA, Beltsville, Maryland |
| Hollowell, E. A. | CRD-USDA, Beltsville, Maryland |
| Huffman, C. F. | Dept. Dairy Husb., Michigan State Univ., East Lansing |
| Hungate, R. E. | Univ. of California, Davis |
| Jackson, H. D. | Dept. of Biochemistry, Purdue Univ., Lafayette, Ind. |
| Jacobson, D. R. | Dairy Dept., Univ. of Kentucky, Lexington |
| Jacobson, N. L. | Dairy Dept., Iowa State Univ., Ames |
| Johnson, R. H. | Iowa State Univ., Ames |
| Johnston, J. E. | Louisiana State Univ., Baton Rouge |
| Jones, L. M. | Iowa State Univ., Ames |
| Juszkiewicz, T. | Iowa State Univ., Ames |
| Kellogg, C. E. | ARS-USDA, Washington, D.C. |
| Lee, A.M. | ADP-USDA, Beltsville, Maryland |
| Leffel, E. C. | Animal Husb. Dept., Univ. of Maryland, College Park |
| Lester, W. L. | Univ. of California, Davis |
| Machlin, L. J. | Monsanto Chemical Co., St. Louis, Missouri |
| Mah, R. A. | Univ. of California, Davis |
| McArthur, J. M. | Canada Dept. of Agr., Summerland, B.C., Canada |
| McCloud, D. E. | CRD-USDA, Beltsville, Maryland |
| Mendel, V. E. | Animal Husb. Dept., Univ. of California, Davis |
| Mitchell, G. E. | Dept. of Animal Science, Univ. of Illinois, Urbana |
| Neumann, A. L. | Dept. of Animal Science, Univ. of Illinois, Urbana |
| Nichols, R. E. | Veterinary Dept., Univ. of Wisconsin, Madison |
| Phelps, R. A. | National Cottonseed Producers Association, Dallas, Texas |
| Pidgen, W. J. | Canada Dept. of Agriculture, Ottawa |
| Pounden, W. D. | Dept. of Veterinary Science, Ohio Agr. Exp. Sta., Wooster |
| Sellers, A. F. | Dept. of Vet. Medicine, Univ. of Minn., St. Paul |
| Stevens, C. E. | Dept. of Vet. Medicine, Univ. of Minn., St. Paul |
| Stewart, W. E. | Dept. Dairy Husb., Univ. of Maryland, College Park |
| Swenson, M. L. | Iowa State University, Ames |
| Sykes, J. F. | AHRD-USDA, Beltsville, Maryland |
| Thompson, C. R. | Western Regional Research Lab., Albany, California |
| Wallace, Volney | Dept. of Biochemistry, South Dakota State College, College Station |
| Whiting, F. | Canada Dept. of Agriculture, Ottawa |
| Wilkins, H. L. | CRD-USDA, Beltsville, Maryland |
| Williamson, J. L. | Ralston-Purina Co., St. Louis, Missouri |

For the purposes of discussion, the program was divided into panels. The identification of the panels and the chairman of each was as follows:

| | | |
|-----------------------|------|-----------------|
| (a) Agronomic | - - | E. H. Hollowell |
| (b) Physio-Pathiology | - - | R. W. Dougherty |
| (c) Microbiology | - .. | W. D. Pounden |
| (d) Animal Management | - - | H. H. Cole |
| (e) Rumen Physiology | - - | C. F. Huffman |

AGRONOMIC PANEL

Effect of Time of Day and of Foliar Spraying on Alfalfa Composition and Bloat - R. S. Allen, A. E. Freeman, Donald Warner and Walter Woods - Iowa State University

In the study on the diurnal changes in composition four series of experimental samples were taken (May 1957; May 1958; August 1958; and September 1958) from prebloom alfalfa (Ranger variety) grown on Colo series, clay loam soil at Ames, Iowa. Each series included samples taken at 6 A. M., 10 A. M., 2 P. M., 6 P. M., and 10 P. M. (C.S.T.) from each of three adjacent sub-plots, employing a stratified, random sampling plan. Each individual sample included six sets of 25 alfalfa tops (approximately four inches in length), each set being taken from a different area within each sub-plot. Samples were frozen and stored in this state until time of analysis.

Each sample was analyzed for dry matter, total nitrogen, nonprotein nitrogen, amino acid nitrogen, reducing sugars, calcium, phosphorus and ash. In addition, the May 1957 series was analyzed for organic acids (total, citric, malonic, and malic); certain other acids were identified on a qualitative scale.

The components which changed most during the period of observation were reducing sugars and dry matter. Dry matter content increased to maximum at 3 P. M., then declined sharply. There was a sharp increase in the level of reducing sugar between 6 and 10 A. M., followed by a gradual decline to 10 P. M. Ash, phosphorus and total nitrogen declined gradually to minima at either 2 or 6 P. M., then increased. Values for calcium, nonprotein nitrogen and amino nitrogen did not follow any specific pattern of change.

All data were subjected to analysis of variance. All sources of variation were considered random variates and appropriate error terms were used. There were significant ($P < 0.01$) differences between series in values for all chemical constituents considered. The only component which showed a significant ($P < 0.01$) sub-plot within series difference was total nitrogen.

There were significant ($P < 0.01$) diurnal changes in dry matter total nitrogen, reducing sugars and phosphorus while ash values differed at the 5 percent level ($P < 0.05$). Including the August 1958 series into the combined analysis did not change any conclusions on diurnal variations drawn from the analysis of all four series.

There were no significant diurnal variations in the levels of total, malonic, malic or citric acids. Moreover, sub-plot differences were non-significant. Succinic, fumaric and glycolic acids were found in small amounts in most samples. Traces of quinic, shikimic and phosphoric acids also were observed in some samples.

No definite relationship between the occurrence of bloat in cattle or sheep and the diurnal pattern in alfalfa composition has been demonstrated.

Sprinkling solutions of urea, glucose, phosphorus as disodium phosphate or potassium as potassium carbonate on chopped alfalfa just prior to feeding to lambs did not increase the incidence of bloat. In a second study the addition of phosphorus or egg white alone or in combination with glucose to chopped alfalfa did not influence the bloat pattern. Glucose additions slightly increased the incidence of bloat.

Foliar spraying of urea on alfalfa at the rate of 40 pounds per acre increased bloat incidence and severity in lambs. In six grazing periods where lambs grazed alfalfa sprayed with urea, the average frequency of bloat was increased by 7 percent and the severity by 9 percent. Spraying of glucose at the rate of 40, 60 or 80 pounds per acre increased the frequency (14 percent) and severity (17 percent) of bloat in lambs grazing alfalfa. Spraying a mixture of glucose and urea on alfalfa pasture increased the bloat frequency (20 percent) and severity (16 percent). Spraying with potassium carbonate did not influence the bloat pattern, but the application of calcium (as calcium carbonate) increased the bloat severity (14 percent).

Chemical analyses were made on sprayed and control alfalfa (4 inch tops). Urea spraying increased total nitrogen and non-protein nitrogen as compared to control plants. Spraying with glucose or calcium carbonate did not appear to affect soluble nitrogen or non-protein nitrogen levels in the alfalfa plants.

The Saponin Content of Legumes as Related to Bloat - B. F. Barrentine -
Mississippi State University

Selected steers, which were bloating well, grazed the legumes morning and afternoon for periods of three to four days. All legumes were compared to Ladino clover. The "bloat index" for Ladino was set at 100 and the "bloat index" for the other legumes calculated from the relative number

of times the steers bloated on the legume under study as compared to Ladino. Forage samples were taken, morning and afternoon, during the period. All samples were dried as quickly as possible using a modified Air Force Ground Heating Unit. Saponin was determined by the puridine-alcohol method. The average saponin content, dry basis, and "bloat index" for the legumes are shown below:

| <u>Legume</u> | <u>Saponin percent</u> | <u>Bloat index</u> |
|----------------|------------------------|--------------------|
| Ladino Clover | 1.84 | 100 |
| Crimson Clover | | |
| Pre-bloom | 1.79 | 75 |
| Full-bloom | 1.70 | 0-12 |
| Hop Clover | 1.23 | 49 |
| Alfalfa | | |
| Immature | 2.35 | 82 |
| Mature | 2.43 | 0 |
| Lespedeza | 1.25 | 0 |

There seems to be little correlation between the saponin content, as determined by the pyridine-alcohol method, and the bloating potential of a forage.

Carbohydrate Content of White Clover and Alfalfa Forage and Rumen Ingesta from Bloating and Non-bloating Steers - H. L. Wilkins, Crops Research Division, USDA

Forage and rumen samples were taken during the spring of 1958 and 1959 from the field and from bloating and non-bloating steers on Ladino white clover and alfalfa pastures at State College, Mississippi. The usual feeding stuffs analyses were made on the A. M. forage samples for both years.

Analyses were made of total sugars, free reducing, and the hydrolyzable sugars by differences, starch and dextrin, "Hemicellulose", and the residue (a product consisting largely of cellulose and protein), total 80 percent alcohol soluble solids, total 80 percent alcohol insoluble solids, and by difference the non-sugar 80 percent alcohol soluble solids. Analyses of the above constituents are available for Ladino clover and alfalfa for 1958 and for about half of the 1959 Ladino trials but less than half of those for alfalfa.

For the most part, the data affords eight comparisons between bloater-non-bloater steers, the forage samples and computational results in 1958 and for 12 similar comparisons in 1959.

The combined data for both seasons and both forages amounts to 34 bloater-non-bloater comparisons. The greatest differences observed were that the rumen ingesta from bloaters was (a) highest in alcohol soluble solids as g./100g., 23 times in the 34 trials; (b) lowest in free reducing sugars, 15 times in 34; (c) lowest in total sugars, 13 times in 34; (d) highest in non-sugar alcohol soluble solids, 23 times in 34.

With respect to the other components, the scores were fairly even in both years, or were high in one and low in the other year.

It must be stated that a large part of the carbohydrate content of these samples has not been accounted for by the above analyses. More complete assays are planned.

Organic Acid Patterns in the Rumen of Bloating and Non-bloating Steers-
J. F. Sykes and H. G. Wiseman, (USDA), C. A. Lindley and C. Shawver and
B. F. Barrentine (Mississippi) and H. L. Wilkins (USDA)

The present preliminary results were accumulated as a result of a joint effort by the Animal Husbandry Research Division, ARS, the Mississippi Agricultural Experiment Station and the Crops Research Branch of ARS to determine possible plant-animal relations related to the incidence of bloat in cattle pasturing on alfalfa or Ladino clover.

The variability in the response of animals grazing similar bloat-producing pastures suggests that the incidence of bloat in any population may be related rather directly to some quirk in animal metabolism which predisposes some animals to bloat. An examination of the organic acid concentrations and patterns in bloat-susceptible and bloat-resistant steers grazing Ladino clover or alfalfa was therefore undertaken.

Procedures

1958 Work - Two steers, which had been characterized as bloaters or non-bloaters, were grazed on both Ladino clover and alfalfa for a period of a week. They were allowed to pasture for approximately two-hour periods twice daily. Rumen samples were obtained by stomach tube immediately after the morning pasture period and just prior to the afternoon pasturing period on all four steers on two successive days. The organic acids were determined chromatographically by the method described by Wiseman (Agr. and Food Chemistry 5: 213, 1957).

1959 Work - The same procedure in general was followed as in 1958 with the exception that more extensive sampling over longer periods of time was undertaken. Samples were again obtained on four steers (two bloaters and two non-bloaters) on two successive days in each of three pasturing periods covering an interval of about three weeks.

Results

The 1958 results were compared for concentrations of organic acids found in rumen contents of bloat-susceptible and bloat-resistant steers. An examination of these data indicates that a distinctly higher concentration of the formic acid (0.22 vs 0.13) and lactic plus succinic acid fractions (0.50 vs 0.27) exists in the bloating steers as compared to the non-bloaters. There is also a tendency for butyric acid to be higher (1.80 vs 1.15). An increase in the ratios of butyric, formic and lactic plus succinic acid fractions to acetic acid is indicated for the bloating steers. These results suggested that a difference existed in the rumen metabolism of bloating versus non-bloating steers.

The results which have so far been obtained in 1959, on the other hand, give no indication that such a difference exists. Both the concentrations of the acids and the ratios of the several acids to acetic acid are remarkably alike for bloating and non-bloating steers. Only about 25 percent of the 1959 samples available to us have so far been analyzed. Whether the remaining analyses will change the situation is anyone's guess. We have more confidence in the 1959 analyses, however, for the formic and lactic plus succinic acid fractions than we have for the 1958 analyses.

Conclusions

Contradictory results on organic acid patterns of rumen contents of bloating and non-bloating steers have so far been obtained in two successive years. It is now extremely doubtful that animal differences in susceptibility to bloat can be explained by differences in rumen acid metabolism.

The Role of Organic Acids of Alfalfa in CO₂ Production - R. E. Nichols - University of Wisconsin, Madison

Rapid production of CO₂ gas by the addition of non-volatile organic acids to samples of ruminal content has been demonstrated. Sodium salts of the same acids and glucose in the same concentrations do not result in a rapid release of CO₂. The rapidity of release of CO₂ is a physical factor resulting in volumes of froth of samples which are greater than normal.

The addition to the rumen of quantities of these acids roughly similar to amounts which we might expect to be consumed in a day's feed does not induce an excessive frothing unless preceded by a similar amount of carbonate, and even then the volumes of froth produced either in the fistulated or intact animal are not of the same magnitude as occasionally occurs during the feeding of fresh legumes.

The Effect of Temperature and Light Intensity on the Organic Acids of White Clover - George Beinhart, Crops Research Division, ARS (USDA)

This paper summarized growth responses and presented data for organic acids in the leaves of plants in two growth chamber experiments. One experiment studied growth with different day/night temperature regimes (50, 62, 74, and 86°F in all combinations as day and night temperatures) for plants receiving 600 foot-candles illumination. In the second experiment, effects of light intensity (000 to 2,000f-c) and night temperature (50 to 86°F) were studied for plants receiving 86° day temperatures.

Nine organic acids were found to be regularly present in clover extracts analyzed by partition chromatography on silicic acid columns. Tentative identifications have been made of eight of these: acetic, pyruvic, succinic, malonic, oxalic, cis-aconitic, malic and citric. The sum of the nine individually measured acids yielded an estimate of total organic acids for each sample.

Total organic acids declined as day temperature increased above 62°F, with the average total acidity at 86° being approximately 75 percent of the averages for 50 and 62 days. A similar trend was apparent for night temperatures, although the magnitude of the effect was not as great as for day temperature. Total acids increased with increasing light intensity for plants at 86 days, while night temperature effects were not very pronounced. Malate seems to be particularly affected by changes in light intensity, while malate, citrate, acetate, and pyruvate (identifications tentative) varied with temperature. These data are being examined in an effort to relate them to the growth patterns for these two experiments.

PHYSIO-PATHOLOGY PANEL

Measurement and Experimental Alterations of Electrical Potentials Between Rumen Ingesta and Blood in Unanesthetized Sheep - A. F. Sellers and A. Dobson, University of Minnesota, and A. T. Phillipson, Rowett Research Institute, Scotland

Summary

Measurements of DC potentials as between rumen ingesta and blood, and of Na and K concentration gradients, obtaining in unanesthetized chronic rumen fistula preparations in 54 experiments involving three dietary variations are summarized. Results of examination of sampling procedures indicated those most valid in representing ingesta studies for the present purpose. On rye grass pasture, fall in reticulo-rumen Na concentration, with accompanying rises in rumen K concentration, and EMF, were seen and were statistically significant. These effects were most marked with first growth material. Correlations observed were such that the effects of the two cation concentrations could not be separated. Concomitant with the above, salivary changes showed the animals to be alternating between sufficiency and deficiency as regards Na, under the conditions imposed.

Effect of Alfalfa Pasture Bloat on Certain Blood Components - L. R. Brown,
R. S. Allen, R. H. Johnson and N. L. Jacobsen, Iowa State University, Ames

This study, conducted during the 1958 and 1959 pasture seasons, was undertaken to determine some of the possible changes that may occur in the composition of the venous blood of cattle during bloat. From 35 to 40 cattle were permitted to graze good alfalfa pasture for two three-hour periods daily. Blood samples were taken from the jugular vein as soon as possible after bloat was rated 3 or more on the 0 to 5 bloat scale proposed previously (J. Animal Sci., 17: 893. 1958). A sample was also taken from a non-bloated animal as soon as possible after the collection of a sample from a bloated animal. Approximately 200 samples were collected with an attempt being made to get an equal number from bloated and non-bloated animals. The blood was analyzed for red cell volume (1959 only), ammonia nitrogen, non-protein nitrogen (1958 only), total phosphorus, plasma inorganic phosphorus and plasma lipid phosphorus.

Although the differences in blood composition associated with bloat were greater the first year, all phosphorus components measured were higher in bloated animals both years. Summarizing all data, values for non-bloated and bloated animals, respectively, (standard error of the mean given in parenthesis) were: plasma inorganic phosphorus, 8.58 and 9.44 mg. percent, (0.13); total phosphorus, 21.1 and 22.2 mg. percent, (0.23); plasma phospholipid phosphorus, 5.98 and 6.33 mg. percent, (0.12); hematocrit values, 40.7 and 41.7 percent, (0.72); ammonia nitrogen, 6.0 and 6.0 mg./ml., (1.50); and non-protein nitrogen 56.5 and 56.4 mg. percent. The differences in nitrogenous components and hematocrit values were not significant at $P = 0.05$, but the differences in phosphorus components were all significant at $P < 0.05$. Using only data from animals with values in both classifications, the differences were somewhat smaller: plasma inorganic phosphorus, 8.79 and 9.25, (0.16); total phosphorus, 20.9 and 21.5, (0.31); and plasma lipid phosphorus, 5.82 and 6.09, (0.17).

An attempt was made to determine the effect of bloat susceptibility on blood phosphorus levels. The levels of blood phosphorus of all animals were measured on dry feed and again on pasture while in the non-bloated state. All animals were ranked on the basis of the average daily maximum bloat scores and the 14 most bloat-susceptible animals were compared to the 14 least bloat-susceptible animals. There was no difference between bloat-susceptible and non-bloat-susceptible animals in total phosphorus, but while on dry feed the more susceptible group was lower in plasma inorganic phosphorus and higher in plasma lipid phosphorus than the less bloat susceptible group. When changed from dry feed to pasture the group with high bloat susceptibility had a considerable increase in plasma inorganic phosphorus and little change in plasma lipid phosphorus whereas the animals with low bloat susceptibility had little change in plasma inorganic phosphorus and a relatively large increase in plasma lipid phosphorus.

Chemistry of a Respiratory Inhibitor Associated with Bloat - H. D. Jackson and R. A. Shaw, Purdue University, Lafayette, Indiana.

Earlier studies at this station have shown that legumes contain a substance(s) which inhibits muscle respiration in vitro (J. An. Sci. 16:711, 1957) and that such a respiratory inhibitor from alfalfa is associated with the occurrence of bloat in cattle (J. An. Sci. 18:158, 1959). The observations reported here were made on samples from the alfalfa pastures grazed in the latter study.

Alfalfa was fractionated by solvent and chromatographic techniques and the steps of the fractionation were followed by analysis of the fractions for their respiratory inhibiting activity in vitro. The inhibitor(s) was a non-lipid neutral substance which was not held on ion exchange columns but which could be held on a carbon column and then removed from it in the methanol eluate. The inhibitor was precipitated by cholesterol and upon hydrolysis lost activity and released five monosaccharides: arabinose, glucose, xylose, galactose, and rhamnose. The inhibitor was found to be a saponin(s) and was compared with other saponin mixtures isolated from the same alfalfa by slightly different procedures. The several saponin preparations were compared by their respiratory-inhibiting activity, specific rotation, infra red spectra, and by the different monosaccharides released on hydrolysis. An isolation procedure including ion exchange, carbon column chromatography, and cholesterol precipitation yielded the saponins with the greatest respiratory-inhibiting activity. Details of this work have appeared in press quite recently (Arch. Biochem. Biophys. 84:405-416, 1959).

These observations support the theory that triterpenoid saponins may be important plant substances in the etiology of bloat.

The Function of the Bovine Omasum in Ingesta Transfer - C. E. Stevens, A. F. Sellers and F. A. Spurrell - University of Minnesota, St. Paul

In a series of five adult cows and five calves motor events in the reticulum and omasum were studied and an attempt was made to correlate these with ingesta flow. Pressure events were recorded by means of open tipped catheters and strain gauges. Ingesta flow was studied by fluoroscopic examination, palpation, a funnel reservoir system and by use of the Pitot tube principle. Results indicated that the omasal canal (vestibulum) undergoes contractions with each primary and secondary contraction of the rumen and that the primary omasal canal contraction is often followed by a contraction of the omasal body. The greatest flow of ingesta from reticulum to omasum appeared to occur directly following the primary omasal canal contraction. It appeared that the canal contractions forced the more fluid portions of its content up among the leaves of the omasal body. The body would then, in turn, contract pumping ingesta into the abomasum. Backflow of large volumes of ingesta from omasum to reticulum were occasionally noted and seemed to occur when the omasal body contracted during closure of the omasoabomasal orifice. This could serve to periodically flush any coarse roughage accumulated in the omasal canal back into the reticulum.

Saponin and Dry Matter Values of Rumen Contents from Steers Grazing Alfalfa - B. F. Barrentine, Mississippi State University, State College

Rumen samples were taken by stomach tube, morning and afternoon, from two bloating and/non-bloating steers grazing alfalfa. Rumen samples were preserved in alcohol, and blended in a Waring Blender with a "polytron head". Saponin was determined by the pyridine-alcohol method. The average saponin content of the alfalfa forage was 2.35 percent, dry matter basis, during the collection period. The average saponin, dry basis, and dry matter values of the rumen contents are shown below:

| | <u>Saponin-percent</u> | <u>Dry Matter-Percent</u> |
|----------------------------|------------------------|---------------------------|
| Bloating steers, A. M. | 0.69 | 8.47 |
| Bloating steers, P. M. | 0.41 | 7.63 |
| Non-bloating steers, A. M. | 0.87 | 6.34 |
| Non-bloating steers, P. M. | 0.39 | 6.16 |

The results indicate considerable metabolism of saponin in the rumen contents, but show little difference between bloating and non-bloating steers.

Studies on Rumen Fermentation in Connection with Bloat in Cattle - D. E. Wright and A. T. Johns, Dept. Sci. Ind. Res., Palmerston North, New Zealand

Studies on the rumen liquor from cows fed on red clover have indicated that there are both foaming and anti-foaming agents present. At bloat the formation of a stable foam could be due to the decrease in the amount of an anti-foaming agent. The main foaming agent from the clover appeared to be cytoplasmic protein while the anti-foaming agent was chloroplast fat. At least part of the action of penicillin in preventing bloat is probably due to the inhibition of bacteria which modify chloroplast fat.

It has been observed that the ciliate Epidinium ecaudatum is the predominant protozoan under bloating conditions. It ingests and digests clover starch and chloroplasts, thereby removing the anti-foaming agents from the rumen liquor. Both rumen bacteria and protozoa have shown to hydrogenate the unsaturated chloroplast lipids which are not triglycerides as had been assumed previously.

Some Chemical Properties and Biological Effects of Legume Saponins - C. R. Thompson, WURDD, ARS, Albany, California

Saponins occur in many plants, especially desert lilies such as the yuccas, agave, etc. Two classes of saponins are recognized, depending upon the characteristics of the fat-soluble nucleus or sapogenin. They are the steroids and triterpenoids. To date all of the sapogenins found and identified in legume forages have proved to be triterpenes. When saponins are hydrolyzed with acids or enzymes, a number of sugars such as glucose, galactose, arabinose, xylose, and rhamnose are split off leaving the nucleus known as the sapogenin.

A summary of our knowledge of the occurrence of saponins in legumes follows:

| <u>Alfalfa</u> | <u>Strawberry Clover</u> | <u>Ladino Clover</u> |
|-------------------|--------------------------------|----------------------|
| Medicagenic Acid | Soyasapogenol B | Soyasapogenol B |
| Lucernic Acid | Unknown Sapogenin | C |
| Soyasapogenol B | | |
| " C | <u>Birdsfoot Trefoil</u> | <u>Burr Clover</u> |
| Unknown Sapogenin | Oleanois Acid as Free Compound | Hederagenin |

The present physiological studies on saponins in forages were started about eight years ago following reports that alfalfa contained a factor which caused growth inhibition in young chickens when it was fed at high levels.

Saponins cause various effects in a number of different biological systems. While no single forage saponin will cause all of the effects noted, the following physiological effects have been observed at various times:

| | |
|--|---------------------------------------|
| Hemolyze red cells | Inhibit chick growth |
| Kill fish, snails | Increase cholesterol in bovine plasma |
| Stimulate or inhibit excised intestinal muscle | Inhibit muscle respiration |
| Free enzyme from red cells | May be involved in ruminant bloat |

Studies with saponins isolated by forming a cholesterol addition product show that hemolysis of red cells occurs at dilutions of 1 - 20,000. Dilute solutions kill fish or other aquatic animals. When the "cholesteride" saponins are added to a muscle bath containing excised rabbit ileum, peristalsis stops. As soon as it is washed out, peristalsis resumes.

Recent studies at the Western Regional Research Laboratory have suggested, but not proved, that the fractions of the alfalfa saponins which form an addition complex with cholesterol in an aqueous solution are those which are most active physiologically. Thus the "cholesteride" saponin fraction from a given lot of alfalfa depresses chick growth to about the same extent as the total saponin although the cholesteride saponins comprise about one-half of the entire amount.

In other studies, 25-mg. portions of purified alfalfa saponins were injected into the jejunum of pentobarbital-anesthetized rats after the abdominal cavity was opened. The rats, except for their heads, were immersed in Locke's solution after injection. Immediate symptoms of hyperemia were observed at the site of injection. Within 15 to 20

minutes the hyperemia extended to the cecum. Also an accumulation of gas in both the small intestine and the cecum was sometimes evident. The gas presumably came from the blood. The possible significance of this effect in ruminant bloat remains to be assessed.

A quantitative analytical method has recently been devised for the determination of total saponins in alfalfa. It may be applicable to other legumes but has not been tested on them to date.

Work is continuing on the legume saponins to determine the identity, occurrence, and possible physiological significance of these interesting compounds in animal nutrition.

Eruption in Insufflated Sheep and its Relation to the Rapid Blood Gas Changes - R. W. Dougherty, W. E. Stoker and Max Nold, Cornell University; Ivan Lindahl, ARS, USDA; and Frank Campeti, Rochester University Medical School; and K. J. Hill, Institute of Animal Physiology, Babraham, England

The report was made in an effort to bring up-to-date what is known about the physiology of eruption in ruminants. This is considered to be a "key" part of the bloat problem.

Previous work has shown that the following events occur during eruption:

1. Two contractions of the reticulum clearing that organ of much of its ingesta.
2. Contractions of the ruminoreticular fold and anterior pillar, holding the ingesta away from the cardia area and preventing the immediate return of ingesta to the relaxed reticulum.
3. These events permit gas to come forward to the region of the cardia so that it is in a position to be erupted.
4. Relaxation of the cardia and prediaphragmatic sphincters, permitting rumen gas to distend the esophagus throughout its entire length.
5. Closure of the cardia and prediaphragmatic sphincters and relaxation of the pharyngoesophageal sphincter followed by an extremely rapid peristaltic wave of the esophageal musculature clearing the esophagus of the contained gas.
6. We also had demonstrated previously that there are receptors in a relatively small area around the cardia which inhibited eruption when this area could not be cleared of liquid, solid or foamy ingesta. These receptors were capable of differentiating between gases and other materials common to rumen ingesta. When this area (around the cardia) is covered for a few minutes with one percent

butyn sulfate and the relatively full rumen is insufflated with gas, decerebrate sheep eructated any material that was in the vicinity of the cardia--gas, liquids, solids or a mixture of these components of rumen ingesta. This indicates that these receptors are fairly superficial and may act as tactile receptors. It must be borne in mind that during rumination the animal must be able to inhibit the inhibitors or regurgitation would be impossible.

A continuation of the work during the past year and especially during the summer and fall has thrown considerable light on pharyngeal events occurring during eructation. These are listed briefly as follows:

7. The soft palate closes against the posterior nares, blocking entrance of the bulk of the eructated gases into the nasal cavities.
8. The mouth and lips remain closed, although not tightly so.
9. Intrapharyngeal pressures rise sharply during eructation.
10. The glottis remains open and even widens during eructation in the decerebrate sheep.
11. Intratracheal pressures rise sharply during the esophageal contraction phase of eructation. These pressures follow patterns similar to those occurring in the esophagus during eructation.
12. Methane is not excreted through the lungs in measurable amounts when it is insufflated into the rumen.
13. When sheep were insufflated with a 60/40 mixture of CO₂ and CH₄, methane was found in the trachea and in the bronchi in appreciable amounts. This indicated that eructated gases get deeply in the lungs.

Recent studies of the intrapharyngeal phases of eructation are considered to be one of the most productive parts of our four-year study of this important physiological process. It has led to our finding that considerable quantities of eructated gases get into the lungs, part of which are absorbed. The implications of this physiological process are rather far reaching. We have already found that this is the chief source of two important off-flavors in milk. It may have considerable influence on what are considered to be normal milk flavors. It can be postulated that the ruminant recycles part of the eructated gas to conserve some of the carbon (CO₂) that would otherwise be wasted. If this is so, then the entire picture of ruminant metabolism may have to be reexamined.

MICROBIOLOGY PANEL

Some Effects of Viscous Materials Upon Foam Stability and Bloat -
E. C. Leffel and R. N. Doetsch, University of Maryland, College Park

In the course of investigations on bloat, experiments were conducted to test the supposition that increased viscosity of the rumen liquor would result in the formation of a stable foam which would tend to block the cardia and interfere with eructation. Substances tested for ability to increase viscosity and/or form a stable foam included guar gum, pectin, polyvinylpyrrolidone, gelatin, egg white, capsular material from Streptococcus bovis and dextran.

Guar gum, which produces a strong gel in aqueous solution due to its galactomannan content, did not increase viscosity of the rumen contents. Investigation of the role of rumen bacteria disclosed a number of obligately anaerobic rumen streptococci capable of hydrolyzing galactomannan contained in the endosperm of Cyanopsis tetragonala. The enzyme(s) associated with the breakdown of galactomannan has been termed "galactomannanase". It is constitutive in rumen bacteria and is found in significant amounts in raw rumen fluid.

Galactomannanase is postulated to be elicited by compounds such as cellulose, cellulose derivatives, and mannagalactan which possess the β -1:4 linkage. A survey of a number of non-rumen bacteria revealed that they do not elaborate galactomannanase. Ability to hydrolyze galactomannan seems confined to a specialized group of anaerobic cocci of the rumen.

Results obtained from in vitro experiments with polyvinylpyrrolidone, gelatin, pectin and dextran may be briefly summarized as follows:

1. A foam was produced in rumen fluid taken from sheep on an alfalfa hay diet by sparging with carbon dioxide, but this foam rapidly deteriorates. The addition of polyvinylpyrrolidone (1-5 percent w/v) to the rumen fluid did not enhance foam formation, despite the fact that the material added produces highly viscous aqueous solutions.
2. Rumen fluid, taken from a sheep on an alfalfa hay diet, was mixed (100 ml 5 percent (w/v) gelatin plus 100ml rumen fluid) in the Waring Blender for one minute and a stable foam was formed. The same sort of stable foam was produced when this mixture was sparged for one minute with 100 percent carbon dioxide.
3. When rumen fluid, as in number 1 and number 2 was mixed with an equal amount of five percent (w/v) pectin, no stable foam was formed in the Waring Blender after one minute's agitation. However, five percent (w/v) pectin added to an equal volume of rumen fluid, and sparged with 100 percent carbon dioxide, formed an extremely stable foam.

4. Dextran (5 percent w/v) produced an extremely viscous aqueous solution, but this solution mixed with an equal volume of rumen fluid and sparged with carbon dioxide did not produce a stable foam.

Results obtained from in vivo experiments may be briefly summarized as follows:

1. The test animal did not bloat when administered a mixture of 1000 ml "Gelsol" (8 percent gelatin), plus 300 ml of 10 percent (w/v) glucose, and sparged in vivo via a fistula with 100 percent carbon dioxide. Results with sheep on a "bloat diet" (61 percent ground barley, 16 percent soybean oil meal, 22 percent ground alfalfa hay and 1 percent NaCl) and on diets of alfalfa hay or alfalfa pellets were similar.
2. An organism tentatively identified as S. bovis was grown in 10 liter quantities, and centrifuged. The animal on an alfalfa hay diet was given two liters of this culture suspended in distilled water (10^{13} capsulated cells). There was approximately 3.3 mg. of carbohydrate (capsular material) per 10 ml. of fully grown culture. There was no in vivo foam formed over an observation period of five hours.
3. Two liters of 10 percent gelatin (w/v) were churned into foam with a whipped cream dispenser. This foam was overlaid on the normal rumen fluid in vivo via a fistula. In addition, 100 percent carbon dioxide was sparged directly into the rumen fluid in vivo. Pressure was maintained at 15-20 ml. Hg. The animal could not eructate the sparged gas under these conditions as well as when no foam was present.
4. When 500 ml. fresh egg white and 200 g. glucose were added to the rumen via an esophageal tube some foam was produced in the rumen. If the egg white was added via the fistula using a whipped cream dispenser, much eructation occurred but no bloat or foam was observed.
5. The administration of five 50 gram doses of dextran to a fistulated and to an intact sheep over a period of 48 hours did not result in observable foam formation or bloat. Microscopic examination of the rumen fluid showed a high degree of encapsulation of the rumen microorganisms. Rumen fluid drawn after feeding dextran for 48 hours formed a very stable foam when sparged with carbon dioxide.

Nitrate Poisoning - G. B. Garner, University of Missouri, Columbia

In studies by workers at the Missouri Station since 1954, certain facts have been related to the overall problem of nitrate poisoning. Classical nitrate or oat hay poisoning is only the more acute response of ruminants to nitrate. The relationship of this problem to soil fertility, species selection, soil moisture and stage of maturity at harvest are important aspects of nitrate poisoning.

In 1958, the addition of 100# N/acre to timothy, bluegrass, fescue and orchard grass resulted in KNO₃ equivalent in the dry forage of 2.85, 1.25, 1.72 and 2.50 percent, respectively. When a complete fertilizer application of phosphate, potash, lime and nitrogen was applied the levels were 0.55, 0.16, 0.39 and 0.21 percent, respectively. Brome and Ladino clover did not accumulate as much nitrate on the nitrogen treatment but did show a decrease with the complete soil treatment.

Species of plants found to accumulate nitrate were ranked in order of decreasing potential. Corn, sudan, small grains, rape and fescue were found to be potentially high accumulators. Alfalfa, Ladino, milo and sorghum were moderate accumulators with the red clover and brome being consistently low.

Seed crops used for forage can be harvested in a late stage of maturity in order to dilute the stem and leaf nitrate by the grain. In general, all nitrogen components of the plant decrease percentagewise with age.

In vitro experiments have shown that the rate of conversion of nitrate to nitrite by rumen fluid is controlled by the ration composition of the donor animal. Sheep rumen fluid from alfalfa-fed sheep reduced nitrate more rapidly than did fluid from sheep fed either alfalfa plus corn or timothy plus cerelose. Glucose added to these in vitro systems did itself increase the rate of nitrite formation. These results have been interpreted to mean that if the amount of nitrate ingesta is small, the bacteria of the rumen can convert the nitrate-nitrogen to protein in the rumen without toxic effects. However, if a large amount of nitrate enters the rumen this conversion cannot take place rapidly enough to prevent the nitrite from passing into the abomasum.

Motility of the rumen microorganism is inhibited by nitrite and the oxidation reduction potential rises with resulting digestive disturbance. Methemoglobin is not an adequate index of nitrate poisoning. Relaxation of smooth muscle, excessive urination, motor weakness and rapid pulse may be seen without a significant rise in methemoglobin.

The level of nitrate intake believed to produce visible symptoms is approximately 0.5 percent KNO₃ equivalent of the dry matter intake. Milk production is lowered, non-thrifty appearance and possible reproductive difficulty results. At one percent KNO₃ intake, a definite loss of milk production, vasodilation, abortion and some deaths may result.

The work of the Missouri Station is compatible with the work of Sund and coworkers from the Wisconsin Station (recently published) concerning abortion in cattle receiving nitrate.

Nitrate poisoning will increase as the efforts to produce greater yields per acre continues. At levels less than one percent KNO_3 equivalent of the dry matter it is a problem of relatively poor nutrition, that is, by increasing the energy content of the ration and maintain satisfactory levels of protein, vitamins and mineral it can be overcome. Above one percent of the toxic nature of nitrate will be difficult to overcome in a dry ration. The effects of nitrate levels in fresh forage utilization are different than in a dry ration and need further work.

Destruction of Penicillin by Rumen Microorganisms - C. K. Smith, June Azukas, R. S. Emery and C. F. Huffman, Michigan State Univ., E. Lansing

Various investigators have used penicillin as a control for bloat, and in so doing, have observed that the efficiency of the treatment decreased as time progressed. This observation suggests that prolonged treatment with penicillin results in induced resistance of the microflora of the rumen with subsequent destruction of the penicillin. Thus, this present investigation was begun.

Fistulated animals were fed penicillin amounting to 100 mg or approximately one unit per ml. of rumen contents daily. Attempts to recover the penicillin showed that in animals receiving penicillin for some time, the penicillin could be detected in the front but not in the back of the rumen for one hour after feeding. This was true of high grain and high hay rations, the lowest detectable level of penicillin being 0.05 unit per ml.

In view of these results, the microflora was taken from an animal on penicillin to determine how quickly and how much penicillin could be destroyed in vitro. This was done by varying the amount of penicillin and qualitatively determining its presence at hourly intervals. It was found that the organisms could destroy 100 unit per ml in 5 hours. Various controls were also run to make certain that the destruction of penicillin was due to microbial metabolism rather than some physical factor. Having ascertained this, the destruction of penicillin was attributed to an induced enzyme, penicillinase. Having assumed penicillinase to be at its highest level at the same time the penicillin disappeared, various fermentations were run which received 10 times the amount of original penicillin at the time the initial penicillin disappeared. Within a total time period of 9 hours, 500 units per ml. of penicillin could be destroyed consistently.

An assay method for penicillin utilizing *Bacillus subtilis* spores is described and results presented. By using this method it was found that penicillinase is not at its highest at the time the penicillin is destroyed but rather three to four hours later. This is true regardless

of the units of penicillin used. This phenomenon has also been observed by Pollock who exposed bacterial cells for a brief period (about one minute) to penicillin at 0° with no energy source and found that penicillinase production would then occur in a penicillin free medium, the total amount increasing linearly with time.

Another method of measuring penicillin is the manometric method. When penicillin is added to penicillinase in the presence of bicarbonate, CO₂ is evolved. If a zero order reaction is maintained, then the rate of reaction is proportional to the concentration of penicillinase. If the reaction is carried out at a pH near 7 and at 25 - 30°C., the nonenzymic destruction of penicillin is negligible. This method was used with the in vitro fermentation liquid but very little activity was demonstrable, probably because the level of penicillinase was too low.

To concentrate and purify the enzyme, two different methods were used. One involves a series of acetone precipitations, dialysis against phosphate buffer and precipitations with ammonium sulfate. This procedure was used on a number of in vitro fermentations with limited success. However, the preparations that showed consistently good activity enabled us to do some preliminary work with inhibitors.

The other method for purification is based on the chance observation by Pollock that the enzyme is not filterable through sintered glass. The enzyme is adsorbed onto powdered glass followed by elution and precipitation with ammonium sulfate. This method theoretically results in 95 percent recovery of the enzyme practically free from other protein. This method has not been successful with us, probably because of the presence of some constituent which inhibits adsorption.

Some limiting factors which yet have to be overcome are: size of fermentation, levels of penicillin that can be used and time of incubation of the fermentation. Of these, perhaps the most important are size of fermentation and time of incubation. Because the organisms are fastidiously anaerobic, it is impossible to obtain a large enough inoculation of viable organisms for, say, 50 liter fermentation. Also, since the fermentations are predisposed to contamination, penicillinase produced in a fermentation run longer than 9 to 12 hours could be attributed to a contaminant.

In summary, then, it has been found that microorganisms of the rumen, when subjected to treatment with penicillin, can produce a principle capable of destroying the penicillin. This principle has been isolated and evidence suggests that it may be a penicillinase. Various procedures for the isolation of the enzyme have been tried but with limited success. Some problems involved are size of inoculum and levels of penicillin that can be used without inhibiting growth.

Application of the Silk Bag Technique for Evaluation of Feed Materials in Cattle - E. S. Erwin and B. R. Taylor, University of Arizona, Tucson

Effect of Diet and Feed Additives on Facultative Anaerobes in the Rumen - P. A. Hartman, R. H. Johnson, L. R. Brown, N. L. Jacobson and R. S. Allen, Iowa State University, Ames

Facultative anaerobic lactobacilli (APT agar) and streptococci (thallus acetate agar) were enumerated on rumen samples from six fistulated cattle during the progress of feedlot bloat investigations and subsequent alfalfa pasture experiments.

When the rumen contents of the animal with the greatest feedlot bloat potential were exchanged with the contents of an animal which did not bloat in the feedlot, intraruminal pH, foaminess and types of lactobacilli present (based on colony morphology) reverted to pre-treatment level in both animals. This indicated that each animal, via inherent physiological processes, gained a high degree of autonomy of rumen flora. Bloat occurred prior to any substantial increase in streptococcus counts; therefore, the import of rumen streptococci in the etiology of feedlot bloat can be questioned. Little success was obtained in attempts to affect the foaminess of one animal by the introduction of cultures isolated from the companion animal. However, feedlot bloat could be induced in the animal which did not normally bloat by administration of 200 gm. of sodium carbonate. Concomitantly, the intraruminal pH (normally below pH 5.8) was elevated for a 5 to 7 day period, changes were noted in the types of lactobacilli present, and greatly increased streptococcus counts were obtained. A single dose of 7 gm. of sodium hydroxide or continued feeding of sodium carbonate (25 gm. per feeding) resulted in subsequent production of intraruminal foam and elevated streptococcus counts, but only temporary effects could be noted on the ruminal pH and numbers and types of lactobacilli present. These results indicate that the ionic environment was of considerable import in the etiology of feedlot bloat and that the microbial balance of the rumen may be altered by regulation of the inorganic constituents of the diet. In addition, intraruminal pH and the types and numbers of lactobacilli present were found to be of little consequence in the etiology of bloat.

The six fistulated animals were also used during alfalfa pasture experiments on the use of antibiotics in bloat prophylaxis in order to estimate the degree of antibiotic resistance developed by the facultative anaerobes of the rumen. Rumen contents from each animal were plated on APT and thallus acetate agars containing no antibiotic, 2.0 units per ml. of penicillin, or 10.0 mcg. per ml. of erythromycin. Marked reductions in viable counts were obtained on the control media for several days following the initiation of penicillin or erythromycin treatment, and, for longer periods, when these antibiotics were fed in combination. This selection of population by penicillin or erythromycin was probably

responsible for an initial increase in apparent bacterial resistance towards one antibiotic in samples obtained from the rumens of animals being fed the other antibiotic. Subsequent development of resistance was specific for the antibiotic which was fed, while the initial "generalized" type of resistance disappeared. Resistant bacteria appeared sporadically in the control animals, which indicated that some transfer of resistant bacteria might occur from treated to control animals. Sometimes the resistant bacteria persisted for several weeks, especially in animals of the treatment groups, but other instances were noted where the resistant forms did not persist for more than several days. Prevalence of resistant forms seemed to be incidental to the efficacy of the antibiotic in prophylaxis of bloat. On the other hand, the ability of an antibiotic or combination of antibiotics to reduce the total numbers of the bacteria enumerated was closely associated with the more effective treatments.

Froth Formation, Surface Tension and Attempts to Alter the Microbial Balance in the Bovine Rumen in Studies Associated with Bloat -

Don R. Jacobson, Ralph F. Wiseman and William M. Miller, University of Kentucky, Lexington

The results indicate that a microbial balance exists in the bovine rumen. Attempts to establish a slime-producing Aerobacter, which was isolated from Ladino clover, in the rumen of Ladino-fed cattle was unsuccessful. It was further observed that pronounced changes in the numbers of paracolon bacteria, lactobacilli, and streptococci did not occur in experiments in which various concentrations of penicillin were added to the rumina either by stomach tube in dairy cattle or directly in fistulated dairy steers. This apparent lack of antibiotic activity was not correlated with the appearance of penicillin-resistant lactobacilli or streptococci since in vitro assays showed that these groups were sensitive to low concentration of the antibiotic. The availability of rumen paracolon bacteria to grow in relatively high concentrations of penicillin suggests that in the rumen this group might have inactivated the penicillin, thus permitting the numbers of penicillin-sensitive Gram positive bacteria to remain unchanged following the administration of the antibiotic. The transfer of the cattle from Ladino clover to hay following the penicillin studies did not markedly modify the numbers of rumen bacteria noted.

The addition of the slime-producing Aerobacter, or the administration of penicillin did not alter the rate of production of total froth or stable froth in the rumen. The stable IVI values on a volume change by weight basis ranged from 0-30. The average surface tension in dynes per sq. cm. surface area was 55.2 on a normal ration, consistently lower (52.6) for fistulated animals on either high or low protein bloat producing rations and 49.2 for an animal not fistulated on a low protein bloat producing ration. Surface tension of 10 samples from animals not bloating on Ladino clover was 54.4.

Bubble Physics and Rumenal Froth - Volney Wallace, South Dakota State College, College Station

The pressure in a simple bubble is equal to environmental pressure plus $2 \gamma/r$, where γ is surface tension and r is the radius of the bubble. The magnitude of this effect is shown here for a solution having a surface tension of 50 dynes per centimeter.

| | | | | | | |
|-------------------------------|--------|-------|------|-------------------|-------------------|-------------------|
| Bubble radius, microns | 1000 | 100 | 10 | 1 | 0.1 | 0.01 |
| Pressure, atmospheres | 0.0005 | 0.005 | 0.05 | 0.5 | 5 | 50 |
| Pressure, cm H ₂ O | 0.5 | 5 | 50 | 5x10 ² | 5x10 ³ | 5x10 ⁴ |

Rumen juice may be regarded as saturated with gas with respect to bubbles of a given radius, r_0 . Larger bubbles grow and smaller bubbles dissolve. The growth pressure of a bubble of radius r is dependent on bubble size, being equal to $2 \gamma (1/r_0 - 1/r)$. These effects may also be derived from the formula relating solubility to particle size.

If a bubble is attached to a surface, but is greater than hemispherical in the shape of the gas/liquid interface, it behaves qualitatively as a simple bubble. If it is less than a hemisphere, dP/dV is positive, and the bubble comes to equilibrium with the solution. These bubbles lose their stability when increasing saturation pressure or spreading of the bubble takes it beyond hemispherical shape. Such nuclei appear to be abundantly present in rumen juice, for this material forms bubbles copiously when placed in a moderate vacuum.

If a bubble is surrounded by an elastic coating, this coating will develop tension as the bubble grows and increase the pressure of the bubble by $2S/r$ where S is the elastic tension. As V increases, dP/dV becomes less negative, then positive. If S does not exceed the tensile strength of the surface layer, the bubble will come into equilibrium with its environment. If the tensile strength is exceeded, the coating will burst and the bubble will continue to grow. The course and extent of this action is a function of bubble size and thickness, modulus of elasticity and yield characteristics of the coating.

If a bubble is out of round, it may be assumed that elastic tension is present in the surface of the bubble acting with or against surface tension. In such a bubble the surface tension (total tension) is directly proportional to the radius of curvature.

Coatings expected to have elasticity are: (1) monomolecular layers of surfactants, (2) gelatinous material (specifically zoogloea matter), (3) mutually adhering particulate matter, and (4) particulate matter bound by matter or fluid occupying the interstices (a cluster of wet glass beads is a model for this).

The existence in frothy bloat of bubbles whose growth has been slowed or stopped by elastic surface coatings is suggested by the following observations by the author:

1. Bubbles in ruminal froth appear under the microscope to be coated with matter, with much particulate matter included in this coating.
2. Bubbles have been observed to rupture, with a bubble growing out of the side of the parent bubble.
3. Bubbles from frothy rumen juice are commonly out of round, with deep constrictions and protruding knobs not uncommon.
4. Matter coating bubbles has been isolated and found to be rich in lipids (12-24 percent) and to contain an abundance of micrococci.

Radiosonde for Measuring Rumen Pressure - Volney Wallace, South Dakota State College, College Station

Radiosondes consisting of a low frequency transistorized oscillator were constructed with the tuning slug of the oscillator attached to a barometric element. These units broadcast a weak signal whose frequency was a function of environmental pressure. The signal was picked up and recorded by a communications receiver (beat frequency oscillator on), an audio frequency meter and a recorder in that sequence. The system was a success in that it permitted continuous monitoring of ruminal activity without disturbing the animal.

The receiving and recording system is extremely precise, but sensitive to noise. A high df/dP in the radiosonde and a standard FM detector in the receiver should give satisfactory precision and less sensitivity to noise.

Use of VHF frequency rather than low frequencies is recommended because of the low level of atmospheric noise at VHF frequencies and the high gain characteristics of VHF receiving antennas.

Battery life is about 10^{-3} watt days per gram. Use of RF power to run the radiosonde may be superior to use of batteries.

The radiosonde should be mounted in a gimble mounting as orientation affects both signal strength and frequency of the signal. The latter effect stems from the fact that the transducer is in effect a weight on the end of a spring.

Extreme precautions should be taken to prevent diffusion of H₂S into the instrument. This gas is very corrosive and goes right through rubber.

Federal Communications regulation should be consulted by those intending to use radio power in excess of 0.1 watt, or for operation in excess of about 1/2 wavelength.

Isolation of the Froth Fraction of Frothy Rumen Contents - Volney Wallace,
South Dakota State College, College Station

Rumen juice from acute cases of bloat on fresh alfalfa were observed to separate on standing with the upper layer being lighter in color and having a marked yellow cast. When the upper layer is mixed with water, it separates again, quite sharply, into a greenish yellow froth layer and green wash water. Repetition of this procedure removes all but a trace of the green color, giving a surprisingly stable yellow froth layer.

The washed froth under the microscope appears as bubbles coated with matter with a considerable amount of particulate matter in the surface layer. The larger bubbles are generally out of round, sometimes possessing major constrictions and proturbances. The bubbles show little tendency to merge. When the cover slip is moved laterally, these bubbles appear to roll. These bubbles are dull in luster and examination of frothy rumen ingesta shows that the large bubbles have a frosty luster and are yellow green in color in contrast to the deep green of the ingested alfalfa.

When washed froth is centrifuged some greenish matter settles to the bottom of the centrifuge tube but the bulk of the solids settle to the top of the tube forming a greenish yellow water repellent layer. Examination of this material has been exploratory rather than systematic with the following data and observations to date. Data are single analysis of separate samples.

Lipid: (percent) - 14.0, 11.8, 24.3, 23.4 and 18.6

Crude protein: (percent) - 14.2 and 22.4

Ash: (percent) - 2.7 and 11.0

Pigment: ca. 0.06 percent carotenoid pigment, with the following pigments tentatively identified: Cryptoxanthin, Xanthophyll, Zeaxanthin and beta-carotene

Solids: Gram positive micrococci most abundant numerically. Also present are gram positive diplococci and short rods, mineral particles and masses of organic matter. When this matter is dried before fat extraction, organisms are imbedded in a solid phase.

Rumen contents were taken from three slaughtered animals from feedlot and a small yield of white froth was obtained by the above procedure. The isolated froth solids were found to contain 5.83, 6.55 and 7.95 percent lipid respectively (average 6.77 percent).

Some of the Biophysical Aspects of Bloat - R. E. Nichols, University of Wisconsin, Madison

A correlation coefficient of 0.83 has been demonstrated to exist between the mass viscosity (V) of samples of ruminal content and the centrifuged volumes of the solids of the same samples (ZV). In animals eating fresh legumes, the fluidity of ruminal content is much less than in animals eating legume hay. When the ratio of the distance between the particulate

elements (d) of a suspension to the diameter of the particles (D) approaches 0.1, the viscosity of the whole suspension begins to increase very markedly. Since ruminal contents are a suspension system, the above phenomenon not only can apply but actually does when a very rapid production of bubbles of CO₂ in ruminal contents of increased viscosity occurs following the feeding of fresh young legumes. During such rapid production of bubbles in the rumen, measured ZV's often increase to 90 percent or better indicating a d/D ratio of < 0.1 and V's often simultaneously increase to two or three times those found before the period of rapid gas production. Following these periods of rapid gas production in animals relieved of excessive ruminal pressure, ZV's and V's decrease rapidly as also does the froth resulting from the rapid gas production.

Observations on the Ruminal Microbial Populations During Bloat -
R. E. Davis, J. Gutierrez and I. L. Lindahl, AHRD, ARS, USDA

The bacterial changes which occurred with the onset of bloat symptoms when animals were adapted to a high grain diet using controlled feeding methods were confirmed. The bloat symptoms were correlated with the appearance in the ruminal fluid of long chained encapsulated streptococci and large sarcina type organisms. When the levels of protein were varied between 13 and 21 percent, the incidence of bloat was not significantly affected. The effect of different levels of carbohydrate on the incidence of bloat is currently under study.

In animals on bloat-provoking clover pastures, gross microbial differences between bloaters and non-bloaters such as are observed in feedlot bloat could not be established. Extreme foaming was a characteristic of rumen samples in both the feedlot type bloat at Beltsville and pasture bloat cases at Mississippi State College. Isolation of saponin-utilizing bacteria in significant numbers from Mississippi bloated steers on Ladino clover pastures was possible by the use of rumen fluid agar medium enriched with 0.5 percent composite alfalfa saponins. The strain characteristics of the bacteria able to degrade saponins were similar to Butyrivibrio sp. which had been isolated earlier from animals on alfalfa pastures at Beltsville.

Total Pectic Substances and Uronic Acids in Pasture Plants and Their Relationship to Incidence of Bloat - H. R. Conrad, W. D. Pounden, A. W. Fetter and R. D. Ramseyer, Ohio Agricultural Exp. Sta., Wooster

Experiments were conducted during two years to determine the possible relationship of pectic substances in legume pasture plants to the formation of stable foam in the rumen and the incidence of bloat in dairy cattle. Four dairy heifers and four dairy steers ranging in age from 8 to 12 months were used for experimental cattle.

During the first year the amount of total pectic substances in the Ladino clover pasture ranged from 1.1 to 6.6 percent. The highest incidence of bloat was found to be during the period when the Ladino clover analyzed

highest in total pectic substances as determined by alcohol precipitation. The following tabulation shows the incidence of bloat in relation to various levels of total pectic substances found in Ladino clover pasture.

| Total pectic substances (mg./g.) | No. of days sampled | Number of animal days | Number of bloat cases | Bloat per animal day |
|-------------------------------------|---------------------|-----------------------|-----------------------|----------------------|
| 11 - 20 | 13 | 104 | 0 | 0.00 |
| 21 - 30 | 7 | 56 | 11 | 0.20 |
| 31 - 40 | 4 | 32 | 24 | 0.75 |
| 41 - 50 | 7 | 56 | 25 | 0.45 |
| 51 and above | 2 | 16 | 5 | 0.31 |

During the second year the pasture forage available remained principally grass throughout the season. Only five cases of bloat were observed. These occurred at peaks in the average pectin content of the mixed forages. Total pectic substances and uronic acids were determined throughout the season. Marked daily variations in the pectin content of alfalfa were observed. The amount of total pectic substance varied from 0.6 percent to 8.9 percent. Variations in the uronic acid content were less marked but were found to be uniformly higher in alfalfa and Ladino than in orchard grass and timothy. Average content of pectic substances and uronic acids was 4.01 percent and 16.3 percent for alfalfa, 2.86 percent and 16.4 percent for Ladino, 2.45 percent and 11.8 percent for birdsfoot trefoil, and 0.26 percent and 8.0 percent for orchard grass.

A positive relationship between incidence of bloat and content of total pectic substances in Ladino clover along with the observed high level of rapid gas producing uronic acids in the bloat producing legumes suggests that the pectic substances and hemicelluloses provide essential plant factors for the occurrence of bloat in cattle.

Studies with the Cellulose Digesting Micro-Organisms from the Rumen -
Burk A. Dehority, Khaled el-Shazly and Ronald R. Johnson, Ohio Agricultural Experiment Station, Wooster

Four sheep on synthetic diets and a fistulated steer on a hay diet were used to determine whether or not the micro-organisms cultured in in vitro fermentations were representative of the in vivo rumen microflora. Using volatile fatty acid production as a criteria the results indicated a definite parallelism in the rates of fermentation of cellulose in vivo and in vitro for the first 12 hours. Further studies on the direct measurement of cellulose breakdown in vivo and in vitro were carried out using the nylon bag technique in vivo. Comparison of the rates of digestion over a 48-hour period revealed a striking similarity between the in vivo and in vitro fermentations. Careful microscopic examination before and after the in vitro fermentation revealed a proliferation of Gram-negative micrococci and very small rods, which were also found in the original inoculum in large numbers. These same organisms were observed to proliferate in vivo.

Concurrently with the work above, a project was started in an attempt to separate out those rumen bacteria responsible for cellulose digestion. Differential centrifugation of rumen fluid revealed that the cellulolytic bacteria remained in suspension after centrifugation at 1500 times gravity but were sedimented at 3000 times gravity. After allowing this fraction to ferment cellulose for 30 hours a tremendous proliferation of the Gram-negative micrococci was observed. All other organisms present in the inoculum had disappeared. Using this technique, enriched cultures of this Gram-negative micrococci were obtained for experimental use in determining the nutritional requirements of these bacteria. At the same time, attempts were made to grow the Gram-negative micrococci on solid media. After three days of anaerobic incubation, a round, smooth, colorless, opaque colony was obtained. On examining a smear of the colony it was found to consist of Gram-negative micrococci. However, when transfers were made from the micrococci colonies on to a similar liquid media no visible signs of cellulose degradation were observed over a 14-day incubation period.

Thus, the nutritional requirements of these Gram-negative micrococci were investigated using the enrichment cultures described above. It was found that neither biotin, PABA or valeric acid alone enhanced cellulose digestion to a very marked degree. When either of these vitamins or both are added in combination with valeric acid, however, a definite increase of cellulose digestion was observed. Further work with washed cells indicated that a combination of both biotin and valeric acid are required for cellulose digestion and the requirement for PABA would appear to be of a secondary nature. The requirement of the enriched culture for biotin was investigated and it was found that approximately .5 gamma per hundred ml. of media was satisfactory.

Numbers of Protozoa in Relation to Bloating Tendency of Cows Fed Fresh Alfalfa - R. A. Mah and R. E. Hungate, University of California, Davis

A comparative estimation of the relative numbers of protozoa present in selected bloating and non-bloating cows disclosed a difference in population, particularly in the protozoa belonging to the genus Ophryoscolex. When cows were bloating on a diet of fresh green chopped alfalfa, Ophryoscolex was present in numbers as high as 1740 per ml. Non-bloating cows on the same diet contained this organism in numbers less than 20 per ml. Variations in the population of Ophryoscolex occurred with a change of feed. On less succulent non-bloating green chopped alfalfa, some bloaters and non-bloaters contained Ophryoscolex in similar numbers. On dried alfalfa hay, both groups of animals showed a decrease in the numbers of Ophryoscolex to a range of 40 to 100 per ml.

Preliminary Experiments on the Microbiology of the Rumen of Cows on an Alfalfa Green-Chop Ration - W. B. Lester, S. Vyas and R. E. Hungate,
University of California, Davis

Availability of the bloating and non-bloating animals used by Mendel and Boda prompted a study of their microbiology. An attempt was made to identify the factors concerned with increased foaming. Rumen contents from bloaters and non-bloaters were inoculated into flask cultures containing various combinations of saliva, alfalfa juice, alfalfa saponin, and sucrose. No differences in the two groups were detected, but the results were inconclusive because during the period of the study the bloat was not marked. Earlier studies when the bloat was more pronounced did show increased foam stability in vitro for the bloaters but this characteristic could not be maintained in vitro.

Culture counts of both groups, using a fresh alfalfa extract medium, disclosed no significant differences in numbers or colony types.

Saponin was fermented by rumen ingesta from bloated and non-bloated animals during periods of active bloat. The attack was relatively slow and maintained over a long period.

ANIMAL MANAGEMENT PANEL

Soil Fertility and the Occurrence of Bloat on Ladino Clover -
H. D. Jackson, R. L. DeVault, G. D. Goetsch and B. W. Hatcher, Purdue University, Lafayette, Indiana

Two adjacent ladino clover paddocks (#2 and #3) of two acres each were used in this study. Soil analyses showed similar fertility in each paddock and revealed approximately 110 lbs. available P₂O₅ per acre. This phosphate level is suboptimum and demanded an additional 100 lbs. P₂O₅ per acre to reach the level recommended for legume crops. Pasture #2 received this phosphate level, and pasture #3 received none. Both pastures were fertilized with 200 lbs. K₂O per acre and provided lush herbage.

Twenty head of Hereford feeder cattle were divided at random into two herds (A and B). The herds grazed each pasture alternately for four experimental eras of four days each. Between these eras the cattle grazed on bluegrass pasture. The cattle were on ladino clover pasture for three grazing periods of one and a half hours each, each day and were in dry lot at other times. The experiment was conducted during June, 1959. A forage sample was harvested from each pasture in the afternoon of the sixteen experimental days.

The animals were scored for bloat when taken off pasture using a four point scale. One hundred and forty-eight cases of bloat were observed. The occurrence and severity of bloat were approximately equal on the two pastures but differed between the two herds. A bloat index was calculated for each forage sample based on the observed bloat (Index = total bloat score + no. of animals x no. of grazing periods).

The forage samples were further analyzed for N, P, Ca, K and for respiratory-inhibiting activity. The levels of these constituents were approximately equal in the forages from the two pastures and did not vary with the bloat index of the forage.

Sustained Prevention of Bloat by Antibiotics Fed in Rotation or in Combination - R. H. Johnson, P. A. Hartman, L. R. Brown, H. H. Van Horn, Jr. and N. L. Jacobson, Iowa State University, Ames

During the 1959 pasture season, 45 dairy and beef cattle (average initial weight, 639 lb.) were utilized for grazing trials. Six were fistulated steers. Following a preliminary test period in early May, the animals were divided into three groups of approximately equal bloat potential by blocking on total bloat scores. Two of the groups then received antibiotics while the third served as a control. The alfalfa pasture caused much serious bloat from May until early August; thereafter

Incidence and severity of bloat decreased noticeably. All animals grazed together twice daily, from 7 to 10 a.m. and from 4 to 7 p.m. Before grazing, each treatment group received antibiotics mixed with ground grain (in the p.m. only) or grain alone at the rate of $1\frac{1}{2}$ lb. of total mixture per animal. Bloat severity was observed continuously during grazing periods and for a time afterward; evaluation was visual using the scale described previously (J. Animal Sci., 17: 374, 1958). Experimental periods were not fixed; each antibiotic was fed until it was proven ineffective, or, if initially effective, until the incidence of serious cases of bloat indicated the development of resistance. Serious cases of bloat were treated with an emulsified soybean oil prepared especially for the purpose; in 94 cases scored 3 or higher, relief was prompt when 100-400 ml. of this product was administered intraruminally. Results were best at the higher levels. One animal died during treatment.

Penicillin and erythromycin fed in combination controlled bloat more effectively and for a longer period of time than did the same antibiotics fed in rotation; control was very good for 26 days in one trial, 23 days in another. Considerable preventive effect was obtained using the combinations after these periods of maximum effectiveness, but serious cases were beginning to occur. With regard to duration of effectiveness when fed individually (in rotations), penicillin (35 mg.) and erythromycin (70 mg.) controlled bloat the longest (about 10 days). Tylosin (105 mg.) was effective about as long but bloat was decreasing toward the end and this may have obscured the true effect of the tylosin. Chloramphenicol (140 mg.) was effective about 7 days during a period of less serious bloat. Novobiocin (70 mg.) was effective for approximately 4 days. Oxytetracycline (140 mg.) reduced bloat substantially for about 4 days, and was less effective thereafter. Neomycin (70 mg.) seemed to reduce bloat under some circumstances, but the results were variable and difficult to interpret. Spontin (210 mg.) may have reduced bloat somewhat. Vancomycin (70 mg.) apparently promoted bloat. It has to be recognized that order of feeding may have affected the effectiveness of individual antibiotics considerably.

Results indicate that the problem of diminishing effectiveness of antibiotics can be avoided by feeding the proper ones in combinations or in rotations. Further work must be done to establish the most effective combinations, appropriate sequences and proper levels. Other antibiotics should be investigated for use in combinations, rotations or possibly in rotations of combinations. Development of water-soluble, stable forms of the antibiotics offers promise as an alternative method of administration.

Bloat Susceptibility of Individual Dairy Cattle -

V. E. Mendel, D. A. Yarns and J. M. Boda, University of California, Davis

It has been noted by several workers that some animals fed a bloat-producing feed are more prone to bloat than others. One report showed that the propensity to bloat is an inherited characteristic in cattle. These observations suggest that some factors are contributed by the animal in determining the development of acute bloat.

In the summer of 1958, 13 head of dry dairy cows were placed on experiment. The cows were housed and fed in individual pens throughout the experimental period of 35 days. They received freshly chopped alfalfa tops ad libitum for 1 $\frac{1}{2}$ hour periods in the morning and afternoon. Feed consumption was measured at the conclusion of each feeding period. Thirty minutes after the commencement of feeding tympanometer readings were made. This was continued at 15-minute intervals until all animals had attained a maximum intra-rumen pressure and returned to near normal pressures.

At the conclusion of these trials, it was noted that several animals had consistently bloated while others had shown little or no tendency to bloat. In order to ascertain the permanency of these bloating characteristics, the 1958 trials were repeated in the spring of 1959 with eight of the same cows. Four additional animals were also included in these trials.

During the course of these studies, it was observed that certain animals would reduce their feed intake to low levels after having experienced severe bloat a few times. That is, they would stop eating as soon as the intra-rumen pressure had reached levels of approximately 6 to 8 mm. Hg. As a consequence, such animals failed to bloat, although from earlier performances it would appear that they had good bloating potential. In order to more accurately evaluate the bloat potential of individual animals, an equation was developed to give a value termed bloat index. Factors in the equation include tympanometer readings and feed consumption.

The application of the procedure to each observation made on an animal gives a clear picture of that animal's tendency to bloat. The results show that some animals are quite consistent bloaters over a relatively long period of time, i.e., 35 days. For example, cow # 37 bloated well for three consecutive years as determined by the large percentage of times bloated, the high maximum intra-ruminal pressure attained and the large, positive bloat index. On the other hand, some animals are equally as consistent as non-bloaters.

Of possibly greater interest than either the consistent bloat or non-bloat is the animal which reverses its bloat susceptibility from time to time. These reversals emphasize the necessity for carefully controlled

bloat characterization trials conducted for reasonably long periods of time when selecting bloating and non-bloating animals for studies on animal factors. Not only do these reversals in bloat susceptibility point out wide variations in the bloating potential of certain animals, they also complicate the interpretation of how animal factors influence the development of bloat. A number of such factors or combination of factors have been proposed as contributing to the development of bloat, e.g., salivary production, rumen motility, microbial slime production, etc. It is evident that any proposed animal factor or combination of factors must explain the bloating and non-bloating condition as well as the reversal from one condition to the other within the same animal.

The Frequency of Occurrence of Bloat in Several Breeds of Cattle Grazing Three Varieties of White Clover - J. E. Johnston, G. A. Hindery and L. E. Foote, Louisiana Agricultural Experiment Station, Baton Rouge

These studies were conducted in an effort to determine whether there were breed differences in bloat frequency or differences in the tendency of animals to bloat on various varieties of white clover. A study of climatic factors which might be responsible for day to day variations in frequency of bloat was conducted concurrently.

An area of approximately 22 acres of Mississippi River bottomland was divided into three plots and seeded to Common White Dutch, Louisiana S-1 White and Ladino clovers during the fall of 1958. On April 1, 1959, 46 head of cattle were placed on the area. They consisted of: 15 aged Brahma cows; 10 aged Angus cows; 4 aged Hereford cows; 9 yearling Hereford steers; 7 two-year-old dairy heifers (5 Holstein, 2 Jersey) and 1 fistulated Holstein cow. Aged cows were 8-9 years old. On April 24 the cattle were divided into three groups so that breed and age groups were represented nearly equally in each group. Tendency to bloat was not considered in the division. During the period from April 24th to May 20th one group was grazed on each of the varieties of clover. Animals were rotated among groups during the study. During the period from May 28 to June 18 the Hereford, Angus and Brahma cows were replaced by a breeding herd of 28 Hereford cows and one bull. During the second period the cattle were grazed on varieties of clover as growth permitted.

Cattle were permitted to graze two hours each morning and two hours each afternoon. They were observed during a one to two hour period following removal from the clover and incidence of bloat recorded on a 0-4 scale. Frequency of bloat was computed as the percentage which the cases of bloat observed were of the total possible cases.

The percentage frequencies of bloat observed on the Ladino, La. S-1 White and Common White Clovers were 70, 62 and 62 respectively. The frequency during the period of study was significantly higher on the Ladino than on the other clovers. This may have been associated with the rate of growth and reproductive status of the clovers since the Ladino produced considerably more forage than the others and displayed very few seed heads during this period. The other clovers were well seeded cut throughout the study.

The percentage frequencies of bloat observed in Brahma, Angus and Hereford cows, Hereford steers and dairy cattle were 45, 74, 68, 51 and 61 respectively. The Brahma cows showed significantly less bloat than all others except the Hereford steers which showed significantly less bloat than the Angus or Hereford cows. It should be noted, however, that despite the lower frequencies in Brahma cows and Hereford steers two of each of these groups of animals died of bloat during the study while no losses were experienced in the other groups. The percentage frequency of bloat in all groups was 54% in the morning and 60% in the afternoon. This difference was not statistically significant.

It would appear that while differences among clovers and animal groups in frequency of bloat were observed the frequency was high enough in all animal groups and on each clover to satisfy experimental requirements. Thus if clover is in the proper stage for induction of bloat any type of cattle may be used as experimental animals without prior screening.

Daily maximum and minimum air temperatures, solar radiation and rainfall were plotted against frequency of bloat during the periods April 21 - May 20 and May 28 - June 17 in an effort to identify changes in one or more of these climatic variables with frequency of bloat. During the period from April 23 - April 27 a rise in minimum air temperature from 42 to 70° F. was associated with an increase in frequency of bloat from 15.6 to 95.6 %. Maximum air temperature increased simultaneously from 74 to 85° F. It is quite probable that growth rate of the clover increased during this period and was responsible for the increase in bloat frequency. Except for this one instance variations in climatic conditions did not appear to show consistent relationships to bloat frequency.

PHYSIOLOGY PANEL

Rumen Metabolism of Natural and Radioactive Lipids -

R. S. Ailen, F. D. Hill, E. G. Hammond and N. L. Jacobson, Iowa State University, Ames

I^{131} - tagged soybean oil (about 1 mc/trial) was poured into the rumen of a steer via a fistula (trials 1 and 2) or fed with concentrate (trials 3, 4 and 5). Samples of rumen ingesta were taken at intervals during the first 24 hours; samples of venous blood and of freshly voided feces were obtained at various times for 7 days. Rumen ingesta samples and selected blood and fecal samples were fractionated to permit measurement of I^{131} activity in neutral fats, fatty acids and iodides. Total I^{131} activity was determined in all blood and fecal samples. Poor distribution of radioactivity in rumen ingesta resulted when labeled oil was administered via fistula, but oral ingestion gave good distribution. I^{131} activity in rumen neutral fats declined sharply; less than half the original activity remained after 4 hours in most trials. Deiodination of the tagged oil was significant. Very little I^{131} was found in the fatty acids fraction. In trials 4 and 5 where alfalfa silage was the major feed, maximum I^{131} activity occurred at 18-24 hours in the blood and at 32 hours in the feces. Virtually no lipid I^{131} appeared in the blood, and only traces were found in the feces. In vitro experiments with I^{131} - labeled soybean oil confirmed the in vivo observations and further suggested that hydrolysis of triglycerides was not extensive.

Additional in vitro studies, employing unlabeled lipids, were performed to estimate the extent of hydrolysis in rumen ingesta. Hydrolysis tended to reach a maximum at about 24 hours in most trials. In various 24-hour trials hydrolysis of soybean oil (1 g./100 ml. ingesta) ranged from 18 to 43% with normal ingesta and was negative with heated or the supernatant fraction of centrifuged ingesta. At pH 5.3, lard oil hydrolysis was essentially the same as that for soybean oil. Oil present in soybean flakes was hydrolyzed to a lesser extent than crude soybean oil. Lard oil hydrolysis was influenced in markedly different fashion by pH than was that for soybean oil. The extent of hydrolysis of soybean oil was affected by the time at which ingesta samples were collected; minimum values were observed after the morning grazing period. Oleic-1-C¹⁴ acid was incorporated into neutral fat to a limited extent (10%) by rumen ingesta, while a significant amount (3%) of the radioactivity was found as non-lipid.

Physiological Studies of Bloat: Methodology and a Preliminary Report on the Secretion of Saliva - V. E. Mendel, D. A. Yarns, J. M. Boda and T. N. Wegner, University of California, Davis

Studies have been conducted in an effort to determine the differences between bloat-susceptible and non-susceptible dairy cows. Equipment required by these studies has been developed.

Six cows were selected for these trials from a group of 17 cattle characterized previously as to their bloat susceptibility. Four were classified as consistent bloaters and two as non-bloaters.

The animals were prepared with 4-inch ruminal fistulas and immediately fitted with semi-pneumatic rubber plugs. Fistulation was carried out by a modification of Schnautz's method (1957, Am. J. Vet. Res. 18:66-73). This modification produces a very uniform fistula which, upon healing, fits the plug well.

The semi-pneumatic plugs employed are a modification of Balch's plug (1948, Vet. Rec. 60:446). They differ by being of one piece rather than two, are made of different types of rubber and are fabricated on the campus rather than commercially. A vulcanizer was developed for the fabrication. These plugs have been found to have many advantages; that is, they do not stretch the fistula since they are quite light and flexible, they do not fall out of the animal under most conditions, leakage of ingesta is minimized, and they can be fabricated easily.

A fully pneumatic fistula plug was also developed for use in rumen motility and gas production trials. This plug was fabricated with the above-mentioned vulcanizer.

After fistulation, a 41-day pilot trial was conducted on a fresh alfalfa-top ration in an effort to determine some differences between the bloat-susceptible and non-susceptible cows. The following factors were considered:

1. Rumen motility
2. Gas production
3. Ingesta
 - a) Nitrogen fractions
 - b) Mucoprotein
 - c) Buffering capacity
 - d) Dry matter
 - e) I.V.I.
4. Salivary secretion
 - a) Resting rate
 - b) Feeding rate
 - c) Composition
5. Water consumption

Rumen motility was recorded on a kymograph by placing balloons, attached to appropriate tambours, in various areas of the reticulo-rumen. The balloons were made from 6-inch sections of bicycle tire tubes, partially filled with lead strips to prevent them from shifting to other areas of the reticulo-rumen. Highly satisfactory tracings were obtained by this method. An apparatus was developed for the purpose of recording eructations. This consisted of a contact microphone strapped to the throat, the signal being fed through an audio amplifier. This enables one to hear the passage of gas as it is eructated, thereby making it possible to distinguish those eructation contractions which result in the expulsion of gas from those which do not.

Methods of gas collection were those previously employed at this station (Cole, H.H. et al., Calif. Agr. Exp. Sta. Bul. 662, 1942) and the methods employed in the ingesta studies were standard methods as reported in the literature.

Concurrent with the pilot trial a group from the Department of Bacteriology, under the supervision of Dr. R. E. Hungate, investigated various microbial factors.

The results from the pilot trial indicated that differences in salivary production offered the most promise for further investigation; therefore, salivary rates and composition as well as various ingesta liquid-solid relationships were investigated in later trials.

Saliva was collected each day from a bloater and a non-bloater, following an 18-hour fast. All animals were kept on a constant intake of 70 lb. green-chop per day. The rumens were evacuated to facilitate collection. A latex rubber cannula was designed for the purpose of collecting resting saliva (fasting saliva) from the cardiac orifice, the collection being carried out with a very low vacuum which was regulated by a vent in the cannula. Collection time was of 20 minutes duration. To eliminate the effect of stimulation produced by insertion of the cannula, the saliva collected during the first 10 minutes of the collection period was discarded. All "resting salivary" data is therefore based on the second 10-minute portion of the collection period.

"Feeding saliva" was then measured by feeding four 500-gm. portions of green-chop. Each 500-gm. allotment was caught in a rubber bag. The weight of the contents of the bag less the 500-gm. green-chop fed represents the amount of saliva produced. The time from the commencement of eating until the last bolus was swallowed, from each 500-gm. feed allotment, represents the time of salivary secretion.

Since there may be differences in salivary flow rates with time in these two kinds of cows, a 20-minute feeding period was employed during which

the animals were fed ad libitum; feed consumption was measured. A second "feeding saliva" collection period was carried out at this time.

These results demonstrate that the rate of salivary flow was greater in the non-bloat-susceptible cows than in the susceptible cows.

New Information on the use of the "Isolated Rumen" and on Saliva Secretion Studies - H. M. Cunningham, Canada Experiment Farm, Nappan, Nova Scotia

The apparatus for temporarily "isolating" the reticulo-rumen of fistulated steers (Can. J. An. Sci. 38:84, 1958) has been further tested and modified. The plug may be made to form a tighter seal with less inflation by using two layers of rubber tubing and dividing only the inner layer into two chambers. When inflated, the plug takes the shape of an hourglass with the outer layer expanding enough at the center to tightly seal the orifice. Preliminary six-hour trials with the "isolated rumen" show that losses of the proximate components of the ingesta are greatest when animals have been fed hay and grain, moderate when fed hay only, and negligible when fasted for 16 hours. Chromic oxide, placed in the rumen the day prior to a test does not mix in well enough with the ingesta to serve as an accurate indicator. Traces of chromic oxide in wet feces may be rapidly detected by fusing a sample in a Parr oxygen bomb with ethylene glycol and observing a green tint in the small beads of ash.

Saliva secretion rate was two to four times higher during the first hour than in the sixth hour of collection. The high initial secretion rate occurred regardless of whether the steer had been fed or fasted, indicating that it may have been stimulated by the collection apparatus.

Acetic acid produced twice as much froth when added to rumen juice obtained before the morning feeding than with juice obtained four hours after feeding. Juice obtained before feeding had approximately the same pH as that obtained after feeding but required 25% more HCl to lower the pH to 6.

Some Aspects of Abomasal Physiology -
Kenneth Hill, Institute of Animal Physiology, Babraham, England

The abomasum is the true secretory part of the ruminant stomach. In the fetal lamb peptic cells containing pepsinogen granules are present in the mucosa from roughly half term and gradually increase in number up to birth. Pepsin can be demonstrated in the fetal abomasal contents but it is in the inactive or pepsinogen form because the pH of the abomasal contents is close to neutrality. There are practically no

acid secreting cells during fetal life, in fact it is not until the first 24 hours of life that parietal (acid secreting) cells are seen in any number. Conditions for peptic digestion are not optimal until 36 hours after birth when the pH of the abomasal contents drops to about 3.0. There is a close correlation between the onset of gastric proteolytic activity and the period when immune globulins are absorbed. This allows the colostral globulins to pass through the stomach unharmed so that they can enter the intestine where they are absorbed.

In ruminants the secretion of gastric juice is more or less continuous, however, the gastric glands do not possess the property of spontaneous secretion, since the secretion of acid gastric juice ceases when ingesta is removed from all four compartments of the ruminant stomach. The cephalic phase of gastric secretion is absent in sheep. Feeding sheep for only a short period each day will increase gastric secretion. This may be due to the increase in motility of the reticulo-rumen with an increased flow of ingesta into the abomasum. The main factor responsible for stimulating gastric secretion is the constant passage of ingesta through the abomasum with the resultant stimulation of the pyloric phase of secretion.

Volatile fatty acid concentration has a marked influence on gastric secretion. As their concentration increases gastric secretion is stimulated. Of the three main V.F.A., acetic acid caused the greatest secretory response. The total volume of gastric juice secreted by the abomasum of a 50 Kg. sheep has been estimated to be 3 liters in a 24-hour period. The total gastric secretion of a two-year old heifer has been estimated to be about 30 liters daily.

Little information is available on the time digesta remains in the abomasum or how much digestion occurs in this compartment. Disintegration of protozoa and bacteria must occur in the abomasum, but there is little or no cultural work on the abomasal contents.

Bloat Versus No Bloat in Identical Twin Cows Fed Identical Rations-
R. S. Emery, C. K. Smith, C. W. Duncan, and H. M. Sell, Michigan State University, East Lansing

Smith et al. (1953) reported that frothy bloat could be produced by feeding 16 lb. of a grain mixture and 4 lb. of unground alfalfa hay. It has since been found that a ration consisting of equal amounts of this grain mixture and hay would sustain the frothing condition once it had been initiated, but would seldom initiate frothy bloat in a normal cow. This fact permitted studying the composition of the rumen liquid

from bloated and non-bloated cows independent of ration effects. During a 3 week preliminary period 1 member of each of 2 pairs of identical twins was assigned to a ration consisting of 16 lb. of the grain mixture of Smith et al. (1953) plus 4 lb. hay while the other member of each pair of twins was given a ration of 20 lb. of hay. The ration of all cows was then changed to 10 lb. of the grain mixture plus 10 lb. of hay for a 2 week period at the end of which samples of rumen liquid were taken via rumen fistulae equipped with plastic cannulae. The cows which had previously received the high grain ration were bloating regularly at this time. The liquid was clarified by centrifuging for 30 minutes at 15,000 R.P.M. on a Servall centrifuge. The short chain fatty acids, pH, sodium, potassium, and ammonia were determined in the clarified liquid. The short chain fatty acids for twins 1 and 2 were determined at 8 intervals relative to feeding. The samples from twins 25 and 26 were taken six hours after feeding. The other determinations were carried out on the dry matter prepared by concentrating the liquid at reduced pressure at 65° followed by freeze-drying. The extractive and carbohydrates were determined by the method of Lagowski et al. (1958). The lipide and nitrogenous components seemed to occur in higher concentrations in the rumen liquid from the bloated cows. The pH was lower in the bloated cows. It has also been observed by Phelps (1958) that a larger amount of magnesium ammonium phosphate containing precipitate could be isolated from the rumen liquid of T-26 (bloated) than from T-25(non-bloated). The role these differences in chemical composition play in the etiology of bloat remains to be determined.