

UNITED STATES DEPARTMENT OF AGRICULTURE  
Agricultural Research Service  
Washington 25, D. C.

February 14, 1962

TO : Participants in Conference on Rumen Function

FROM : H. W. Marston, Chairman of Conference, Agricultural Research  
Service, U. S. Department of Agriculture, Washington 25, D. C.

SUBJECT: Report on Sixth Conference on Rumen Function

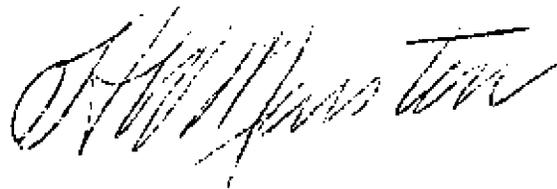
Attached is a list of participants and a copy of the abstracts of papers presented at the Conference on Rumen Function held at the Maryland Hotel, Chicago, Illinois, on November 29-30, 1961.

The Conference was convened at 9:00 AM on November 29 and was concluded at approximately 1:30 PM on November 30.

The Chairmen of the several panels organized the program for their respective panels. They, together with the persons who presented papers, are commended for the excellent material made available to the group. Those attending the Conference are also commended for the lively and interesting discussions that followed the presentation of the various papers. The Conference was most fortunate to have the following persons participate in the program and to bring some fresh viewpoints and techniques to the attention of North American workers: Dr. K. J. Hill, Institute of Animal Physiology, Babraham, England; Dr. C. S. W. Reid, Plant Chemistry Division D.S.I.R., Palmerston North, New Zealand; and Dr. Alan Dobson, The Rowett Research Institute, Bucksburn, Aberdeen, Scotland. It is sincerely hoped that they may join us at other Conferences.

The Chairman raised the question as to the desirability of continuing the Conference on its present schedule and the question of the most desirable place for holding future Conferences. It was agreed that the Conference should be continued at approximately the same time and place.

Enclosure



REPORT ON  
CONFERENCE ON RUMEN FUNCTION  
held at  
Maryland Hotel, Chicago, Illinois  
November 29-30, 1961

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

<u>NAME</u>	<u>ORGANIZATION</u>
Allen, R. S.	Dept. Biochemistry & Biophysics, Iowa State Univ., Ames
Bartley, Erle E.	Dairy Science, Kansas State Univ., Manhattan
Bechtel, H. Ernest	Daves Laboratories, 4800 S. Richmond St., Chicago 32, Illinois
Blackburn, T. H.	Dept. Bacteriology, Univ. California, Davis
Boda, J. M.	Dept. Animal Husbandry, Univ. California, Davis
Brown, R. E.	Dairy Science, Univ. Illinois, Urbana
Crump, M. A.	Dept. Veterinary Science, Univ. Wisconsin, Madison
Davis, Carl	Dairy Science, Univ. Illinois, Urbana
Davis, R. E.	Animal Husbandry Res. Div., ARS, USDA, Beltsville, Md.
Deese, D. C.	Dept. Veterinary Science, Univ. Wisconsin, Madison
Dobson, Alan	Rowett Research Institute, Aberdeen, Scotland
Dougherty, R. W.	National Animal Disease Laboratory, Ames, Iowa
Durbin, Charles G.	Food & Drug Administration, Washington 25, D. C.
Dziuk, H. E.	Univ. Minnesota, St. Paul
Elam, C. J.	Animal Husbandry Res. Div., ARS, USDA, Beltsville, Md.
Ellis, N. R.	Animal Husbandry Res. Div., ARS, USDA, Beltsville, Md.
Emery, Roy	Dairy Dept., Michigan State Univ., E. Lansing
Erwin, E. S.	Monsanto Chemical Co., St. Louis, Missouri
Essig, R. W.	Animal Husbandry Dept., Mississippi State Univ., State College
Fina, Louis R.	Dept. Bacteriology, Kansas State Univ., Manhattan
Foote, L. E.	Dept. Veterinary Science, Louisiana State Univ., Baton Rouge
Garner, George B.	Agricultural Chemistry, Univ. of Missouri, Columbia
Gessert, Roland A.	The Upjohn Co., Kalamazoo, Michigan
Gutierrez, J.	Animal Husbandry Res. Div., ARS, USDA, Beltsville, Md.
Hartman, Paul A.	Dept. Bacteriology, Iowa State Univ., Ames
Hill, K. J.	Institute of Animal Physiology, Babraham, Cambridge, England
Hoernicke, Heiko	Animal Husbandry Res. Div., ARS, USDA, Beltsville, Md.
Hungate, R. E.	Dept. Bacteriology, Univ. California, Davis
Jackson, H. D.	Dept. Vet. Physiology & Pharmacology, Purdue Univ., Lafayette, Indiana
Jacobson, Don R.	Dairy Science, Univ. Kentucky, Lexington
Jacobson, N. L.	Dept. of Animal Husbandry, Iowa State Univ., Ames
Johnson, R. H.	Daves Laboratory, Modesto, California
Kearley, Edward O.	Department Vet. Physiology, Univ. Missouri, Columbia
Lee, A. M.	Animal Disease & Parasite Div., ARS, USDA, Beltsville, Maryland
Lloyd, L. E.	Animal Science, Macdonald College, Canada
Marston, H. W.	Agricultural Research Service, USDA, Washington 25, D.C.

<u>NAME</u>	<u>ORGANIZATION</u>
Macleod, G. K.	Dept. Animal Husbandry, Ontario Agri. College, Gulph, Canada
Marco, Gino J.	Monsanto Chemical Company, St. Louis, Missouri
McArthur, J. M.	Research Branch, Canada Dept. Agr., Summerland, British Columbia, Canada
McCarthy, Robert J.	Geigy Chemical Corp., Ardsley, New York
McGilliard, A. Dare	Dept. Animal Husbandry, Iowa State Univ., Ames
McNairy, S. A. Jr.	Dept. Biochemistry, Purdue Univ., Lafayette, Indiana
Means, T. M.	Agri. Research Lab., Eli Lilly & Co., Greenfield, Indiana
Mendel, J. E.	Univ. of Alberta, Animal Science, Edmonton, Alberta, Canada
Miltimore, J. E.	Research Branch, Canada Dept. of Agri., Summerland, British Columbia, Canada
Nichols, R. E.	Veterinary Science, Univ. Wisconsin, Madison
Phillips, G. D.	Univ. of Manitoba, Winnipeg, Canada
Pidgen, W. J.	Animal Research Institute, CEF, Canada Dept. of Agri., Ottawa, Canada
Pounden, W. D.	Dept. Veterinary Science, Ohio Agri. Expt. Station, Wooster
Pressey, Russell	Dept. Biochemistry & Biophysics, Iowa State Univ. Ames
Reid, C. S. W.	Plant Chemistry Div., DSIR, Palmerston North, New Zealand
Richards, Clyde R.	Cooperative State Experiment Stations Service, USDA, Washington 25, D. C.
Rohens, Jane F.	Food & Drug Administration, HEW, Washington 25, D. C.
Roberts, W. K.	Univ. of Manitoba, Winnipeg, Canada
Ryan, R. K.	National Animal Disease Laboratory, USDA, Ames, Iowa
C. K. Smith	Dept. Microbiology, Michigan State Univ., E. Lansing
Stevens, C. E.	Dept. Veterinary Physiology, Cornell Univ., Ithaca, New York
Stone, Edward	Dept. Dairy Science, Louisiana State Univ. Baton Rouge
Stowe, C. M.	Dept. Veterinary Physiology and Pharmacology, Univ. Minnesota, St. Paul
Stustin, Francis H.	Div. Physiology and Pharmacology, Univ. Minnesota, St. Paul
Sullivan, J. T.	Crops Research Div., ARS, USDA, University Park, Pennsylvania
Troelsen, J. E.	Experiment Farm, Swift Current, Saskatchewan, Canada
Van Horn, Harold	Dept. Dairy Husbandry, Iowa State Univ., Ames
Vetter, Richard L.	Hess & Clark, Ashland, Ohio
Wass, Wallace M.	College Veterinary Medicine, Univ. of Minnesota, St. Paul
Williams, Walter F.	Dairy Dept., Univ. of Maryland, College Park
Williamson, J. L.	Ralston Purina, 835 S. 8th St., St. Louis 2, Missouri
Woods, Walter	Dept. Animal Husbandry, Iowa State Univ., Ames

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

(a) Microbiology	- -	W. D. Pouden
(b) Animal Management	- -	J. M. Boda
(c) Rumen Physiology	- -	N. L. Jacobson
(d) Agronomic	- -	D. E. McCloud
(e) Physio-Pathology	- -	R. W. Dougherty

#### MICROBIOLOGY PANEL

Digestion of C<sup>14</sup> Labeled Alfalfa in the Bovine - L. R. Fina, I. S. Yadava, E. E. Bartley, C. L. Keith and E. L. Sorensen, Kansas Experiment Station, Manhattan

Alfalfa uniformly labeled with C<sup>14</sup> was used in this work. Measured quantities were placed in the in vivo artificial rumen (IVAR) or directly into the rumen of fistulated cows. Reactions in the IVAR apparently closely follow those of the actual rumen. It was also demonstrated that the bulk of cellulose digestion in alfalfa occurs after the first 24 hours.

Upon addition of alfalfa C<sup>14</sup> to the rumen of fistulated animals there are low peaks of activity in the VFA of the rumen at 1, 7 and 11 hours; however, the major peak does not occur until after the 40th hour. Within minutes activity appears in blood with peaks initially, then at 9, 13 and 37 hours, and remaining until the 45th hour. In the urine and feces there is residual activity until the 95th hour. Carbon-14 first appears in the urine at the 2nd hour and in the feces at the 7th hour.

"Visking" and "Millipore" membranes tested for use in the IVAR were found to have suitable diffusibility properties for volatile fatty acids (C<sub>2</sub>-C<sub>6</sub> tested) and were not subject to decomposition even after 120 hours when placed in the rumen of fistulated cows or steers. All IVAR C<sup>14</sup> work reported herein was done using "Visking" membranes. When sterile assembled IVAR apparatuses, filled with distilled water, were placed in the rumen of fistulated animals for 4 or 5 days, the fluid in the interior reaction chamber remained crystal clear, was light amber colored, and was completely free of any bacteria.

Experiments on the Attachment of Cellulolytic Bacteria to Solid Particles of Rumen Contents - R. E. Hungate and Bannelore Storz, University of California, Davis

It has been assumed previously that the cellulolytic bacteria of the rumen cling closely to the fibers of cellulosic plant material undergoing digestion and that the numbers in which these bacteria could be demonstrated by cultural methods might not be as large as the actual number of viable cells in the rumen. Experiments on the relative proportions of cellulolytic bacteria in the liquid and solids fractions of rumen contents of a fistulated cow on an alfalfa hay ration were undertaken. Counts of total bacteria were made using an alfalfa extract rumen fluid agar medium and of cellulolytic bacteria using a cellulose rumen fluid agar medium. Particles were broken up by using a Waring blender for one minute. The solids fraction was separated from the liquid by sedimentation and viable counts for each were determined both before and after blending, on media for counts of total and cellulolytic bacteria.

Waring blending did not significantly increase the total count for the liquid fraction. The count for the solids was increased about tenfold by blending. The proportion of cellulolytic colony-producing units in the Waring blended liquid was only slightly less than in the blended solids. The proportion in the unblended solids was not as much greater than the proportion in the blended solids as would be predicted from the average number of bacteria per particle. The results are interpreted as indicating that a few particles contain a great many bacteria in proportions not much different from those for the cellulolytic and total bacteria in the liquid. There was little evidence that the cellulolytic bacteria were firmly attached to the plant particles to a greater extent than were the non-cellulolytic bacteria.

The results obtained apply only to the most numerous cellulolytic bacteria encountered in the cultures and these were of the general Butyrivibrio and Ruminococcus. These bacteria do not digest the cellulose in low dilutions of cellulose agar inoculated with rumen contents yet they do digest cellulose in similar liquid cultures. The difference is assumed to be due to an achievement by the cellulolytic bacteria in a liquid culture of a favorable position with respect to the cellulosic substrates whereby they gain from their cellulase enough sugar to permit them to grow and synthesize more cellulase. In solid medium they cannot achieve a preferential position near the plant fibers and the numerous accompanying non-cellulolytic bacteria take so much of the sugars from cellulose digestion that the cellulase-elaborating cell does not receive the yield from its cellulase necessary to permit it to manufacture more enzyme. In low dilutions the cellulose in cellulose agar cultures does not disappear for this reason. In the more diluted cultures the cellulolytic bacterium has a better position relative to cellulose, since its neighbors are more distant, and colonies with clearings of the cellulose can develop.

Bacteroides succinogenes is able to move through the agar in a cellulose culture, provided the concentration of the agar is not too high, and it is able to digest cellulose and form clearings even in the low dilutions of rumen contents.

The results of the study indicate that with the exception of Bacteroides succinogenes, which was not abundant in the rumen studied, the cellulolytic bacteria are not firmly attached to the particles of rumen digesta but in some fashion are able to get close enough to derive a yield of sugar from their cellulase which permits them to grow. It is doubtful that they are able to capture all of the sugar formed by their cellulase, however, and some of it probably is absorbed by accompanying non-cellulolytic forms. This may account for the fact that the proportion of cellulolytic bacteria in the rumen is smaller than the fraction which cellulose constitutes of the material digested in the rumen. This appears to be a more probable explanation of the low percentages of cellulolytic bacteria than is the hypothesis that culture counts do not reflect the true numbers because of close attachment to fibers.

Protelysis in the Sheep Rumen - T. H. Blackburn, University of California, Davis

Previous investigations have indicated that ingestion, which is a function of live protozoa, might be an essential step before digestion could occur.

Washed, bacteria-free Entodinia suspensions were obtained using a bicarbonate buffer pH 6.5 incorporating 50 µg chloramphenicol per ml of buffer to inhibit

bacterial activity. Incubations were carried out in 100 ml Erlenmeyer flasks under CO<sub>2</sub> with periodic shaking, in the presence of 1.0% casein. From a number of experiments the following points emerged; clean, washed entodinia made up to the rumen concentration hydrolysed 39% of the casein in three hours under the conditions described but only hydrolysed 14% when incubated under toluene. Clean washed suspensions of holotrich protozoa had no proteolytic activity.

It thus seemed probable that oligotrich protozoa might contribute significantly to protein breakdown in the rumen and might even be the chief agents of this activity. That this is not always true was demonstrated by showing that the rumen fluid from lambs which had been reared in isolation from birth, and which contained no protozoa, had the same proteolytic activity as other sheep. In three hours 36% of a 1.0% casein solution was hydrolysed. The rate at which casein was hydrolysed in the rumen of two sheep which had been maintained on a diet containing casein as the main nitrogen source was known. Proteolysis occurred very rapidly in the rumen, approximately 60% of the casein was digested in one hour. This rate could never be matched in vitro by adding casein to rumen fluid. The proteolytic activity of the rumen fluid was related to the microbial content of the rumen fluid. The smaller bacterial content fluctuated very little on these different rations unlike the protozoa and in general the level of proteolytic activity seemed to be related to the numbers of protozoa present. These fractionations were not precise in that the protozoal fraction contained a variable number of the larger bacterial species. The digests for the estimation of proteolytic activity were performed under toluene for 24 hours at 37°C using rumen fluid at 1/10 concentration. Shorter term incubations were performed under the conditions described earlier for the Entodinia suspensions leaving out the chloramphenicol. The rumen fluid was fractionated, the protozoal and bacterial fractions washed twice and suspended at 10 times their rumen concentration in the buffered casein 1.0% and incubated at 37°C. These concentrated protozoal and bacterial fractions were able to accomplish only 25% and 20% hydrolysis respectively of the 1.0% casein in two hours, considerably less activity than they exercised in the rumen. The addition of starch to these digests produced a light inhibition of proteolysis. Starch was the main carbohydrate in the sheep ration. It would thus seem likely that protozoa under certain circumstances might contribute significantly to protein hydrolysis in the rumen but bacteria undoubtedly can fulfil this function in the absence of protozoa and probably always are responsible for a major portion of the hydrolysis. Holotrich protozoa were never found to have any proteolytic function.

The isolation of casein degrading bacteria from these sheep was investigated since it was known that such bacteria must be present. A medium was developed consisting of Hungate's salt solution, 10% w/v clarified rumen fluid, 0.3% w/v 'Bacto' tryptose, 0.05% w/v cysteine hydrochloride, 0.0001% w/v pheno safranin 0.5% w/v sodium bicarbonate, 0.5% w/v casein and 2.5% w/v agar. Some special precautions were employed to ensure that the pheno safranin remained reduced. The medium in roll tubes had a slight opacity due to the presence of casein. This opacity was cleared in the area immediately surrounding a colony of proteolytic bacteria. The extent of the clearing differed with different isolates but in all cases the clearing was preceded by an increased opacity in the casein. On experiment with carbohydrate additives it was found that maltose in this medium brought up a very active proteolytic flora. It was found that this was specifically due to the presence of Bacteroides amylophilus, whose presence in these sheep was no doubt due to the high level of starch in their diet.

An examination was made of the effect of various carbohydrates on the type and numbers of proteolytic bacteria which could be cultured. All carbohydrates were included at a 0.3% w/v level.

From a limited number of identified isolates from some sheep on different rations the following points emerged. The types of proteolytic bacteria isolated were Bacteroides amylophilus, Butyrivibrio species, Selenomonas species, Lachnospira multiparus, Bacteroides ruminicola, Gram negative cocci bacilli and Gram positive cocci.

None of these species was found exclusively on any particular diet. Maltose in the isolation medium encourage the growth of Bacteroides amylophilus as did starch but it also grew on media containing carbohydrates which it did not utilize, provided its presence was not masked by more rapidly growing species. A good variety and number of proteolytic bacteria could be cultured on media containing no carbohydrate or a less readily available one such as carboxymethyl cellulose after 6-10 days incubation. The reason for this seemed to be that the proteolytic flora seldom predominated in the rumen and that they could easily be outgrown by non-proteolytic types, when a readily available carbohydrate was present. Some colonies which produced little or no detectable change in the casein of roll tubes, would on subculture break casein down in fluid culture.

Proteolysis occurs at a very rapid rate in the rumen. This rate cannot be matched in in vitro experiments. Oligotrich protozoa have been shown to be associated with proteolytic activity and in vitro can account for 50% of this activity. A number of proteolytic bacterial species have been isolated, most of which would appear to be types which have been associated with other rumen metabolic activities.

Isolation of a Slime from the Rumen Fluid of Animals on a High Grain Ration - J. Gutierrez and R. E. Davis, Animal Husbandry Research Division, ARS, USDA, Beltsville, Maryland

During the past three years we have been following the microbial changes which occurred with the onset of bloat symptoms when animals are fed a high grain diet. In conjunction with these studies an ethanol-precipitable slime has been isolated from the rumen fluid of animals on a feedlot bloat type of diet. Changes in the rumen bacteria and in the appearance of the slime can be studied at the onset of bloat, and a large percentage of the bacteria which have been isolated are of the slime-producing type. An analysis of the slime showed the material contained 97% water, and on a dry matter basis the slime gave values of 27% ash, 35% crude protein, 10% carbohydrate and 6% phosphorus. Paper chromatography techniques demonstrated the nucleic acid derivatives, adenine, guanine, cytosine and thymine were also present in the slime.

Slime harvests were obtained from animals during different stages of bloat and these data are shown in the following table:

Weight of Ethanol-Precipitable Slime at Different Stages of Bloat in Various Animals

Animal Number	Bloat Symptoms	Slime (Wet Wt.)	(Dry Wt.)
3	severe	452 mg.	60 mg.
27	olight	389	52
18	slight	335	38
26	slight	388	117
9	slight	1342	300

None of the viscid slime was obtained from the non-bloaters, but the appearance of the slime was well correlated with the onset of bloat symptoms. When the ethanol-precipitable slime was washed and suspended in water, the viscosity of the water was doubled.

The changes in the viscosity which occurred in the rumen fluid at various times are shown in the following table. The time is given in seconds and is the interval required for 30 ml. of the fluid to travel through a measured capillary.

Viscosity Changes in Rumen Fluid as Related to Bloat in Different Animals (30 ml.)

Date	Animal		Time
		Water	33 sec.
	steer	centrifuged rumen fluid, hay diet	34 sec.
	heifer A	"	35 sec.
	heifer B	"	35 sec.
3/30/60	488	rumen fluid, slight bloat	70 sec.
4/6/60	488	rumen fluid, moderate	89 sec.
4/11/60	488	rumen fluid, severe	96 sec.
4/6/60	79	rumen fluid, moderate	88 sec.
4/13/60	79	rumen fluid, severe	102 sec.

Increases in the viscosity of the rumen fluid were correlated with the onset of bloat, with the appearance of frothy foam in the ingesta, and with a buildup of the slime material in the rumen fluid.

Studies with Germfree Ruminants - C. K. Smith and P. C. Trexler, Michigan State University, East Lansing, University of Notre Dame, Notre Dame, Indiana

Thirteen germfree ruminants have been produced using hysterectomy and caesareotomy techniques. Delivery by caesareotomy is more satisfactory as this overcomes the losses from respiratory failure and has lowered the rate of contamination. These ruminants have been reared for varying periods of time up to 127 days. The diets used have been whole homogenized milk or 50 per cent whole homogenized milk and 50 per cent skimmed milk. Both of the above diets have been supplemented with vitamins of the B-complex and trace minerals. The rate of growth in the germfree animals is not as fast as in the conventional control animals fed the same diet. The organs of the alimentary tract have the greatest difference in weight and tissue response. The rumen, reticulum, abomasum, jejunum and cecum of the germfree animal has less connective tissue development and fewer leukocytes in the epithelium. The pH values of the contents of the intestinal tract of a germfree ruminant approach those of a conventional animal.

The Mode of Action of Penicillin in Preventing Uncomplicated Legume Bloat - W. M. Miller and D. R. Jacobson, Department of Dairy Science, University of Kentucky, Lexington

Much data obtained from bovine animals that grazed or were stall fed bloat-producing and non-bloat-producing forage over the past several years has rather conclusively shown that uncomplicated legume bloat occurs only when there is a high rate of stable froth or foam formation in the rumen which interferes with normal eructation.

The high rate of stable foam formation occurred when the fermentation rate was high.

The fermentation rate was significantly higher on the bloat-producing than on the non-bloat-producing forage.

Penicillin exerted its bloat-preventive effect by reducing the fermentation rate and hence the rate of stable foam formation in the rumen following the consumption of bloat-producing forage.

Approximately 30 animals grazed the pasture while 4 to 8 animals were stall fed clipped forage from the same pasture to obtain rumen samples for treatment and analyses.

#### ANIMAL MANAGEMENT PANEL

Combination of Antibiotics for Bloat Prevention - H. W. Essig, C. B. Shawver and L. W. Williams, Mississippi State University, State College

This study was conducted to compare the bloat controlling value of a supplement containing a combination of antibiotics and a supplement containing no antibiotics for bloat control as well as to determine the length of time a combination of antibiotics were effective in controlling bloat incidence and severity. Paddocks of Ladino clover were used as grazing. The paddocks were rotationally grazed, clipped and irrigated as needed. All steers were rated for severity and incidence of bloat twice daily, by visual observations, as they were removed from grazing. The ratings for severity were: non-bloat (0); slightly bloated (1); moderately bloated (2); and severely bloated (3). Eighteen selected bloating steers were grazed for 1½ hours morning and afternoon. They were divided into two groups, one group was fed supplement A containing no antibiotic and the other group was fed supplement B containing a combination of antibiotics. Supplement A consisted of a pelleted mixture of 90% wheat middlings and 10% molasses. Supplement B was the same as A plus the following antibiotics: 40 mg. penicillin, 70 mg. erythromycin, 70 mg. tylosin and 70 mg. streptomycin (per lb. of supplement). The supplements were fed at the rate of 1 lb. per head daily immediately before the afternoon grazing period.

This 63 day study consisted of the following periods: (1) 5.5 day control, in which all animals were fed supplement A thus allowing each animal to serve as his own control; (2) 48-day treatment period; (3) 9.5-day reversal period. The average daily incidence of bloat for steers on treatment A increased from 1.45, during the control period, to an average of 1.62 for the 48-day treatment period, whereas the steers on treatment B decreased from 1.60, during the control period, to an average of 0.67 for the treatment period. The average daily severity for steers on treatment A increased from 2.63 (during the control period) to 3.93 for the treatment period. The average daily severity of the steers on treatment B decreased from 3.33 for the control period, to 1.47 for the 48-day treatment period. At the end of 48 days, four steers on supplement A were switched to supplement B and four steers on B were switched to A. During the reversal period, the average daily incidence for those steers receiving antibiotics decreased from 1.68 to 0.21 and increased for those not receiving antibiotics from 0.89 to 1.24. The average daily severity for the reversal period decreased from 4.64 to 0.34 for those receiving antibiotics and increased from 1.75 to 2.63 for those not receiving antibiotics.

The incidence and severity of bloat for animals on treatment B does not reflect a true picture of the actual bloat control since two animals did not consume the supplement at the start of the trial; therefore, they continued to bloat creating the appearance that the antibiotic combination was not completely effective in controlling bloat for a limited time. There was no incidence or severity of bloat for 18 days in the seven steers which consumed the antibiotic supplement, after this time the incidence and severity steadily rose until the 41 day on which the severity and incidence were the same for the antibiotic treated and non-treated steers.

Evaluation of the Effectiveness of an Antibiotic Supplement in Controlling Bloat - J. E. Johnston, C. P. Braidenstein, A. J. Guidry, P. B. Brown, and W. H. Willis, Louisiana State University, Baton Rouge

A total of 51 cattle, 28 yearling steers, 16 two year old steers, 3 Jersey cows and 4 fistulated dairy cows were initially available for this experiment. All animals except 2 fistulated cows had been grazing clover for a period of 1 to 4 weeks prior to the initiation of the experiment on May 3, 1961.

Clover pastures utilized consisted of a 22 acre field divided into 3 approximately equal plots, each planted to Ladino, White Dutch or Louisiana S-1 white clover. Each variety of clover was further sub-divided into 2 equal plots by means of an electric fence. Irrimeters were used to indicate a deficiency of moisture and when necessary approximately 2 acre inches of water was applied to each plot.

The cattle were maintained as one group, pastured on clover, and fed the control supplement (without antibiotics) for an 8 day preliminary period. Percentage incidence of bloat was computed by animal for this period and 6 animals were lost during the time. Severity of bloat of each animal was evaluated and ranked 0, 1, 2, 3, and 4; the latter being the most severe. The percentage of incidence of bloat was computed by animal for this period and the animals were ranked on this basis.

Alternate animals were then placed into either the control or treated group. The control group was fed a supplement without antibiotics. The treated group was fed the same supplement but which contained penicillin, erythromycin, tylocin and streptomycin. The two groups (20 animals each) were maintained separately for the rest of the experiment which was terminated June 26, 1961. Each group was placed on clover pasture 2 hours each morning and 2 hours each afternoon. Each group when not on pasture, was maintained in a lot with a water trough and was fed the respective supplement in a feed trough just prior to the afternoon grazing period at the rate of one pound per animal.

The overall incidence of bloat was 37.26% for the control group and 10.53% for the treated group. The severity of bloat was greater in the control group than in the treated group. Three animals died in the control group but none were lost in the treated group. The incidence of bloat was higher when animals grazed Ladino than when they grazed other clovers.

Effects of Feeding Penicillin, Erythromycin, Tylosin, and Streptomycin in Combination for Prevention of Pasture Bloat - H. H. Van Horn, Jr., N. L. Jacobson, P. A. Hartman, and A. D. McGilliard, Iowa State University, Ames

In bloat research conducted during the 1961 grazing season, emphasis was placed on field testing a combination of penicillin (P), erythromycin (E), tylosin (T), and streptomycin (S). PETS as used herein, represents P-40, E-70, T-70 and S-70 (the numbers indicating milligrams per animal per day). The carrier for PETS was 1 lb. of pellets consisting of 90% wheat middlings and 10% molasses unless otherwise indicated. All control animals received basal pellets of 90% wheat middlings and 10% molasses.

One part of these studies was conducted at Ames in the same manner as previous studies. Seventy-five dairy and beef steers were divided into five groups of 15 animals each on the basis of body weights. These cattle were allowed to graze alfalfa pasture 3 hours in the morning and 3 hours in the evening and were retained in dry lot the remaining time. The five groups received the following treatments, respectively: Control pellets; PETS in loose salt initially and then in grain; PETS pellets; PETS pellets fed intermittently; PETCO pellets initially and then PETCO in grain. PETCO represents P-40, E-70, T-70 chloramphenicol-100, and oxytetracycline-100. Field trials were also conducted using cattle on two other Iowa State University farms and 12 herds of cattle owned by farmers who cooperated with the University in these studies. These herds of cattle were divided into control and treatment groups (at least 15 animals in each group) and allowed to graze separately on comparable plots of legume forage. In most cases the animals were on pasture continuously. The treated group received 1 lb. of PETS pellets per head per days. In some instances levels were later increased to 2 lb. per head daily. A total of 362 control animals and 474 PETS treated animals were utilized. Evaluation of bloat severity was visual, using a scale from 0 (no bloat) to 5 (terminal).

Bloat control was excellent in most of the herds; two farmer cooperators, however, experienced considerable difficulty in getting their animals to eat the PETS pellets. After the animals had refused to eat the prescribed amount of pellets for several weeks, they were changed to PETS in grain. The latter was eaten readily but did not prevent bloat. When number of animals and frequency and severity of bloat are considered, the feeding of PETS resulted in a 67% reduction of bloat. In the Ames trial, PETS in loose salt, PETS pellets and at the 1 lb. level, and PETCO pellets greatly reduced bloat but did not eliminate all serious bloat. Changing to PETS mixed daily in grain, increasing PETS pellets to 2 lb. per head daily, and changing to PETCO mixed daily in grain in the respective groups resulted in excellent control of bloat for the remainder of the season. Assays of antibiotic activity in the pellets indicated that during storage there was a rapid decline in erythromycin activity. This may have accounted for the need of increased levels of PETS pellets. The animals received PETS and PETCO for 128 days without any indication of loss of effectiveness.

Weight gains were determined in the three herds owned by the University. PETS resulted in increased weight gains, an average of 0.16 lb. per animal daily, in all trials; ( $P < .05$  in one trial).

Upon initiation of treatment the cattle usually consumed PETS pellets readily for 1 to 2 days but after that time many refused pellets. Usually about 1 week elapsed before the animals consumed the prescribed amount of PETS pellets again. This refusal to eat was sometimes accompanied by transient diarrhea and in about 5% of the females by local swelling of the vulva. These adverse effects usually disappeared within 1 to 2 days.

Palatability studies comparing PETS and PET (P-40, E-70, T-70 and P-56, E-97, T-97) combinations were conducted with additional cattle on pasture. The PET combinations did not cause as great a drop in eating rate on the days following first administration as did PETS but the problem was not completely eliminated by omitting streptomycin. No other adverse effects were observed with PET while some diarrhea was noticed with PETS. In studies of microbiological changes in the rumen following PETS administration, steers receiving green-chopped alfalfa-grass forage were given up to eight times the normal level of antibiotics (2 gm. total antibiotics) without any ill effects. The bacterial flora of all these animals were predominantly gram negative. No appreciable changes in total counts or numbers of gram positive bacteria were observed in these animals after antibiotic administration. Since the antibiotics in PETS are primarily active against gram positive bacteria, a decrease in gram positives might be expected.

Most of the adverse effects in the field trials occurred in animals which were receiving 6 to 12 lb. of grain per head daily. To explore this further, four Holsteins weighing approximately 500 lb. and receiving 10 lb. of grain per animal daily plus alfalfa hay free choice were fed PETS. Four similar animals receiving a diet high in alfalfa hay (where the grain was 1 lb. per animal daily) were also fed PETS in grain. About 1 day after antibiotic feeding was initiated, animals fed the high grain diet exhibited symptoms ranging from depressed appetite to diarrhea. In striking contrast, there were no ill effects in animals fed the high-hay diet. Bacteriological counts showed that the animals on the high-grain diet had higher gram positive counts before the antibiotics were administered. No appreciable changes in counts of gram positive bacteria were observed after antibiotic administration; however, at least one type of microorganism (a large, gram positive sarcina which tended to form chains) was absent in rumen samples of antibiotic treated animals.

Motility of the Rumen in Cattle of Known Bloat Susceptibility - V. E. Mendel, Univ. of Alberta, Edmonton, Alberta, Canada; J. M. Boda, D. A. Yarns and H. E. Cole, Univ. of California, Davis

Four non-lactating Jersey cows, of known bloat susceptibility, were alternately fed succulent alfalfa tops and sudan grass hay in an effort to assess individual differences and ration effect on rumen motility.

Seventy pounds of fresh alfalfa tops, four inches in length, were fed daily for three weeks, after which rumen motility studies of 3 hours duration were conducted with each animal for three consecutive days. Immediately following these motility studies fifteen to twenty pounds of choice sudan grass hay was fed for three weeks and motility studies were again conducted. These procedures were repeated once more on both rations. Methods of measuring rumen motility were those previously described by this station.

Intra-rumen pressure was subatmospheric in all animals except one when slight bloat occurred, thus contraction frequency and amplitude were not influenced by changing intra-rumen pressures. No correlation between rumen motility and bloat susceptibility was found among the four animals tested. Thus differences in bloat susceptibility do not appear to be the result of variations in receptor sensitivity to scabrous material.

Average values were obtained from both rations and these show that frequency and amplitude of rumen contractions are significantly less during the consumption of dry feed than during that of green feed. On the other hand, little difference was found between rations after 12 hours of fast which suggests that fermentation eliminated either differences in the physical nature of the feed or other factors associated with it.

On the basis of these results it is suggested that rumino-reticular motility of adult cattle is not increased by the physical nature of scabrous forage per se but by factors as yet unknown.

Further Observations on the Relationship of Bloat to Respiratory Inhibiting Saponins.- H. D. Jackson, S. A. McNairy, Jr., B. W. Hatcher and G. D. Goetsch, Department of Biochemistry, Purdue University, Lafayette, Indiana

Saponins have been reported as causative agents in the production of bloat in ruminants grazing legume pastures. Several years ago, it was reported by our laboratory that the substance(s) in legumes which inhibits muscle respiration is a saponin(s) and that the presence of this substance(s) is correlated with the incidence of bloat in cattle grazing alfalfa. However, a correlation between respiratory inhibition and bloat was not found in cattle grazing Laino clover pastures.

Since these findings showed a difference in the correlation of this respiratory inhibitor with the incidence of bloat, further investigations were carried out with cattle grazing two varieties of alfalfa, Culver and Vernal.

The inhibitory effect on tissue respiration in vitro by extracts of the two varieties of alfalfa was compared with the bloat index of the animals that grazed the particular forage each day. Although differences in the severity of bloat were observed and the inhibitory effect of different extracts on tissue respiration varied, data show that there was no consistent correlation between the inhibitory effect of different alfalfa extracts and the bloat index of the forage.

RUMEN PHYSIOLOGY PANEL

Salivary Secretion Rates of Identical Twin Cattle - J. M. Boda and A. T. Johns, University of California, Davis and Plant Chemistry Division, DSIR, Palmerston North, New Zealand

Previous studies at Davis indicated an association between individual bloat susceptibility and salivary secretion rate in that selected bloat susceptible cattle secreted less saliva than nonsusceptible cattle under similar conditions. During the past year we had an opportunity to investigate whether the procedures used in the above mentioned studies would accurately assess individual salivary secretion rates of cattle by comparing secretion rates between and within two sets of identical twin cattle. Saliva was collected before and during fresh clover feeding. It was assumed that differences in flow rates between individuals of the same twin set would be small or nonexistent if the procedure used measured the inherent capacity to secrete saliva; differences between sets would be great or small depending on the particular sets. The effects of such variables as individual feeding rates and bolus size on salivary flow during feeding were also determined.

Means and standard errors were obtained for salivary flow rates for the individual animals of the twin sets during the consumption of fresh, red clover. Salivary flow expressed either in g. per minute or in g. per 100 g. dry matter of clover consumed during salivary collection was similar in individuals of the same twin set but significantly different between twin sets. This suggests that the methods employed were measuring the inherent ability of the individuals to secrete saliva under the conditions of collection.

During the collection of boluses for estimating feeding salivary flow, apparent individual day-to-day variations in the rate of feed consumption were observed. The data were analyzed to see whether the rates of food consumption had an influence on absolute salivary flow rates. These relationships, that is the correlations between salivary flow rate per minute and the rates of fresh clover intake and dry matter intake, are inconsistent except in the case of one cow where there appeared to be definite and positive correlations between salivary flow and the rates of both fresh clover and dry matter consumption. Thus, in certain animals at least, it appears necessary to take into account individual differences in feeding rate in order to evaluate the significance of differences or similarities in salivary flow rates between animals.

It was thought that the size of the bolus might have an influence on the rate of salivary flow. Considering all animals, however, there was no consistent relationship between bolus size and rate of salivary flow, although in two cows correlations approach statistical significance but are of opposite sign. In one cow, such a positive correlation would be expected since bolus size tends to increase with an increase in rate of feeding and in this particular animal, salivary flow increased with increasing feeding rate. In the case of another cow, in which there was no relationship between feeding rate and salivary flow, there tended to be an inverse correlation between salivary flow and bolus size.

In view of the influence of rate of feeding on salivary flow in some animals, flow rates were adjusted for individual variations of feeding rate by analysis of covariance. The unadjusted and adjusted means and standard errors of the

means show that, although differences between animals of the same twin set were increased, such differences were not great and were insufficient to vary the conclusion that there were significant differences between twin sets but not within twin sets.

The "resting" salivary flow rates, that is the rates in g. of saliva per minute were obtained by cannulation of the cardia before feeding. The flow of cardiac saliva before feeding was similar in one set of twin cows but differed greatly between the animals of the other twin set. One cow had a significantly higher, resting flow rate than any other animal whether in the same twin set or not. This difference rules out any decision on the relative importance of differences between or within pairs. Thus, the method used for collecting cardiac saliva is probably of little value in assessing the inherent rate of flow of an individual. Resting flow rates are probably of little value in predicting the rates of flow during feeding in most animals. In all animals, except one cow, there were no significant correlations between resting flow rates and subsequent flow rates during feeding. In the exception, a highly significant positive correlation between resting and feeding flow rates was observed.

In conclusion, the data suggest that the methods used for collecting saliva during feeding measure the inherent individual capacity of the animal to secrete saliva under the particular conditions of collection. In some animals, rates of feed intake influence salivary flow rate and this factor must be taken into account to evaluate differences or similarities between individuals. Methods used for measuring resting salivary flow from the cardia generally cannot be used to estimate or predict subsequent salivary flow rates during feeding. The results support indirectly previous data demonstrating an association between individual bloat susceptibility and salivary flow rate during feeding, in that they indicate the collection procedures used measured the inherent individual abilities of the cows to secrete saliva during feeding.

Effects of Feeding Artificial Saliva Salts and Excessive Sodium Chloride on Bloat Incidence and Ruminant Characteristics in Cattle - C. J. Elam and R. E. Davis, Animal Husbandry Research Division, ARS, USDA, Beltsville, Maryland

The importance, in relation to bloat, of the diluting effect of saliva on ruminal contents or of the inorganic salts present in saliva has not been firmly established. The objectives of this experiment were to determine the effects of increased water intake and the consumption of salivary salts by cattle on the incidence of bloat and on various ruminal characteristics.

Eight Hereford steers, averaging 1050 lb. each, were randomly assigned to the columns of two 4 x 4 Latin-squares. The four treatments included: 1) a basal bloat-producing ration composed of ground barley, 61%; dehydrated alfalfa, 22%; SOM, 16%; and salt, 1%. 2) basal ration with an extra 5% sodium chloride added. 3) basal ration with 39 lb. of an artificial saliva salt mixture added per 1000 lb. feed, and 4) basal ration with both extra sodium chloride and saliva salts added. The composition of the saliva salts was a modification of the mixture suggested by McDougall. The salts were fed at a level to approximate the amount in 20 liters of saliva daily. The extra 5% salt was expected to increase water consumption about 20 liters daily. All steers had previously bloated on the basal ration. They were each fed 13 lb. of the pelleted rations in two equal portions daily. Water was available ad libitum and the amount consumed recorded during the four 28-day periods.

Steers were scored for bloat severity at 1 and 1½ hours following each feeding. The highest value was considered the score for each feeding period. Two ruminal samples were taken from each steer during the third and fourth weeks of each period using a stomach tube. The pH of the samples was determined upon reaching the laboratory with a Beckman Model H pH meter. Surface tension of centrifuged ruminal fluid was measured with a Du Nouy tensiometer. Microbial activity was determined in a Warburg respirometer using the technique described by Hungate. Volatile fatty acids were determined using a silicic acid column.

Feeding excessive sodium chloride or a mixture of synthetic saliva salts to steers did not influence the incidence of feedlot bloat. Water consumption was increased by the saliva salts and increased further by feeding 5% sodium chloride in the ration. The ruminal samples were more alkaline when artificial saliva salts were fed. There was no correlation between ruminal pH and bloat. Surface tension was neither altered by treatment nor correlated with bloat. Microbial activity of ruminal samples was decreased by feeding the combination of sodium chloride and saliva salts. There was no correlation between bloat and microbial activity. Neither the total concentration of ruminal fatty acids nor the proportion of the acids was affected by treatments. There was, however, a negative correlation ( $r = -.47$ ) between bloat and the concentration of fatty acids in the rumen. In addition, bloat and the molar percentage of acetate were positively correlated ( $r = +.55$ ).

The Role of Saliva in Bloat - Erle E. Bartley and L. R. Fina, Kansas State University, Manhattan

Previous studies with identical-twin cows suggested that the susceptibility to bloat in animals is inherited. Using fistulated animals, it soon became apparent that the consistency of rumen contents was similar within sets of twins but differed between sets of twins. Twins with watery rumen contents were not liable to bloat so readily as twins with drier ruminal ingesta.

Several experiments were initiated to determine the role of saliva in bloat. The effect of saliva on the release of trapped gas from frothing rumen contents obtained from bloating animals was tested in vitro. Adding saliva to incubated frothing rumen contents permitted greater quantities of gas to escape than when no extra saliva was added.

Since saliva is a solution of mucin and various mineral salts, it was conjectured that mucin in saliva might be the active antifoaming agent. Numerous plant mucilages and animal sources of mucin were used in experiments to screen a variety of products for possible use as bloat preventives. Linseed meal mucilage had an effect similar to saliva in releasing gas from frothing rumen contents. A simple screening procedure, based on the antifoaming action of various test materials on alfalfa saponin foams in vitro, was developed. The addition of saliva or linseed meal mucilage to an alfalfa saponin solution almost completely prevented the formation of foam when the solution was aerated. The plant mucilages tested, except linseed meal mucilage, were found to be inefficient antifoaming agents. Two animal mucin products (precipitated from fresh bones and extracted from hog stomachs) effectively prevented the formation of stable saponin foam.

On the basis of the *in vitro* results, several of these materials were tested on alfalfa saponin foams *in vivo*. The plant mucilages which were ineffective *in vitro* were also ineffective *in vivo*. Feeding linseed meal to cows before pasturing appeared to reduce the incidence of bloat. However, it is so difficult to extract mucilage from linseed meal that studies on the bloat preventive action of linseed meal mucilage were abandoned.

The two animal mucin products found effective *in vitro* were tested *in vivo* using four sets of fistulated identical twin cows. These products were given via the fistula immediately before pasturing the animals on bloat provoking alfalfa. These products prevented bloat for two to four hours.

Animal mucin products appear to have promise as practical bloat preventives. However, unless their protective action can be extended, they cannot be recommended for field application. During attempts to extend the protective action of mucin the question was raised whether bacteria capable of degrading mucin exist in the rumen. Consequently a search for rumen mucinolytic bacteria was undertaken. Several organisms were isolated from the rumen and tested for mucinolytic activity. Five were found capable of breaking down salivary mucin and utilizing free sialic (neuraminic) acid. When cultures of these organisms were introduced into the rumina of fistulated cows grazing a mature nonbloat-provoking pasture, bloat resulted in the majority of instances.

Three sets of fistulated identical-twin cows were fed a feed-lot-bloat-producing ration. After frothy bloat developed, the minimum amount of coarse, long hay needed to prevent bloat was determined. When bloat ceased, one member of each twin pair was inoculated with a mucinolytic rumen organism. This resulted in a marked increase in the degree of bloat over the uninoculated controls. Thus, it may be postulated that if there is reduced salivation during the consumption of bloat provoking diets, or if mucin is destroyed due to an excessive concentration of mucinolytic flora, bloat may result.

There is ample evidence that rumen microflora are in some way related to bloat. Rumina of fistulated identical twin cows were emptied, washed with water, and replaced with (a) 4 liters of natural rumen fluid or (b) 4 liters of autoclaved rumen fluid. Both kinds of rumen fluid were from bloating animals, and both groups of identical-twin cows were pastured on succulent alfalfa. Cows receiving the natural fluid bloated the second day. Cows receiving autoclaved fluid bloated the fourth day, even though consuming almost optimum amounts of bloat-producing feed the second and third days. These results, plus a bacteriological study also made, suggested that bloat does not result from a simple physical breakdown of feed, but that bloat may depend on establishing a certain concentration of microorganisms.

Microorganisms may be related to bloat in other ways. Slime production by rumen microorganisms may contribute frothing factors to legume or feedlot bloat. Control of bloat by antibiotics further suggests an association between microorganisms and bloat. Antibiotics may prevent bloat by destroying mucinolytic bacteria, slowing down gas production, and by inhibiting bacterial processes involved in the formation of intrarumen foam.

Since there appears to be a relationship between saliva production and the molar proportions of volatile fatty acids in the rumen, studies were initiated to determine the changes in VFA ratios during bloat. The ratio of rumen VFA in fistulated identical-twin cows fed alfalfa hay was: acetic 68, propionic 18, butyric 11, valeric 3. After grazing alfalfa the VFA ratio was acetic 61,

propionic 20, butyric 15, valeric 4. During a 90-day period the VFA ratio did not change significantly even though the cows were rotated from young succulent alfalfa to mature alfalfa. There was no correlation between VFA ratio and bloat. Since fatty acids are surface-active materials and are known to affect the stability of foams, it appears that an alteration in the normal proportions of these acids might either directly affect foaming or indirectly contribute to bloat by affecting the rumen microflora.

It is obvious that bloat is a complex mechanism. It is necessary for several factors to operate simultaneously before bloat will result. Saliva and mucinolytic bacteria are apparently two factors in this complex. The bloat complex may be explained as follows: Bloat is a problem of the development of foam in the rumen. Foam develops because of the consumption of feeds containing foaming agents such as proteins and saponins. Gas that is a normal product of rumen fermentation becomes trapped in the ingesta to form a stable foam which probably inhibits eructation. This accumulation of gas that the animal cannot belch results in bloat. Excessive gas production in the rumen when frothing agents are not present is not a problem since the cow can rid herself of the gas by belching. However, if frothing compounds are present, then excessive gas production, particularly that occurring from readily metabolizable carbohydrates in very young legume pastures or in grain in feedlot rations, will serve to provoke bloat. Also, the existence of frothing compounds in a plant does not necessarily mean that bloat will occur. Alfalfa hay made from young legumes usually does not provoke bloat even though the hay contains the same froth producing compounds in nearly the same concentrations as the green forage. Adding to the complexity of the situation is the knowledge that during the consumption of bloat producing rations there are changes in the ratios of rumen volatile fatty acids and the pH of the rumen. These changes may affect the nature and extent of the foam and probably alter the rumen microflora. An increase in numbers of slime producing rumen microorganisms may contribute frothing factors to legume or feedlot bloat. Finally a lack of coarse roughage results in an insufficiency of the antifoaming factor mucin due to reduced salivation and/or mucinolytic bacterial destruction. Removal of the natural antifoaming agent mucin permits bloat to result.

The Absorption of Methane and Carbon Dioxide from the Rumen as Influenced by Kind and Amount of Feed - H. Hoernicke, W. F. Williams, D. R. Waldo and W. P. Flatt, Animal Husbandry Research Division, ARS, USDA, Beltsville, and University of Maryland, College Park

A separate collection of rumen and expired gas was made in tracheostomized Jersey cows by means of a special cannula. The gases have been analyzed by gas chromatography. The following rations have been fed in amounts ranging from 1 to 2 times maintenance: I - late cut orchard grass pellets; II - a feed lot bloat ration (60% barley, 22% alfalfa, 16% soya oil meal, 1% NaCl); III - long alfalfa hay. Because the results with rations I + III (hay rations) have been similar, the figures have been combined and compared with those of II (grain ration).

Methane was always present in the expired air in concentrations from 0.02 to 0.05 Vol. %. The total volume of  $CH_4$  exhaled per hour was 0.4 - 1.3 L before feeding and 0.6 - 2.0 L, 0-6 hours after feeding. This amounts to 25-96 (mean 21) percent of the total  $CH_4$  production before and to

8-53 (mean 21) percent after feeding which was exhaled, the remainder being eructed. The absorption rate (ml/hour/mm Hg partial pressure gradient between rumen gas and blood) was greater with the grain ration (7.0 before, 8.2 after feeding) than with the hay ration (6.5 before, 5.1 after feeding). This indicates that in addition to the partial pressure gradient other factors, probably blood flow, influence the methane absorption from the rumen.

The carbon dioxide absorption from the rumen has been calculated from the radioactivity in the expired and the eructed gas during the continuous infusion of <sup>14</sup>C- labelled carbonate into the rumen. Twelve to 99.8% of the radioactivity excreted was found in the expired air. This corresponds to a net carbon dioxide transfer from the rumen to the lungs of 0.5 - 9.7 L/h before and 1.8 - 29.5 L/h after feeding. The net absorption rate (ml/h/mm Hg) after feeding was 34 on grain and 43 on the hay rations. This is 4.1 and 8.4 times higher, respectively, than transport of methane at the same concentration gradient. The smaller rate on the grain ration is one other indication of the increasing retention of bicarbonate in the rumen with increased absorption of VFA.

Rates of Production of Individual Volatile Fatty Acids in the Rumen of Lactating Cows - R. E. Huugate, R. A. Mah, and Mogens Simonsen, University of California, Davis

In conjunction with some experiments on lactating cows by Dr. Kleiber and his group in animal physiology an attempt was made to determine accurately the rate of production of the individual volatile fatty acids during the time that tracer experiments were being conducted. The zero-time rate method was used but with a greater accuracy, obtained by duplicate sampling, subsampling, and analysis. The method of Wiseman and Irwin for chromatographic estimation of the acids was employed. Manometric methods were utilized to estimate acid production independently as well as to determine carbon dioxide and methane formed.

Smooth curves of the shape expected for a gradually deteriorating rate were obtained in five out of the eight rate measurements. Exposure to oxygen and a fall in temperature were believed responsible for failure to achieve expected curves in three cases. From the five experiments the rate of production of each acid at zero time was estimated graphically and for the other three the average rate during the incubation period was utilized for estimating total production. The volume and dry matter of the rumen contents were determined at the same hour as the experiment but on another day when the animal was subjected to the same feeding regimen.

The average estimated rates of volatile acid production for the four experiments were, in mols per day: butyric 10.56, propionic 12.72, and acetic 40.08, or 9.6 pounds in all. From 23 to 36 per cent of the carbon dioxide respired by the cow was calculated to have arisen as fermentation carbon dioxide from the rumen fermentation. The carbon in the fermentation acids and carbon dioxide accounted for 68 to 105 per cent of the carbon in the milk and respiratory carbon dioxide of the experimental animal.

In one case a sudden shift in the proportion of volatile fatty acids produced was found, with a relatively greater production of propionic acid. It was correlated with a decrease in the amount of methane found, as would be expected if methane is formed from hydrogen and carbon dioxide.

The results of the experiments indicate that the rates of production of the volatile fatty acids can be determined with a fair degree of accuracy on a single animal in a particular experiment, and that the rumen fermentation accounts for the major part of the carbon turnover in the lactating cow.

Effect of a Combination of Antibiotics on Chloroplasts and Volatile Fatty Acids in the Rumen - A. D. McGilliard and H. H. Van Horn, Jr., Iowa State University, Ames

Studies have indicated that feeding antibiotics in combination is an effective means of controlling pasture bloat. Since the mode of action of the antibiotics is not clear, studies were conducted to take a preliminary look at the two following proposed mechanisms of action:

1. Antibiotics reduce fermentation rate in the rumen as indicated by decreased fatty acid levels and lowered gas production.
2. Antibiotics act indirectly as antifoaming agents by inhibition of bacteria which normally destroy or alter chloroplast lipid, thus removing a natural defoamer from the rumen.

Volatile acid levels and ratios were determined in 10 steers fed hay, and changes in these levels and ratios were followed after the animals were changed to alfalfa pasture. Five of the steers received a combination of antibiotics (penicillin 40 mg., erythromycin 70 mg., tylosin 70 mg. and streptomycin 70 mg.) in 1 lb. of a pelleted feed (90% wheat middlings and 10% molasses) daily beginning with the first day on pasture. Five steers served as controls. Each sample of rumen fluid taken for analysis was obtained by suction strainer immediately following a 3-hour grazing period in the morning. Total volatile acid levels were determined by steam distillation and titration; volatile acid ratios were determined by gas chromatography. Total rumen volatile acid levels increased sharply in the control group after the animals were transferred to pasture. Levels in the antibiotic group were depressed initially but reached levels similar to those of the control group in about 5 days. No apparent differences in volatile acid ratios were observed between groups. The initial reduction in volatile fatty acid levels of the group fed antibiotics suggests a reduction of the microbial population in the rumen and/or an alteration in the rate of fermentation. It is difficult to reconcile the transient depression of volatile fatty acid levels, however, with the continued administration of antibiotics and their effectiveness in controlling bloat. Moreover, subsequent microbiological studies indicated that administration of the combination of antibiotics did not alter total, gram positive nor gram negative bacterial counts in the rumen.

Concentrations of chloroplasts, chloroplast fragments and cell-free lipid were measured in samples of rumen fluid from five animals receiving 1 lb. of the pellets containing antibiotics daily and from five controls. The time and method of sampling were the same as those used in obtaining samples for the volatile fatty acid analyses. Separation of whole chloroplasts and chloroplast fragments was accomplished by selective centrifugation. These fractions were extracted with 80% acetone and their relative concentrations determined, photometrically, as chlorophyll. Cell-free lipid was extracted from cell-free rumen fluid by the Majonnier procedure and determined gravimetrically. Chloroplasts and chloroplast fragments were found in greater

concentrations in the rumen fluid of antibiotic-treated animals. The differences between groups were highly significant. No difference between groups, however, was observed in the cell-free lipid levels. The higher chloroplast concentrations in the rumen fluid of antibiotic-treated animals lends some support to the second mechanism proposed above but it is still not clear why chloroplast concentrations are increased. It would appear that increased chloroplast concentrations could be caused either by reduced utilization of chloroplasts, resulting in their accumulation, or by a more rapid breakdown of the legume plant, releasing more chloroplasts into the rumen fluid.

Effects of Penicillin and Several Other Antibiotics on Rumen Fermentation -  
R. S. Emery, T. R. Lewis, R. L. Baldwin, H. N. Jordan, Michigan State University, East Lansing

Inclusion of 2.5 ug./ml. of penicillin in 3 hr. in vitro fermentations with rumen fluid has caused the following:

1. A 10% increase in gas formation without change in composition.
2. A several fold increase in lactic acid production with a slight decrease in utilization.
3. Little or no change in fatty acid production or glucose utilization.
4. Some inhibition of ammonia utilization.
5. Some decrease in the carbohydrate content of bacterial cells.

Neomycin, Virginiamycin, Tylosin, Erythromycin, and Hyamine have a similar effect regarding items 2 and 3. The increased lactic acid production is the most marked and repeatable of these observations.

Influence of Calcium and Magnesium on the Occurrence of Bloat in Lambs -  
Walter Woods and Keith J. Smith, Iowa State University, Ames

The effect of different minerals on the occurrence of bloat was studied using wether lambs during the 1960 and 1961 summer months. Lambs grazing alfalfa treated with a foliar application of magnesium carbonate (3.9 lb./acre and 7.8 lb./acre), calcium carbonate (40 lb./acre) and calcium phosphate (50.3 lb./acre) bloated more severely than lambs grazing untreated alfalfa. The increase in bloat observed appeared to be similar between the different compounds applied to the alfalfa. Lambs drenched with magnesium carbonate at the rate of 10 gm. per day, immediately prior to grazing alfalfa, bloated more severely than control lambs. The effect of magnesium carbonate applied as a foliar spray or as a drench appeared to be similar with respect to the bloat syndrome. Drenching lambs with 5 gm. of either calcium carbonate or magnesium carbonate increased the severity of bloat to a similar degree. The minerals were administered in the mornings and the lambs were grazed in both the morning and afternoon. The minerals were as effective in increasing bloat in the afternoon as in the morning grazing period.

The administration of ethylene diamine tetraacetic acid (EDTA) and diethylenetriamine pentaacetic acid (DTPA) significantly decreased the amount of bloat observed. The drenching of lambs with  $2\frac{1}{2}$  gm. of DTPA once daily decreased bloat in lambs to a greater extent than drenching with 5 or 10 gm. of EDTA. The maximum bloat index for the control lambs was 1.72 as compared to 1.51 for lambs drenched with 5 gm. of EDTA, 1.42 for lambs drenched with 10 gm. EDTA

and 1.23 for lambs drenched with  $2\frac{1}{2}$  gm. of DTPA. Drenching lambs with magnesium carbonate or DTPA singly or in combination indicated that magnesium carbonate increased the amount of bloat and DTPA reduced the severity of bloat.

No correlation was found between the day to day analyzed total calcium and magnesium content of untreated alfalfa and the degree of bloat in lambs. There was considerable variation in the calcium and magnesium content of the alfalfa plant. The average calcium and magnesium content on a dry matter basis was 1.49% and .45% respectively. The cell-free soluble calcium and magnesium of the rumen contents did not increase as the level of bloat observed increased with control lambs on the same forage. Drenching lambs with magnesium carbonate increased the cell-free ionic magnesium in the rumen, but had no effect on calcium. Drenching lambs with calcium carbonate had no effect on the rumen contents of calcium and magnesium. The time of sampling was about 4 pm following the drenching of the lambs at 7 am. The results might be different depending on the time of sampling following drenching. The oral administration of 5 or 10 gm. of EDTA reduced the amount of ruminal ionic calcium and magnesium in lambs grazing alfalfa. The average ruminal cell-free calcium for the control lambs, and for lambs treated with magnesium carbonate, calcium carbonate and EDTA was 19.7, 18.2, 17.5 and 13.6 mg.%, respectively. The average ruminal cell-free magnesium for the control lambs, and for the lambs treated with magnesium carbonate, calcium carbonate and EDTA was 24.2, 32.2, 22.5 and 20.6 mg.%, respectively.

The Utilization of Pectic Substances by Rumen Microorganisms - R. Pressey, J. D. Fetty, and R. S. Allen, Iowa State University, Ames

Earlier work has demonstrated that the water-soluble pectic substances of alfalfa are rapidly extracted and utilized in the rumen, while the water-insoluble fraction is extracted and utilized only slowly. *In vitro* incubation studies with pectin and rumen ingesta from different animals indicated that the rate of utilization varies from animal to animal, diurnally, and also from day to day. Although the rumen protozoa are capable of hydrolyzing pectin, only the bacteria can completely utilize pectin.

The observation that pectin was utilized much more readily than galacturonic acid by rumen bacteria led to a detailed study of the metabolic pathway involved. The utilization of pectin, and even di-galacturonic acid, but not galacturonic acid itself, appeared to be a new example of the utilization of a disaccharide, and higher analogues, but not the monosaccharide. It was suspected that phosphorylation rather than hydrolysis may be involved, and this was supported by the observation that arsenate inhibited the utilization.

Cell-free enzyme extracts were made from washed-cell suspensions of rumen bacteria. Packed cells were diluted several fold with 0.16M saline and disintegrated with a Raytheon sonic oscillator or a French pressure cell. The enzyme solution was stored in the deep-freeze.

On incubation of the enzyme solution with one per cent pectin in phosphate buffer, apparent hydrolysis occurred with formation of galacturonic acid, accompanied by the formation of an unknown product. Incubation of galacturonic acid resulted in no reaction. Di-galacturonic acid was degraded to galacturonic

acid, and also was converted to tri-galacturonic acid and tetra-galacturonic acid, indicating a transferase action. Tri-galacturonic acid was hydrolyzed to mono-galacturonic acid with traces of di-galacturonic acid and larger quantities of tetra-galacturonic acid. In contrast, tetra-galacturonic acid was only hydrolyzed to the lower oligo-uronides, with no formation of penta-galacturonic acid.

Traces of the unknown product were formed from tetra-galacturonic acid, and readily from pectic acid, indicating that the ester group of pectin is not essential but that a fairly long chain length may be necessary. The reaction occurred only in the presence of phosphate, but the unknown product was not a phosphate derivative. Chromatographic studies indicated that the unknown is not tagaturonic acid, but that it is probably a monomer. Treatment of the unknown with  $\text{LN N}_2\text{H}$  at room temperature for 1/2 hour transforms it completely to galacturonic acid and tagaturonic acid. The evidence suggests that the unknown may be an unsaturated derivative of galacturonic acid.

There are only a few instances in which the metabolism of pectin by bacteria has been studied completely. The first few steps of the observed metabolic pathway consist of hydrolysis of pectin to galacturonic acid followed by its isomerization to tagaturonic acid, which is subsequently cleaved, and the pyruvate and triose phosphate formed link the pathway to the glycolytic cycle.

Galacturonic acid and tagaturonic acid are therefore intermediates in such a scheme. The metabolism of pectin by rumen bacteria does not involve these intermediates, but probably proceeds by a pathway involving an unsaturated derivative of galacturonic acid.

In Vitro Studies of the Digestibility of Certain Plant Fractions Using the Nylon Bag Technique - E. J. Stone, A. J. Guidry and J. B. Frye, Jr., Louisiana Agricultural Experiment Station, Baton Rouge

A  $2 \times 2 \times 2 \times 4$  factorial experiment replicated six times was run to determine the relative digestibilities of dry matter and cellulose. Four rumen fistulated cows, two maintained on bloat producing clover pasture and two on dry lot with alfalfa hay as the sole roughage were the experimental host animals for nylon bags. One animal of each pair was given one pound of a control pelleted grain ration through the rumen fistula, while the other animal was given one pound of the same control ration containing a bloat inhibitor PETS. Into each animal was placed 4 bags of freshly plucked clover and 4 bags of ground alfalfa hay. At periods of 1, 2, 4, and 24 hours, samples of clover and alfalfa were removed from each cow for their respective analyses.

Analysis of variance of the data for dry matter digestibility indicates that the mean value for clover, 43.3 per cent, was significantly higher ( $P < .01$ ) than alfalfa, 37.6 per cent, when all other factors were considered simultaneously. There were no differences attributable to dietary roughages. As was expected there were highly significant differences in digestibility of dry matter among time intervals. The addition of antibiotic had a significant effect ( $P < .05$ ) on dry matter digestibility. Where PETS was added to the ration of the host animal the ultimate dry matter digestibility of both clover and alfalfa hay was significantly less ( $P < .05$ ) than without the antibiotic added for the respective roughage substrates. There were no significant interactions for dry matter. Furthermore there were no differences among replications.

For cellulose, significant differences were found for time intervals ( $P < .01$ ), dietary antibiotic ( $P < .05$ ), and the three factor interaction between dietary ration X dietary antibiotic X substrate, ( $P < .05$ ). When clover was the dietary roughage there was a significant decrease in cellulose digestibility over time and replications. There was no corresponding decrease for alfalfa. When alfalfa was the dietary roughage the picture was reversed with a decrease in cellulose digestibility being greater for alfalfa.

A comparison of the best fitting curves for the various treatments and variables indicates that the FETS anti-bloat treatment was effective in reducing the rate of digestion over the first four hours (normal bloating period) for dry matter digestibility. The slopes of the curves for the digestibility of cellulose were not significantly different over this same period. The curves indicate that some fraction other than cellulose is being digested rapidly where FETS was not fed to the host animal.

Physiological Action of the Nitrate Ion - G. B. Garner, R. A. Bloomfield, C. Welsch, J. Hersey, E. O. Kearly and M. E. Muhrer, University of Missouri, Columbia

The physiological significance of the nitrate ion has been presented in most texts as of little importance. Today, the interest is heightened by the various physiological phenomena which are associated with dietary intakes above 0.3%  $\text{NO}_3$  of the dry matter of the ration. At least five areas are involved, and once the nitrate ion is in the circulation, species differences are lessened. The areas are:

1. Iodine-chloride metabolism and concentrations in extra-cellular fluids
2. Thyroid function.
3. Non-protein nitrogen utilization.
4. Production loss (milk and gain).
5. Vitamin A deficiency and abortion.

With rats, it has been observed that the % of dose uptake of  $\text{I}^{131}$  is diminished as the dietary level of nitrate increases. Nitrate has also been shown to inhibit  $\text{I}^{131}$  uptake by bovine thyroid slices in an in vitro system. Sheep fed 1.5%  $\text{KNO}_3$  showed a marked reduction in circulating  $\text{I}^{131}$  and an equal reduction in  $\text{PBI}^{131}$ . The blood nitrate level in the serum was 1.83 mg. %. Thus it is not surprising to find enlarged thyroid glands in rats receiving 2.5% dietary potassium nitrate and under cold stress. How much of these data can be applied to the bovine is now under study. No difference in rat weight response was noted until cold stress was applied. Therefore this is highly suggestive of the field picture we have observed in dairy herds.

Since nitrate interferes with iodine it can be expected to affect chloride metabolism. Some data indicate that the major drop in milk production following nitrate dosing is not due to methemoglobinaemia and one can only ask if chloride could be involved. This too is under study.

The effect of vitamin A metabolism appears to be primarily a prevention of storage. Missouri experiments have demonstrated this in rats and sheep and recent Illinois and Arizona work indicate this same finding in cattle.

The utilization of NPN by sheep fed dietary nitrate indicates that the nitrogen balance is reduced and that urea-ammonia nitrogen is not metabolized by the rumen bacteria or the host animal in the normal manner. This can be shown by in vitro studies in which bacterial ammonia incorporation into protein is reduced before nitrite is detectable. With higher blood ammonia or urea levels the tissue probably reduces less nitrate to ammonia and thus the toxicity is increased.

In order to study the possible interaction of nitrate and urea, an experiment was designed solely to determine the nitrate/urea space of sheep and cattle by intravenous injection of each and in combination.

The urea space (% of body weight) was 51-59% for cattle, 67-78% for sheep. The nitrate space (% of body weight) was 23-35% for sheep and 20-33% for cattle. The nitrate space increased to the higher levels when both urea and nitrate were injected simultaneously. Blood clearance rates for both urea and nitrate were lessened in combination as compared to single injection. The recovery of nitrate in 7 hours indicates that nitrate is metabolized by the tissue or secreted into the rumen by way of saliva in considerable quantity and metabolized by the bacteria.

The purpose of this report is to point out the physiological action of the nitrate ion as we now know it.

Organic Acid Composition of Rumen Content of Bloaters and Non-Bloaters Steers -  
H. W. Essig, C. B. Shawver and L. B. Waymack, Mississippi State University,  
State College

This study consisted of three trials in which selected bloater and non-bloater steers were allowed to graze Ladino clover for 1½ hours, morning and afternoon. Rumen samples were taken by stomach tube before and after grazing. The rumen contents were strained through cheese cloth, then made acid (pH 1) and frozen in a dry ice-alcohol bath. The frozen samples were then stored at 0°F. until analyzed for acetic, propionic, butyric and higher acids, using a celite partition chromatographic technique. In trial I, rumen samples were taken at 7:30 and 9:30 a.m. from three bloater and three non-bloater steers on two dates giving a total of six observations for each group of bloaters and non-bloaters. When the VFA data (milliequivalents of VFA's per 100 ml. rumen fluid) were subjected to a statistical analysis the total VFA concentrations from bloaters were significantly higher than those from non-bloaters. Significantly more total VFA's were present in samples taken at 9:30 than those taken at 7:30. There was a highly significant difference in VFA's present in the rumen fluid. There were significantly more total VFA's in the 9:30 bloater rumen samples than in the 9:30 non-bloater samples. In trial II, rumen samples were removed at 9:00 a.m., 11:00 a.m. and 2:00 p.m. from two bloaters and two non-bloaters on two dates to give four observations for each group of bloaters and non-bloaters. Total VFA concentration for bloaters was highly significantly greater than those for non-bloaters. There was a significant difference within VFA's. There was a significantly greater total concentration of VFA's for 9:00 a.m. bloaters than for 9:00 a.m. non-bloaters. In trial III, rumen samples were removed from four bloaters and four non-bloaters at 9:00 a.m. and 2:00 p.m. on two dates to give eight observations for bloaters and non-bloaters; however, one observation for bloaters and non-bloaters was not included in the statistical analysis due to incomplete chemical analysis of the rumen samples. Total VFA concentrations for bloaters and non-bloaters

were again significantly different in this trial. There was a significant difference in time of sampling and within VFA's. There was a significantly higher total VFA concentration for the 9:00 a.m. bloaters than for the 9:00 a.m. non-bloaters. There was no difference in the 2:00 p.m. bloater and non-bloater total VFA concentrations. In all three trials there was a significantly greater total concentration of VFA's for bloaters than for non-bloaters. In all trials the total concentration of VFA's was higher for the bloaters immediately after grazing than for the non-bloaters, indicating that either the bloaters were producing greater quantities of VFA's or that the VFA's were not absorbed as readily in the bloaters as in the non-bloaters.

#### AGRONOMIC PANEL

##### Some Environmental Factors Associated with the Accumulation of Nitrate in Corn - R. H. Hageman, University of Illinois, Urbana

Experiments initiated in 1956 demonstrated that increasing levels of artificial or competitive (self and sib) shading were associated with accumulation of nitrate, and lower amounts of carbohydrates in the plant as well as decreased yields of grain and stover. Varietal response under competitive or artificial shade treatment was similar and varietal differences were noted. Varieties (Hy2 X Oh7 or Hy2 X Oh41) recommended for high density plantings outperformed WF9 X C103 under the artificial shade treatments.

In general, nitrogen metabolism was more adversely affected by decreasing light intensity than was carbohydrate metabolism. The data obtained would not support the conclusion that carbohydrate metabolism was limiting nitrate assimilation but implicated lack of light as a causal factor.

Subsequent experiments demonstrated that young corn plants placed in complete darkness for 48 hours lost 90% of their nitrate reductase activity. The activity was quickly restored when the plants were returned to the light. Artificial shade experiments in field and greenhouse demonstrated that nitrate reductase activity decreased in rough proportion to the amount of shading. It was shown that both light and nitrate (substrate) are necessary for the formation of the enzyme nitrate reductase. Diurnal variations under field conditions were observed for nitrate reductase activity, nitrate, and water soluble protein content of the plant. The enzyme activity correlated negatively with the nitrate content and positively with the protein content. Variety Hy2 X Oh7 under wide variations in environmental conditions has consistently shown a higher level of nitrate reductase activity than WF9 X C103. Droughty conditions and actual wilting of plants is associated with a dramatic loss of extractable nitrate reductase.

Current experiments show that with four varieties there is a correlation between nitrate reductase activity and their yield potential at high density plantings. Field surveys over the past two seasons have revealed that inbred strains differ in their extractable nitrate reductase activity and can be grouped into high, medium or low categories.

The nitrate content of any tissue at time of harvest is the resultant of two factors: rate of supply and rate of utilization. Rate of supply is dependent on nitrate content in the soil, available water to move the nitrate in the soil and plant, and accumulation and transport properties of the plant concerned. The rate of utilization is dependent on the activity of the enzyme nitrate reductase. The activity of this enzyme is affected by a wide array of environmental factors, light, nitrate concentration, drought (plant wilting) and temperature.

The Problem of Predicting Digestibility from Chemical Composition -  
J. T. Sullivan and Donald Burdick, Crops Research Division, U.S.D.A.,  
University Park, Pennsylvania

Many methods have been proposed for predicting the digestibility of forages from one or more chemical constituents, such as crude fiber, protein, lignin, etc. The prediction is based on a statistical analysis of data from experiments in which feeds of known chemical composition have been fed to animals under more or less controlled conditions. This paper is concerned with the question: What shall we analyze for in order to relate our findings to the digestibility of forages?

We have been studying the composition of forages and their digestibility by ruminants. We have studied about 200 samples, most of them from pure stands, either grass or alfalfa, a small number are mixtures and a small number of silages. Most of them are from first cuttings and relatively few are second or later cuttings. All had been fed to either cattle or sheep without added concentrate. They were donated to us by stations which had conducted digestion trials. With them we received some analytical data and also made some analyses ourselves. The data were studied for the purpose of selecting or designing a procedure of chemical analysis which may be used to evaluate the forage or to predict its digestibility.

The most digestible portion of the forage is that comprising the cellular contents and the least digestible is the cell wall substance. The problem is therefore to determine by chemical means how much of the cell wall substance is digestible. Can that be determined by chemical analyses alone?

The crude fiber method is the oldest of attempts to measure the digestibility of forage. The isolated crude fiber fraction contains a large portion of the cellulose and a large portion of the lignin. Forages of a high crude fiber content are believed to be of low digestibility.

In grass and in alfalfa there is a negative correlation between the crude fiber percentage and the digestibility of the dry matter. The latter can be predicted from the crude fiber percentage with an error of 4.1 in grass and of 3.5 in alfalfa. The positive correlation between crude fiber and digestibility in samples containing both grass and alfalfa is less common. A plotting of the regression equations for grass and alfalfa would show that there is a fundamental difference in the composition of grass and alfalfa in relation to digestibility and that while the crude fiber content may be used as a predictant in one or the other, the same regression equation cannot be applied to both or to a mixture.

Other fiber methods have been studied, the normal acid fiber, a 5 percent acetic acid fiber, the alcohol-insoluble portion of the forage, the protein-free alcohol-insoluble portion, and cellulose in a number of different forms. The quantity of any of these has some relationship to digestibility but the relationship does not seem close enough so that one of them can be used as a reliable predictant in all forages.

Protein is another common index of the digestion value. In grass that is growing normally the intake of nitrogen does not keep pace with the deposit of carbohydrates so that in time the percent protein steadily decreases and the proportion of cell wall material increases. Positive correlations result therefore between the percentage of protein and some digestion coefficients. The standard errors in the prediction of digestibility from protein content are high.

Lignin is intimately associated with the other cell wall constituents and where it occurs in large quantity the rumen microorganisms have difficulty in breaking down the cellulose. It is therefore negatively correlated with digestibility. The error of prediction of the digestibility of dry matter from the lignin content is less than similar error with crude fiber or protein but it is still too high for our purpose. Better correlations were obtained in previously published data with a small number of samples which had been obtained from stations with long experience in carrying out digestion trials. The error was close to 2.0 in these other cases. Here with a larger population from a greater number of sources there is a greater error.

If the regression equations for grass and for alfalfa are plotted it will again be evident that there is a fundamental difference in the composition of grass and alfalfa in its relation to digestibility and that the lignin content is not a reliable predictant for mixtures. For a reliable prediction in mixtures it would be necessary to know the proportion of grass and alfalfa in the mixture and the lignin content of each. As most of our forages occur as mixtures we are faced with the problem of finding a common denominator for their evaluation.

A method which is being studied in our laboratory is based on the ease of hydrolysis of hemicellulose. The most abundant constituents of the cell wall are cellulose and hemicellulose, and their digestion coefficients are of the same order. While cellulose is readily attacked by rumen microorganisms, it is difficult to break down in the laboratory. Hemicelluloses, on the other hand, are relatively easy to solubilize and to hydrolyze by laboratory means. They are broken down by mild acid hydrolysis into a number of sugars, as rhamnose, galactose, glucose, arabinose, and xylose, and to one or more uronic acids. Xylose is the most abundant of the products of hydrolysis, sometimes amounting to 70 percent of the total hydrolytic production. These products may be separated by paper chromatography and determined quantitatively but the relative errors are large for those which occur in minor quantities. Because of its abundance xylose can be determined more accurately. One of these sugars, glucose, may be a contaminant in hemicellulose hydrolyzates, as is fructose also, and they may be removed by fermentation by yeast. Two methods being used for the quantitative determination of the hemicellulose constituents after hydrolysis are one involving chromatographic separation and another the measuring

Of the reducing power of the nonfermentable portion. Complete hydrolysis as measured by the maximum yield of products is obtained by subjecting a sample (after extraction with benzene-alcohol) to 1.0 N  $H_2SO_4$  for 6-8 hours at 100°C. Hydrolysis takes place more slowly with a weaker acid, as 0.1 N, and the degree of partial hydrolysis which has taken place at any given time may be measured by analyzing the products obtained at that point. It has been observed that the different sugar components are not released from the parent hemicellulose at the same rate. For example, most of the arabinose and galactose are released quickly, and xylose more slowly, and this feature of slow release of xylose lends itself to the measurement of the degree of partial hydrolysis. The pattern of release of the various sugars by acid hydrolysis resembles that obtained by in vitro rumen action. It has been noted further that the amount of hydrolysis which has occurred at a given period of time (i.e., one hour) is positively related to the digestibility of the forage as determined by conventional means.

An empirical set of conditions was chosen to measure the ease of hydrolysis of hemicelluloses. Xylose was determined chromatographically in two hydrolyzates, that of 0.1 N acid in one hour and that of 1.0 N in 8 hours, the latter to represent the total xylose of the forage. The proportion of the total xylose found in the first hydrolyzate is compared with the digestion coefficients of dry matter of 11 forages of a number of different species of grass and of alfalfa. There results a correlation of +0.96 ( $P < .01$ ) between these two values. This appears a promising method in that different species fell on the same regression line,  $44.0 + 1.08 x$ . A correlation of -0.82 occurred between the proportion of xylose released and the lignin content of the forages. These data show that forages of high digestibility, when subjected to conditions of partial hydrolysis, release a greater proportion of their total xylose than do forages of lower digestibility, under the same conditions of treatment. We do not recommend as yet that this method be used to predict the digestibility of forages. Perhaps the empirical conditions chosen here will not prove to be the ones most suitable but it seems that the principle of this approach is good and that some conditions may be found which will be successful.

Foaming Properties of Alfalfa and Their Relationship to Bloat - R. Pressey, S. H. Synhorst, Janet Bertram, and R. S. Allen, Iowa State University, Ames

The following procedure was used to study the foaming properties of alfalfa extracts and their relationship to bloat. Twenty-five grams of fresh four-inch tops of alfalfa were blended with 60 ml. of buffer in a Serval OMNIMixer for two and one-half minutes. The blending was done intermittently to prevent excessive heating. The macerated material was squeezed through cheese cloth and then centrifuged at 1000g for five minutes. Fifty ml. of the supernatant were placed into the foam meter which was immersed in a constant temperature bath. The instrument is essentially a graduated column with a sintered glass plate at the bottom. Passage of nitrogen at a constant rate through the sintered glass plate into the solution converted the solution to foam. The volume of solution remaining in the foam after given periods of time following the passage of nitrogen was measured and used as an index of the foaming properties of the solution.

The foaming properties of an extract were observed to be dependent on the pH and temperature of the macerating medium. Maximum stability was obtained when maceration was done at about 20°C and in a buffer of pH 5.5. Maceration at higher temperature and low pH resulted in decreased foam stability. Subsequent to extraction, variation in pH resulted in only small changes in foam stability.

The dependence of the foaming properties of an extract on maceration conditions may be explained by regarding the plant material as a complex system of not only foaming agents but also foam inhibitors and foam stabilizers. Thus, the low stability obtained when maceration is done at high temperature and low pH may be the result of maximum extraction of a foam stabilizer under these conditions.

That such a system does exist in the plant material is supported by the observation that the pectic substances, which occur in substantial quantities in alfalfa, are effective foam stabilizers. Evidence was also obtained for the presence of one or more foam inhibitors in alfalfa. Extraction of lyophilized alfalfa extracts with alcohol at low temperature increased the foam stability. The solubility properties of this foam inhibitor suggest that it may be a conjugated lipid in the plant material. Further evidence for the presence of foam inhibitors was obtained from fermentation studies. When washed suspensions of rumen bacteria were incubated with alfalfa extracts, the foam stability increased during the first two hours and then remained constant. It is known that certain foam stabilizers, like the pectic substances, are rapidly degraded by rumen bacteria, but the observed increase in foam stability suggests that destruction of foam inhibitors has an important effect on the system.

The variation of foaming properties of alfalfa with stage of growth consisted of an increase as growth progressed with a decrease near the bloom stage. Deviations from this pattern were common, and changes occurred gradually during several days or rapidly during a single day.

The foaming properties of alfalfa samples from plots grazed by experimental animals were compared to the occurrence of bloat. The peaks in bloat corresponded to high foam stability, but a direct relationship was not always observed. The results suggest the importance of conditioning of the animal in its susceptibility to bloat.

#### PHYSIOPATHOLOGY PANEL

##### Physiological and Biochemical Effects of Experimental Bloat in Calves - C. M. Stowe and A. L. Good, University of Minnesota, St. Paul

In an effort to better understand the pathogenesis and cause of death in acute bloat, anesthetized calves were insufflated to 20, 30, and 40 mm Hg pressure with nitrogen, oxygen, and a gas mixture containing carbon dioxide, methane, hydrogen sulfide and hydrogen. The following parameters were studied in each animal before anesthesia, during an anesthesia control period, and during rumen insufflation to various pressures: Cardiac output, blood pressure, arterial and venous blood oxygen, carbon dioxide, oxygen consumption, blood pH, and arterial and venous blood lactate from various portions of the circulatory system.

In general, it was found that cardiac output decreased during bloat, as did the blood oxygen values, blood pH, and oxygen consumption. The anaerobic metabolic rate increased, and the calculated oxygen debt rose steadily during the bloating period, as did the lactate-pyruvate ratios. Although the L/P ratios and excess lactate increased in the general blood pool, there was a relatively greater rise in the post-caval and femoral vein blood posterior to the rumen distention. In animals given oxygen at a rate of 5 liters per minute via a tracheal catheter during insufflation, the changes noted above were greatly modified. These experiments suggest a rather severe hypoxia, systemic acidosis and a deterioration of the cardio-vascular system during experimental bloat.

Some Undesirable Affects Observed in Cows Fed a Combination of Antibiotics -  
R. S. Emery, Michigan State University, East Lansing

A combination of antibiotics was fed to several herds in such a manner that each animal received daily 40 mg. procaine penicillin plus 70 mg. each of Tylosin, Erythromycin and Streptomycin. A total of six out of 50 cows receiving the antibiotic produced dark colored feces containing mucus casts. One of the cows vomited several times and there was some discoloring of the urine. All 6 cows dropped to about 1/2 of their normal milk production. None of these symptoms were displayed by any of the 50 cows in the control group. In another herd, no symptoms were noted in the 20 heifers on the antibiotic group, but the junior herd sire obtained access to the feed bunks where the heifers were being fed the antibiotics plus grain. He was found dead on pasture. A post was conducted on this bull and death was due to severe enteritis. The rumen flora was very abnormal in microscopic appearance. Two types of rods were the only apparent flora. Antibiotics assays using a B Subtilis spore suspension was conducted. The rumen contents apparently contained antibiotics activity equivalent to about 1/10 of a unit of penicillin per ml. The rumen contents also contained 12 mg.% lactic acid. Since the contents were not neutralized in any way, it is possible that some of the inhibition zone was due to acidity in the rumen contents. Pathological examination of the liver, kidneys, and gut was inconclusive due to postmortem decomposition. No undesirable reactions have been observed in another group of 50 heifers and in 22 cows although the preparation was unpalatable to the cows.

Digestive Secretions in the Duodenum of the Sheep - F. A. Harrison and K. J. Hill, Institute of Animal Physiology, Babraham, Cambridge, England

Passage of digesta from the fore-stomachs of the ruminant is a continuous process and is reflected in the continuous nature of the secretory behaviour of the digestive glands. Quantitative data are however required on the flow of digesta and digestive secretions in the abomasum and the remainder of the tract before a complete assessment of the digestive events in these regions is possible. Attempts have been made to obtain some information on these topics by measuring the rates of flow of bile, pancreatic juice and duodenal secretion under different conditions and relating these to the passage of digesta through the duodenum. The relationship of gastric, pancreatic and duodenal secretory activity and the secretion of bile, to the reaction of the digesta in the duodenum has also been investigated.

The Use of Partial Exteriorizations of the Reticulum and Rumen for Motility Studies - C.S.W. Reid, Physiological Laboratory, Cambridge, England

Surgically created partial exteriorizations of the reticulum and of different regions of the rumen provide a convenient means of observing the contractions of the stomach wall in conscious animals. The advantages offered by this technique are: 1) visual observation of both tonic and co-ordinated motility is possible, at any time, and without physical interference with the animal; 2) direct mechanical recording of the activity is possible; and since this does not impede simultaneous visual observation, one can be checked against the other; 3), multiple recording can be carried out from well-defined points which are readily accessible; 4) the recording procedures cause little if any disturbance to gastric sensory innervation; 5) there is no interference with the passage of the gastric contents nor with the processes of digestion; observation and recording may therefore be continued for long periods without risk of development of abnormal circumstances.

A preliminary investigation of the movements of the reticulum and rumen during fasting, feeding and rumination has been carried out in sheep using this method. As well as differences in the frequency of the movements, characteristic differences in their form (the magnitude, duration, complexity and temporal inter-relationships of the contractions of the various compartments) have been found. These observations have allowed a more precise definition of the sequences of contraction in the reticulum and rumen, particularly of the events in the ventral regions of the rumen.

The Effect of Absorption on the Acidity of Rumen Contents - Alan Dobson, The Rowett Research Institute, Bucksburn, Aberdeen, Scotland

When a neutral solution of a short chain fatty acid is placed in the isolated reticulorumen, for every two moles of fatty acid absorbed about one mole of bicarbonate appears in the lumen. The epithelium is, however, permeable to both carbon dioxide and bicarbonate. The concentration of bicarbonate in the rumen solution at which it passes neither in nor out of the lumen is raised when acetate is absorbed, whereas that of carbon dioxide is lowered. This indicates that part of the acetate is absorbed as the free acid even at neutral pH. This absorption mechanism appears to be of an importance similar to the production of saliva in allowing the plasma to neutralize the fatty acid produced in the rumen.

Quantitative Studies of Eructated Gas Entering the Lungs During Normal Eructation in Cattle: The Possible Physiological Significance of Plumonary Absorption of Eructated Gas - R. W. Dougherty and H. M. Cook, National Animal Disease Laboratory, Ames, Iowa

Two well-trained cows having rumen and tracheal fistulas were used in this series of experiments.

A face mask and a cuffed endotracheal cannula passed through the mid-cervical tracheal fistula and directed towards the larynx made it possible to measure the eructated gas leaving the mouth and the gas entering the trachea.

In all experiments, the greatest percentage of the gas entered the trachea with each eructation. The difference was not as much during rumination.

Physiological implications were discussed.