

ELEVENTH CONFERENCE ON RUMEN FUNCTION

held at

Midland Hotel, Chicago, Illinois

December 1-2, 1971

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

- | | | |
|------------------------------------|-----|-------------------------------------|
| (a) Physiopathology and Physiology | --- | R. W. Dougherty
A. D. McGilliard |
| (b) Microbiology | --- | M. P. Bryant |
| (c) Agronomic | --- | J. C. Burns |
| (d) Nutrition | --- | J. T. Huber |

PHYSIOPATHOLOGY AND PHYSIOLOGY PANEL

The Effect of Absorption of Carbon Dioxide on the Composition of Blood Draining the Rumen Epithelium - A. Dobson, Department of Veterinary Clinical Studies, School of Veterinary Medicine, Cambridge.

Observations were made on anesthetized sheep with the trachea cannulated and the ruminoreticulum isolated by ligatures from the rest of the gut. The organ contents were removed, and suitable solutions introduced through a permanent cannula in the dorsal sac of the rumen. These solutions were adequately stirred by passing mixtures of carbon dioxide and nitrogen, humidified and heated to body temperature, through a sintered glass tube in the ventral sac. The sheep breathed through a Ruben valve to permit the measurement of inspired volume, and collection of expired gases. By measuring the carbon dioxide content of the expired air the rate of exhalation of carbon dioxide could be calculated.

The rate of addition of carbon dioxide to the ruminal epithelial blood was deduced from the increment in exhaled carbon dioxide accompanying an increase in the proportion of carbon dioxide in the gas stirring the ruminal solution once steady conditions were reached. Simultaneously the blood flow to the epithelium was estimated by observing the clearance of tritiated water from the ruminal solution (Dobson, Sellers, and Thorlacious. Am. J. Physiol. 220, 1337-1343, 1971). By dividing the absorption rate of carbon dioxide by the blood flow, the increase in total carbon dioxide in the blood perfusing the epithelium was estimated. When 100% CO₂ was present in the rumen, it was concluded that the acidity of the blood leaving the rumen was well below PH 7 and its osmotic pressure was raised by 10%.

These findings could account for the apparently anomalous movements of water between the contents of the ventral sac of the cow and its blood in the presence of carbon dioxide (Dobson, Sellers, and Shaw. J. Appl. Physiol. 28, 100-104, 1970).

(Supported by a Fellowship from the Wellcome Trust)

Relationships between Time Length of All-Concentrate Feeding, Performance, and the Development of Rumen and Liver Pathology in Cattle - W. M. Wass, Department of Veterinary Clinical Science and A. E. Ledet, Department of Veterinary Pathology, Iowa State University, Ames, Iowa.

The feeding trial was conducted to study the effects of time length of all-concentrate feeding on performance and incidence of liver abscess and rumenitis of beef cattle. One hundred ninety eight Hereford steers (wt. approx. 215 kgms) were selected for the study. The animals were given a starter ration for fourteen days and then were re-alloted to 18 pens with 11 steers in each pen. They were assigned to six different treatments with varying time length of concentrate feeding and with each treatment having three replicates. All animals were weighed throughout the study to determine progressive effects of all-concentrate feeding. Rumens and livers were graded at slaughter to evaluate pathologic changes.

The data obtained suggest that removal of hay from rations tends to increase the incidence of rumenitis and liver abscess and to decrease performance. The time length of all concentrate feeding is also an important factor.

A Convenient Method for Examining Ruminal Contents - Ana N. Alonso, School of Veterinary Medicine, University of Pennsylvania.

Summary:

The evaluation of ruminal activity is important in the study of ruminants. Many researchers have approached the subject from the strictly nutritional point of view but relatively little is known about the interactions between disease and ruminal digestion.

To facilitate study of these interactions under clinical circumstances, a convenient routine method for examining ruminal contents has been developed. It has been tested in normal animals and applied to a variety of clinical cases.

A 200-250 ml sample is taken by esophageal tube. It is immediately examined for consistency, color, odor, and potentiometric measurement. Ammonia concentration percent is measured if pH is above 7.0.

The sample is divided into two containers, each with a layer of liquid paraffin (mineral oil) on the surface in order to keep anaerobiosis. Both samples are utilized for microscopic observations.

One container is used for:

- a. - Fresh Giemsa stain for protozoa motility observation and counting.
- b. - Iodine stain for protozoa family identification, starch metabolism and iodophilic - aniodophilic bacteria ratio interpretation.
- c. - Gram stain for grouping bacteria.
- d. - Counting microorganisms (optional).
- e. - Velocity of sedimentation.
- f. - Gas production.
- g. - Complex carbohydrate digestion.

The second container has about 20 ml glycerin added to suspend protozoa. It is used for identification of protozoal species and yeast or molds if present by special stainings.

Interpretation of the examinations will depend at first on a knowledge of normal situations and incorporates more associations with abnormal situations as the study progresses.

Thiamin Inadequacy, Amprolium Toxicity, and Ruminant Polioencephalomalacia^{1/} - F. M. Loew and R. H. Dunlop, Department of Veterinary Physiology, University of Saskatchewan, Saskatoon, Canada.

Polioencephalomalacia (PEM), a disease of cattle and sheep characterized by necrosis in the cerebral cortex, is thought to result from an inadequacy of thiamin (vitamin B₁), a vitamin not usually considered to be a dietary requirement of mature ruminants.

Field studies were conducted to evaluate the thiamin status of ruminants in various disease states including PEM. Blood total thiamin concentrations in cattle with PEM did not differ significantly from those in cattle with other clinically similar diseases. A field outbreak was studied in detail and cattle with the disease had thiamin-destroying activity in their rumen contents. Three of four apparently normal cattle in another herd had no such activity; one did.

The effects of chronic intraruminal administration to sheep of amprolium, a structural analog of thiamin, were studied. Cardiac output, blood total thiamin concentration, erythrocyte transketolase activity and thiamin pyrophosphate (TPP) effect, blood pyruvate and total lactate concentrations, blood and rumen amprolium concentrations, and several serum electrolytes were measured weekly.

Of seven sheep given amprolium intraruminally at a daily dose of 400 mg/lb body weight, three survived approximately six weeks and developed the clinical signs and histological lesions of PEM. The remaining four sheep survived approximately three weeks and, based on clinical signs and post-mortem lesions, did not develop PEM. Although TPP inadequacy was inferred from terminally depressed erythrocyte transketolase activity and increase TPP effect in the sheep which developed PEM, their blood total thiamin concentrations decreased significantly only after the first and second weeks of amprolium administration and then

^{1/} Based on a Ph.D. Thesis by F. M. Loew. 1971.

stabilized at a level not associated with deficiency in other species. The four non-PEM sheep did not have terminally depressed transketolase activity or increased TPP effect, but did have a suggestion of a greater lowering of blood thiamin. Death of the non PEM sheep was associated with much higher blood and rumen amprolium concentrations and amprolium:thiamin molar ratios than in the sheep developing PEM. It was speculated that the non-PEM animals died from a toxic effect of amprolium unrelated to TPP.

In neither group did cardiac output change during amprolium administration.

It was concluded that amprolium-induced PEM was not associated with the high cardiac output cardiac failure reported in human beriberi. The data suggested an inadequacy of TPP but not of total thiamin in amprolium-induced PEM. The field disease was concluded to be more likely to result from chronically inadequate supplies of intraruminally-synthesized thiamin than from the production of thiamin analogs in the rumen by the action of thiaminase I, as has been suggested by others.

Acknowledgements: Thanks are given to the National Research Council and the Medical Research Council of Canada, and to Merck Sharp & Dohme Canada Ltd.

Pharmacokinetics in the Ruminant Animal - Lloyd E. Davis, Carol A. Neff, and J. Desmond Baggot, Division of Comparative Pharmacology, College Veterinary Medicine, Ohio State University, Columbus.

Species differences in the effects of drugs are due to differences in disposition and fate of the drugs in the body rather than being due to variations in the nature of drug receptors. The ruminant animal appears to be unique from a pharmacokinetic standpoint as most of the drugs which we have investigated are eliminated rapidly as compared to elimination rates in monogastric animals.

We studied the pharmacokinetics of a number of commonly used drugs in domestic goats. The drugs included six acidic drugs (salicylate, pentobarbital, phenylbutazone, oxyphenbutazone, phenol, and sulfanilamide) and six basic drugs (pentazocine, chloramphenicol, quinidine, amphetamine, antipyrine, and tolazoline). Parameters measured or computed included: elimination rate constants, drug concentrations in plasma, volumes of distribution and extent of drug-plasma protein binding.

The rumen potentially can alter the disposition of drugs by serving as a compartment for sequestration of certain drugs. Furthermore, the efficacy of certain orally administered drugs may be diminished as a result of poor absorption and/or biotransformation by the ruminal microflora. The rumenologist should be aware of these factors when using drugs for experimental or therapeutic purposes in ruminant animals.

Cannulization of the Bovine Abomasum. A Surgical Technique - F. Rafael Alonso, William G. Donawick, and Elaine Hammel, Department of Clinical Studies, University of Pennsylvania, Kennett Square, Pennsylvania.

Direct access to the lumen of the abomasum facilitates studies of abomasal physiology and disorders as well as nutritional studies that require by-passing of the rumen.

Two different cannulas were surgically implanted. One was a thin (ID 0.6 cm., OD 1.0 cm.), 12 cm. long, hard plastic cannula while the other had a larger diameter (ID 2.5 cm., OD 3.0 cm.), 18 cm. long and made of a flexible material. Both types were similar in shape, with a base in the shape of a wide ring (8 cm. diam.) fixed around the cannula. A similar ring secured the cannula from the outside. The first type was implanted in 5 steers (4 Aberdeen Angus, 1 Holstein) while the other was implanted in 16 steers (Hereford). All animals were approximately 8-10 months of age.

Following the trial of several different approaches, it was concluded that the paracostal incision, with the animal tilted 20 degrees from the horizontal was best. After exteriorization of the abomasum, the cannula was located into the lumen of the fundic part, secured by a modified purse-string suture. A plastic mesh was sutured over the parietal surface of the abomasum, surrounding the neck of the cannula which was then exteriorized through a stab incision. An outer ring secured the cannula to the skin.

Excessive granulation was observed 3 to 4 months after surgery in some cases, but receded after local antibiotic therapy. No other untoward effect related to the surgery has been observed in the follow-up of a year.

Reversal of the Reticular Complex of the Rumino-Reticular Cutaneous Potential in Sheep - A. R. Graham, Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Changes in the cutaneous electrical potential associated with rumino-reticular movements were recorded from conscious sheep having cannulated permanent fistulae of the dorsal rumen. Simultaneous records were obtained from transthoracic and thoracic-abdominal leads. In routine records reticular motility appeared as a biphasic deflection during non-rumination and as an essentially triphasic deflection during rumination; in both cases the polarity of the individual deflections was similar. Complete reversal of the polarity of the reticular complex accompanied by increased amplitude of the individual deflections occurred in separate experiments following the individual addition to the rumen of simple solutions of 3.0 M calcium chloride, 6.0 M sodium acetate, 4.0 M sodium chloride, and 3.4-4.0 M potassium chloride. In one experiment potassium chloride produced a transient augmentation of the conventional reticular complex; this effect was reversed by sodium chloride.

Supported in part by funds received from the Ontario Department of Agriculture and Food.

Motility and pH Changes in the Sheep's Digestive Tract, Following Experimental Grain Engorgement - R. W. Dougherty, M. J. Allison, I. M. Robinson, J. L. Riley, H. M. Cook, and J. A. Bucklin.

These data are taken from 5 "overfed" sheep. After normal studies were made, three pounds of coarsely cracked wheat followed by one liter of warm water were poured into the rumens through surgically established cannulas. The same procedure was repeated 24 hours later.

All rumen and gut motility was recorded using radio telemetric methods. Small, wafer pressure type transducers were placed in surgically implanted cannulas and were connected to the transmitters.

Because of the low power output of the transmitters the signals were received by antennas mounted in the animal rooms. The signals were fed into standard FM receivers through 75 ohm coaxial cables. The outputs of the FM receivers were transmitted to subcarrier discriminators. The outputs of the subcarrier discriminators were recorded on a multi channel direct writing recorder.

The following deductions were made from representative samples of the recordings:

- a. The pH of the normal rumens varied between 6.4 - 6.8. Motility of the normal rumen was quite rhythmic. Motility of the cecum, abomasum, and small intestine was measured. There were periods of strong activity followed by periods of rest. The pH of the cecum was alkaline varying between 7.5 and 8.0. After overfeeding the pH of the cecum dropped to approximately the same level as that of the rumen.
- b. There was rough correlation between motility and pH of the rumen and cecum. In some experiments fairly good motility occurred when the pH of ingesta did not drop below 5.0.
- c. The cecal pH returned to normal much quicker than did the rumen pH. Cecal motility also returned to normal activity when the pH reached normal levels. The same could be said of the rumen except for the longer time factor. Although there is an increased relationship between pH and motility of the rumen and cecum, it appears to be non-linear.

Adrenergic Agonist and Antagonist Effects on Feeding of Sheep and Steers - C. A. Baile, C. W. Simpson, L. F. Erabill, and F. H. Martin, Smith, Kline, and French, West Chester, Pennsylvania, and Monell Chemical Senses Center, University of Pennsylvania, Philadelphia.

The medial hypothalamus of rats is hypothesized to contain α -adrenergic receptors which when activated suppress the medial neural inhibition of the lateral hypothalamus and results in eating. The lateral hypothalamus may contain β -adrenergic receptors which when activated inhibit feeding. We have tested several aspects of this proposed catecholamine coded system in sheep and steers. Animals were each prepared with bilateral lateral ventricular

cannulas. They were fed pelleted diets ad libitum and had access to water at all times. Prior to each test, animals were given additional feed and allowed to eat one hour, after which each animal was given a ventricular injection of 1 ml of synthetic cerebrospinal fluid. Drugs were added to the fluid on treatment days, and fluid alone was injected on control days. Load cells and a data acquisition system were used to measure feed intakes at intervals following injections. Norepinephrine (NE) injections (183 μ g) into sheep resulted in feeding for about 30 min (309 g vs. 59 g for control $P < .01$). An α -adrenergic antagonist, dibenzyline (100 μ g), alone had no effect on feeding, while it blocked the effect of NE. The β -adrenergic agonist, isoproterenol (137 μ g), suppressed spontaneous feeding for 2 hr (12 g vs. 112g; $P < 0.05$). A β -adrenergic antagonist, dl-propranolol, alone did not affect feeding, but it blocked the inhibitory action of isoproterenol. In contrast, steers given isoproterenol (250 μ g) ate 1062 g vs. 393 g ($P < 0.05$) control during the 60-min after injection. Furthermore, NE injections (as little as 500 μ g, $P < 0.05$) resulted in a significant decrease in intake for at least 60 min. Further studies showed that $\frac{1}{2}$ bilateral injections containing 40 μ g of NE into the medial hypothalamus resulted in eating within 5 min in satiated sheep; intakes in 30 min were 100 g vs. 34 g ($P < .01$). Our results to date show that the CNS chemical coding for feeding is probably similar in sheep and rats but is different in steers. More specific tissue injections may help clarify this apparent unexpected difference.

Feeding behavior as Related to the Hypnotic Activities of A Series of Barbiturates Injected into the III Ventricle of Sheep - J. R. Seosne, C. A. Baile, and R. L. Webb, Smith, Kline, and French Animal Health Products Division, and Monell Chemical Senses Center, University of Pennsylvania, Philadelphia, Pa.

The effects on feeding behavior of sheep of a series of barbiturates of differing hypnotic activities was studied. Crossbred wethers were prepared with a stainless steel cannula placed in the III Ventricle of the brain so that barbiturates could be injected in close proximity to the medial hypothalamus and limit their possible action on higher brain centers. Half a ml was injected into the III Ventricle during 160 seconds (approx. 0.2 ml/min). The barbiturates were chosen according to their length of hypnotic action, ranging from the ultrashort acting thiamylal, secobarbital (short), pentobarbital (short-intermediate), amobarbital (intermediate), phenobarbital (long) and barbital (long). The feeding response was closely related to the length of action and biological half-life of each barbiturate. Long acting barbiturates produced a consistent and sustained feeding, for as long as two hours, ($P < 0.01$) while short acting barbiturates did not affect feed intake significantly. The 24 hr. feed intake was increased approximately 30% following injections of the long acting sodium barbital ($P < 0.05$). We suggest that barbiturates act by suppressing the activity of inhibitory fibers acting on the feeding center of the lateral hypothalamus, thus resulting in feeding.

MICROBIOLOGY PANEL

Inorganic Nutrient Requirements of Ruminal and Non-Ruminal Bacteroides Species - Daniel R. Caldwell, Caryn M. Arcand, and Richard F. Hudson, Division of Microbiology and Veterinary Medicine, University of Wyoming, Laramie.

Although considerable evidence indicates that inorganic nutrients are critically important to a variety of quantitatively important ruminal processes, knowledge concerning the nature, specificity, and functions of the inorganic requirements of pure cultures of predominant rumen bacteria is relatively sparse. The present study was initiated to expand knowledge concerning the inorganic requirements of predominant ruminal bacteria, and to contrast the requirements of ruminal representatives of the genus Bacteroides with the requirements of taxonomically related organisms isolated from non-ruminal environments. The organisms were grown in a defined medium containing either glucose or maltose as energy sources, ammonium sulfate as the major nitrogen source, a B-vitamin mixture, a volatile fatty acid mixture, ferrous sulfate, hemin, methionine, resazurin, either cysteine or sodium sulfide as a reducing agent, a bicarbonate-carbon dioxide buffer system, and a mineral mixture containing Na^+ , K^+ , PO_4^{-3} , Cl^- , Mg^{+2} , Ca^{+2} , Mn^{+2} , and Co^{+2} . Single deletion of these ions from the medium, under isotonic conditions, showed that all of the species studied, which included Bacteroides ruminicola subsp. ruminicola, Bacteroides ruminicola subsp. brevis, Bacteroides amylophilus, Bacteroides succinogenes, Bacteroides fragilis, Bacteroides oralis, Bacteroides ovatus, Bacteroides vulgatus, Bacteroides hypermegas, Bacteroides fundiliformis, Bacteroides thetaiotaomicron, and Bacteroides diastonicus, required K^+ , PO_4^{-3} , and Mg^{+2} . Single deletion of Ca^{+2} drastically reduced growth of Bacteroides succinogenes S 85, confirming previous demonstrations of a Ca^{+2} requirement for this organism, but none of the remaining species were affected by Ca^{+2} deletion in media containing Mg^{+2} , Co^{+2} , and Mn^{+2} . Single deletion of Mn^{+2} or Co^{+2} also failed to affect the growth of any of the species studies.

The divalent cation requirements of species within the genus Bacteroides appear somewhat complex, as shown by studies in which various combinations of Co^{+2} , Mn^{+2} , Ca^{+2} , and Mg^{+2} were added back to media from which all four cations were deleted. Single addition of Mg^{+2} to divalent cation-deficient media resulted in greatest total growth. However, in many cases, the single addition of either Ca^{+2} or Mn^{+2} to divalent cation-deficient media permitted some growth of strains for which no effect for these ions could be shown through single deletion. Furthermore, combinations of two (e.g., Ca^{+2} and Mg^{+2} , Ca^{+2} and Mn^{+2} , and Mg^{+2} and Mn^{+2}) often permitted higher total growth than was obtained by addition of either component separately. These results suggest that interactions exist (e.g., sparing effects) among the effects of divalent cations on the growth of Bacteroides species.

Striking differences were found among Bacteroides species with regard to their responses to sodium and related monovalent cations. Bacteroides amylophilus, Bacteroides ruminicola, and Bacteroides succinogenes all

displayed obligate, sodium requirements. Additional work has shown that most predominant rumen bacteria other than Streptococcus bovis, D. elsdenie, and Selenomonas ruminantium display an apparently obligate sodium requirement. Li^+ , Cs^+ , and Rb^+ , not only failed to replace Na^+ , but were toxic to all three rumen Bacteroides species at sodium concentrations. The potassium requirement of these organisms could be replaced by Rb^+ , while Li^+ and Cs^+ were either inactive or toxic at potassium replacing concentrations.

Bacteroides species of non-ruminal origin displayed diverse responses to Na^+ and K^+ . No requirement for sodium could be demonstrated for Bacteroides hypermegas or B. oralis strain 7CM, whereas an obligate Na^+ requirement was found for B. oralis strain J1 and for Bacteroides diastonis. B. oralis J1 displayed an obligate K^+ requirement. The remaining non-ruminal Bacteroides species displayed partial requirement for Na^+ and K^+ . The drastic reduction in growth observed when both Na^+ and K^+ were deleted from the medium as opposed to only a partial reduction in growth observed when either ion was deleted alone indicates that Na^+ and K^+ can at least partially replace each other as nutrients for these organisms. Monovalent cations chemically related to Na^+ and K^+ also affected non-ruminal Bacteroides species diversely. Na^+ could be partially replaced by Li^+ for B. fragilis 8560, B. oralis J1, and B. diastonis, but Na^+ replacement by Li^+ markedly reduced growth rate. Sodium could not be replaced by Rb^+ , Cs^+ or Li^+ for any of the remaining organisms. Rubidium could partially replace K^+ as a growth factor for B. fragilis strains H and 8560, both B. oralis strains J1 and 7CM, B. ovatus, B. vulgatus, B. fundiliformis, and B. diastonis. The rate of growth of B. vulgatus decreased when Rb^+ replaced K^+ . Cs^+ and Li^+ also appeared to partially replace the K^+ requirements of B. fragilis 8560, B. vulgatus, and B. diastonis. Cs^+ , but not Li^+ , or Rb^+ also appeared able to replace the K^+ requirement of B. hypermegas. The extent of replacement of Na^+ and K^+ by related monovalent cations varied from species to species and, in some cases, among strains within species.

All of these results indicate that diversity exists in the effects of inorganic nutrients on the growth of various species within the genus Bacteroides, both with respect to the specificity of their inorganic requirements, and with respect to the nature of the effects observed. The ruminal Bacteroides species appear more similar to each other than to non-ruminal Bacteroides species, particularly with respect to their monovalent cation requirements. An obligate requirement for Na^+ appears to be a general requirement of ruminal Bacteroides species, and apparently among many other predominant rumen bacteria. A K^+ requirement replacable by Rb^+ only also appears to be of frequent occurrence among ruminal bacteria. The significance of the differences observed in the inorganic nutrient requirements among Bacteroides species, and, in particular, the metabolic and physiological functions of Na^+ and K^+ for the rumen bacteria remain to be determined.

The Endogenous Lipids of Several Rumen Bacteria - Joseph E. Kunsman, Jr.,
University of Wyoming, Laramie.

Several strains of rumen bacteria were grown on a chemically defined lipid free medium. The cells were isolated by centrifugation and the lipid extracted. Four strains of Bacteroids ruminicola exhibited quite similar lipid content. The major non-polar lipids were triglycerides, diglycerides, and free fatty acids. The major polar lipid was composed of phosphatidyl ethanolamine, sphingolipid type compounds and phosphatidic acid. Strain, B, 4 differed from the other three strains, (118B, B, 18, GA33) in that the major lipid component was phosphatidic acid. Phosphatidyl ethanolamine was the major lipid component in the other three strains. The fatty acid concentration for all four strains was similar with the major acids being C15:0 and C15:1. No difference was noted in the fatty acids for individual lipid components except the sphingolipid was found to be higher in C16:0. The lipids of B. ruminicola were compared with the reported values for B. melaninogenicus, B. amylophilus, and B. succinogenes. The sphingolipids were found only in B. ruminicola and B. melaninogenicus. Butyrivibrio fibrisolvens was grown to various growth levels and the cells harvested as above. Cultures harvested in the log arithmetic phase of growth showed higher concentrations of phosphatidic acid and lower concentrations of phosphatidyl ethanolamine than cultures harvested during the stationary phase. The cultures harvested at the stationary phase of growth also exhibited a higher concentration of short chain fatty acids (C10:1 to C14:0) than the log phase cultures.

Anaerobic Degradation of Phloroglucinol by Rumen Bacteria - G. A. Jones and
Chii-Guany Tsai, Agricultural Microbiology Section, Department of Dairy and
Food Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Anaerobic enrichment cultures of the rumen microflora containing 0.01 M phloroglucinol (1,3,5-trihydroxybenzene) as sole added energy source in a medium with rumen fluid completely degraded the substrate within 72 hours. Nine strains of obligately anaerobic bacteria capable of degrading phloroglucinol were isolated from the enrichments. All were Gram-positive streptococci; biochemical reactions and analysis of products of glucose and maltose fermentation showed three strains to be Peptostreptococcus sp. and six to be Streptococcus sp. Washed cell suspensions of one strain, Peptostreptococcus sp. Pe₅ degraded phloroglucinol only if prepared and incubated under rigorously maintained conditions of anaerobiosis. Analysis of products of phloroglucinol degradation by both growing cultures and washed cell suspensions of this strain showed that acetate and CO₂ were produced. No phenolic intermediates could be detected.

Studies on the Ciliate Fauna of Alaskan Ruminants (Reindeer, Caribou, Musk-Ox, Moose, and Dall Mountain Sheep) - B. A. Dehority, Department of Animal Science, Ohio Agricultural Research and Development Center, Wooster, Ohio.

Samples of rumen contents were obtained from Alaskan reindeer, caribou, moose, musk-ox, and Dall mountain sheep. Total numbers of ciliate protozoa, generic distribution, and occurrence of various species was determined. The ciliate fauna of a herd of semi-domestic reindeer, originally obtained from the Seward peninsula, was qualitatively similar whether the animals were fed alfalfa

pellets, lichen, browse and native pasture, or dried lichen. Total numbers were considerably higher however, for those animals feeding on lichen, browse and native pasture. Their fauna differed quite markedly from other reports, from caribou living in the same locality supposedly feeding on similar vegetation and from a herd of wild reindeer living on Umnak Island. When wild reindeer and domestic sheep grazed the same pasture, no transfaunation of protozoal species supposedly specific to the reindeer was observed. The musk-ox, although feeding on bromegrass pasture, had a fauna somewhat similar to Alaskan wild reindeer and caribou. Both the moose and Dall mountain sheep appear to have faunas quite characteristic to the species. Four tentative new species of protozoa were observed, one in moose and three in the Dall sheep.

The Effects of Changes in Feed Level, Starvation and Level of Feed After Starvation upon Total Rumen Protozoa Numbers - E. L. Potter and B. A. Dehority, Ohio Agricultural Research and Development Center, Wooster, Ohio.

Four rumen fistulated sheep were used in 3 experiments to investigate the effect of feed level upon changes in the protozoa numbers. The sheep were fed once daily 650 gms of a pelleted diet composed of corn cobs 45%, alfalfa meal 35%, oats 12.6%, cane molasses 5%, urea .4%, and vitamins and minerals 2%. Total protozoa numbers showed that the diurnal cycle reached minimum and maximum concentration at 5 and 22-1/2 hrs. after feeding respectively. Total protozoa concentration between 5 and 21 hrs. post-feeding were used to estimate apparent generation rates. Apparent Generation rates/Hr = $\text{natural log } \frac{T_{21}}{T_5}$. Results showed that as the ratio of feed to protozoa increased generation rate increased. The feed to protozoa ratio was altered by changing the protozoa population through starvation and by changing the level of feed (200 to 900 gm/day). A decrease in the generation rate occurred with the 900 gram feed level. This may be the result of an increased rate of passage from the rumen.

Glucose Fermentation by Mixed Continuous Cultures of Ruminococcus albus and Vibrio succinogenes - D. Kafkewitz, E. L. Iannotti, M. J. Wolin, and M. P. Bryant, University of Illinois, Urbana, Illinois.

V. succinogenes, which utilizes only H_2 or formate as energy sources, grew in glucose-limited, fumarate-containing, complex medium because R. albus produced H_2 which V. succinogenes used as an energy source (H_2 +fumarate succinate). This permitted an evaluation of the effect of complete H_2 -utilization on glucose fermentation by R. albus. The kinetic parameters for the mixed culture were similar or slightly higher than those for R. albus cultivated alone, i.e., K_s for glucose (mixed culture) was $1.7 \times 10^{-3}M$ and maximum growth rate was $0.73-0.94 \text{ hr}^{-1}$. Glucose incorporation into cells, measured with a pulse of ^{14}C -glucose, was 21 and 24% of glucose used at dilution rates of 0.12 and 0.36 hr^{-1} , respectively. In contrast to R. albus cultures alone, no ethanol was produced in the mixed system. Acetate, CO_2 and succinate were the sole fermentation products (C recovery, 74%; O/R index, 0.77). Acetate increased in the mixed cultures above the amount obtained with R. albus alone by an amount equivalent

to the ethanol in the R. albus culture. The electron deficit caused by the ethanol to acetate shift in the mixed system appeared as an increase in the amount of succinate produced above the amount expected from H₂ production by R. albus alone. Thus, the H₂-using V. succinogenes causes a significant change in the fermentation products formed from glucose by R. albus.

Glucose Fermentation by Continuous Cultures of Ruminococcus albus - E. L. Iannotti, D. Kafkewitz, M. J. Wolin, and M. P. Bryant, University of Illinois, Urbana, Illinois.

Glucose accumulated in increasing amounts with increasing dilution rates in glucose-limited continuous cultures. The medium also contained salts, trypticase, yeast extract, cysteine, fumarate, small amounts of isobutyrate, isovalerate and DL-2-methylbutyrate, and bicarbonate, and the initial atmosphere was 100% CO₂. Glucose concentration and growth rate data were used to calculate a K_s of 1.2 x 10⁻³M. Maximum growth rate was 0.60-0.65 hr⁻¹. Glucose incorporation into cells was measured, using a pulse of ¹⁴C-glucose, at growth rates of 0.16 and 0.36 hr⁻¹ and was 15% of the glucose used. Fermentation products in moles of ethanol, acetate, H₂ and CO₂ per 100 moles glucose fermented were 69, 74, 237, and 143, respectively, with a C recovery of 72% and O/R index of 0.76 (values corrected for incorporation of glucose into cells). CO₂ was not measured but production was assumed to be equivalent to that of C₂ units. No formate, lactate, succinate, acetoin, diacetyl, or other volatile acid or dicarboxylic acids were detected. The data are means from 10 experiments in which flow rates were varied from 0.17 to 0.59 hr⁻¹. No consistent changes in fermentation balance which correlated with changes in flow rate were apparent. Batch culture experiments showed that formate was a significant fermentation product only when growth was not limited by glucose.

Microbial Changes in the Sheep Cecum Caused by Overfeeding with Wheat - M. J. Allison, I. M. Robinson, J. A. Bucklin, and R. W. Dougherty, Ames, Iowa.

Adult sheep were prepared with ruminal and cecal fistulas. Bacteria were cultured from the rumen and cecum before and after intra-ruminal dosage with 1.35 Kg of cracked wheat on two successive days. Differential media were employed to permit enumeration of anaerobic lactobacilli, streptococci, Clostridium perfringens, and coliforms. A non-selective rumen fluid medium was also used to estimate numbers of "total" viable anaerobic organisms.

For the most part, changes in cecal microbes after overfeeding were similar to changes seen in the rumen. The notable exception to this was the pattern of change observed with organisms presumptively identified as Clostridium perfringens. The concentrations of C. perfringens in the cecum were invariably higher than in the rumen.

Numbers of cecal C. perfringens increased significantly within 24 hours after the first dosage with wheat but a second and greater increase was seen about 72 hours after the first dosage. At this time, C. perfringens numbers reached

10^8 /g in cecal contents from 2 sheep and 10^6 /g in the other sheep studied. This increase coincided with a rather rapid rise in cecal pH from less than 5 to more than 7 while rumen pH remained less than 5.

The significance of the microbial changes seen in the cecum is yet to be determined. Experiments are in progress to test the hypothesis that the C. perfringens strains that become so numerous, produce an enterotoxin and that this is responsible, in part, for the diarrhea that accompanies acute acid indigestion.

Ration Digestibility and Preliminary Studies on the Bacteria Occurring in the Rumen of Lambs Born and Reared in Isolation - J. R. Males and B. A. Dehority, Department of Animal Science, Ohio Agricultural Research and Development Center, Wooster, Ohio.

Six lambs were born and reared in strict isolation from other ruminants. They were housed in a completely sealed room, that had a positive pressure and was entered through a double door arrangement. All feed and equipment entering the room were either sterilized or disinfected. Lambs were reared to weaning on an artificial milk replacer. Total bacterial counts, cellulolytic bacterial counts, cellulose digestibility, dry matter digestibility and rumen volatile fatty acids were measured. After the lambs had been on a corn, alfalfa, soybran flake diet for two months, dry matter digestion was 63.5% and cellulose digestibility was 28.4%. At this time, there were 18.9 billion total bacteria per gram of rumen contents, 137 thousand cellulolytic bacteria per gram, and 65.6 μ m of total V.F.A's per ml. of rumen fluid, with 76.5 molar % C_2 and equal proportions of C_3 and C_4 , were produced. None of the major cellulolytic digesting bacteria of the rumen were observed from the isolates made in cellulose agar roll tubes at this time.

Shortly after the above data was collected, contamination with a Ruminococcus type organism occurred and cellulose digestibility increased to 44.5% and numbers of cellulolytic bacteria to 250 million per gram.

The establishment of Bacteroides succinogenes A3c was attempted at two separate times; however, no organism that morphologically resembles A3c has been isolated.

The effect of the presence or absence of B-vitamin supplements on isolated lambs was also investigated.

Interspecies Hydrogen Transfer - D. Kafkewitz, E. Iannotti, M. J. Wolin, and M. P. Bryant, University of Illinois, Urbana, Illinois.

A theory was developed to explain how H_2 -using bacteria, e.g., methane-forming bacteria, can affect fermentation product formation by carbohydrate-fermenting bacteria. Reduced nicotinamide adenine dinucleotide (NADH) produced during carbohydrate fermentation is reoxidized during pure culture fermentations by

reactions leading to products such as ethanol and lactate. If an NADH-linked hydrogenase is present in the carbohydrate-fermenting bacterium, reoxidation of NADH via H_2 formation will not be easily expressed in pure culture because of the unfavorable equilibrium of the NADH to NAD and H_2 reaction. A H_2 -using bacterium, however, can pull the reaction in the direction of NAD formation by using up H_2 . Products such as ethanol and lactate would not be formed. The rationale for an experiment to test the theory was explained. Ruminococcus albus, which ferments glucose to acetate, ethanol, H_2 and CO_2 in pure culture in a chemostat was mixed in a chemostat with *Vibrio succinogenes*, an organism that uses H_2 to reduce fumarate. The prediction was that no ethanol and increased amounts of acetate would be formed in the mixed culture.

AGRONOMIC PANEL

Reed Canarygrass---Alkaloids, Palatability, and Potential of a Problem Forage - G. C. Marten, U.S. Department of Agriculture, PSRD, Agronomy and Plant Genetics Department, University of Minnesota.

Reed Canarygrass (Phalaris arundinacea L.) is often the highest-yielding cultivated perennial grass of northern United States and southern Canada. Its digestibility by ruminants is equal to the best of the adapted perennial forages of this region (including alfalfa). However, it is relatively less palatable to livestock than most of the other perennial grasses and legumes. Whether this means it has lower intake potential than other species is a subject of much discussion but very little experimental evidence.

Relative palatability (a plant characteristic eliciting a proportional choice by an animal among two or more forages offered simultaneously) should be clearly distinguished from voluntary intake (units of forage consumed per animal unit when no choice is offered). Research is currently in progress at the University of Minnesota to determine whether relative palatability differences significantly affect animal performance potential of forage species and of genotypes within reed canarygrass.

We have clearly demonstrated that relative palatability of reed canary genotypes is highly negatively associated with concentration of total basic alkaloids^{1/}. Type of alkaloid (gramine, N,N-dimethyltryptamine, or 5-methoxy-N, N-dimethyl-tryptamine) is not critical in this relationship.

Genotype is apparently much more influential than environment as a determinant of alkaloid concentration in reed canary. In cooperative studies with personnel at Lafayette, Indiana, and State College, Pennsylvania, we have found excellent repeatability of alkaloid patterns in specific clones among locations.

In all of six trials, we found no association between alkaloid concentration (inherent in the forage) and in vitro digestibility of reed canary. In four of five additional trials, concentration of added reagent alkaloids (comprising zero to 3% of dry matter) was not significantly correlated with in vitro digestibility of a variety of forage substrates. However, in one trial, high concentrations (3% of DM) of 5MeoDMT significantly depressed digestibility of two clones of reed canary.

Improving the Nutritive Value of Tall Fescue through Ryegrass X Tall Fescue Hybridization - L. P. Bush and R. C. Buckner, Department of Agronomy, University of Kentucky and PSRD, ARS, U.S. Department of Agriculture.

Tall fescue (Festuca arundinacea Schreb.) is a well adapted, widely used pasture species in the southeastern United States. Although it has many good qualities as a pasture grass, it has been criticized for lack of palatability and nutritional

^{1/} Simons, A. B., and G. C. Marten. 1971. Relationship of incole alkaloids to palatability of Phalaris arundinacea L. Agron. J. 63:Nov.-Dec. issue.

value. Animal performance has not been consistent with the forage quality as measured by chemical constituents. Annual ryegrass (*Lolium multiflorum* Lam.) and perennial ryegrass (*L. perenne* L.), highly palatable and nutritious forages, have been hybridized with tall fescue to combine the palatable and nutritious qualities of the ryegrass with the good features of tall fescue. The F_1 of annual ryegrass x tall fescue is male sterile but the amphiploids of this cross are fertile and several lines have been obtained by crossing the male sterile F_1 with the fertile amphiploid. Selections were made from these annual ryegrass x tall fescue hybrid derivatives that have the agronomic qualities of tall fescue and that approach the palatability and nutritive qualities of the ryegrass. These hybrid derivatives showed greater yield of dry matter/acre, were more acceptable to livestock when grazed free-choice and had a higher NVI than tall fescue varieties. There was difference between the hybrid material and the tall fescue varieties in protein, sugar, acid-detergent fiber and lignin content. Perloline content of the hybrid material was less than that of the tall fescue varieties. The perloline content is genetically controlled and levels from 0.0 to 0.5% have been observed in the annual ryegrass-tall fescue hybrid derivatives. Perloline has been shown to inhibit in vitro ruminal cellulose digestion and in vitro volatile fatty acid production. Objectives of present and future investigations are to determine the in vivo effect of the alkaloid fraction on animal performance and to develop a ryegrass-tall fescue cross with a low alkaloid content but of high palatable and nutritive value with good agronomic characteristics.

Tannin in Sericea Lespedeza Affects Forage Quality - E. D. Donnelly, Auburn University (Alabama) Agricultural Experiment Station.

Sericea lespedeza, *Lespedeza cuneata* /Dumont/ G. Don, is a perennial, summer legume well adapted to the climate and soils of the southeastern U. S. A. It generally is recognized as being low in palatability and nutritive value. We began a breeding program in 1950 to improve this crop. Earlier workers had found that sericea was high in tannin and thought that tannin and coarse stems contributed to its relatively low quality.

In a "free choice" grazing study, using cattle and spaced plants, fine-stemmed plants and low-tannin plants were most heavily grazed. As a result of this study, the fine-stemmed but normal or high-tannin variety 'Serala' was developed. In a grazing study it was shown to be good grazing crop in the Piedmont area of Alabama.

In cooperation with Dr. W. B. Anthony, Department of Animal and Dairy Sciences, low-tannin sericea plants were found to be 12% higher in digestible dry matter (DDM) than high-tannin plants as indicated by the in vivo nylon bag method.

In another study steers weighing approximately 250 kg were grazed on high-and low-tannin sericea. The Cr_2O_3 -chromogens technique was used. In addition to W. B. Anthony, J. W. Langford cooperated in this study. Crude protein was higher than 16% during three 28-day grazing periods from May through mid-September. Steers on low-tannin forage consumed less dry matter per day than did steers on high-tannin forage. Even so, there was no difference in daily DDM intake between the two forages. The higher digestibility of

low-tannin forage was sufficient to overcome lower intake of this forage. Chromogens estimated DDM was higher for low-tannin forage than for high-tannin. This was substantiated by a lower crude protein percentage of feces from cattle on low-tannin forage than from cattle on high-tannin sericea. Feces from cattle on high-tannin sericea contained 22% more crude protein than did that from cattle on low-tannin forage. Also, feces from steers on low-tannin forage was higher in per cent in vitro digestibility than that from steers on high-tannin forage.

Data showed that the dry matter and crude protein of low-tannin sericea were more highly digestible than that in high-tannin sericea. Thus, low-tannin varieties should be more nutritious than present varieties. A grazing experiment designed to measure weight change in cattle, among other things, will be necessary to determine the relative merits of low-tannin and high-tannin sericea.

The Use of Lignin and Mineral Values to Estimate Forage Nutritive Value -
J. W. Thomas, Yu Yu, and D. Hillman, Michigan State University, East Lansing, Michigan.

Alkali solubilized forage lignins exhibit characteristic direct and difference absorption phenomena in the ultraviolet region. Absorptivity at selected wavelengths and other values characterizing the spectra have been quantitated and related to digestibility and intake values of some forages. Two populations of alfalfa samples plus one of bromegrass and one of fescue having known sheep intake and digestibility were used for this study. Multiple regression equations were developed using a least squares deletion program that can be used to predict digestibility, intake and lignin content of the forages. The resulting equations contained 4 to 8 easily measured variables and estimated digestibility of alfalfa with a R^2 varying from .53 to .81. R^2 value for grasses were .94 to .99. Estimations of intake were less accurate with R^2 values of .47 to .99. Lignin content of alfalfa, corn silage and grasses was also accurately estimated with R^2 values of .86 to .95.

The mineral content of these samples was also highly related to digestibility. Reasonable equations with R^2 values of .41 to .99 were developed.

Typical equations obtained are: bromegrass digestibility = $46.01 - 281X_4 + 168X_{12} - .74X_{30} + 1.04X_{21}$ where respective X values represent absorbency at 250 μ /1 g ADF; at 337; at 270/g lignin and distance in μ of peaks at +350 and +248 on difference spectra; $R^2 = .98$. Digestibility of fescue = $24.52 + 16.3\% K - 551\% P + 27.2\% Ca$; $R^2 = .91$.

In Vitro Forage Fiber Digestion Rates - L. W. Smith, U.S. Department of Agriculture, Animal Science Research Division, ARS, Beltsville.

Linearity of semilog plots of remaining digestible cell walls on time and their high r^2 of .978 indicated first-order digestion kinetics for each of 112 forages

differing widely in maturity, composition and rates of fiber digestion. Forage samples of total dry matter or isolated cell walls, approximately half gram, were incubated with ruminal fluid for 3, 6, 12, 18, 24, 30, 36, 48, and 72 hours or less and was, thus, considered complete at 72 hours as was reported earlier. Rates of cell wall digestion were regressions of the ln percentages potentially digestible remaining on hours of fermentation.

Cell wall digestion rates generally decreased with increasing forage maturity. Several chemical constituents were highly correlated with cell wall digestion rates within particular forage species. However, soluble dry matter was the only parameter which remained highly correlated with cell wall digestion rate when all samples of the fifteen species were considered in a single analysis. Even though soluble dry matter was highly correlated and shown to be the most suitable general predictor of cell digestion rates, soluble dry matter was shown not to directly contribute to the faster cell wall digestion rates.

Only 52% of the variation in cell wall digestion rates can be attributed to change in soluble dry matter content of forages. Thus, changes in fiber with forage maturation that are not detectable by chemical analyses used in these studies may be partially limiting the rate of cell wall digestion.

Nutrient Cycling in Grazed Pasture - Philip F. May, CSIRO Pastoral Research Laboratory, Armidale 2350 NSW Australia, Postdoctoral Program, Biomathematics Department, NCAU, Raleigh, North Carolina.

The use of radio-isotopes to study quantitative aspects of the utilization and recycling of sulphur in pastures grazed by sheep is described. The research employed a conceptual model of the sulphur cycle, reduced to minimum dimensions necessary to satisfactorily reproduce the observed behavior of the integrated grazed system.

Investigations of the major features of the cycling system were made more objective by the relatively stringent requirements of quantitative modelling.

Employment of criteria of goodness of fit of the model provided a basis for estimated of intercompartment transport rates in a simplified recycling network, and the interpretation of the effects of imposed treatments in terms of modifications to kinetics.

The long term multidisciplinary nature of the research was emphasised.

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NUTRITION PANEL

The Effect of Dietary Protein Level and Type of Dietary Protein Source on Plasma and Tissue Free Amino Acids in Growing Sheep - W. G. Bergen, Department of Animal Husbandry, Michigan State University, East Lansing, Michigan.

When ruminants are fed rations containing high levels of non-protein nitrogen (NPN) growth is often depressed and plasma levels of leucine, valine and isoleucine decline while plasma levels of non-essential amino acids (NEAA) tend to increase. This shift in plasma amino acid (PAA) patterns is similar to PAA shifts observed in growing non-ruminants when fed a diet containing inadequate levels of protein but adequate levels of calories. In the work, described below the hypothesis was tested that PAA shifts noted for various rations when fed to ruminants are due to the amount of protein reaching the lower gut (either microbial or bypassed protein of dietary origin). Sheep were fed for 60 days rations containing (air dry basis) 5%, 10%, 15%, and 15% crude protein respectively containing soy protein, zein and corn gluten, urea, and fish meal as supplemental proteins respectively. Sheep receiving the 5% basal ration gained no weight while the other 4 groups gained on the average 200 gm daily. There were no difference between dietary treatments in muscle free amino acid content in the gastrocnemius muscle at the end of the feeding trial. Liver free amino acid content was markedly lower in sheep fed the 5% CP ration; however, there were no differences between the other 4 dietary treatments. PAA analysis revealed that the 5% CP group had lower leu, ile and val levels than the other 4 groups. Branched chain amino acid levels were lower in the urea ration group than in the soy, zein and corn gluten and fish meal rations groups. Plasma lys was lower and leu was higher in sheep fed the zein and corn gluten ration. These results indicate that: 1. When a poorly balanced protein which is not readily degraded in the rumen is fed to ruminants, PAA shifts will reflect the amino acid pattern of such proteins. 2. When inadequate protein reaches the lower gut, plasma branched chain amino acids levels will decline and this PAA shift is dependent on total available amino acids for tissue metabolism. Thus all additions to NPN rations that stimulate ruminal microbial cell synthesis would be expected to cause a rise in branched chain amino acid levels in the plasma.

Kinetic Simulation Model of Ruminant Nitrogen Metabolism - I. D. Hume and R. L. Baldwin, University of California, Davis.

Computer - simulation techniques are being used to summarize and integrate information currently available on many different aspects of ruminant nitrogen metabolism into a single, unified model, with the object of making better use of available data in the planning, conduct and interpretation of future research in this area. To date, the model contains a total of 73 reactions describing metabolic transformations of nitrogen in the rumen and the remainder of the digestive tract, and in the liver and extra-hepatic tissues. The reference animal was a 40 kg adult wether fed 100 g casein and 850 g carbohydrate once daily. Initial metabolite pool sizes and initial fluxes for each

reaction were written for the pre-feeding state. Under these conditions 115 g microbial protein were produced in the rumen/day, and the nitrogen balance of the reference animal was 1.96 g/day.

In order to challenge the model, the reference animal was fed the same daily ration in equal portions at 2-hour intervals. By the end of 3 simulated days most metabolite pools approached a steady state, but protein production (100 g/day) and nitrogen retention (1.41 g/day) were reduced. When the reference animal was fed 100 g of the relatively insoluble protein zein and 850 g carbohydrate once daily, the rumen ammonia pool fell sharply, and only 47 g microbial protein were produced/day. However, the portal amino acid pool fell only slightly, due to the digestion of the zein which passed out of the rumen undegraded, and nitrogen balance remained positive (3.85 g/day). When 50% of the zein nitrogen was replaced with urea the rumen ammonia pool remained at a level similar to that obtaining when casein was fed, and protein production increased to 96 g/day. Nitrogen retention, however, was similar (3.73 g/day).

It is concluded that, in most respects, the response of the kinetic simulation model to several different dietary inputs was realistic, even though the model is still in the early stages of development and in a relatively simple form. Future efforts will be directed toward representation of specific aspects of the model in greater detail. This should result in better regulation, and improve the predictive power of this whole animal model.

Further Studies on Adaptation and Deadaptation to Biuret - R. R. Johnson and E. T. Clemens. Department of Animal Sciences and Industry, Oklahoma State University, Stillwater.

The adaptation of ruminants to biuret as a source of non-protein nitrogen has previously been studied primarily by the means of classical digestion and nitrogen metabolism trials. The results from most of these trials were equivocal and suggested that more precise methodology would be needed to describe the adaptation to and utilization of biuret. The adaptation and loss of adaptation to biuret was studied in both sheep and cattle consuming high roughage rations. The ability to metabolize biuret was measured by an in vitro biuretolytic test in which whole rumen contents were incubated in vitro with biuret solutions. Biuretolytic activity was followed by both disappearance of biuret and the appearance of ammonia nitrogen. In the first trial, biuret was compared to cottonseed meal and cottonseed meal-urea mixtures as nitrogen supplements to a poor quality bermudagrass hay ration for sheep. Significant biuretolytic activity was not apparent until 42 days of supplementary feeding. Maximum biuretolytic activity was not observed until some time between 60 and 80 days on supplement. In trial 2, biuret was used as a supplementary source of nitrogen for a poor quality hay ration fed to three fistulated steers. Significant biuretolytic activity was apparent by 30 days on supplement. When the biuret was removed from the ration, the ability to hydrolyze biuret in vitro was lost within two days after removal. In trial 3, biuret was used as a nitrogen supplement in 60% concentrate rations. With the high concentrate ration, biuretolytic activity was already high after 10 days of supplementation and was maximum by 21 days on feed. Again, when biuret was removed from the ration, the biuretolytic activity was lost within two days after removal.

Effect of 2-Methylbutyrate and Phenylacetate on Acetate Production in the Rumen - R. M. Cook and J. T. Huber, Department of Dairy Science, Michigan State University, East Lansing, Michigan.

It is established that certain VFA and phenylacetic acid stimulate the growth of some rumen cellulolytic bacteria in pure culture. These chemicals may increase the rumen fermentation rate in vivo when urea is the major source of supplemental crude protein. In the first experiment lactating cows fitted with rumen fistulas were fed a basal ration of corn-silage and corn grain supplemented with: 1) SBOM; 2) Urea; 3) Urea-2-methylbutyrate; or 4) Urea-Phenylacetate. The design was a 4 x 4 Latin square using 4 lactating Holstein cows with 4 week periods. Phenylacetate, 2-methylbutyrate and urea were added directly to the rumen twice daily. The last day of each treatment period rumen acetate production was measured using an isotope dilution technique and polyethylene glycol to determine total rumen fluid volume.

In a second experiment a basal ration of corn silage was supplemented with: 1) nothing; 2) soybean meal; 3) urea; 4) urea + phenylacetate and 2-methylbutyrate; and 5) phenylacetate plus 2-methylbutyrate was fed. The design was a 5 x 5 Latin square using 5 Jersey heifers, for 2 week periods. On the last day of each treatment period, acetate production was measured. In the first experiment rumen volume ranged from 70 to 127 liters. Acetate production rates ranged from 32 to 68 moles/day/rumen. The mean acetate production rates for the 1 to 4 treatments were 47.2, 49.6, 52.4, and 37.5, respectively.

In the second experiment acetate production rates, expressed in moles/liter of rumen fluid/12 hrs/lb silage, varied from 3.0 to 14.6. The mean acetate production rates for the respective treatments (1 through 5) were: 5.5, 6.8, 4.8, 4.7, and 5.4.

For both experiments there was considerable variation in rumen acetate production rates within treatments and between treatments. The study did not show a consistent effect of phenylacetate or 2-methylbutyrate on rumen acetate production.

Illustration of an Automatic Feeding Device for Studying Steady State Functions in the Rumen - R. R. Johnson, Department of Animal Sciences and Industry, Oklahoma State University Stillwater.

In order to properly interpret measurements made on kinetic systems in the rumen it is necessary to have a steady state condition. To produce this, an automatic feeder was used which could be set to feed the animals at short intervals throughout the day. The feeder consisted of a small commercial vibratory feeder (Model No. FBV-212, Eriez Magnetics, Asbury Road at Airport, Erie, Pennsylvania) actuated by a programmed signal timer (Model 5701, Tork Time Controls, Inc., Mount Vernon, New York). In our studies with high concentrate rations, beef steers have been fed hourly and were found to train quickly to such a feeding regime. This system not only allowed for steady state calculations during infusion studies but we also found that steers consumed more daily ration and stayed on feed better when fed high concentrate rations by this method.

Effect of Level of Feed Intake on Rate of Passage of Digesta through the Intestines of Sheep - W. L. Grovum and G. D. Phillips, University of Manitoba, Winnipeg, Manitoba, Canada.

Gradients of digesta retention times exist along the small and large intestines of the sheep. In the small intestine, the digesta was retained from 10 to 17 times longer in the terminal ileum than in the duodenum. In the large intestine, the rate of passage was slowest in the cecum-proximal colon and fastest in the spiral colon.

The retention time of digesta in the large intestine was decreased by increasing the level of alfalfa intake however that for the small intestine was not significantly affected.

A Kinetic Analysis of Digesta Movement through the Alimentary Tract of the Sheep - W. L. Grovum and G. D. Phillips, University of Manitoba, Winnipeg, Manitoba, Canada.

The digestive tract of the sheep does behave as a kinetic system with regard to the passage of radioactive markers. The half times of markers in the abomasum were substantially smaller than those for the rumeno-teticulum or the cecum-proximal colon. The entire concentration curve of marker excretion in sheep resulting from a single injection of marker into the rumen was predicted using one equation and five parameters derived from the concentration data. The three parameters relating to marker passage through the rumeno-reticulum, intestines and cecum-proximal colon should be of use in future studies of the rates of passage of digesta in sheep.