UREASE IMMUNITY IN RUMINANT ANIMALS

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CHAPTER I

INTRODUCTION

Protein is the most limiting nutrient for man and domestic animals. Protein requirements of non-ruminants are met by the inclusion of preformed proteins or amino acids in their diets. However, ruminants have microflora in the rumen which can convert non-protein nitrogen (NPN) into microbial protein which is utilized. Preformed proteins are expensive and are in great demand for the feeding of non-ruminants. There is also a great need to preserve proteins for the ever-increasing human population. Decreased supplies of proteins have drawn the attention of research workers to the use of NPN sources for ruminants. Urea is the most widely used NPN source for ruminants. Its usage in ruminant rations is increasing and it is projected that in the United States over 210 thousand metric tons of feed grade urea will be used for this purpose by 1970.

Urea is hydrolyzed by ruminal urease into ammonia and carbon dioxide. Ammonia can be used in the synthesis of microbial protein. One limiting factor in urea utilization concerns the fact that it is usually hydrolyzed so rapidly that much ammonia is absorbed into the body and thus, escapes incorporation into microbial protein. Induction of jackbean urease immunity in ruminants and non-ruminants has been shown to decrease the release of ammonia from urea and result in

increased rate and efficiency of growth in animals. The present study was designed to investigate the effect and mode of action of jackbean urease immunity on the performance of ruminant animals.

CHAPTER II

REVIEW OF LITERATURE

Urease

Urease (urea amidohydrolase, EC 3.5.1.5) which catalyzes the hydrolysis of urea to ammonia and carbon dioxide was isolated from jack-bean (Canavalia ensiformis L.) by Sumner (1926). Sumner and Somers (1953) reported that it occurs in over 200 species of bacteria, in several species of yeast, in fungi and in large numbers of higher plants. Its presence was observed in blue-green algae (Berns et al., 1966) and in some species of protozoa (Hunter, 1957, 1959).

Sumner (1926) described urease as a crystallizable globulin protein. It was isoelectric at pH 5.0 to 5.1 (Sumner and Hand, 1929; Wills, 1952; Creeth and Nichol, 1960). It could polymerize and the polymers could dissociate into enzymatically active subunits (Creeth and Nichol, 1960; Gorin et al., 1962; Sehgal, 1964; Gorin and Chin, 1967). Creeth and Nichol (1960) and Gorin et al. (1962) reported that polymers of urease were formed by intermolecular disulfide cross linkages. The 19 S protein was found to be the main and most stable component of urease (Sumner et al., 1938; Sehgal, 1964). The molecular weight of urease was found to be 483,000 (Sumner et al., 1938). Reithal et al. (1964) reported that the molecular weight of a structural subunit of urease was 83,000, whereas Hand (1939) stated that a dissociated particle of molecular weight 17,000 could still be enzymatically active.

Gorin and Chin (1967) observed that urease molecular weight 480,000 could dissociate into two subunits in 0.1 M acetate buffer, pH 3.5. Each subunit was a 9.8 S protein with a molecular weight of 240,000.

Urease was considered absolutely specific for urea (Sumner, 1951), however, Fishbein et al. (1965) and Fishbein (1967) reported that urease catalyzed hydrolysis of hydroxyurea and dihydroxyurea to yield ammonia, carbon dioxide and hydroxylamine.

Wall and Laidler (1953) studied the kinetics of Jackbean urease catalyzed hydrolysis of urea in trishydroxymethylaminomethane sulfate (tris) buffer at 21° C. They found that the optimum pH was 8.0, and the K_m at this pH was 4.0×10^{-3} M.

In urease catalyzed hydrolysis of urea the formation of ammonium carbamate was observed by many research workers (Sumner, 1951). Gorin (1959) found that carbamate was directly formed from urea and was an intermediate in the enzymatic hydrolysis of urea to ammonia and carbon dioxide.

Ruminal Urease

Urease activity is found in rumen mucosa (Luck, 1924) and rumen contents (Pearson and Smith, 1943). Urease activity of rumen contents is attributed to ruminal bacteria (Jones et al., 1964a). As about 60 percent of urease activity present in rumen mucosa was removed by washing, and kinetic properties of urease preparations made from ruminal bacteria or rumen mucosa were not different, Rahman and Decker (1966) concluded that urease in rumen mucosa originated from bacteria. Inhibition of urease activity of rumen mucosa by the use of antibacterial agents (Houpt and Houpt, 1968) further supported this conclusion. Houpt

and Houpt (1968) postulated that some ruminal urease penetrates the rumen mucosa.

Jones et al. (1964b) observed that ruminal urease prepared from mixed rumen microflora was stimulated by Mn^{++}, Mg^{++}, Ca^{++}, Sr^{++} and Ba^{++}, and inhibited by Na^{+}, K^{+} and Co^{++}. However, Jones (1965) reported that the urease produced from an isolated rumen bacteria showed inhibition with Mn^{++}, stimulation with Mg^{++} and no effect with Ca^{++}. On the basis of varying effects of Mn^{++}, Mg^{++} and Ca^{++} on urease activity of the rumen isolate and the uniform stimulatory effects of these ions on urease activity of mixed rumen microorganisms, Jones (1965) suggested that the bacterial species responsible for ruminal urease activity may differ in behavior to divalent cations.

Rahman and Decker (1966) studied the kinetics of ruminal urease catalyzed hydrolysis of urea in tris buffer at 20^{o} C. They found that the optimum pH was 8.5, and the K_m at this pH was 1.5×10^{-3} M.

Abou Akkada and Howard (1962) demonstrated that rumen protozoa, Entodinium caudatum, possessed no urease activity. Jones et al. (1964a) observed that about 1.8 percent of rumen urease activity was associated with the protozoal fraction; however, they suggested a possible contamination of their suspensions of protozoa by ureolytic bacteria.

Gibbons and Doetsch (1959) isolated from bovine rumen fluid an obligately anaerobic, gram-positive rod, which is related to Lactobacillus bifidus. Carroll (1960) isolated two urease strains of Lactobacillus bifidus in numbers of 10^{7} per ml. from a steer rumen. Isolation of a urease positive strain of Aerobacter aerogenes from sheep was reported by Sosnovskaja (1959). Urease activity in other isolates from the rumen was reported in facultative anaerobic gram-positive

micrococci (Mann et al., 1954), coliform organisms (Mackay and Oxford, 1954), gram-negative rod type organisms, presumably proteus spp. and some micrococci (Appleby, 1955), actinomycetes (Carroll, 1960), strains of butyrivibrio gram-negative motile rods (Abou Akkada and Blackburn, 1963), gram-positive cocci (Blackburn and Hobson, 1962; Abou Akkada and Blackburn, 1963), and a facultative gram-negative coccus (Jones et al., 1964a).

Jones (1967) stated that some of the urease positive rumen isolates, described by many workers, are not true rumen bacteria because they enter the rumen only as "passengers" from feed and soil. Among these passengers he considered were the proteus strains of Appleby (1955), actinomycetes of Carroll (1960), and coliforms of Mackay and Oxford (1954). He believes that the passenger bacteria could play some part in metabolism in the rumen.

None of the washed cell suspensions of anaerobic ureolytic rumen isolates was active enough to account for the total urease activity observed in rumen fluid. Thus, it seems logical to conclude that either the present cultural methods are not adequate to isolate all urease producing organisms (Carroll, 1960) or low levels of urease must be present in many bacterial species rather than a few active urea hydrolysers (Carroll, 1960; Hungate, 1963).

Urea Metabolism in the Ruminant

Hydrolysis in the rumen of both exogenous and endogenous urea to ammonia and carbon dioxide appears to be the first step in its utilization. Ammonia concentration in the rumen represents a balance between utilization by rumen bacteria, metabolism in the rumen wall, absorption

into the portal vein and passage to the omasum. Ruminal ammonia concentration varies from 0-130 mg./100 ml. (Johns, 1955).

Ammonia Utilization by Rumen Microorganisms

Utilization of ammonia for synthesis of bacterial proteins depends primarily on the capacity of rumen bacteria to metabolize it. Ammonia is an essential nutrient for the growth of Bacteriodes succinogenes, Ruminococcus flavefaciens, Ruminococcus albus, Bacteriodes amylophilus, Methanobacterium ruminantium, and Eubacterium ruminantium (Bryant, 1963; Hungate, 1966). Addition of nitrogen sources yielding ammonia stimulated in vitro digestion of cellulose (Belasco, 1954; Chalupa et al., 1963; Little et al., 1963) and starch (Acord et al., 1966; 1968). Brüggemann et al. (1962) reported that both cellulolytic and amylolytic activities in vitro of mixed rumen microorganisms were increased when urea replaced soybean meal as a crude protein supplement for cattle. Thus, it appears that ammonia is important in the nutrition of both cellulolytic and amylolytic rumen bacteria. Additional evidence was provided by el-Shazly et al. (1961), who found that inhibition of cellulose digestion by dietary starch was primarily due to a competition for nitrogen between cellulolytic and amylolytic bacteria. This condition may be alleviated by urea supplementation. The effect of dietary urea on rumen microorganisms was studied by Gall et al. (1951) and Briggs et al. (1964). The former workers reported an increase in total counts of bacteria when casein of a semi-purified diet was replaced by urea. Briggs et al. (1964) reported that when urea replaced soybean meal, it increased counts/ml. of entodinia, flagellates, total bacteria, amylolytic bacteria and cellulolytic bacteria.

Synthesis of amino acids from ammonia by rumen microorganisms requires the use of ammonia, "carbon skeletons" and energy. Utilization of carbon from carbohydrates (McNaught, 1951; Hoover et al., 1963) CO_2 (Huhtanen et al., 1954; Otagaki et al., 1955), isovaleric acid (Allison et al., 1959), acetate and other volatile fatty acids (Hoover et al., 1963) indicates that carbon from a wide variety of sources could be used for synthesis of amino acids. However, synthesis of leucine from isovalerate (Allison et al., 1966), isoleucine from 2-methyl butyrate (Hungate, 1966), valine from isobutyrate (Allison and Bryant, 1963), phenylalanine from phenylacetate (Allison, 1965) and tryptophan from indole-3-acetate (Allison and Robinson, 1967) reveals the requirement of certain specific carbon skeletons for synthesis of certain amino acids.

Energy for amino acid synthesis is provided by dietary carbohydrates or other organic compounds. Hogan and Weston (1967) calculated that approximately 15 gm. microbial protein was synthesized for each 100 gm. of organic matter utilized in the rumen. This value roughly agrees with that determined by Bloomfield et al. (1964) and one calculated by Walker (1965). Hungate (1966) estimated that about 1.1 gm. of microbial nitrogen is assimilated for each 100 gm. of carbohydrate fermented.

Details of biochemical steps involved in amino acid synthesis by rumen bacteria remain obscure but some discussion seems warranted. Presence of nicotinamide-adenine dinucleotide and nicotinamide-adenine dinucleotide phosphate-linked glutamic acid dehydrogenases in rumen microorganisms (Burchall et al., 1964; Palmquist and Baldwin, 1966; Hoshino et al., 1966) indicates possible involvement of amination reaction in the fixation of ammonia. It is possible that some amino

acids may be synthesized in the rumen by transamination reactions. Alpha keto acids required for transamination reactions were detected in rumen liquor of cattle after feeding them hay, grasses or glucose (van der Horst, 1961). Synthesis of glutamine by rumen microorganisms was also observed by Hoshino et al. (1966).

Ammonia Anabolism in Rumen Mucosa

McLaren et al. (1961, 1962) found that the utilization of ammonia for synthesis of L-glutamate was the result of reductive amination in the rumen mucosa. Demonstration of nicotinamide-adenine dinucleotide-linked glutamic acid dehydrogenase activity in rumen mucosa by Hoshino et al. (1966) adds support to this idea. Hoshino et al. (1966) also observed the ability of rumen mucosa to synthesize and hydrolyze glutamine. They postulated that glutamine serves as a storage form of ammonia in rumen mucosa.

Ammonia Absorption and Its Conversion to Urea

Absorption of ammonia from the rumen, reported by McDonald (1948), is influenced both by concentration gradient (Lewis et al., 1957; Hogan, 1961) and pH (Bouckaert and Oyaert, 1952; Hogan, 1961; Bloomfield et al., 1963). Ammonia is a weak base with a pK_a from 8.80 to 9.15 (Bromberg et al., 1960; Bloomfield et al., 1963; Visek, 1968). An increase in pH causes the NH_4^+ ion to be converted to NH_3, which is rapidly absorbed (Coombe et al., 1960; Hogan, 1961; Bloomfield et al., 1963).

Absorbed ammonia is carried via portal circulation to the liver, where it is converted to urea. In general, only small quantities of ammonia are found in the peripheral blood. Lewis et al. (1957) reported that the liver was able to convert all absorbed ammonia into urea until

the concentration of ammonia in portal blood reached a level of 0.8 mM which was observed with rumen fluid concentration of 55 to 60 mM.

Urea Recycling

Endogenous urea enters the rumen via saliva and by diffusion across the rumen wall (McDonald, 1948; Houpt, 1959; Somers, 1961a,b,c,d; Packett and Groves, 1965; Juhász, 1965; Cocimano and Leng, 1967; Houpt and Houpt, 1968). Diffusion through rumen epithelium seems to be the main route of entry (Houpt, 1959; Moir and Harris, 1962; Juhász, 1965) but blood urea is also diffused into the abomasum and the intestines (LeBars, 1967; Cocimano and Leng, 1967).

Houpt and Houpt (1968) suggested that urea which diffuses into rumen fluid from the blood was almost completely hydrolyzed before it entered the rumen fluid. A possible explanation concerns the penetration of ruminal urease into rumen epithelial layers and the possibility that ammonia produced here diffused more rapidly than urea through the remaining layers of rumen epithelium; the net result of this effect could greatly increase transfer of urea nitrogen as ammonia into rumen fluid. In accord with this postulation, transfer of urea nitrogen across the rumen wall would also depend on the concentration gradient of ammonia.

Somers (1961a,b,c) reported that urea represented 60 to 70 percent of the total nitrogen in sheep saliva and that there is a positive relationship between level of nitrogen intake and amount of urea secreted in saliva. Salivary glands and kidneys play important roles in the conservation of nitrogen by ruminants. At the time of great need for nitrogen, secretion of urea in saliva is increased (Somers, 1961d) and excretion of urea in urine is decreased (Schmidt-Nielsen et al., 1957;

Schmidt-Nielsen and Osaki, 1958). Schmidt-Nielsen et al. (1957) reported that in the camel, on normal nitrogen intake, 40 percent of the urea filtered by the glomeruli was excreted in urine, but this value fell to 1 to 2 percent when a nitrogen-deficient ration was fed. A similar phenomenon was observed in sheep (Schmidt-Nielsen and Osaki, 1958).

Recycled urea is hydrolyzed by ruminal urease to ammonia, which may be used to maintain an active microbial population (Moir and Harris, 1962). Houpt (1959) calculated that 52 percent of the injected urea was not recovered in urine and body fluids and thus presumably was utilized in the synthesis of bacterial proteins. Dietary carbohydrates improved the utilization of recycled urea (Houpt, 1959; Packett and Groves, 1965).

Urealysis Inhibition in the Rumen

Bloomfield et al. (1960) reported that rate of urea hydrolysis was four times greater than the corresponding uptake of ammonia by rumen microorganisms suggesting that it might be possible to improve urea utilization by decreasing the rumen urease activity.

Prescott (1953) reported that addition of aureomycin, terramycin, bacitracin or penicillin reduced in vitro hydrolysis and utilization of urea by rumen microorganisms. Cahill and McAleese (1964) found that inclusion of auredmycin in rations containing urea improved performance and nitrogen retention in fattening lambs. Merino and Raun (1964) observed no effect of chlortetracycline on ruminal urease activity in sheep.

Gibbons and Doetsch (1959) stated that inclusion of a small amount of urea in the medium for a urease-positive strain reduced the production of urease. Dietary urea reduced ruminal urease activity (Merino and Raun, 1964; Caffrey et al., 1967). The reduced rumen urease activity observed with added dietary urea was associated with a decreased liveweight gains and a tendency towards lowered feed efficiency (Merino and Raun, 1964); however, Caffrey et al. (1967) observed that in vitro rate of ammonia assimilation by rumen microorganisms was high when bacteria inoculum was taken from urea-fed lambs.

Barbituric acid (malonylurea) inhibited in vitro cellulose digestion and when fed to sheep it decreased gain and feed efficiency (Harbers et al., 1962). Clifford et al. (1968) observed that inclusion of barbituric acid in high roughage rations increased fecal nitrogen losses and thereby reduced nitrogen retention in lambs. They also found that neither copper nor nitrates, when employed as urease inhibitors in urea-containing diets, improved performance of ruminants. Neither inhibitor reduced ruminal urease activity as measured by these workers.

Brent and Adepoju (1967) initiated studies on the use of acetohydroxamic acid (AHA) as a rumen urease inhibitor. They found that AHA inhibited rumen urease in vitro as well as in vivo. Administration of 3 gm. AHA per feeding (6 gm./day) lowered rumen fluid ammonia levels. Streeter et al. (1968) reported that administration of AHA to wethers lowered ammonia concentration of rumen fluid, increased digestibility of dry matter and improved retention of nitrogen. However, Moore et al. (1968) observed no increase in nitrogen retention of AHA administered steers.

Jackbean Urease Immunity

Parenterally administered crystalline jackbean urease induced the production of a urease antibody (antiurease) in rabbits, chicks, rats, mice, humans, guinea pigs, swine, cattle and sheep (Kirk and Sumner, 1931; Howell, 1932; Dang and Visek, 1960, 1964; Thomson and Visek, 1963; Kornegay et al., 1964; Harbers et al., 1965; Glimp and Tillman, 1965). Irradiated urease lacked the ability to produce antiurease (Pillemer et al., 1938). Active and passive immunization of animals against urease provided protection to lethal doses of urease (Kirk and Sumner, 1931; Howell, 1932; Dang and Visek, 1966, 1968) and lethal levels of gamma irradiation (Visek and Dang, 1966). Human patients with liver insufficiency showed increased tolerance to dietary proteins following urease immunization (Thomson and Visek, 1963).

Kirk and Sumner (1934) observed that urease precipitated by anti-urease retained up to 70 percent of its original activity. The results of Marucci and Mayer (1955) and Visek et al. (1967) supported this idea and indicated that antigen and catalytic sites of urease were not identical. They stated that enzymic inhibition by antiserum was due to steric hindrance. Visek et al. (1967) reported that maximum total inhibition approached 70 percent when antigen-antibody ratio was slightly less than 0.6. Marucci and Mayer (1955) observed that although urease-antiurease system fixed complement, neither destruction nor addition of complement affected inhibitory effectiveness of rabbit antiserum.

Urease immunity increased rate and efficiency of growth in rats and chicks (Dang and Visek, 1960). This improved performance was accompanied by low urease activity of gastrointestinal contents of

immunized animals. Wagner et al. (1963), however, failed to observe any effect of urease injections on the performance of chicks and rats. Their findings are open to criticism since their animals were injected with commercial preparation of urease and no efforts were made to determine the presence of antiurease titers.

Kornegay et al. (1964) reported that urease immunity decreased urease activity in the intestines of immunized swine, but the gains were improved only in one out of three trials. Glimp and Tillman (1964) observed that urease injections showed no effect on the performance of swine. Unfortunately, cases of atrophic rhinitis were found in their animals and the controls also were positive for antiurease. They did not exclude the possible relationship between antiurease titers and atrophic rhinitis.

Harbers et al. (1965) indicated that the change in growth rate of urease immunized calves was related to the level of circulating antibodies. Glimp and Tillman (1965) observed that urease immunity increased rate and efficiency of growth in lambs.

CHAPTER III

GENERAL MATERIALS AND METHODS

Urease and Antiurease Assay Procedures

Urease

Thrice crystalline urease from jackbean meal was prepared by the procedure of Mamiya and Gorin (1965) and assayed for urease at 25° C. by the alkalimetric method of Gorin and Chin (1966). The enzymic activity was expressed as an International Union of Biochemistry (IUB) unit.[1]

Antiurease

Procedures used to assay antiurease were enzyme inhibition technique, hemagglutination technique, precipitin ring test and gel immunodiffusion technique. A brief description of each is given.

Enzyme Inhibition Technique. Two tenths ml. of the sample was incubated at 37° C. for 20 minutes with 1 ml. of standard urease in 0.02 M phosphate buffer (pH 7.0). The blank consisted of 0.2 ml. of sample and 1 ml. of 0.02 M phosphate buffer. The enzymic activity after incubation with sample was determined at 25° C. by the alkalimetric method of Gorin and Chin (1966). The loss of urease after incubation was taken to represent antiurease activity of the sample; however, for

[1] International Union of Biochemistry (IUB) unit is defined as the amount of enzyme which catalyzes the hydrolysis of 0.5 u mole of urea and liberates 1 u mole of ammonia in one minute.

the purpose of this study it is reported as units of urease recovered after incubation of standard amount of urease with the sample.

Hemagglutination Technique. Sheep red blood cells (RBC) were prepared according to Stoffer (1960). They were tanned, sensitized, and suspended in normal rabbit serum (NRS) as described by Kabat and Mayers (1967). Each sample tested for antiurease was incubated at 56° C. for 30 minutes to destroy any complement present. The heat-inactivated sample was thoroughly mixed with an equal volume of washed packed sheep RBC and incubated at room temperature for 10 minutes to remove the heterophile antibodies. The controls included were NRS plus tanned RBC, NRS plus sensitized RBC, undiluted antiserum plus tanned RBC, and undiluted antiserum plus sensitized RBC. Hemagglutination titers were recorded after incubation at room temperature for 12 hours.

Precipitin Ring Test. This test was performed in small bore (5 mm. internal diameter) serological tubes according to the procedure described by Burrell and Mascoli (1966). Two controls consisting of undiluted serum plus urease, and undiluted antiserum plus urease were incorporated in each test. The concentration of urease suitable for detecting antiurease was 90 IUB units of urease per milliliter. Therefore, urease of this concentration was used to determine antiurease titers of samples diluted by double dilutions.

Gel Immunodiffusion Technique. The technique for gel immunodiffusion carried on slides was adopted from Gelman procedures[2] (1968). Urease, 90 IUB units per milliliter was used to detect antiurease in undiluted samples.

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[2]Gelman Procedures, Techniques, and Apparatus for Electrophoresis. Gelman Instrument Co. (Ann Arbor, Michigan, 1968) p. 55.

Experiment 1

The objectives of Experiment 1 were to determine the lowest level of injected urease promoting growth response and to explore the mode of action of urease immunity on growth. Response criteria included the effect of urease injections on antiurease production, growth performance, digestibility, and plasma urea and ammonia levels in lambs.

The composition of the diet used in all trials of this experiment is shown in Table I.

Trial 1-1

A 92-day growth trial was conducted to determine the effect of urease injections on antiurease production and to find the lowest level of urease which promotes the maximum growth response. Thirty-five lambs which were about 90 days of age were assigned at random to seven treatments involving zero, 1650, 3300, 4950, 6600, 8250 and 9900 total IUB units of urease. The enzyme was injected subcutaneously using the schedule shown in Table II. Lambs were housed in individual and elevated wooden pens having wooden slatted floors. Feed and water were provided ad libitum. Residual feed was weighed and discarded at weekly intervals. Body weights were recorded initially and at the 14-day intervals. All lambs were bled via a jugular puncture on the 49th day (14 days after last urease injection) and 92nd day (last day of trial). Serum antiurease assays were made by the enzyme inhibition technique.

Trial 1-2

A metabolism trial was conducted to determine effects of urease injections on digestion of dry matter, organic matter and nitrogen.

TABLE I

COMPOSITION OF THE DIET IN EXPERIMENT 1

Ingredient	%
Glucose[a]	25.84
Starch (corn)	25.84
Cellulose[b]	15.00
Alfalfa meal	10.00
Grain sorghum (ground)	10.00
Urea[c]	4.20
Mineral mix[d]	4.00
Corn oil[e]	3.00
Polyethylene resin[f]	2.00
Choline chloride	0.10
Vitamin A and D mix[g]	0.02
Total	100.00

[a]Cerelose. Corn Products Co., Argo, Illinois.

[b]Solka-Floc. B-W20. Brown Co., Berlin, New Hampshire.

[c]Crystalline urea, courtesy of John Deere Chemical Co., Pryor, Oklahoma.

[d]Same as in Oklahoma Purified Diet; Clifford et al., 1967. J. Animal Sci. 26:400.

[e]Mazola. Santoquin was added to give 0.0125% in total diet.

[f]Alathon. E. I. DuPont de Nemours, Inc., Wilmington, Delaware.

[g]Quadrex, containing 20,000 and 2,500 U.S.P. units/gm. of vitamin A and D, respectively. Product obtained from NOPCO Chemical Co., Harrison, New Jersey.

TABLE II

SCHEDULE OF UREASE INJECTIONS IN TRIAL 1-1

Injection No.	Day	Urease level[a], IUB units					
		1650[b]	3300	4950	6600	8250	9900
1	0	110[c]	220	330	440	550	660
2	7	220	440	660	880	1100	1320
3	14	330	660	990	1320	1650	1980
4	21	440	880	1320	1760	2200	2640
5	35	550	1100	1650	2200	2750	3300

[a]Injected subcutaneously.

[b]Each control lamb was injected with 5 ml. saline.

[c]Urease was added to physiological saline and assayed prior to each injection. Each lamb received 5 ml.

Upon completion of Trial 1-1, lambs which had received zero, 4950, 6600 and 8250 units urease were given a "booster" injection of zero, 2000, 2500 and 3000 units of urease, respectively, and were transferred to metabolism stalls. The sampling and preparation of feces for analysis were as described by Tillman and Swift (1953). On the 14th day of the 28-day collection period blood samples were taken from all lambs for serum antiurease assays determined by the enzyme inhibition technique. Chemical analyses on feed and feces were determined by methods of the A.O.A.C. (1960).

Trial 1-3

Three control and three urease-injected (6600 units) lambs from Trial 1-2 were randomly selected to determine effects of urease injections on plasma urea and ammonia-nitrogen levels. Two weeks before this trial the three control lambs were injected with saline and the remaining three received booster injections of 2700 IUB units of urease. Feed was withheld for 16 hours before the animals were anesthetized with sodium pentobarbital.[3] The jugular and ruminal veins were cannulated according to the procedure of Glimp and Tillman (1965). A solution containing 10 gm. of urea and 50 gm. of dextrose in 100 ml. of distilled water was infused directly into the rumen. Blood samples were taken immediately before and at hourly intervals for six hours afterwards. Urea and ammonia levels in plasma were determined by procedures described by Conway (1962).

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[3]Diabutal obtained from Diamond Laboratories, Inc., Des Moines, Iowa.

Experiment 2

The objectives of Experiment 2 were to determine effects of urease immunity on general performance, urease activity of gastrointestinal fluids, and antiurease activity in bile of lambs.

The composition of the diet used in all trials of this experiment was the same as in Experiment 1.

Trial 2-1

As no antiurease activity was found in urease-injected lambs on the 92nd day of Trial 1-1, a second growth trial was conducted in which a booster injection of urease was made on the 56th day of the trial. Twenty lambs averaging 120 days of age were randomly placed on two treatments involving zero and 6600 total IUB units of urease. The schedule of urease injections is given in Table III and other details were as described in Trial 1-1. Blood samples were taken on the 35th, 70th, and 92nd days of the trial to assay for serum antiurease activity by the enzyme inhibition technique.

Trial 2-2

Six control and six urease-injected lambs from Trial 2-1 were randomly selected to determine the effect of urease injections on urease activity of gastrointestinal fluids. Two weeks prior to sacrificing, the control and urease-injected lambs were given a booster injection of zero and 3000 IUB units of urease, respectively. All lambs were sacrificed and contents of the rumen, mid-ileum and proximal colon were sampled. The liquid samples from the rumen were strained through four layers of cheese cloth directly, whereas the samples from the ileum and

TABLE III

SCHEDULE OF UREASE INJECTIONS IN TRIAL 2-1

Injection No.	Day	Urease Level[a], IUB units
		6600[b]
1	0	900[c]
2	7	1400
3	14	1900
4	21	2400
5[d]	56	2700[d]

[a]Injected subcutaneously.

[b]Each control was injected with 5 ml. saline.

[c]Urease was added to physiological saline and assayed prior to each injection. Each lamb received 5 ml.

[d]Booster injection of urease.

colon having greater consistency, had to first be suspended in 0.02 M phosphate buffer.

In pre-experimental assays the effect of chloramphenicol[4] on jack-bean urease was determined. It showed no effect on urease activity as shown in Table IV. Therefore, in order to prevent microbial growth in gastrointestinal fluids 1 ml. of chloramphenicol (1000 ug/ml.) was added per 20 ml. of each strained fluid. Urease activity of fluids was determined according to Gorin and Chin (1966).

Trial 2-3

The objective in this trial was to test for the possible presence of antiurease in the bile of urease-immunized lambs. Gall bladder bile samples from four control and four urease-injected lambs were collected at the time of slaughtering lambs in Trial 2-2. Each sample was centrifuged at 7500 rpm at 4° C. for 10 minutes to remove the particulate matter. Antiurease assays on each supernate of bile samples were made by the enzyme inhibition technique. The effect of bile on immune serum titers was determined by precipitin ring test.

The gamma globulin fraction prepared from the supernate of bile from a urease-immunized lamb was assayed for antiurease by hamagglutination technique. The procedure followed to prepare this fraction is reported herein: one-half volume of saturated ammonium sulfate was added to the supernate of bile. The precipitated fraction was obtained by centrifuging at 7500 rpm at 4° C. for 10 minutes. It was suspended in 1.0 percent NaCl solution whose volume was one-half as compared to the initial bile sample. The suspension was dialyzed against several

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[4]Chlormycetin (chloramphenicol) sodium succinate obtained from Parke, Davis and Co., Detroit, Michigan.

TABLE IV

EFFECT OF CHLORAMPHENICOL[a] ON JACKBEAN UREASE

Item	Chloramphenicol added[b], ug./ml. 0	50	250	500	SE[c]
Urease, IUB/ml.	29.4	29.1	29.3	29.9	0.38

[a]Chloromycetin (chloramphenicol) sodium succinate obtained from Parke, Davis and Co., Detroit, Michigan.

[b]Three observations per treatment.

[c]Standard error.

changes of 0.85 percent sodium chloride. The suspension was then poured in a graduated cylinder and dialyzed against moist polyethylene glycol[5] until a 20-fold concentration as compared to the initial bile sample volume was obtained.

Experiment 3

The objectives of Experiment 3 were to determine the effect and possible mechanism of urease immunity on urease activity in the rumen. Response criteria included urease activity of rumen fluid, antiurease activity in saliva, and reaction between antiserum and ruminal urease of sheep.

The composition of the diet used in all trials of this experiment is shown in Table V.

Trial 3-1

The objective in this trial was to determine the effect of urease injections on urease activity of rumen fluid. Eight rumen-fistulated sheep averaging about one year in age were randomly placed on two treatments involving zero and 6800 IUB units of urease. The schedule of urease injections is given in Table VI and other details were as described in Trial 1-1. All animals were bled via a jugular puncture on the 31st day of trial and serum samples were tested for antiurease by the enzyme inhibition technique, precipitin ring test and gel immunodiffusion technique. Rumen fluid samples were collected on the 33rd and 40th days of trial. They were prepared and assayed for urease as described in Trial 2-2. Total rumen fluid urease activity was

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[5]Polyethylene glycol obtained from Matheson Coleman and Bell Manufacturing Chemists, Norwood, Ohio.

TABLE V

COMPOSITION OF THE DIET IN EXPERIMENT 3

Ingredient	%
Starch (corn)	32.00
Cellulose[a]	30.00
Glucose[b]	22.65
Isolated soya protein[c]	6.32
Mineral mix[d]	4.92
Urea[e]	2.00
Corn oil[f]	2.00
Choline chloride	0.10
Vitamin A and D mix[g]	0.01
Total	100.00

[a]Solka-Floc. B-W20. Brown Co., Berlin, New Hampshire.

[b]Cerelose. Corn Products Co., Argo, Illinois.

[c]Purina Assay Protein. RP-100, Ralson Purina Co., Saint Louis, Missouri.

[d]Same as in Oklahoma Purified Diet; Clifford et al., 1967. J. Animal Sci. 26:400.

[e]Crystalline urea, courtesy of John Deere Chemical Co., Pryor, Oklahoma.

[f]Mazola. Santoquin was added to give 0.0125% in total diet.

[g]Contained 40,000 and 5,000 I.U./gm. of vitamin A and D, respectively.

TABLE VI

SCHEDULE OF UREASE INJECTIONS IN TRIAL 3-1

Injection No.	Day	Urease Level[a], IUB units
		6800[b]
1	0	900[c]
2	4	1400
3	10	2000
4	17	2500

[a]Injected subcutaneously.

[b]Each control was injected with 5 ml. saline.

[c]Urease was added to physiological saline and assayed prior to each injection. Each lamb received 5 ml.

calculated. Rumen volumes were determined according to the procedure of Smith (1958).

Trial 3-2

Two control and two urease-injected sheep from Trial 3-1 were randomly selected to determine the effect of urease injections on the possible presence of antiurease in saliva. Two weeks before the trial the control and urease-injected sheep were given a booster injection of zero and 3000 IUB units of urease, respectively. Saliva samples were collected by parotid cannulas fitted by a surgical procedure described later. Each sample of saliva was concentrated 10-fold by dialyzing against moist polyethylene glycol. Blood samples were obtained one day prior to collection of saliva samples. Antiurease activity of serum and cencentrated saliva samples was determined by the enzyme inhibition and hemagglutination techniques.

Parotid Duct Cannulation. Twelve cm. of polyethylene tubing (A) suitable to fit tightly into the sheep parotid duct was used. It was cut at an angle of slightly greater than 60 at one end and square at the other (Figure 1-A). A second polyethylene tubing (B) with a slightly larger internal diameter than tubing A and of sufficient length to facilitate collection of saliva was selected. One end of tubing B was flared with a flame as is done for connecting polyethylene adapters. With a hot 22 gauge needle, four holes were punched equidistantly around the circumference of the flare (Figure 1-B). The square end of tubing A was inserted into the flared end of tubing B (Figure 1-C). A drop of plastic glue[6] was applied to the square end of tubing A prior to

[6]Dab obtained from R. M. Hollingshead Corp., Sunnyvale, California.

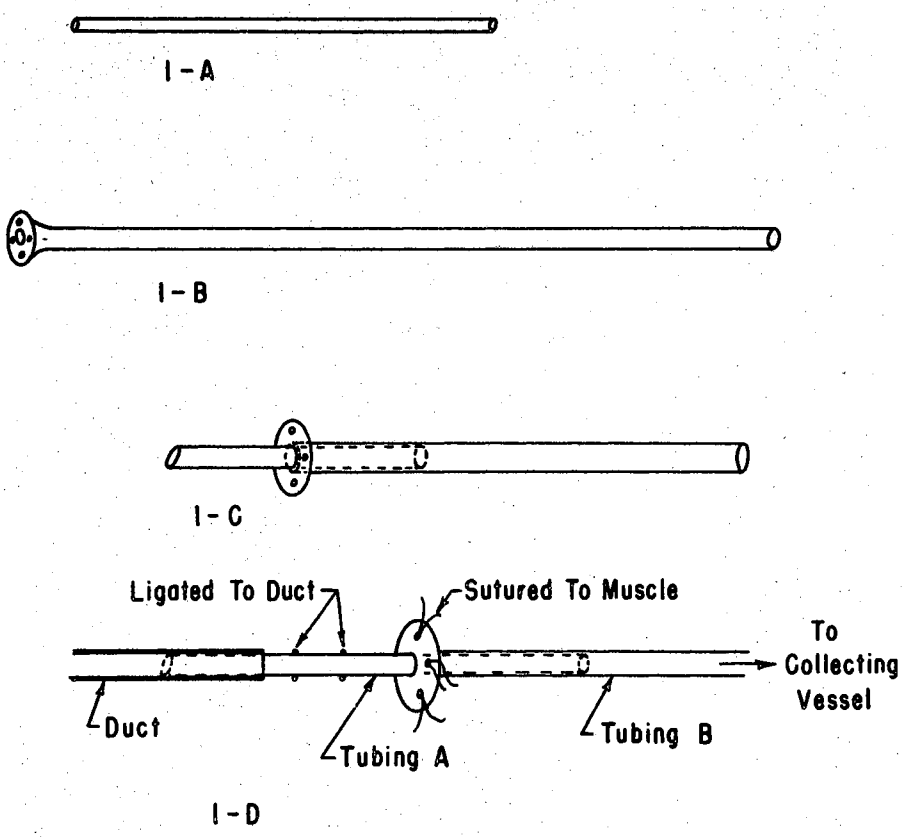

Figure 1. Diagrammatic Sketch of Cannulation of Parotid Duct

insertion. The connection was allowed to dry for 20 minutes. Sterilization with chlorhexidine[7] solution did not affect this glued junction.

Feed and water were withheld for 16 hours prior to the operation. Anaesthesia was induced by intravenous injection of sodium thiamylal[8] and maintained by halothane.[9] An 8 cm. long skin incision was made and the parotid duct was exposed and incised. The angular end of tubing A was inserted into the duct which was ligated to the tubing. The flare of tubing B was sutured to surrounding muscles (Figure 1-D) and the skin incision was repaired.

Trial 3-3

The objective in this trial was to test for possible reaction between ruminal urease and antiserum. Ruminal urease was prepared from mixed microorganisms obtained from the rumen of a control fistulated sheep as follows: approximately 400 ml. of rumen fluid was withdrawn through the rumen fistula. It was strained twice through four layers of cheese cloth. The strained rumen fluid was centrifuged at 15000 X G at room temperature for 10 minutes. The sediment was suspended in 200 ml. 0.02 M phosphate buffer (pH 7.0). The suspension was again centrifuged in a similar manner. The sediment obtained was resuspended in 100 ml. 0.02 M phosphate buffer. The microbial cells were disintegrated

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[7]Nolvasan solution. 1,1'-hexamethylenebis [5-(p-chlorophenyl) biguandide] diacetate obtained from Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

[8]Surital obtained from Parke, Davis and Co., Detroit, Michigan.

[9]Fluothane (2-bromo-2-chloro 1:1:1-trifluoroethane) made by Ayerst Laboratories, Inc., New York, New York.

by using an ultrasonic disintegrator[10] for 3 minutes. A cell free extract (ruminal urease) was obtained by centrifuging at 15000 X G at 4° C. for 30 minutes; the material contained urease activity in vitro. The reaction between ruminal urease and antiserum was tested by precipitin ring test.

Data from all experiments were subjected to statistical analyses according to applicable procedures as outlined by Steel and Torrie (1960).

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[10]Branson Sonifier (Model LS-75) manufactured by Ultrasonic Power Division, Branson Instrument Co., Stamford, Connecticut.

CHAPTER IV

RESULTS AND DISCUSSION

Antiurease Production and Animal Performance

The results of Trial 1-1 are presented in Table VII. Gains and feed efficiencies were not affected by urease injections and these results appear to be at variance with those of Glimp and Tillman (1965) and Harbers et al. (1965). Antiurease activities were affected ($P < .01$) by treatment on the 49th day and the effect of the level of urease did not differ from linearity ($P < .01$). However, blood samples taken on the 92nd day had no antiurease activity. It is possible that the disappearance of urease-antibodies from the animal body explains the apparent discrepancy of these results from those reported by Glimp and Tillman (1965). Harbers et al. (1965) also noted that antibody titer was reduced with time and that growth response was obtained when the titer in cattle was high but that this effect was reduced when the titer was low.

The results of Trial 2-1, which are shown in Table VIII, confirm these earlier results. When a booster injection was made on the 56th day of the trial the antiurease activity remained high, average daily gain was improved ($P < .01$) and feed efficiency was apparently improved ($P < .10$).

The results of Trial 1-1 did not identify the lowest level of urease giving maximum response; however, the level of 6600 IUB units

TABLE VII

EFFECT OF UREASE INJECTIONS ON GAIN, FEED EFFICIENCY AND SERUM ANTIUREASE ACTIVITY (TRIAL 1-1)

Item	Urease level[a], IUB units							SE[b]
	0	1650	3300	4950	6600	8250	9900	
Initial wt., kg.	27.36	28.09	29.45	26.45	26.36	26.18	27.18	
Av. daily gain, gm.	133	123	93	128	144	122	126	13.9
Gain/feed	0.10	0.10	0.09	0.11	0.12	0.11	0.11	0.01
Serum antiurease[c]								
49th day[d]	41.3**	35.2	35.1	26.8	27.8	33.7	25.1	2.1
92nd day	41.6	43.1	41.2	40.9	41.9	42.5	38.6	1.8

[a]Five lambs per treatment.

[b]Standard error.

[c]Antiurease is expressed as urease recovered after incubation of 44.8 IUB units of the enzyme with serum.

[d]The effect of level of urease did not differ from linearity ($P < .01$).

**$P < .01$.

TABLE VIII

EFFECT OF UREASE INJECTIONS ON GAIN, FEED EFFICIENCY AND SERUM ANTIUREASE ACTIVITY (TRIAL 2-1)

Item	Urease level[a], IUB units		SE[c]
	0	6600 + 2700[b]	
Initial wt., kg.	25.76	26.37	
Av. daily gain, gm.	138	167*	8.6
Gain/feed	0.10	0.12[e]	0.004
Serum antiurease[d]			
35th day	34.2	28.5**	0.8
70th day	36.6	25.8**	1.8
92nd day	33.8	26.3**	1.3

[a]Ten lambs per treatment.

[b]Booster injection given on the 56th day.

[c]Standard error.

[d]Antiurease is expressed as urease recovered after incubation of 36.6 IUB units of the enzyme with serum.

[e]$P < .10$.

*$P < .05$.

**$P < .01$.

gave slightly, though not significant, better gains than other levels. This level seems reasonable since Glimp and Tillman (1965) found that a level over 9900 IUB units caused lameness in some lambs and showed poorer performance than a level of approximately 4950 IUB units. The level (6600 IUB units) is equivalent to injecting subcutaneously a total of 250 IUB units of urease per kg. initial body weight over a period of 3 to 4 weeks.

Digestion Coefficients

The results of Trial 1-2 are presented in Table IX. The booster injections produced significant ($P < .05$) antiurease activity in treated lambs. There was no significant ($P > .05$) effect of urease immunity on digestion of dry matter, organic matter or nitrogen; however, all mean values of digestion coefficients of dry matter and organic matter were apparently higher in immunized animals than the controls.

Plasma Urea and Ammonia

The results of Trial 1-3 are presented in Figures 2 and 3. Post infusion change in plasma ammonia-nitrogen of blood from the ruminal vein was consistently low ($P < .05$) in the immunized animals (Figure 2). Differences between treatments in the case of the plasma ammonia-nitrogen of blood from the jugular vein were not significant ($P > .05$); however, the trend favored the treated animals. These results are in close agreement with the results reported earlier (Glimp and Tillman, 1965).

TABLE IX

EFFECT OF UREASE INJECTIONS ON DIGESTION OF DRY MATTER, ORGANIC MATTER AND NITROGEN

Item	Urease level[a], IUB units				SE[c]
	0	4950 +2000[b]	6600 +2500[b]	8250 +3000[b]	
Digestion coefficients, %					
Dry matter	75.1	75.4	76.8	76.0	1.0
Organic matter	76.2	76.3	80.5	77.1	1.6
Nitrogen	75.9	75.8	78.5	76.3	0.9
Serum antiurease[d]	43.4[e]	32.7[f]	27.9[f]	28.1[f]	3.3

[a]Five lambs per treatment.

[b]Booster injection.

[c]Standard error.

[d]Antiurease is expressed as urease recovered after incubation of 44.8 IUB units of the enzyme with serum.

[e,f]Figures bearing different superscripts are different ($P < .05$).

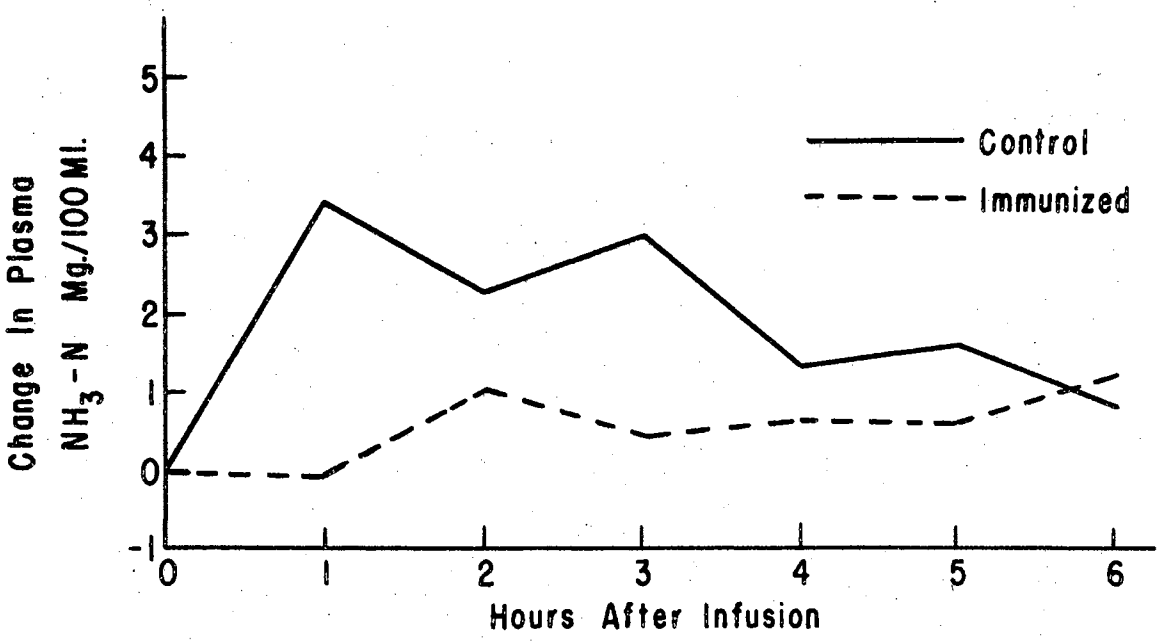


Figure 2. Effect of Jackbean Urease Immunity on Ruminal Vein Plasma Ammonia-Nitrogen

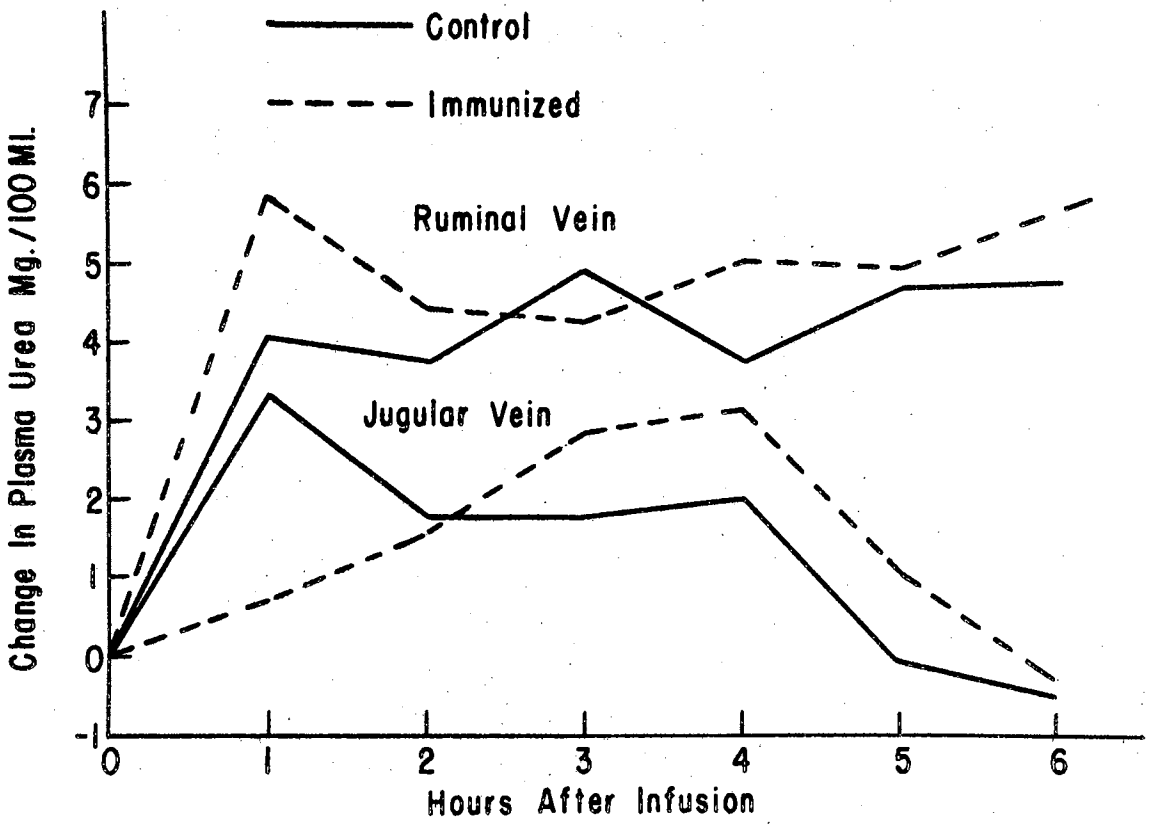


Figure 3. Effect of Jackbean Urease Immunity on Ruminal and Jugular Veins Plasma Urea

Post infusion changes in plasma urea from both ruminal and jugular blood are shown in Figure 3. Differences between ruminal venous plasma urea values were not significant at any sampling time; however, the greatest difference was found at the end of the trial and these results agree with those of Glimp and Tillman (1965). Jugular vein plasma urea values were not different ($P > .05$) when immunized animals were compared to the controls; however, in agreement with the results of Glimp and Tillman (1965), the values appeared to be higher in immunized animals at all times from two hours after dosing.

Since ammonia is absorbed through the rumen wall (McDonald, 1948) and its concentration in the rumen is associated with its level in portal blood (Lewis et al., 1957; Hogan, 1961), the results of Trial 1-3 indicate a decrease in the hydrolysis of urea in the rumen of immunized sheep.

As blood urea concentration follows the change in rumen fluid ammonia concentration (Lewis, 1957), the jugular blood urea results also support the idea that the rate of urea hydrolysis was decreased in the rumen of the immunized lambs.

The results of Houpt and Houpt (1968) indicate that some ruminal urease penetrates rumen epithelial layers to some unknown distance. If this is true, it is possible that urease-antiurease reaction can take place in rumen epithelium and result in a decreased rate of urea hydrolysis in the rumen of immunized lambs.

Gastrointestinal Fluid Urease Activity

The results of Trial 2-2 are presented in Table X. As found in previous trials, the booster injection of urease produced significant

TABLE X

EFFECT OF UREASE INJECTIONS ON UREASE ACTIVITY OF GASTROINTESTINAL FLUIDS

Item	Urease level[a], IUB units		SE[c]
	0	6600 + 5700[b]	
Urease IUB units/ml.			
Rumen fluid	34.5	31.9	0.9
Ileum fluid	22.2	16.2	2.2
Colon fluid	36.2	24.9*	3.0
Serum antiurease[d]	35.6	24.6*	2.6

[a]Six lambs per treatment.

[b]First booster injection (2700 IUB units) was given during Trial 2-1 and the second (3000 IUB units) was given as indicated in text.

[c]Standard error.

[d]Antiurease is expressed as urease recovered after incubation of 36.6 IUB units of the enzyme with serum.

*$P < .05$.

antiurease activity. Urease activity of colon fluid was reduced ($P < .05$). Immunization did not affect urease activity of fluid from the rumen or ileum; however, these differences approached significance ($P < .10$).

The results of Trial 3-1 are shown in Table XI. Serum antiurease as determined by the enzyme inhibition technique was affected ($P < .05$) by treatment. Serum antiurease was also detected by gel immunodiffusion technique (Figure 4) and precipitin ring test. Immunized sheep had lower ($P < .05$) total rumen urease activity than the controls.

The results of Trials 2-2 and 3-1 indicate decreased urease activity in the rumen, colon and perhaps in ileum of immunized animals. These results support the observations made in rumen infusion Trial 1-3. Data are also in agreement with results obtained with non-ruminants (Dang and Visek, 1960, 1964; Kornegay et al., 1964).

Antiurease in Digestive Secretions

The results of Trial 2-3 are shown in Tables XII and XIII. Antiurease was not found in the bile of immunized lambs by the enzyme inhibition technique (Table XII) The pooled bile sample from immunized lambs raised the precipitin titer of antiserum, whereas the pooled bile sample from control animals showed no such effect (Table XIII).

The gamma globulin prepared from a sample of bile of an immunized lamb showed a hemagglutination titer of 160.

Though the evidence is very limited these results indicate the possible presence of antiurease in the gall bladder bile of immunized lambs.

The results of Trial 3-2 are shown in Table XIV. Serum antiurease

TABLE XI

EFFECT OF UREASE INJECTIONS ON UREASE ACTIVITY IN THE RUMEN

Item	Urease level[a], IUB units		SE[b]
	0	6800	
Total rumen urease, IUB x 10^4	32.4	21.6*	2.99
Serum antiurease[c]	33.6	27.4**	0.72

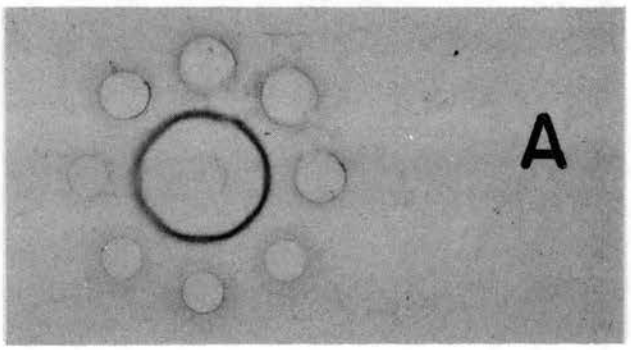
[a]Four sheep per treatment and two observations per sheep.

[b]Standard error.

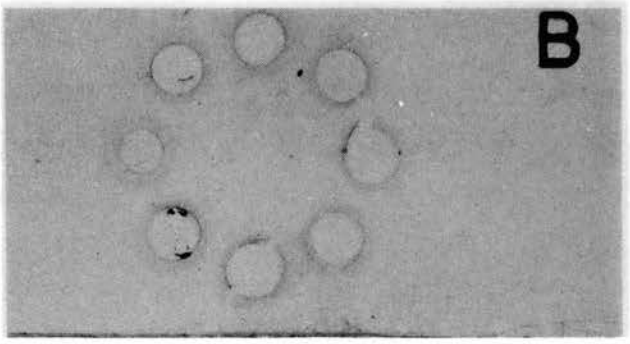
[c]Antiurease is expressed as urease recovered after incubation of 33.0 IUB units of the enzyme with serum.

*$P < .05$.

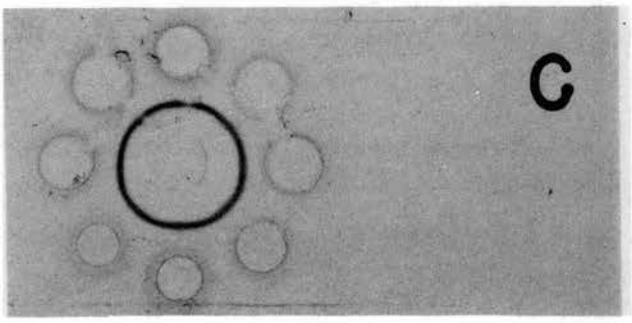
**$P < .01$.



A - Urease-Injected



B - Control



C - Urease-Injected

Figure 4. Detection of Antiurease in Serum by Gel Immunodiffusion.

TABLE XII

EFFECT OF UREASE INJECTIONS ON ANTIUREASE ACTIVITY IN BILE

	Urease level[a], IUB units		
	0	6600 + 5700[b]	$S\bar{d}$[c]
Antiurease[d]			
Bile	35.8	33.2	2.6
Serum	35.6	25.4*	3.4

[a]Four lambs per treatment.

[b]First booster injection (2700 IUB units) was given during Trial 2-1 and second booster (3000 IUB units) was given during Trial 2-2.

[c]Standard deviation appropriate to the difference between the sample means.

[d]Antiurease is expressed as urease recovered after incubation of 36.6 IUB units of the enzyme with bile or serum.

*$P < .05$.

TABLE XIII

EFFECT OF BILE FROM CONTROL AND UREASE-IMMUNIZED ANIMALS ON IMMUNE SERUM PRECIPITIN TITER

Diluent for antiserum	Precipitin titer
Saline	1:40
Pooled[a] bile from control lambs	1:40
Pooled[a] bile from immunized lambs	1:160

[a]Four lambs per treatment.

TABLE XIV

EFFECT OF UREASE INJECTIONS ON ANTIUREASE ACTIVITY IN PAROTID SALIVA

Item	Urease level[a], IUB units		$S\bar{d}$[c]
	0	6800 + 3000[b]	
Antiurease[d]			
Saliva[e]	35.5	33.1[f]	1.0
Serum	35.8	28.0*	2.2
Hemagglutination titer			
Saliva	——	1:20	...
Serum	——	1:1280	...

[a]Two lambs per treatment.

[b]Booster injection.

[c]Standard deviation appropriate to the difference between the sample means.

[d]Antiurease is expressed as urease recovered after incubation of 35.8 IUB units of the enzyme with saliva or serum.

[e]Tenfold concentrated saliva.

"——" indicates negative.

[f]$P < .10$.

*$P < .05$.

activity was affected by treatment ($P < .05$). Concentrated saliva from immunized lambs apparently inhibited ($P < .10$) jackbean urease. Hemagglutination titer of concentrated saliva and serum of urease-injected sheep were 1:20 and 1:1280, respectively.

The results of Trial 3-2 support the finding of other workers (Stoffer, 1960; Kraus and Konno, 1963; and Tourville et al., 1968) that antibodies are secreted in saliva.

Reaction Between Ruminal Urease and Antiserum

Reaction between ruminal urease and antiserum studied in Trial 3-3 gave a precipitin titer of 160. This is in agreement with the finding of Visek (1962) that serum of immunized rats inhibits the urease activity of phosphate buffer extract of gastrointestinal contents.

General Discussion

This investigation confirms the finding of Glimp and Tillman (1965) that injected jackbean urease induces the production of antiurease in sheep. However, serum antiurease activity, as determined by the enzyme inhibition technique, disappeared in less than 92 days. Incorporation of urease with some suitable adjuvant should have a desirable effect on the duration of antiurease activity and it should be studied.

Induction and maintenance of urease immunity improved the rate and efficiency of growth in lambs. This is in agreement with work done with non-ruminants and ruminants (Dang and Visek, 1960; Harbers et al., 1965; Glimp and Tillman, 1965). The mode of action of urease immunity on growth appears to be complex and may be the result of several factors.

Induction of urease immunity was accompanied by growth response and low urease activity in the rumen, ileum and colon. The results of present rumen infusion studies and those reported by Glimp and Tillman (1965) also indicate a decrease in urease activity in the rumen of immunized lambs. This is in close agreement with low urease activity found in gastrointestines of immunized rats, mice and guinea pigs (Dang and Visek, 1960, 1964) and in intestines of immunized swine (Kornegay et al., 1964). Low urease activity in the rumen is probably due to urease-antiurease reaction, since antiurease was found in immunized sheep saliva and the antiserum (antiurease) showed positive precipitin ring test with ruminal urease. Low urease activity in intestines of immunized animals may be the result of inhibition of intestinal urease by antiurease, possibly present in digestive secretions. Although the present results are based on very limited evidence, it appears that antiurease is present in the gall bladder bile of immunized ruminants. In this connection Visek et al. (1962) detected antiurease in the feces of immunized non-ruminants. Possible penetration of ruminal urease to some unknown distance into rumen epithelial layers was indicated by the results of Houpt and Houpt (1968). The possibility of urease-antiurease reaction taking place in either the epithelial lining of the rumen or the mucosal lining of the intestines is not yet known and should be studied.

It appears that improved performance of immunized lambs is related to reduced urea hydrolysis in the gastrointestinal tract. It is possible that decreased urease activity in rumen fluid resulted in the improvement of urea utilization since the rapid rate of urea hydrolysis limits its utilization.

Holtzman and Visek (1965) suggested that cellular renewal in gastrointestinal mucosa is altered by ammonia production. According to their suggestion, substances which accelerate growth by virtue of antibacterial action may do so by reducing urea hydrolysis in the gastrointestinal tract; thereby also reducing nutrient requirements for cellular renewal. In the present investigation decreased urease activity in ileum and particularly colon of immunized animals was observed. Therefore, if the above postulation is true, urease immunity can accelerate growth by controlling ammonia production in intestines.

Feeding urea and its liberation of ammonia leads to profound histological changes in the intestines (Visek, 1968). The role of urease immunity in this area needs to be explored.

Visek et al. (1968) observed a reduction in reduced pyridine nucleotides level in the liver during acute ammonia intoxication. They suggested that niacin might be limiting in animals fed diets which promote elevated blood ammonia levels. In view of the synthesis of niacin by rumen microorganisms this effect of ammonia on reduced pyridine nucleotides may not be of much importance in ruminant animals.

Chalupa et al. (1968) reported low activities of urea cycle enzymes in the liver of sheep fed urea as compared to soy protein. They also stated that a greater than normal hepatic load of ammonia possibly caused partial damage of liver in urea fed animals. A study of urea and ammonia metabolism in the liver of urease-immunized ruminants, on a urea rich diet, may provide additional leads regarding the mechanism of urease immunity on growth.

Complete physiological and biochemical details involved in increased growth response shown by urease-immunized animals remains obscure but it appears that it is related to reduced urea hydrolysis in the gastrointestinal tract.

CHAPTER V

SUMMARY AND CONCLUSIONS

Three experiments involving nine trials and 63 lambs were conducted to determine the effect of subcutaneous injections of jackbean urease on antiurease production, general performance, digestion coefficients, plasma urea and ammonia levels, gastrointestinal fluids urease activity, and digestive secretions antiurease activity in sheep. Antiurease activity was observed in serum of all urease-injected lambs. Significant response in average daily gains was associated with production and maintenance of antiurease activity in blood. A total dose of 250 (International Union of Biochemistry, IUB) units of urease per kilogram initial body weight, given by subcutaneous administration over a period of 3 to 4 weeks was considered suitable for this response. There was no significant effect of urease immunity on digestion of dry matter, organic matter and nitrogen; however, all mean values of digestibility of dry matter and organic matter were apparently higher in immunized animals than the controls.

In rumen infusion studies post infusion change in plasma ammonia-nitrogen of blood from ruminal vein was consistently lower in immunized animals than in the controls. Differences between the immunized and controls for ruminal venous plasma urea values were not significant. Jugular vein plasma urea peak values appeared to be obtained later in immunized lambs than in the controls. Urease activity in fluids from

the rumen, ileum and colon was low in immunized sheep. Antiurease was found in saliva from the parotid duct of immunized sheep. Limited evidence suggested the possibility of presence of antiurease in the gall bladder bile of immunized lambs. Low urease activity in gastrointestinal tract was attributed to the presence of antiurease in saliva, bile and possibly other digestive secretions. The possible association of improved growth performance of lambs to a reduction of urea hydrolysis in gastrointestinal tract is discussed.

An improved surgical technique to cannulate the parotid duct of sheep is described.

LITERATURE CITED

Abou Akkada, A. R. and T. H. Blackburn. 1963. Some observations on the nitrogen metabolism of rumen proteolytic bacteria. J. Gen. Microbiol. 31:461.

Abou Akkada, A. R. and B. H. Howard. 1962. The biochemistry of rumen protozoa. 5. The nitrogen metabolism of Entodinium. Biochem. J. 82:313.

Acord, C. R., G. E. Mitchell, Jr. and C. O. Little. 1968. Combinations of nitrogen sources for starch digestion by mixed rumen bacteria. J. Dairy Sci. 51:1249.

Acord, C. R., G. E. Mitchell, Jr., C. O. Little and M. R. Karr. 1966. Nitrogen sources for starch digestion by rumen microorganisms. J. Dairy Sci. 49:1519.

Allison, M. J. 1965. Phenylalanine biosynthesis from phenylacetic acid by anaerobic bacteria from the rumen. Biochem. Biophys. Res. Commun. 18:30.

Allison, M. J. and M. P. Bryant. 1963. Biosynthesis of branched-chain amino acids from branched-chain fatty acids by rumen bacteria. Arch. Biochem. Biophys. 101:269.

Allison, M. J., M. P. Bryant and R. N. Doetsch. 1959. Conversion of isovalerate to leucine by Ruminococcus flavefaciens. Arch. Biochem. Biophys. 84:245.

Allison, M. J., J. A. Bucklin and I. M. Robinson. 1966. Importance of the isovalerate carboxylation pathway of leucine biosynthesis in the rumen. Appl. Microbiol. 14:807.

Allison, M. J. and I. M. Robinson. 1967. Tryptophan biosynthesis from indole-3-acetic acid by anaerobic bacteria from the rumen. Biochem. J. 102:36p.

Appleby, J. C. 1955. The isolation and classification of proteolytic bacteria from the rumen of the sheep. J. Gen. Microbiol. 12:526.

Association of Official Agricultural Chemists. 1960. Official Methods of Analysis (9th ed.). Association of Official Agricultural Chemists. Washington, D. C.

Berns, D. S., P. Holohan and E. Scott. 1966. Urease activity in blue-green algae. Science 152:1077.

Belasco, I. J. 1954. New nitrogen feed compounds for ruminants--A laboratory review. J. Animal Sci. 13:601.

Blackburn, T. H. and P. N. Hobson. 1962. Further studies on the isolation of proteolytic bacteria from the sheep rumen. J. Gen. Microbiol. 29:69.

Bloomfield, R. A., G. B. Garner and M. E. Muhrer. 1960. Kinetics of urea metabolism in sheep. J. Animal Sci. 19:1248. (Abstr.).

Bloomfield, R. A., E. O. Kearley, D. O. Creach and M. E. Muhrer. 1963. Ruminal pH and absorption of ammonia and VFA. J. Animal Sci. 22:833. (Abstr.).

Bloomfield, R. A., R. P. Wilson and G. B. Thompson. 1964. Influence of energy levels on urea utilization. J. Animal Sci. 23:868. (Abstr.).

Bouckaert, J. H. and W. Oyaert. 1952. Fate of some nitrogen compounds in the rumen. Zootechnia 1(6):21.

Brent, B. E. and A. Adepoju. 1967. Effect of acetohydroxamic acid on rumen urease. J. Animal Sci. 26:1482. (Abstr.).

Briggs, M. H., T. W. Heard, A. Whitcroft and M. L. Hogg. 1964. Studies on urea-fed cattle. 1. Chemical and microbiological properties of rumen fluid. Life Sci. 3:7.

Bromberg, P. A., E. D. Robin and C. E. Forkner, Jr. 1960. The existence of ammonia in blood *in vivo* with observations on the significance of the $NH_4^+ - NH_3$ system. J. Clin. Invest. 39:332.

Brüggemann, J., D. Giesecke and K. Drepper. 1962. Influence of addition of different sources on composition and output of rumen flora. Z. Tierphysiol. Tierernähr. Futtermittelk. 17:162.

Bryant, M. P. 1963. Symposium on microbial digestion in ruminants: Identification of groups of anaerobic bacteria active in the rumen. J. Animal Sci. 22:801.

Burchall, J. J., R. A. Niederman and M. J. Wolin. 1964. Amino group formation and glutamate synthesis in *Streptococcus bovis*. J. Bact. 88:1038.

Burrell, R. G. and C. C. Mascoli. 1966. Experimental Immunology. Burgress Publishing Co., Minneapolis, Minnesota.

Caffrey, P. J., E. E. Hatfield, H. W. Norton and U. S. Garrigus. 1967. Nitrogen metabolism in the ovine. 1. Adjustment to a urea-rich diet. J. Animal Sci. 26:595.

Cahill, D. and D. M. McAleese. 1964. The effect of aureomycin supplementation on urea utilization in the rations of growing-fattening lambs. Sci. Proc. Roy. Dublin Soc. B. 1:123.

Carroll, E. J. 1960. Urea-utilizing organisms from the rumen. Ph.D. Dissertation, University of Missouri, Columbia, Missouri.

Chalupa, W., J. H. Clark, P. Opliger and R. Lavker. 1968. Enzyme systems for ammonia metabolism in ruminant liver. J. Dairy Sci. 51:979. (Abstr.).

Chalupa, W., J. L. Evans and M. C. Stillions. 1963. Nitrogen source availability and activity of rumen microorganisms. J. Dairy Sci. 46:1431.

Clifford, A. J., J. R. Bourdette and A. D. Tillman. 1968. Effect of urease inhibitors on sheep fed diets containing urea. J. Animal Sci. 27:1073.

Clifford, A. J., R. D. Goodrich and A. D. Tillman. 1967. Effects of supplementing ruminant all concentrate and purified diets with vitamins of the B complex. J. Animal Sci. 26:400.

Cocimano, M. R. and R. A. Leng. 1967. Metabolism of urea in sheep. British J. Nutr. 21:353.

Conway, E. J. 1962. Microdiffusion Analysis and Volumetric Error (5th ed.). Crosby Lockwood and Son Ltd., London.

Coombe, J. B., D. E. Tribe and J. W. C. Morrison. 1960. Some experimental observations on the toxicity of urea to sheep. Australian J. Agr. Res. 11:247.

Creeth, J. M. and L. W. Nichol. 1960. Evidence for the chemical interaction of urease in solution. Biochem. J. 77:230.

Dang, H. C. and W. J. Visek. 1960. Effect of urease injection on body weights of growing rats and chicks. Proc. Soc. Exp. Biol. Med. 105:164.

Dang, H. C. and W. J. Visek. 1964. Some effects of urease administration on laboratory animals. Am. J. Physiol. 206:731.

Dang, H. C. and W. J. Visek. 1966. Antagonism of urease toxicity by chemical agents and antisera. Toxicol. Appl. Pharm. 8:318.

Dang, H. C. and W. J. Visek. 1968. Some characteristics of blood in normal and immune rabbits after urease injection. Am. J. Physiol. 215:502.

el-Shazly, K., B. A. Dehority and R. R. Johnson. 1961. Effect of starch on the digestion of cellulose in vitro and in vivo by rumen microorganisms. J. Animal Sci. 20:268.

Fishbein, W. N. 1967. Urease: A three substrate enzyme. Fed. Proc. 26:578. (Abstr.).

Fishbein, W. N., T. S. Winter and J. D. Davidson. 1965. Urease catalysis. I. Stoichiometry, specificity, and kinetics of second substrate: Hydroxyurea. J. Biol. Chem. 240:2404.

Gall, L. S., W. E. Thomas, J. K. Loosli and C. N. Huhtanen. 1951. The effect of purified diets upon rumen flora. J. Nutr. 44:113.

Gelman Instrument Co. 1968. Gelman Procedures, Techniques, and Apparatus for Electrophoresis. Gelman Instrument Co., Ann Arbor, Michigan.

Gibbons, R. J. and R. N. Doetsch. 1959. Physiological study of an obligately anaerobic ureolytic bacterium. J. Bact. 77:417.

Glimp, H. A. and A. D. Tillman. 1964. Effect of jackbean urease injections and chlortetracycline on rate of gain and feed efficiency in swine. J. Animal Sci. 23:963.

Glimp, H. A. and A. D. Tillman. 1965. Effect of jackbean urease injections on performance, anti-urease production and plasma ammonia and urea levels in sheep. J. Animal Sci. 24:105.

Gorin, G. 1959. On the mechanism of urease action. Biochim. Biophys. Acta. 34:268.

Gorin, G. and C. C. Chin. 1966. Urease. VI. A new method of assay and the specific enzymic activity. Anal. Biochem. 17:49.

Gorin, G. and C. C. Chin. 1967. Activity and dissociation of urease in acetate of pH 3.5. Fed. Proc. 26:605.

Gorin, G., E. Fuchs, L. G. Butler, L. S. Chopra and R. T. Hersh. 1962. Some properties of urease. Biochemistry 1:911.

Hand, D. B. 1939. Molecular weight and association of the enzyme urease. J. Am. Chem. Soc. 61:3180.

Harbers, L. H., A. D. Tillman and W. J. Visek. 1962. Effect of barbituric acid on urea diets for ruminants. J. Animal Sci. 21:754.

Harbers, L. H., A. D. Tillman, W. J. Visek and H. A. Glimp. 1965. Some effects of jackbean urease immunity in young calves. J. Animal Sci. 24:102.

Hogan, J. P. 1961. The absorption of ammonia through the rumen of the sheep. Australian J. Biol. Sci. 14:448.

Hogan, J. P. and R. H. Weston. 1967. The digestion of two diets of differing protein content but with similar capacities to sustain wool growth. Australian J. Agr. Res. 18:973.

Holtzman, J. L. and W. J. Visek. 1965. Growth of rats fed chlortetracycline or an exchange resin. J. Nutr. 87:101.

Hoover, W. H., E. M. Kesler and R. J. Flipse. 1963. Carbon sources for in vitro protein synthesis by rumen bacteria. J. Dairy Sci. 46:733.

Hoshino, S., K. Sarumaru and K. Morimoto. 1966. Ammonia anabolism in ruminants. J. Dairy Sci. 49:1523.

Houpt, T. R. 1959. Utilization of blood urea in ruminants. Am. J. Physiol. 197:115.

Houpt, T. R. and K. A. Houpt. 1968. Transfer of urea nitrogen across the rumen wall. Am. J. Physiol. 214:1296.

Howell, S. F. 1932. Antiurease formation in the hen. Proc. Soc. Exp. Biol. Med. 29:759.

Huhtanen, C. N., F. J. Carleton and H. R. Roberts. 1954. Carbon dioxide utilization by rumen microorganisms. J. Bact. 68:749.

Hungate, R. E. 1963. Symbiotic associations: The rumen bacteria. In P. S. Nutman and B. Mosse (ed.) Symbiotic Associations. Thirteenth Symp. Soc. Gen. Microbiol. Cambridge University Press.

Hungate, R. E. 1966. The Rumen and Its Microbes. Academic Press, Inc., New York.

Hunter, N. W. 1957. Intracellular localization of some hydrolases and iron and copper porphyrin enzymes of Opolina carolinensis. Trans. Am. Microscop. Soc. 76:36.

Hunter, N. W. 1959. Enzyme systems of Stylonychia pustulata. II. Miscellaneous systems (hydrases, hydrolases, and dehydrogenases). J. Protozool. 6:100.

Johns, A. T. 1955. New Zealand J. Sci. and Technol. A 37:323, cited by R. E. Hungate. 1966. The Rumen and Its Microbes. Academic Press, Inc., New York.

Jones, G. A. 1965. Effect of divalent cations on the activity of bacterial urease. Can. J. Microbiol. 11:120.

Jones, G. A. 1967. Ureolytic rumen bacteria. In M. H. Briggs (ed.) Urea as a Protein Supplement. Pergamon Press, New York.

Jones, G. A., R. A. MacLeod and A. C. Blackwood. 1964a. Ureolytic rumen bacteria. I. Characteristics of the microflora from a urea-fed sheep. Can. J. Microbiol. 10:371.

Jones, G. A., R. A. MacLeod and A. C. Blackwood. 1964b. Ureolytic rumen bacteria. II. Effect of inorganic ions on urease activity. Can. J. Microbiol. 10:379.

Juhász, B. 1965. Endogenous nitrogen cycle in ruminants. Acta. Vet. Acad. Sci. Hung. 15:25.

Kabat, E. A., M. M. Mayers. 1967. Kabat and Mayer's Experimental Immunochemistry (2nd ed.). Charles C. Thomas, Publisher, Springfield, Illinois.

Kirk, J. S. and J. B. Sumner. 1931. Antiurease. J. Biol. Chem. 94:21.

Kirk, J. S. and J. B. Sumner. 1934. The reaction between crystalline urease and antiurease. J. Immunol. 26:495.

Kornegay, E. T., E. R. Miller, D. E. Ullrey and J. A. Hoefer. 1964. Effects of urease immunization of growing pigs upon performance and blood and intestinal ureolysis. J. Animal Sci. 23:688.

Kraus, F. W. and J. Konno. 1963. Antibodies in saliva. Ann. N. Y. Acad. Sci. 106:311.

LeBars, H. 1967. The endogenous urea cycle of the ruminant. In M. H. Briggs (ed.) Urea as a Protein Supplement. Pergamon Press, New York.

Lewis, D. 1957. Blood-urea concentration in relation to protein utilization in the ruminant. J. Agr. Sci. 48:438.

Lewis, D., K. J. Hill and E. F. Annison. 1957. Studies on the portal blood of sheep. I. Absorption of ammonia from the rumen of sheep. Biochem. J. 66:587.

Little, C. O., W. Burroughs and W. Woods. 1963. Nutritional significance of soluble nitrogen in dietary proteins for ruminants. J. Animal Sci. 22:358.

Luck, J. M. 1924. Ammonia production by animal tissues in vitro. II. The demonstration of urease in animal body. Biochem. J. 18:825.

Mackay, E. S. M. and A. E. Oxford. 1954. Some facultatively anaerobic gram-negative rods from the rumen of the calf and the sheep. J. Gen. Microbiol. 11:472.

Mann, S. O., F. M. Masson and A. E. Oxford. 1954. Facultative anaerobic bacteria from the sheep's rumen. J. Gen. Microbiol. 10:142.

Mamiya, G. and G. Gorin. 1965. Urease. V. Some observations on the procedure for its isolation. Biochim. Biophys. Acta. 105:382.

Marucci, A. A. and M. M. Mayer. 1955. Quantitative studies on the inhibition of crystalline urease by rabbit anti-urease. Arch. Biochem. Biophys. 54:330.

McDonald, I. W. 1948. The absorption of ammonia from the rumen of the sheep. Biochem. J. 42:584.

McLaren, G. A., G. C. Anderson, W. G. Martin and W. K. Cooper. 1961. Fixation of ammonia nitrogen by rumen mucosa. J. Animal Sci. 20:942. (Abstr.).

McLaren, G. A., W. K. Cooper and G. C. Anderson. 1962. Factors influencing glutamate synthesis by rumen mucosa. J. Animal Sci. 21:1005. (Abstr.).

McNaught, M. L. 1951. The utilization of non-protein nitrogen in the bovine rumen. 7. A qualitative and quantitative study of the breakdown of carbohydrate which accompanies protein formation in bovine rumen contents during in vitro incubation. Biochem. J. 49:325.

Merino, H. and N. S. Raun. 1964. Effect of chlortetracycline and urea on ruminal urease activity in sheep. J. Animal Sci. 23:884. (Abstr.).

Moir, R. J. and L. E. Harris. 1962. Ruminal flora studies in the sheep. X. Influence of nitrogen intake upon ruminal function. J. Nutr. 77:285.

Moore, M. J., W. R. Woods and T. J. Klopfenstein. 1968. Nitrogen metabolism as influenced by acetohydroxamic acid. J. Animal Sci. 27:1172. (Abstr.).

Otagaki, K. K., A. L. Black, H. Goss and M. Kleiber. 1955. In vitro studies with rumen microorganisms using carbon-14-labeled casein, glutamic acid, leucine and carbonate. J. Agr. Food Chem. 3:948.

Packett, L. V. and T. D. D. Groves. 1965. Urea recycling in the ovine. J. Animal Sci. 24:341.

Palmquist, D. L. and R. L. Baldwin. 1966. Enzymatic techniques for the study of pathways of carbohydrate utilization in the rumen. Appl. Microbiol. 14:60.

Pearson, R. M. and J. A. B. Smith. 1943. The utilization of urea in the bovine rumen. 2. The conversion of urea to ammonia. Biochem. J. 37:148.

Pillemer, L., E. E. Ecker, V. C. Myers and E. Muntwyler. 1938. Chemical and immunological studies of the effects of radiant energy and of oxidation on crystalline urease. J. Biol. Chem. 123:365.

Prescott, J. M. 1953. Effect of diet and antibiotics on utilization of non-protein nitrogen. J. Agr. Food Chem. 1:894.

Rahman, S. A. and P. Decker. 1966. Comparative study of the urease in the rumen wall and rumen content. Nature 209:618.

Reithal, F. J., J. E. Robbins and G. Gorin. 1964. A structural subunit molecular weight of urease. Arch. Biochem. Biophys. 108:409.

Schmidt-Nielsen, B. and H. Osaki. 1958. Renal response to changes in nitrogen metabolism in sheep. Am. J. Physiol. 193:657.

Schmidt-Nielsen, B., K. Schmidt-Nielsen, T. R. Houpt and S. A. Jarnum. 1957. Urea excretion in the camel. Am. J. Physiol. 188:477.

Sehgal, P. P. 1964. Ontogenetic, physical and chemical studies on urease in Canavalia ensiformis (L.) DC. Ph.D. Dissertation. Duke University, Durham, North Carolina.

Smith, R. H. 1958. The development and function of the rumen in milk-fed calves. J. Agr. Sci. 52:72.

Somers, M. 1961a. Factors influencing the secretion of nitrogen in sheep saliva. 1. The distribution of nitrogen in the mixed and parotid saliva of sheep. Australian J. Exp. Biol. Med. Sci. 39:111.

Somers, M. 1961b. Factors influencing the secretion of nitrogen in sheep saliva. 2. The influence of nitrogen intake upon blood urea nitrogen and upon the total nitrogen and urea nitrogen in the parotid saliva of sheep. Australian J. Exp. Biol. Med. Sci. 39:123.

Somers, M. 1961c. Factors influencing the secretion of nitrogen in sheep saliva. 3. Factors affecting the nitrogen fractions in the parotid saliva of sheep with special reference to the influence of ammonia production in the rumen and fluctuations in level of blood urea. Australian J. Biol. Med. Sci. 39:133.

Somers, M. 1961d. Factors influencing the secretion of nitrogen in sheep saliva. 4. The influence of injected urea on the quantitative recovery of urea in the parotid saliva and the urinary excretions of sheep. Australian J. Exp. Biol. Med. Sci. 39:145.

Sosnovskaja, E. A. 1959. Rumen microflora of sheep on different rations. Zivotnovodstvo. 7:61.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York.

Stoffer, H. R. 1960. The production of salivary antibodies in experimental animals. M. S. Thesis. University of North Carolina, Chapel Hill, North Carolina.

Streeter, C. L., R. R. Oltjen, L. L. Slyter and W. M. Fishbein. 1968. Urea utilization in wethers receiving acetohydroxamic acid. J. Animal Sci. 27:1177. (Abstr.).

Sumner, J. B. 1926. The isolation and crystallization of the enzyme urease. J. Biol. Chem. 69:435.

Sumner, J. B. 1951. Urease. In J. B. Sumner and K. Myrbäck (ed.) The Enzymes--Chemistry and Mechanism of Action. 1:873. Academic Press, Inc., New York.

Sumner, J. B., N. Gralen and I. Eriksson-Quensel. 1938. The molecular weight of urease. J. Biol. Chem. 125:37.

Sumner, J. B. and D. B. Hand. 1929. The isoelectric point of crystalline urease. J. Am. Chem. Soc. 51:1255.

Sumner, J. B. and G. F. Somers. 1953. Chemistry and Methods of Enzymes (3rd ed.). Academic Press, Inc., New York.

Tillman, A. D. and R. W. Swift. 1953. The utilization of ammoniated industrial by-products and urea in sheep. J. Animal Sci. 12:201.

Thomson, A. and W. J. Visek. 1963. Some effects of induction of urease immunity in hepatic insufficiency. Am. J. Med. 35:804.

Tourville, D., J. Bienenstock and T. B. Tomasi, Jr. 1968. Natural antibodies of human serum, saliva, and urine reactive with Escherichia coli. Proc. Soc. Exp. Biol. Med. 128:722.

van der Horst, C. J. G. 1961. Keto-acids and amines in rumen liquid of cattle. Nature 191:73.

Visek, W. J. 1962. Studies on urea hydrolysis in birds and mammals. Am. J. Vet. Res. 23:569.

Visek, W. J. 1968. Some aspects of ammonia toxicity in animal cells. J. Dairy Sci. 51:286.

Visek, W. J. and H. C. Dang. 1966. Protection against lethal doses of gamma irradiation with urease immunity. Res. Rpt. Cornell Univ. Agr. Exp. Sta., Ithaca, New York.

Visek, W. J., M. E. Iwert and W. Burrows. 1962. Detection of antibody to urease by hemagglutination. Proc. Soc. Exp. Biol. Med. 109:54.

Visek, W. J., M. E. Iwert, N. S. Nelson and J. H. Rust. 1967. Some immunologic properties of jackbean urease and its antibody. Archiv. Biochem. Biophys. 122:95.

Visek, W. J., R. L. Prior and A. J. Clifford. 1968. Enzyme and coenzyme in urea fed and ammonia intoxicated animals. Proc. Cornell Nutr. Conf. p. 79.

Wagner, G. R., E. L. Nichols, R. H. Kohlmeier, S. L. Balloun and V. W. Hays. 1963. Lack of effect of urease injection on growth rate and feed efficiency of chicks and rats. J. Nutr. 81:30.

Walker, D. J. 1965. Energy metabolism and rumen microorganisms. In R. W. Dougherty and others (ed.) Physiology of Digestion in the Ruminant. Butterworths, Washington, D. C.

Wall, M. C. and K. J. Laidler. 1953. The molecular kinetics of the urea-urease system. IV. The reaction in an inert buffer. Arch. Biochem. Biophys. 43:299.

Wills, E. D. 1952. Enzyme inhibition by suramine and the measurement of the isoelectric points of some enzymes. Biochem. J. 50:421.

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