

INFLUENCE OF AUREOMYCIN ON SYNTHESIS AND DIGESTION IN  
THE RUMEN OF CATTLE FED NATURAL AND PURIFIED RATIONS

By

CHARLES MARION CHANCE

A THESIS

Submitted to the School of Graduate Studies of Michigan  
State College of Agriculture and Applied Science  
in partial fulfillment of the requirements  
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# ABSTRACT

Two steers, each with a rumen fistula fitted with a plastic plug, were used to investigate the influence of aureomycin on the synthesis and digestion in the rumen when the steers were fed natural and purified rations. The animals were fed a natural ration of 15 pounds of second-cutting alfalfa-brome hay and four pounds of corn once daily throughout the feeding trials. Aureomycin was fed at each level (0, 0.5 and 1.0 gram per day) for 15 days before changing to the next highest level. The period in which the steers received no aureomycin served as the control period for comparison with those in which aureomycin was fed.

The samples of the rumen contents were collected by completely evacuating the rumen before feeding, 0-hour and at 6- and 12-hours after feeding the ration. Determinations for dry matter, crude fiber, crude protein and ether extract as well as nitrogen-free extract were made on both the rumen contents and the ration. Microbiological methods of assay were used for the determination of the 10 essential amino acids, riboflavin, nicotinic acid and pantothenic acid.

Neither of the steers showed any signs of anorexia or diarrhea at any time during aureomycin supplementation when the natural ration was fed. Steer 714 went off-feed just prior to the addition of 0.5 gram of aureomycin to the puri-

fied ration and did not regain its appetite during the period that aureomycin was included in the ration.

The pH declined for about six hours after feeding with only a slight increase toward alkalinity at the end of 12 hours. When the aureomycin was included in the natural ration, the pH did not become as acid as that observed when no aureomycin was fed; however, the response was in the same direction in each case.

A definite increase in the total bacterial count of the rumen contents and the feces occurred when aureomycin was included in the ration. The number of rumen streptococci decreased when aureomycin was fed, while the number of coliform organisms in the rumen remained approximately the same in one animal and increased in the other.

The rate of removal of dry matter, crude fiber, crude protein, nitrogen-free extract, non-protein nitrogen, the 10 essential amino acids and riboflavin from the rumen was the highest when 0.5 gram of aureomycin was fed. There was an accumulation of ether extract, nicotinic acid and pantothenic acid in the rumen when aureomycin was included in the ration.

There was an accumulation of dry matter and crude fiber in the rumen at the 0-hour when 1.0 gram of aureomycin was fed which indicated that a slight depression of digestibility of these constituents may have occurred.

The pH and the total bacterial count were approximately the same for the periods when no aureomycin and 0.5 gram of aureomycin was added to the purified ration. The synthesis of the amino acids was lower when 0.5 gram of aureomycin was included in the purified ration. A definite decrease in the synthesis of riboflavin, nicotinic acid and pantothenic acid also occurred when 0.5 gram of aureomycin was added to the purified ration.

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## INTRODUCTION

The use of antibiotics in animal nutrition was incidental to the development of vitamin B<sub>12</sub> concentrates from by-products of antibiotic manufacture. Some of the by-products were more effective in stimulating growth than others and this extra effect was traced to the residual antibiotic.

Many investigations with swine and poultry have shown that various antibiotics were beneficial in promoting growth, less digestive disturbances and an increased feed efficiency. Recent reports have shown favorable growth responses when aureomycin was fed to young calves. However, several investigators reported that aureomycin produced adverse effects, such as anorexia and diarrhea, when fed to steers and lambs. Also, there was a decreased digestibility of dry matter and crude fiber by the steers and the lambs lost weight when aureomycin was included in the ration.

This investigation was undertaken to study the influence of aureomycin on the digestion and synthesis of certain ration nutrients in the rumen, the synthesis, if any, of the essential amino acids and some of the B vitamins in the rumen. The influence of aureomycin on the bacterial population of the rumen and the feces also was determined in an attempt to correlate these findings with the digestion studies.

## REVIEW OF LITERATURE

### Introduction

The term antibiotic is defined in pharmaceutical and medical practice as a metabolic product of one microorganism that is detrimental to the life activities of other microorganisms. Antibiotics have been known, if not in pure form, at least by their effects for centuries; but have only recently assumed the dominating position in clinical medicine and in the pharmaceutical industry that they now occupy. Most of the research and development in the field of antibiotics has developed following Fleming's observation of the action of Penicillium sp. on Staphylococcus aureus in 1929. During the early years of World War II, sulfa drugs were the only chemotherapeutic agents available for the treatment of bacterial infections and these drugs had several undesirable characteristics. Therefore, there was need for new antibacterial agents that might not have these undesirable properties.

By definition, sulfa drugs cannot be called antibiotics; however, their action is similar to that of an antibiotic. An occasional reference is made to some sulfa compound merely to show some of the earlier attempts to arrive at a solution of the same problem that antibiotics are being used for at the present time.

Some of the literature concerning the use of antibiotics with poultry, swine and other animals is included in this review because most of the antibiotic research has been done with those animals.

### Antibiotics in Poultry Nutrition

Moore et al. (1946) fed streptomycin with an experimental purified diet which stimulated growth of chickens. These workers were interested in finding a drug or combination of drugs that would completely inactivate all bacteria in the intestinal tract in order to study vitamin requirements uncomplicated by vitamins or toxic substances. Sulfasuxidine and streptomycin, singly or in combination, increased the growth of chicks supplemented with adequate amounts of folic acid.

Oleson and coworkers (1950) found that animal protein factor (APF)-depleted chicks showed no response to aureomycin in the absence of B<sub>12</sub> in the diet; however, there was a marked response to the antibiotic when B<sub>12</sub> was present in suboptimal or optimal amounts.

Berg et al. (1950) observed a cessation of the accelerated growth response when APF was removed from the ration. When this material was added to a ration for chicks which had not received any during <sup>the</sup> first 4.5 weeks of age, there was an immediate acceleration of growth. The same effect was observed

when crystalline aureomycin was used. They concluded that the mode of action of the antibiotic in promoting growth is dependent on the continuous presence of the material in the diet.

Similar results in growth were obtained by Groschke and Evans (1950) who reported that the best gains occurred when synthetic vitamins or vitamin B<sub>12</sub> were fed in combination with streptomycin or aureomycin. These workers also injected aureomycin but failed to get an increase in growth.

Stokstad and Jukes (1951) studied the effect of graded levels of vitamin B<sub>12</sub> on growth in chicks in the presence and absence of aureomycin. A growth-promoting effect for aureomycin was noted both in the presence and absence of vitamin B<sub>12</sub>. In some of their trials, a "sparing effect" of aureomycin upon vitamin B<sub>12</sub> was observed; but in others, no effect was observed. The mortality of deficient chicks on the diet containing no added vitamin B<sub>12</sub> was markedly reduced by aureomycin.

Biely and March (1951) have observed considerable differences in response of chicks to aureomycin. When they fed rations which contained vitamins above levels recommended by the National Research Council, no increase in growth occurred following the addition of aureomycin to the experimental ration. If riboflavin, nicotinic acid and folic acid were omitted individually, a significant decrease in growth re-

sulted. The addition of aureomycin to these vitamin deficient rations stimulated growth in each case so that the weights attained were similar to those fed the complete basal ration.

Kratzer et al. (1951) fed droppings from month-old chicks which had been receiving a normal diet or a diet supplemented with aureomycin to day-old chicks for three days. The droppings (fresh or autoclaved) from chicks fed aureomycin showed less growth depression than droppings from chicks receiving the normal ration.

While most investigators were concerned mostly with growth, Slinger and coworkers (1951) reported a condition in turkeys known as "white feathers". This condition occurred when the turkeys received rations containing sunflower seed oil meal but was not shown when they received soybean oil meal. The addition of APF to the sunflower oil meal ration increased the severity of the white feather condition. These same authors (1950) had shown that one per cent lysine was sufficient to prevent white feathers with a ration containing 20 per cent protein. When the ration containing 20 per cent protein and 1.2 per cent lysine was supplemented with penicillin, aureomycin or APF concentrates, a very high incidence of white feathers resulted. These results indicate that the antibiotics and APF concentrates increased the lysine requirement of the poult for normal feather pigmentation. The

authors suggested that the requirement of lysine for growth takes precedence over the requirement for feather pigmentation because an increased growth occurred simultaneously with an increase of white feathers.

Couch (1951), Halick and Couch (1951) and Elam, Gee and Couch (1951a) conducted several experiments using antibiotics and vitamin B<sub>12</sub> to study the effect on the life cycle of the chick, egg production, and hatchability of the eggs. The feeding of antibiotics alone assisted in the depletion of the birds of vitamin B<sub>12</sub> and possibly the factor in liver "L". Elam et al. (1951a) observed that birds injected with penicillin after 14 weeks of age grew very well but no live chicks were obtained from pullets injected with penicillin. In this study it was observed that the vitamin B<sub>12</sub> content of liver, kidney, and egg yolk was directly related to the injection of this vitamin into the birds. However, Halick and Couch (1951) fed a practical all-mash ration and found that the hatchability of eggs from birds on this ration was higher than from birds fed the experimental ration and injected with vitamin B<sub>12</sub> even though the vitamin B<sub>12</sub> content of the eggs was lower than those from birds injected with vitamin B<sub>12</sub>.

Using another antibiotic, Sieburth (1951) found that terramycin stimulated an increase in the body weight of turkeys.



### Antibiotics in Swine Nutrition

Using a corn and peanut meal ration, Cunha et al. (1949a) showed that Lederle's APF and vitamins were different in their response in the pig. The addition of the APF supplement had considerable effect, especially with lighter pigs, while the addition of vitamin B<sub>12</sub> concentrate was of no apparent help, even when doubled. Burnside et al. (1949) observed that Lederle's APF increased the feeding value of peanut meal and soybean oil meal so that these plant supplements were similar in feeding value to fish meal. Again, with the same corn and peanut meal ration, Cunha and coworkers (1949b) found that Lederle's APF and vitamin B<sub>12</sub> spared the methionine needs of the pig. This latter study also indicated that Lederle's APF contains vitamin B<sub>12</sub> plus some other factor.

Luecke and coworkers (1950a) observed that streptomycin increased growth in pigs fed a ration of corn and soybean oil meal. Later, however, they reported that streptomycin produced no increase in growth while aureomycin and penicillin produced a significant response over the B vitamin supplemented basal ration (1950b). These same authors (1950c) studied the effect of the addition of low levels of antibiotics on the stimulation of growth when the corn and soybean oil meal ration contained inadequate amounts of niacin, pantothenic acid, and riboflavin. Again, streptomycin without added B vitamins did not significantly increase the rate of growth of young

pigs; however, there was some increase when B vitamins were fed with streptomycin.

Jukes et al. (1950), using a corn and peanut meal ration, and Carpenter (1950), using a corn and soybean oil meal ration, demonstrated that the growth stimulation obtained by feeding Lederle's APF supplements to pigs was due to the aureomycin they contained. Carpenter (1951) found that aureomycin, terramycin, penicillin, streptomycin, and chloromycetin were effective in stimulating growth in pigs fed a corn and soybean oil meal ration. Pigs supplemented with aureomycin, terramycin, and penicillin made the greatest growth response even though streptomycin and chloromycetin caused some increase in growth over the pigs fed the basal ration. Similar results were observed by Brown and Luther (1950) who used the same antibiotics, except for chloromycetin, as Carpenter. Cunha et al. (1950a, 1951) reported that penicillin was of no benefit in stimulating growth of swine fed a ration of corn and peanut meal and that streptomycin was not as effective as a growth stimulant as aureomycin. These authors stated that the growth-promoting effect of various antibiotics varied with the species of animal and the nature of the basal ration.

Edwards and coworkers (1951) observed that vitamin B<sub>12</sub> gave the greatest growth response in pigs that had been depleted of this vitamin for six weeks. They treated Lederle's APF with 1.0 N NaOH for 30 minutes to destroy vitamin B<sub>12</sub> and most of

the antibiotic properties of aureomycin and fed some of the remaining material to pigs. The data indicate that the alkali-treated material contained a factor(s) which increased the rate of growth of pigs. The factor from the alkali-treated APF promoted growth and may have had a sparing effect upon the vitamin B<sub>12</sub> needs of the pig.

Wahlstrom and Johnson (1951a) found that aureomycin caused an increase in daily gain over the basal group when synthetic rations were fed; however, the addition of aureomycin to the ration of pigs not getting vitamin B<sub>12</sub> did not prevent vitamin B<sub>12</sub> deficiency symptoms. The injection of vitamin B<sub>12</sub> to these pigs after 49 days resulted in an increase in average daily gain and feed efficiency.

Shefchik et al. (1950) and Nesheim et al. (1950) observed that aureomycin and streptomycin stimulated growth in two-day old pigs fed a "synthetic" milk. Shefchik stated that a combination of streptomycin and aureomycin gave the best results. Nesheim et al. reported that streptomycin stimulated growth when alpha-protein was used in the "synthetic" milk; but when vitamin-free casein was used, penicillin, aureomycin or streptomycin did not stimulate growth significantly above the basal ration.

Speer et al. (1950) reported an experiment in which the addition of aureomycin (5 or 10 milligrams per pound) had no effect on the growth rate. The authors explain this lack of

response to aureomycin by the "disease level" theory in which healthy pigs do not respond to aureomycin as well as unthrifty pigs in unsanitary surroundings. The pens had been cleaned daily to reduce coprophagy.

Cunha et al. (1950b) report that pigs fed a 19.6 per cent protein ration without B vitamins did not gain as rapidly as pigs fed 12.2 per cent protein ration plus APF and B vitamins. They suggested that the accepted values for protein requirements of swine may need to be re-evaluated by using adequate amounts of vitamin B<sub>12</sub> in the ration as well as other factors present in Lederle's APF supplement.

Miller and coworkers (1951) obtained about the same growth response by feeding sulfathalidine as was obtained from a combination of vitamin B<sub>12</sub> and streptomycin. Growth in both cases was much higher than that obtained with pigs by the addition of vitamin B<sub>12</sub> alone.

Wahlstrom and Johnson (1951b) reported that the addition of aureomycin to an alpha-protein "synthetic milk" diet, which was used to produce vitamin B<sub>12</sub> deficiency, did not give a beneficial response, either before or after the deficiency existed. The injection of 20 milligrams of cortisone daily into vitamin B<sub>12</sub> deficient pigs enhanced the deficiency state rather than having any curative effect.

### Antibiotics in Ruminant Nutrition

Sulfa drugs are used for treating various ailments in the ruminants and are usually given orally. Stableforth and Hignett (1942) observed with dairy cows that sulphanilamide caused sleepiness, anorexia, some incoordination, diarrhea and a loss of milk production, if the dosage level was greater than one gram per ten pounds of body weight. Oyaert, Quin and Clark (1951) used various amounts of sulphanilamide in in vitro and in in vivo studies with sheep and ruminal ingesta. The in vivo work confirmed the in vitro studies by showing that sulphanilamide depressed cellulose digestion and this in turn was reflected in decreased appetite. The cotton thread technique was used for digestion of cellulose in vitro and sulphanilamide caused retardation of cellulose digestion almost linear to the amount of sulphanilamide added. Extra glucose exerted an antagonistic action to that of sulphanilamide. Johnson et al. (1947) observed that nicotinic acid was excreted in the urine of two newborn calves fed a nicotinic acid-free diet. The addition of one per cent sulfathalidine had no effect on the growth of the animals or excretion of nicotinic acid over a 2.5-week period even though the bacterial count of the feces decreased about 80 per cent. Teeri et al. (1950) used the same drug and noted a slight decrease in the fecal excretion of thiamine but the total excretions of thiamine and pantothenic acid were greater than the dietary intakes, indicating rumen or intestinal synthesis of these vitamins.

Antibiotics and APF supplements have proved to be very successful in swine and poultry nutrition. Consequently, investigations have been conducted or are in progress to determine the growth-stimulating properties of these materials in calves and older cattle. Rusoff and Haq (1950, 1951a) did not observe an increase in growth when APF (Merck) was added to the ration of dairy calves weaned at 28 days of age. Similar observations were made by Williams and Knodt (1951) with slightly younger calves and by Hibbs and Pounden (1950) who fed APF to calves which had been rumen-inoculated with cud material.

Loosli and Wallace (1950) obtained an increase in growth when Lederle's APF or crystalline aureomycin was included in the diet of young dairy calves. The incidence and severity of diarrhea also was reduced in the calves. The authors suggested that the responses may be largely the result of an antibiotic effect of aureomycin. Loosli et al. (1951) observed that the Lederle antibiotic supplement fed to young calves at the rate of two per cent of the ration caused an increase in growth over that obtained with the controls. The calves getting the supplement used 40 per cent more feed but required less total digestible nutrients to make one pound of gain. Studies of the rumen microflora failed to reveal any striking difference in the total count or morphological types.

Rusoff (1951b) fed Lederle's APF at two per cent level to 14-week old calves and obtained a 35 per cent increase in

growth over the controls for the next six weeks, after which the daily gains for both groups were the same.

Murley et al. (1951a) and Rusoff et al. (1951c, 1951d) fed crystalline aureomycin to newborn calves. There was an increase in the rate of gain and feed utilization in the supplemented groups over that of the controls. Rusoff et al. (1951c, 1951d) observed that the Jersey calves receiving either Lederle's APF or crystalline aureomycin gained 25 per cent more than the controls while the Holstein calves only showed a 15 per cent increase over the controls.

Jacobson et al. (1951) and Bartley et al. (1951) observed an increase in the growth of calves when aureomycin was included in the ration. Bartley et al. fed one 16-week old calf 2,500 milligrams of aureomycin daily for four weeks without any deleterious effects on feed consumption, rumination or growth.

Voelker and Cason (1951) obtained increases in the rate of growth of experimental calves over control groups with both terramycin or aureomycin (Aurofac).

Neumann and coworkers (1951) did not observe any beneficial effect when aureomycin, either in crystalline form or as Aurofac, was fed to yearling beef heifers for 150 days. There was a slight reduction in the appetite for a few days.

Murley et al. (1951b) found no difference in the urinary content of reducing sugars and nitrogen or the fecal content

of dry matter, nitrogen, reducing sugars, ether extract and ash of calves fed aureomycin.

Bell et al. (1951) fed 0.6 grams of crystalline aureomycin daily to yearling steers and observed a marked anorexia and diarrhea within 48 to 72 hours after feeding. This condition persisted for several days after the aureomycin was discontinued. Some of the steers showed these symptoms even when the amount fed daily was reduced to 0.2 grams. The authors conducted a digestion trial and found that the ingestion of 0.2 grams of aureomycin daily apparently appeared to decrease the digestibility of dry matter 15 per cent and crude fiber 50 per cent.

Using aureomycin, penicillin, and streptomycin in feeding trials with fattening lambs, Colby et al. (1950) found that lambs fed 100 milligrams of aureomycin daily went almost entirely off-feed and lost weight. The lambs fed penicillin went off-feed and had diarrhea for a week but gradually recovered. Streptomycin was the least severe of the antibiotics; however, all groups fed antibiotics gained less than the controls. Jordan and Bell (1951) also fed aureomycin to suckling and fattening lambs but could not confirm the results of Colby et al. The lambs receiving the aureomycin made faster and more efficient gains than the controls.

Wasserman et al. (1952) made an in vitro study of cellulose digestion with rumen microorganisms in the presence of penicillin, streptomycin, neomycin or chloromycetin. In the



concentrations used, penicillin stimulated cellulolytic rumen microorganisms at the lower concentrations, neomycin was stimulatory in all concentrations, streptomycin was slightly stimulatory in the lowest concentration and chloromycetin adversely affected the microorganisms. As the cellulolytic activity increased, a corresponding increase in the bacterial count was observed.

#### Antibiotics in Other Nutritional Studies

Emerson and Smith (1945) produced a biotin deficiency in rats by the oral administration of streptomycin. The rats responded to biotin therapy. Miller (1945) observed signs of nutritional deficiency in rats which could be corrected by feeding biotin and folic acid. He used a purified diet with all the known growth factors added and then added succinylsulfathiazole or phthalylsulfathiazole to the diet. Lower levels of biotin, folic acid and pantothenic acid were excreted in feces of rats fed the diets containing the drugs than were excreted by the rats on the control diet. Grundy et al. (1947) found a decreased fecal excretion of the L. casei factor and biotin from human beings fed sulfathalidine. In the above studies a decrease in the number of coliform organisms occurred simultaneously with the nutritional deficiency or lowered excretion of the vitamin studied.

Waisman and coworkers (1951) found that 5 to 10 milligrams of aureomycin per 100 grams of purified diet were effective in

overcoming toxic signs of folic acid deficiency produced by including aminopterin in the diet. Aureomycin had no effect on this deficiency when it was caused by the use of sulfa-phthalidine in the diet. These workers found that the citrovorum-factor concentrates were more efficient than folic acid in overcoming the deficiency caused by aminopterin or sulfa drugs. Schwarz (1951) noted that cecal contents from rats on the basal diet had a higher proportion of the citrovorum factor in the inactive form than in the active form; however, when aureomycin was added to the diet, only the active citrovorum factor was found. The same relationship was observed in the liver on both diets as was observed in the cecal contents.

According to Linkswiler et al. (1951), aureomycin caused a marked increase in growth of rats fed pyridoxal or pyridoxamine, in fact, growth was approximately the same for the three forms of vitamin B<sub>6</sub> when fed in presence of aureomycin.

Swick, Lih and Baumann (1951) and Lih and Baumann (1951) obtained an increase in the growth of rats receiving limited amounts of thiamine, riboflavin or pantothenic acid by the addition of penicillin, aureomycin or streptomycin to the diet. The antibiotics were most effective in diets which contained only enough vitamins for one-half maximum growth and were about equal to the growth obtained when the vitamin content was doubled or trebled in the absence of the antibiotic. The

effectiveness of the antibiotic varied with the vitamin deficiency: penicillin gave the best response in thiamine deficiency, penicillin and aureomycin gave the same results in riboflavin deficiency, while aureomycin and streptomycin were superior to penicillin in pantothenic acid deficiency.

In the microbiological assay for nicotinic acid with Lactobacillus arabinosus, Teeri and Josselyn (1949) and Teply et al. (1943) found that the results were not affected by sulfa drugs if the medium contains p-amino benzoic acid. In the case of higher concentration of the sulfa drugs, the effect of the drugs could be prevented by increasing the amount of p-amino benzoic acid in the basal medium.

Marx and Wainfan (1951) observed that approximately 15 per cent of the cholesterol fed to mice on a high cholesterol diet was destroyed or modified. When one per cent sulfasuxidine and 0.04 per cent streptomycin were added to the diet, the total cholesterol ingested was recovered within limits of error of the method. Results indicate that microorganisms in the intestine are primarily responsible for the destruction or modification of cholesterol.

Davis and Chow (1951) using Co<sup>60</sup> in the diet of rats found that aureomycin caused an increase in the radioactivity and microbiological activity or the vitamin B<sub>12</sub> content of the feces.

Meites and Ogle (1951) studied the effect of penicillin, neomycin and streptomycin in counteracting the retardation

of growth caused by feeding Protomone in thyrotoxic amounts. Penicillin and neomycin, but not streptomycin, were effective in overcoming the growth inhibition when the ration was adequate in vitamin B<sub>12</sub>; however, all three antibiotics were only partially effective when the ration was deficient in vitamin B<sub>12</sub>. Protomone increased basal oxygen consumption 74 per cent, whereas the antibiotics were ineffective.

Sutherland and coworkers (1951) observed that the ingestion of therapeutic levels of aureomycin by normal dogs and rats did not cause fatty liver. Histological studies were made from needle biopsy in dogs and necropsy from rats. They also fed aureomycin to three healthy human beings and did not find any liver abnormality as shown by liver function tests.

#### Effect of Antibiotics on Bacteria

In 1945, Miller observed that either succinylsulfathiazole or phthalylsulfathiazole caused a noticeable drop in the number of coliform microorganisms in the feces of rats; although, there was not a significant decrease in the total bacterial population. Johnson and coworkers (1947) noted that the bacterial count of feces from calves decreased about 80 per cent when one per cent sulfathalidine was added to the ration. The sulfathalidine added to the ration had no effect on the growth of the animal or on the excretion of nicotinic acid. Grundy et al. (1947) also observed a marked and immediate

decrease in the number of coliform microorganisms in human beings when sulfathalidine was included in the diet. Along with the decrease in the number of coliform microorganisms, there was a decreased bacterial synthesis of folic acid and biotin.

Moore et al. (1946) produced a marked reduction of coliform bacteria in the feces of chickens by the oral administration of streptomycin. Emerson and Smith (1945) observed a similar effect in their studies with rats; however, the coliform organisms increased gradually to normal within a few days, indicating a development of fastness to the drug. Spaulding et al. (1949) found that bacteria readily developed resistance to streptomycin, even in presence of sulfathalidine. Kane and Foley (1947) observed that E. coli could be eliminated from the stool of human beings after two days by the oral administration of streptomycin; also, it could be kept free of these microorganisms as long as streptomycin was present. The microorganisms would reappear as soon as the administration of the antibiotic ceased. A similar observation of this latter fact was made by Hamburger and Berman (1950).

Bierman and Jawetz (1951) found that aureomycin caused a prompt disappearance of coliform microorganisms from the human stool with a marked reduction in the usual flora, but a normal flora returned within 24 to 48 hours after discontinuing the antibiotic. Williams et al. (1951), using chickens, observed that the total numbers of coliform and lactic acid

bacteria were not significantly changed but the total numbers of anaerobic bacteria were reduced 150- to 200-fold by aureomycin. Hemolytic clostridia were eliminated almost completely from the intestinal contents and feces of chicks by feeding aureomycin. McVay and coworkers (1951), using patients with cecostomies and transverse or sigmoid colostomies, found that aureomycin appeared rapidly in the intestine and maintained a significant intestinal bacterial flora. Only Streptococcus faecalis was not significantly reduced by the administration of aureomycin. This fact also was reported by Metzger and Shapse (1950). Bartley et al. (1951) fed aureomycin to calves but could not find any consistent microscopic differences between the rumen microflora in the control and aureomycin-fed calves. Neumann and coworkers (1951) noted that the total counts were about the same for the control as for the aureomycin-fed heifers, but the types found in the heifers receiving aureomycin were much less diverse, suggesting that the normal rumen flora had been disturbed. Wahlstrom and Johnson (1951) observed that chloramphenicol decreased the number of coliform bacteria in feces from pigs; but had no apparent effect after three weeks. They also noted that aureomycin, streptomycin, and penicillin appeared to have no effect on the coliform, lactobacilli or yeast cell counts. Sborov et al. (1951) found that aureomycin or terramycin were effective in inhibiting urobilinogen formation in the bile, urine and feces of human

beings. This inhibition was associated with a disappearance of coliform microorganisms and a reduction or disappearance of clostridia in the feces; consequently, the data lend support to the bacterial concept of urobilinogen formation.

Elam et al. (1951a) and Couch and coworkers (1951) observed an increase in the total number of intestinal microorganisms when penicillin was included in the diet of chicks. Jawetz et al. (1950) made some in vitro studies of the combined action of penicillin with streptomycin or chloromycetin on enterococci. They observed that streptomycin failed to inhibit the growth of enterococci, while penicillin decreased the growth of enterococci for 48 hours and then gradually increased but stayed below the controls for the drug. Combination of streptomycin and penicillin produced complete sterilization of the medium which suggests there may be a synergism of the two drugs; that is, increase in rate of bactericidal action beyond the optimum obtained with penicillin alone. A penicillin and chloromycetin combination was not as effective as the combination of penicillin and streptomycin.

#### Theories Proposed for the Action of Antibiotics

Many investigations have been carried on during the past several years using antibiotics. The investigators observed and commented on their results, but they did not have a real explanation to what actually happened. In 1949, Waksman, one

of the pioneers in the antibiotic field, proposed several theories to explain the action of antibiotics: first, antibiotics may interfere with some of the metabolic processes of the bacterial cell by substituting for one of its essential nutrients; second, may interfere with various enzymatic systems, especially the respiratory mechanism of the bacterial cell; third, may prevent the synthesis of some essential metabolite by the bacterial cell; and fourth, may act as a detergent and affect the surface tension of the bacterial cells.

Since these theories were first proposed by Waksman, reports have appeared in the literature, which confirm to some extent the theories he proposed. In fact, Moore et al. (1946) suggested that sulfasuxidine and streptomycin increased growth in chicks by inhibiting the intestinal bacteria that were either producing toxic materials or were rendering certain dietary vitamins unavailable for utilization. Sieburth and coworkers (1951) also stated that antibiotics promote growth by inhibiting or preventing the production of bacterial toxins in the intestine. Their data supported this theory by showing a suppressed growth of Clostridia perfringens which has been shown to cause enterotoxemia in sheep. Biely and March (1951), Swick et al. (1951), Linkswiler et al. (1951), and Bratzler and Black (1951) postulated that antibiotics exert their growth-promoting effect by reducing the bacterial flora which might compete with the host for nutrients, thereby making more nu-



trients available for utilization by the host. Biely and March (1951) also stated that the antibiotic at proper levels may permit the proliferation of microorganisms which synthesize the vitamins required by the animal. Groschke and Evans (1950) and Slinger et al. (1951) suggested that antibiotics may aid in the establishment of more favorable types of bacteria which stimulate growth of the host as a result of intestinal synthesis of unidentified factors required by the chick.

The inhibition of the formation of certain adaptive enzymes in E. coli was reported by Hahn and Wisseman (1951) who also thought this effect probably pointed toward a general interference with protein metabolism of the organism. Loomis (1950) suggested that aureomycin may exert its antibacterial activity through an inhibition of aerobic phosphorylation. He observed that aureomycin depressed phosphorylation without inhibiting respiration while penicillin, chloromycetin, and sulfadiazine were inactive when tested in a similar fashion. Weinberg (1952) observed excellent growth of bacteria when phosphate was included in the medium and that terramycin, aureomycin, chloramphenicol and streptomycin inhibited growth while penicillin and bacitracin were inactive in the presence of phosphate. When phosphate was omitted from the medium, growth of the bacteria was moderate and aureomycin became inactive while streptomycin and chloramphenicol remained antagonistic to the bacteria. This suggested that the aureomycin

exerted its antibiotic effect by acting on the phosphorylation system of the bacteria.

Lichstein and Gilfillan (1951) found that streptomycin markedly inhibited growth of S. fragilis when it was growing in a synthetic medium containing B-alanine as the precursor for pantothenate, but no effect on growth was observed when pantothenic acid was included in the medium. From this fact, they postulated that streptomycin may inhibit the coupling of B-alanine and pantoyl lactone to form pantothenic acid.

Ely (1951) found certain surfactants would produce an increased growth response in chicks similar to that observed when antibiotics are used. Luecke et al. (1952) observed that Ethomid C/15, a surface active agent, stimulated growth in swine equal to that obtained when aureomycin was included in the ration.

Berg et al. (1950) stated that the effect of the antibiotic in promoting growth is dependent on the material continuously present in the diet. He observed a cessation of the accelerated growth response when aureomycin was removed from the ration of the chicks. Cunha and coworkers (1951) reported that the growth-promoting effect of various antibiotics will vary with the species of animal and the nature of the basal ration.

Elam et al. (1951b) surmised that the antibiotic molecule or a fragment of it might act as a metabolite within the body of the chick, since parenteral administration of antibiotics

and autoclaved penicillin increased the rate of growth and had little effect on the fecal microflora count.

#### pH of Rumen Contents

Phillipson (1942a) found that the rate of production of volatile acids in the rumen of sheep depended on the diet. Changes in pH reflected the fluctuations in the quantities of organic acids that accumulate in the ingesta as a result of fermentation of the food in the rumen. The greatest fluctuations were observed when the diet was high in soluble carbohydrates and least when the diet was fibrous. Gall et al. (1949) observed that the type of ration had some effect on the pH of the rumen contents in cattle. The pH values obtained for a fattening ration were 6.3 to 6.7, a breeding ration 7.0 to 7.3, and a dairy ration 6.8 to 7.3. Kick et al. (1938) reported that the pH of rumen ingesta varied from 5.5 to 7.7 depending on the ration. The ingesta was most alkaline when alfalfa was fed alone and the pH decreased as the amount of corn was increased in the ration. Similar results were noted by Hunt and coworkers (1943). Smith (1941) also obtained lower pH values when beet pulp was fed with alfalfa hay than when the hay was fed alone. When the ratio of starch to roughage was increased in the ration, Burroughs et al. (1949) found lower rumen pH values in a shorter time following feed consumption. Kick et al. (1938), Monroe and Perkins (1939), Wegner et al. (1941), Smith (1941) and Myburgh and Quin (1943)

reported that the pH declined for about four hours with a gradual increase toward alkalinity following the decline. This cycle is repeated after each feeding.

Jacobson et al. (1942) working with cattle and Phillipson (1942a) working with sheep observed that the rumen contents were more acid when the animals were grazing than when they were stall-fed. Gall et al. (1949) did not notice any difference in the pH when the animals were on pasture or were on the winter ration.

Roine and Elvehjem (1950) stated that the pH was not determined by the food but by the kind of microflora which developed in the digestive tract. Gall et al. (1949) observed that the fast-growing organisms usually ferment glucose with the production of high acidity and turbidity while the slow-growing cultures seldom exhibit much turbidity or lowered pH. Huhtanen and coworkers (1951) found the most common types of organisms in calves' rumens were fast-growing lactic acid forming bacteria which produce a low pH and heavy turbidity. The older cattle had types which grew more slowly with little turbidity.

Coop (1949) working with sheep and Stone (1949) working with cattle observed that during fasting, the pH and the production of the volatile fatty acids in the rumen ingesta decreased. Coop also reported that it took from 12 to 24 hours for sheep to recover to normal ruminal activity following starvation for several days. Jacobson et al. (1942) using an

in vitro method observed that the rumen contents of cattle were very alkaline and gas production was low when the feed was withheld for 24 hours.

Meites et al. (1951) while studying in vitro digestion of cellulose by rumen microorganisms found that the optimum pH lies between 4.53 and 7.35. Above pH 7.35, digestion dropped sharply. Oyaert et al. (1951), Clark et al. (1951a) and Clark and Lombard (1951a) observed a decrease in the motility of the sheep's rumen as the alkalinity of the ruminal ingesta increased. When the pH reached 7.5 to 7.8, a complete rumen paralysis resulted which could be relieved by administering dilute acetic acid either orally or by intravenous injection. Clark et al. (1951a) also observed a sharp rise in pH and an increase in ammonia when the sheep were dosed with urea.

Monroe and Perkins (1939) and Smith (1941) noted that the pH values varied in different regions of the rumen, especially soon after feeding. Clark and Lombard (1951b) found that the pH values of samples taken through the fistula were lower than those taken by stomach tube.

Phillipson and McAnally (1942b) observed that volatile fatty acids were the result of fermentation of carbohydrates in the rumen. Glucose, fructose and cane sugar fermented more rapidly than maltose, lactose and galactose, while the fermentation of starch and cellulose was much slower and the production of volatile fatty acids was prolonged. Elsdon et al.

(1946) observed that volatile fatty acids (acetic, propionic and butyric acids) occur in the rumens or large intestines of seven species of animals. Gray (1948) found that both acetic and propionic acids are absorbed readily from the rumen at an acid reaction. Acetic acid was not absorbed from an "isolated" rumen when the reaction was made slightly alkaline by a solution of sodium acetate.

Barcroft et al. (1944) stated that the volatile fatty acids were absorbed through the rumen wall in order of acetic > propionic > butyric. Johnson (1951) showed the rate of absorption of these acids to be in the order of butyric > propionic > acetic.

Myburgh and Quin (1943) noted that the rumen material was well buffered between pH 6.8 to 7.8 against N HCl or N NaOH, whereas beyond this range, both on alkaline and acid side, the efficiency of the buffering action was distinctly reduced. Reid and Huffman (1949) found that the pH of saliva was 8.53 to 8.71. They believed that this secretion is responsible for the maintenance of a medium which appears to be optimal for microbial activity and chemical changes in the rumen. Clark and Lombard (1951b) stated that the normal regulation of pH of ruminal ingesta depends on the interaction between organic acids from microbial activity of carbohydrate and sodium bicarbonate of saliva or by selective absorption of volatile fatty acids from the rumen.

### Rate of Passage of Materials Through the Digestive Tract

The length of time a feed remains in the rumen would appear to influence its eventual utilization. Various attempts have been made to study this factor. Fish (1923) and Reed et al. (1928), in studies with cattle, found that Sudan III dye first appeared in the feces within 15 to 17 hours and that 50 to 60 hours were required for complete passage through the tract. Balch (1951a) using stained particles noted that the marker first occurred in the feces 12 to 24 hours after feeding. Approximately 80 per cent of the stained residues appeared within 70 to 90 hours while it took seven to ten days to complete the excretion of all of the stained particles. Iron oxide and rubber disks were used by Moore and Winter (1934) who observed that most of these materials had passed through the tract within 36 to 40 hours while it took five to six days to complete the elimination of the materials. Hoelzel (1930) noted that the rate of passage was found to be more or less proportional to the specific gravity of the test materials, the heavier materials required more time than the lighter materials. Also, the rate of passage varied considerably with different species and individuals. Amadon (1926) stated that the weight of the feed determined the route to be followed; in that, the light food went to the back part of the rumen while a portion of the heavy food went directly into the reticulum. Mitchell et al. (1928) suggested that the rate of feeding may be an

important factor in determining the length of time required for food to pass through the digestive tract.

Burroughs et al. (1946) studied two methods for measuring the rate of passage of food through the rumen of cattle. The first method was a mathematical approach which was based upon the nutrient composition of the feeds ingested and the total weights of various nutrients found in the rumen at a given time. The second method consisted of the physical separation of the dried rumen contents by fanning or by floatation with water.

Phillipson (1948) using sheep with a duodenal cannulae observed from quantities of digesta collected for 1.5 hours at different intervals throughout the day that there is no simple relation between total dry matter of the food and the flow of the digesta.

Quin and Van der Wath (1938) found that after three to four days of complete starvation of well nourished animals, a complete cessation of ruminal movement may take place at any time. On subsequent feeding of such starved animals, rumen motility usually reappeared after considerable delay, as the appetite itself may be seriously disturbed after prolonged fasting. Coop (1949) observed that the type of ration had an influence on the rate of activity in the rumen.



### Microorganisms in the Rumen

The ruminant is unique from other animals in that it has four stomachs. It relies mainly upon its fermentation vat for the nutrients which are made available by bacterial fermentation and decomposition of feedstuffs in this chamber.

Numbers and types. Baker (1942a) and Gall et al. (1947) stated that the number of bacterial cells in each milliliter of rumen fluid is expressed in billions. These values were obtained by the microscopic examination of formalized rumen-fluid samples. Baker (1947) stated that both pre-cultural and direct microscopic methods of evaluating bacterial counts were superior to the plate method which gave an unreliable estimate of the kinds and numbers of organisms concerned. Gall et al. (1947) and Moir and Williams (1950) observed that samples of rumen juice obtained by use of the stomach tube gave consistently lower bacterial counts than samples taken directly from the rumen through a rumen fistula. Moir and Williams stated that the method was constant and compares favorably with the fistula method.

Henneberg (1922) was the first investigator to apply direct microscopy to detect the microorganisms concerned in the digestion of structural cellulose. He stained the organisms with iodine and called the organisms that gave a blue color, iodophiles. Of the iodophilic organisms, Baker (1942a,

1943) has distinguished at least five species by their large size:

1. Oscillospira guillermondi - A colorless spore-forming oscillarian.
2. A giant Spirillum - Divided by transverse septa into spherical or ovoidal compartments.
3. Large Sarcina Packets.
4. An unidentified navicular organism forming rosette-shaped oscillations of 5 to 30 units.
5. Coccoid chains of 2 to 8 units.

In more recent investigations Bryant (1951) found 50 to 60 different kinds of bacteria in rumen contents. The morphological types included various rods, cocci, oval-shaped and spirochetes. There was considerable variability in the frequency of occurrence of the different kinds of bacteria but about one-fourth were found with some regularity. Gall and Huhtanen (1951) designed some criteria for determining a true rumen organism. These are: (a) anaerobiosis, (b) presence in numbers of one million or more per gram of fresh rumen contents, (c) isolation of a similar type bacterium at least ten times from at least two animals, (d) isolation from animals in at least two geographical locations, and (e) production by the organisms of end-products found in the rumen from substrates found in the rumen. They also observed that at least 99 per cent of the organisms isolated from the rumen were anaerobes. Huhtanen et al. (1951) found nine organisms characteristic of calves' rumens that almost never occur in the rumen of healthy

adult cattle maintained on a balanced practical ration. The 11 organisms usually found in the rumen of adult cattle begin to appear in the rumen of calves as early as two months of age, depending on the ration, and become more prominent as the calf approaches maturity. The most common types found in the calves' rumens were fast-growing lactic acid-forming bacteria which produce low pH and heavy turbidity while the adult types grew more slowly with little turbidity and a higher pH.

Baker (1942a, 1943), Hoflund and Hedstrom (1948), Johnson et al. (1944) and Uzzell et al. (1949) found that protozoa are another group of organisms that are normal inhabitants of the rumen.

#### Effect of Various Rations on Rumen Microorganisms

Bortree et al. (1946) found by taking samples every two hours for 10 to 12 hours that an increase in the numbers of bacteria occurred about two hours after feeding and remained high for several hours after which there was a gradual return to normal. The addition of glucose to the ration caused the counts to increase about 100 per cent over those on hay alone. These workers only counted iodophiles, therefore, the changes do not reflect changes in the total population. The addition of grain or a readily fermentable carbohydrate decreased the time required to reach the peak of the bacterial population in the rumen. In 1948, Bortree et al. found that an iodine staining "giant spirillum" occurred in the rumen when methion-

ine was added to a ration consisting of corn, starch, glucose and minerals.

Pounden and Hibbs (1948a, 1948b, 1949, 1950b) were successful in establishing certain microorganisms in the rumen of young calves by inoculating them with cud material obtained from mature animals. The inoculations assisted in the establishment of protozoa in the rumens of calves eating hay alone or both hay and grain. No protozoa or hay organisms were present in calves on grain alone. Very small gram-negative organisms and a moderate number of protozoa were prevalent in calves on rations containing hay alone or high proportions of hay. Small gram-positive short rods or cocci were observed in increasing proportions after the addition of grain to rations of hay. Rations high in grain or low in roughage depressed the numbers of rumen microorganisms which were characteristically associated with relatively high roughage ingestion. They also found that the rumen microorganisms can be established in young calves on pasture if grain is not fed in excessive amounts. Conrad et al. (1950) observed that inoculated calves digested a significantly higher percentage of cellulose and dry matter than the calves not inoculated.

Gall (1949a) and Gall et al. (1949c) noted that animals on pasture had higher bacterial counts than animals fed in the barn. Gall et al. (1949c) postulated that the fast-growing organisms which break down glucose use starch and other soluble

carbohydrates in the rumen; while slower growing bacteria, which clump on cotton, might be cellulose digesters. Gall (1949a) found that cobalt-deficient sheep showed a simpler flora and lower bacterial count than sheep on the same ration plus cobalt. Gall et al. (1951) studied the effect of purified diets upon the rumen flora and found that sheep fed the urea-without-sulphur ration supported a rumen bacterial population consisting almost entirely of facultative anaerobes in place of the obligate anaerobes usually found in the rumen. Different physiological types of bacteria were found in the rumen of sheep fed casein than in sheep fed urea plus sulphur.

Burroughs et al. (1950a, 1950d, 1950e, 1951a, 1951b) and Arias et al. (1951) used an artificial rumen to study the factors affecting cellulose digestion by rumen microorganisms. They (1950a) found that a complex salt solution, ash of alfalfa extract, autoclaved rumen liquid and an autoclaved water extract of manure were beneficial in aiding rumen microorganisms to digest cellulose. There was a decrease in bacterial population and a decrease in individual size of bacteria without any noticeable change in predominating types in the flasks promoting poor cellulose digestion. In recovery experiments in which any one of the above substances was added to the flask, cellulose digestion progressively improved; the size and the numbers of bacteria increased but the types of bacteria were the same. Burroughs et al. (1950d) found that protozoa were always present in the flasks showing some cellulose digestion.

Burroughs et al. (1950d, 1951a) stated that rumen microorganisms had three general nutrient requirements; first, related to energy which is the motivating force for rumen bacteria to digest compounds like cellulose; second, related to protein or elements such as nitrogen which are eventually synthesized to protein; and third, inorganic nutrients which are involved in enzymes or enzyme systems of the organism. Burroughs et al. (1951b) found that phosphorus and iron were effective in stimulating urea utilization and cellulose digestion by rumen microorganisms. Arias et al. (1951), using six different carbohydrate sources for energy, observed that small amounts of a readily available carbohydrate aided in cellulose digestion which in turn increased urea utilization; whereas, large amounts of such materials inhibited cellulose digestion.

Bentley et al. (1951) used the artificial rumen technique, similar to that devised by Burroughs, to study the effect of feeding poor quality hay on the biochemical functions of the rumen microorganisms. They found that the activity of the rumen microorganisms from a steer receiving a poor hay ration had decreased ammonia utilization by 25 per cent at the end of one week and the rate of cellulose digestion had dropped 90 per cent at the end of four weeks. Volatile fatty acid production dropped 30 per cent and riboflavin synthesis was reduced 14 per cent. When the steer was allowed free choice of a mineral mixture consisting of two parts of steam bonemeal, two parts of ground limestone and one part of salt, the activity of the

microorganisms was similar to that observed when alfalfa hay was fed. When the mineral mixture was removed from the ration, the activity of the microorganisms was lowered again. Preliminary evidence indicated that the low phosphorus content of the poor quality hay was a limiting factor in cellulose digestion. Burroughs et al. (1950b), from their study with corncobs, suggested that the quality of roughages as applied to animal feeding may be dependent to a large extent upon the mineral makeup of the roughage supplying nutrients for the rumen microorganisms involved directly in roughage digestion. Burroughs et al. (1950c) found a decided decrease in the bacterial counts when starch was added to the roughage, but these counts increased when casein was added to the ration. These findings lend support to their theory that protein aids roughage digestion by furnishing an essential nutrient for the bacteria concerned directly in roughage digestion.

McNaught et al. (1950a) observed that 1,000 parts per million of iron, 25 parts per million of cobalt, 1,000 parts per million of copper and 2,000 parts per million of molybdenum definitely inhibited growth of rumen bacteria. It was shown that one to two parts per million of iron in rumen liquid produced good bacterial growth.

Depending on concentration used, Weinstein and McDonald (1945) found that both urea and urethane exert a bacteriostatic and bactericidal action on gram-negative bacteria and to a lesser degree on gram-positive organisms.

Thomas et al. (1951) observed that lambs on a sulfur-deficient ration gradually lost appetite, declined in body weight and finally died. There were marked changes in the types and numbers of rumen flora in the sulfur-deficient lambs.

Reed et al. (1949) found that sheep fed dry feed had coccal and oval cells and some giant iodophiles, while lambs receiving green feed had a greater proportion of rod-forms and an increase in spiral-forms. Moir and Williams (1950) observed a very high correlation between the levels of protein intake and the number of microorganisms in the rumen.

Quin (1943) and Quin et al. (1951) found that fasting caused a marked decrease in the ability of the rumen microflora to ferment glucose. Quin et al. (1951) observed that starvation caused a decrease in cellulose digestion; also, it affected the free organisms in the rumen liquid before those embedded in the plant particles were affected. They also observed that sheep automatically regulate the intake of protein to correspond with the ability of the flora to metabolize it.

Balch and Johnson (1951b) observed that the rate of breakdown of cellulose in the rumen of cattle was much faster in the ventral sac than the dorsal sac. They also noted that a low dry matter content of the ration favored the rapid breakdown of cellulose.



### Functions of Rumen or Intestinal Microorganisms

In 1939, Baker described a process of bacterial decomposition by formation of zones of erosion around the responsible microorganisms. The zones were studied by double refraction and the loss of double refraction indicated the region had been used up. Baker (1942b) made a direct microscopic examination in polarized light to study the decomposition of cellulose by iodophilic organisms on the surfaces and in the interstices of vegetable fragments or, in the case of starch, the organisms concerned would be lodged on the surface of the starch granule.

Hungate has made some very good studies concerning the ability of the rumen microorganisms to digest cellulose (1942, 1943, 1944, 1946, 1947, 1950). He (1944, 1946) postulated that the bacteria secreted an enzyme, cellulase, which diffused into the substrate, attacked the substrate to produce sugars which diffused back to the bacterial cell. A clear area developed around each colony which indicated that the cellulase was not free to diffuse until the substrate immediately adjacent was completely dissolved. Hungate (1947, 1950) was successful in isolating some microorganisms found in the rumen in pure culture. He isolated a cocci which was important in the decomposition of crude fiber in the rumen. He also isolated several of the rod-type organisms. One of these, a non-spore-forming rod, actively fermented cellulose with the forma-

tion of acetic and succinic acids. Another rod attacked many carbohydrates, including hemicelluloses, to form butyric acid. He also observed that some of the rods did not show an iodophilic reaction and stated that this feature alone was not an adequate index of the cellulose digesting ability of the microorganisms. Hungate (1942, 1943) found that the Diplodinium protozoa secreted cellulase and were capable of digesting cellulose while the Entodinium, Qsatricha, Dasytricha and Blitschlia do not. Burroughs et al. (1950d) using the artificial rumen technique always found protozoa present in the flasks showing some cellulose digestion.

The protozoa digest the bacteria in order to synthesize their own protein (Baker 1942b, 1942c, 1943). Johnson et al. (1944) observed a symbiotic relationship between bacteria and protozoa in the rumen. The greatest number of bacteria and fewest number of protozoa were present one hour after feeding, whereas the bacterial population gradually decreased and the protozoan population increased for about 16 hours. Their data are in agreement with the hypothesis that the nitrogen of the food is first synthesized into bacterial protein; then the protozoa use bacterial protein for their own use; and finally, the host digests protozoan protein and the remaining bacterial protein. Pounden et al. (1950a) examined contents from various parts of the digestive tract of cattle for the presence of four types of bacteria normally present in the rumen. They ob-

served that the large coccoids were found in all parts of the digestive tract, although fewer in the large intestine; the cigar-shaped organisms disappeared in the abomasum; the large or small rods disintegrated gradually as they reached the posterior part of the digestive tract; and protozoa were destroyed in the abomasum. Uzzell et al. (1949) observed that the stomach and small intestine of calves were devoid of protozoa at birth. They found 18 species of protozoa in 12 animals and agree with Baker and Pounden as to the fate of the protozoa in the abomasum.

Van der Wath (1948) observed that bacterial disintegration of starch began within five hours after feeding and was completed in 18 to 20 hours when sheep received starch regularly, but it took seven hours for disintegration to begin and 8 to 10 hours longer for disintegration to be completed in sheep not used to receiving starch. He also stated that the rate of breakdown of different carbohydrates depend upon the complexity of the molecule. Baker et al. (1950) observed that corn starch was broken down more readily than potato starch and in a shorter time in ruminants.

The ability of the ruminant to synthesize some of the vitamin B complex was first noted by Bechdel and Honeywell (1927). Hoflund and Hedstrom (1948) stated that the fungi, another type of organism in the rumen, synthesized the B vitamins and had the ability to synthesize amino acids. They also stated that the bacteria in the rumen were concerned primarily

with carbohydrate decomposition, particularly cellulose; while the infusoria (protozoa) assimilated vegetable protein and converted it into animal protein more suitable for the host.

More complete information on the role of microorganisms in the rumen may be obtained from the reviews by Hastings (1944), Elsdon and Phillipson (1948) and Johansson and Sarles (1949).

#### Formation of Protein in the Rumen

Zuntz (1891) first suggested that non-protein nitrogen might be converted to protein by the bacteria, which in turn was used by animals. Armsby (1911) concluded that non-protein nitrogen could serve as a partial substitute for protein for maintenance, milk production and growth when the level of protein in the ration was low and the other conditions were favorable. He stated, however, that non-protein nitrogenous substances were inferior in nutritive value to protein of an equivalent nitrogen content.

In 1939, a series of experiments using urea as the principal source of non-protein nitrogen were begun by Hart et al. and continued through the war years by Wegner et al. (1940a) and Mills et al. (1942). Using growing calves, Hart et al. (1939) found that urea and ammonium bicarbonate could be used as a partial supply of nitrogen in the ration. They also observed that the most efficient utilization came when some soluble sugar, such as corn molasses, was included in the

ration. Wegner et al. (1940a) using an in vitro technique presented evidence to show that rumen bacteria convert inorganic nitrogen to protein. They noted that the extent of disappearance of inorganic nitrogen depended on the amount of carbohydrate in the medium and not the source, except cellulose; also the decrease in ammonia could be accounted for by an increase in protein nitrogen.

Wegner et al. (1941a, 1941b) observed that the urea-nitrogen would always be hydrolyzed to ammonia within one hour after feeding. These workers used an animal with a rumen fistula from which the samples could be removed whenever desired. Wegner et al. (1941b) noted that the rate of conversion of urea-nitrogen to protein decreased whenever the protein level of the rumen ingesta became greater than 12 per cent. Mills et al. (1942) observed that the hydrolysis of urea to ammonia was delayed when urea was fed with hay and about one-half of the urea was found in the rumen six hours after feeding. When starch was added to the hay and urea ration, the urea was hydrolyzed within one hour after feeding and the ammonia formed from the hydrolysis would disappear from the rumen in six hours. As the ammonia level decreased, the protein level of the rumen increased. Mills et al. (1944) found that starch was superior to molasses in promoting protein synthesis in the rumen. The starch was less soluble and remained in the rumen longer than the molasses. Bell et al. (1951), using different

carbohydrate feeds in digestion studies with steers receiving urea nitrogen, found that nitrogen retention in the steers was greater when corn was added than when molasses was added to the ration.

Harris and Mitchell (1941a, 1941b) observed that urea added to the low nitrogen ration improved the digestibility of cellulose and itself was digested to the extent of 88 per cent. On calculation, they found that the biological value of urea nitrogen was 62, while the value for casein nitrogen was 79. Harris and Mitchell (1941b) found that as the amount of urea was increased to produce rations of higher protein equivalent, the average biological value decreased. Harris et al. (1943) observed that more true protein was found in the rumen of steers receiving urea than in those subsisting on the same low protein ration without urea. Johnson et al. (1942) noted that the protein is digested by the ruminant as it passes through the abomasum in the same manner as any other preformed protein. In this manner, the ruminant would actually digest approximately the same type or quality of protein irrespective of the sources of nitrogen, provided the total nitrogen consumed did not exceed the maximum amount which the microorganisms could utilize. They also stated that the biological value of nitrogen in rations containing 10 to 12 per cent protein is about 60. Moir and Williams (1950) found that about 50 per cent of the ingested protein was converted to bacterial protein.

They also noted that as the amount of protein in the ration increased, a definite decrease in biological value occurred.

Loosli and Harris (1945) obtained an increase in the rate of gain and nitrogen stored when urea was added to the basal ration. The addition of methionine to the urea ration increased the rate of gain and nitrogen retention to the same level as the linseed oil meal ration. They stated that it appeared likely that the protein formed in the rumen by bacterial action was deficient in methionine or that the diet was deficient, which could limit the quantity of protein synthesized. Reed et al. (1949) separated bacterial cells from rumen juice by use of a Sharples centrifuge. The rumen samples were obtained from sheep that were fed either dry or green feed. Both samples had about the same cysteine content but the lambs fed the green feed had a higher level of methionine. They concluded that the rumen bacterial protein must be regarded as low in digestibility, relatively high in biological value, but mildly deficient in methionine. Thomas et al. (1951) observed that lambs fed a sulfur-deficient ration containing urea lost appetite and body weight, became emaciated and finally died; also, the urea nitrogen was apparently not utilized, since the deficient lambs were consistently in negative nitrogen and sulfur balance. Block and Stekol (1950) and Block et al. (1951) fed radioactive sodium sulfate ( $S^{35}$ ) to a cow and a goat. They observed that both cystine and methion-

ine found in the cow's milk contained radioactive sulfur in appreciable amounts. In the case of the study with the goat, the radioactivity was the same for methionine and cystine in the milk, serum albumin and rumen. The results indicated that methionine and cystine were synthesized in the rumen at approximately the same rate and were used by the tissues to make new protein in quantities needed.

Loosli et al. (1949) working with sheep and Agrawala (1950) using steers found that the animals can synthesize considerable quantities of the essential amino acids in the rumen. In both instances a purified ration was used as the basal ration with urea as the sole source of nitrogen. Loosli et al. (1949) also reported that animals on a purified ration containing glycine as the only source of nitrogen synthesized the amino acids at a lower level.

Since the rumen contents are part of a moving system, heterogenous in character, and therefore difficult to sample, some investigators have developed in vitro techniques for conducting studies of rumen problems. Pearson and Smith (1943a) observed that the total nitrogen content of the rumen ingesta varied as much as 20 per cent when taken from four different locations in the rumen; while there was 16 per cent difference when the samples were taken at different depths. Pearson and Smith (1943a, 1943b, 1943c) filtered the rumen contents through muslin to remove the coarse particles and the resulting liquid was incubated at 39°C. for three to four hours under conditions



similar to those in the rumen. When the temperature was increased to 50° or 60°C., hydrolysis of the protein occurred. It was believed, therefore, that the changes which occurred in the incubated material during the first few hours after removal from the rumen closely resembled those which occur in vivo. They also stated that the synthesis of protein was microbiological as shown with toxic substances such as boric acid, quinone and sodium fluoride. When the concentration of these increased, hydrolysis of the protein occurred. McNaught et al. (1951b) separating bacterial cells in a Sharples centrifuge observed that 58 per cent of the bacterial protein was present in the liquid when it was removed from the rumen. The other 42 per cent was synthesized during incubation. Smith (1945) stated that the greatest part of synthesis occurred within the first two hours of incubation. Pearson and Smith (1943b) stated that the urease activity of rumen ingesta was so great that all the urea ever likely to be fed would be readily converted to ammonia in one hour. This fact has actually been observed by Wegner (1941a, 1941b) in their studies with the rumen fistula animal.

#### B-Complex Vitamin Synthesis in the Ruminant

Bechdel and Honeywell (1927) observed that cows maintained on a ration deficient in the vitamin B complex from growth through completion of the first lactation produced milk with vitamin B potency equal to that of the herd milk from cows

receiving a good winter ration. Bechdel et al. (1928) stated that the vitamin B complex was produced in the rumen by bacterial fermentation. Gall et al. (1951) have been successful in isolating some organisms responsible for the synthesis of some of the B-vitamin complex. Type RO-575 was the predominating organism and was found to be able to synthesize several members of the B-vitamin complex. This type was found mostly in urea plus sulphur ration and occasionally in the other rations. Type RO-T also was present in the sheep fed urea plus sulphur but it required several of the B vitamins for growth rather than synthesizing any. Type RO-C8 was found in animals receiving the urea plus sulphur and casein ration and was capable of synthesizing several of the B vitamins.

Thiamine synthesis. Hunt et al. (1941, 1943) observed a slight increase in the quantity of thiamine in the rumen about four hours after feeding but a decrease 16 hours after feeding. By increasing the amount of corn in the ration, a slight increase in thiamine in the rumen could be observed. Lardinois et al. (1944) could not find evidence for the synthesis of thiamine in the rumen. McElroy and Goss (1939, 1941a) found that thiamine was synthesized in the rumen of the sheep, but no thiamine could be detected in two cows with rumen fistulas fed the same deficient ration as the sheep. However, they did detect thiamine in the rumen contents of an intact cow and suggested that an artificial opening into the rumen may alter

the conditions in such a way as to make it unfavorable for the growth of rumen organisms capable of synthesizing thiamine. Johnson et al. (1941) observed that the rumen contents from goats, sheep and calves fed the same thiamine-deficient ration contained some thiamine. Teeri et al. (1950, 1951a, 1951b) noted that the thiamine excretion of heifers was always greater than the dietary intake. Kesler and Knodt (1950, 1951a, 1951b, 1951c) working with young dairy calves found that the concentration of thiamine in the digestive tract, especially the rumen, was greater than the feed consumed on a dry matter basis. After the calves were eight days old, the concentration of the vitamin in the digestive tract did not change with age. Also, no effect could be observed between the time of the last feeding and time of slaughter following the feeding. Wegner et al. (1940b) observed that thiamine added to the rumen was not destroyed and that there was an apparent stimulation of the other factors when thiamine was added.

Riboflavin synthesis. McElroy and Goss (1939, 1940a) found that the riboflavin content of the rumen ingesta in sheep increased 100-fold over that of the feed. Further evidence of riboflavin synthesis was presented by Wegner et al. (1940b, 1941c) and Hunt et al. (1941). Wegner et al. (1941c) observed that the riboflavin values of rumen contents decreased as the nitrogen level of the ration increased. Hunt et al. (1941) observed no increase in riboflavin when steers

were fed hay alone, but that riboflavin increased when corn was included in the ration. However, Lardinois et al. (1944) found that the addition of urea to a ration containing a readily fermentable carbohydrate definitely increased the synthesis of riboflavin in the rumen. Teeri et al. (1951b) found that cows fed a low quality of late-cut hay had a decreased excretion of riboflavin. Also, the excretion values of riboflavin indicated that silage and cane molasses favor the rumen or intestinal synthesis of this vitamin. Kesler and Knodt (1950, 1951a, 1951c) observed that in the case of calves the riboflavin concentration was highest in the small intestine.

Loosli and McCay (1943) observed that the riboflavin content of the organs and edible meat was not increased by feeding supplements of B vitamins to calves.

Several investigations have been conducted to study the influence of ration on the riboflavin content of milk. Whitnah et al. (1938) noted a breed difference in the riboflavin content of milk; Jersey milk was the highest and Ayrshire was the lowest. Also, the riboflavin content of milk was fairly constant between 15 days and 10 months after freshening. Kramer et al. (1938) found that colostrum was richer in riboflavin than milk produced later in the lactation. Whitnah et al. (1938), Kramer et al. (1939) and Hand and Sharp (1939) observed that cows on pasture produced milk higher in ribo-

flavin than cows on a winter ration. Johnson et al. (1941) found that when cows were changed from pasture to a ration low in riboflavin the riboflavin content of the milk decreased 25 per cent. They also observed that goats fed purified rations continued to secrete large amounts of riboflavin in the milk; which indicated that riboflavin was not a dietary essential for lactation in the goat. Agrawala (1950) observed a very high synthesis of riboflavin in the rumen of steers fed a purified ration.

Nicotinic acid synthesis. Wegner et al. (1940b) obtained first proof of synthesis of nicotinic acid. These workers found a three- to four-fold increase in the concentration of this vitamin in the dried rumen contents as compared with the feed. Lardinois et al. (1944) observed that urea plus a readily fermentable carbohydrate increased the level of nicotinic acid in the rumen. Johnson et al. (1947) found that nicotinic acid excretion remained normal throughout the experiment while the calves were on a nicotinic acid-free ration, even though one per cent sulfathalidine was included in the ration. They stated that the nicotinic acid was synthesized in the body tissue rather than in the rumen or intestinal tract of these animals. Kesler and Knodt (1950, 1951a, 1951c) observed that nicotinic acid, like riboflavin, was in the highest concentration in the small intestine. Teeri et al. (1951a) found that wood molasses and cane molasses were comparable with respect to nicotinic acid synthesis in the rumen.

Lindhahl and Pearson (1951) observed that the excretion of niacin from sheep getting a ration containing casein was higher than on a low protein ration. They believed that tryptophan was converted to niacin by sheep. As in the case of riboflavin, Agrawala (1950) observed a very high synthesis of this vitamin in the rumen of steers fed a purified ration.

Pantothenic acid synthesis. Ruminal synthesis of pantothenic acid was demonstrated in sheep by McElroy and Goss (1939). These same authors (1941b) observed that rumen contents from sheep and cows receiving a low B complex vitamin ration had a 25-fold increase of pantothenic acid over the ration fed. Wegner et al. (1941c) and Lardinois et al. (1944) have observed that pantothenic acid was synthesized in the rumen. Teeri et al. (1950, 1951a) observed that the excretion of pantothenic acid was greater than the dietary intake, especially when molasses was included in the ration. Agrawala (1950) observed that there was appreciable synthesis of pantothenic acid in the rumen when the synthetic ration was fed, but not as great as that when the steers received a normal ration.

Synthesis of other B-vitamins. McElroy and Goss (1939, 1940b) and Lardinois et al. (1944) have reported the synthesis of pyridoxine in the rumen of sheep and cattle. Lardinois et al. (1944) stated that pyridoxine showed a definite increase when a fermentable carbohydrate was fed with urea. The same authors (1944) found folic acid in the rumen of a

cow receiving a ration which contained little or none of this vitamin.

McElroy and Jukes (1940c) observed that biotin was synthesized in the rumen of a cow. Within four hours after feeding, Wegner et al. (1940b) found a five-fold increase in biotin of the rumen contents as compared with the feed.

Abelson and Darby (1949) feeding radioactive cobalt to sheep noted that large amounts of vitamin B<sub>12</sub> occurred in the sheep feces. Lindahl and Pearson (1951) observed an increase in the synthesis of vitamin B<sub>12</sub> in sheep receiving an all-hay ration as compared to a purified ration.

#### Summary of the Review of Literature

Many investigators have reported that antibiotics produce increased growth, less digestive disturbances and an increase in feed efficiency in swine and poultry. The antibiotics used in these studies were, namely; aureomycin, penicillin, streptomycin and terramycin. Favorable growth responses have been obtained when aureomycin was fed to young calves; however, adverse effects have been reported by several investigators when this antibiotic was fed to beef steers, heifers and lambs.

There are conflicting reports in the literature concerning the effect of the antibiotics on the intestinal microorganisms; nevertheless, whether the antibiotic caused an increase or decrease in the number of microorganisms, the effect of the

antibiotic appeared to be dependent on the concentration used, the type of ration and the species of animal.

Most of the theories which have been proposed for the action of antibiotics are as follows: Antibiotics reduce the bacterial flora which may compete with the host for nutrients; antibiotics inhibit intestinal microorganisms that are either producing toxic materials or are rendering certain dietary essentials unavailable for utilization; antibiotics at proper levels permit proliferation of microorganisms which synthesize vitamins and unidentified factors required by the animal. The inhibition of bacterial growth by antibiotics is probably the result of some interference with the metabolic processes of the bacterial cell, or the interference with various bacterial enzymatic systems, or by preventing the synthesis of some essential metabolite by the bacterial cell, or may act as a detergent and affect the surface tension of the bacterial cell.

As many as 50 to 60 different types of bacteria have been observed in the rumen contents. The total number of the microorganisms was very large and was usually expressed in billions per milliliter of rumen fluid.

The type of ration that the animal received had a definite influence on the type of organisms present in the rumen and also on the number and size of the bacteria. The pH of the rumen contents was dependent upon the type of ration fed and the stage of activity of the microorganism in the rumen.



Bacterial synthesis of amino acids and B vitamins on natural and purified rations has been demonstrated in vitro and in vivo. Most of the investigations concerned with the formation of protein have used urea as the non-protein nitrogen supplement.

## EXPERIMENTAL PROCEDURE

### Animals Used in Experiment

Two-year old steers, 707-a Guernsey and 714-a Holstein, which were fitted with plastic rumen fistula plugs were used in these experiments. The plugs were easily removed when samples of rumen contents were desired.

### Rations Used

The steers were fed a natural ration which consisted of 79 per cent second-cutting, alfalfa-brome hay and 21 per cent corn. The chemical composition of these feeds is shown in Table 1. The composition of the purified ration fed to 714 is shown in Table 2.

TABLE 1

CHEMICAL COMPOSITION OF THE CORN AND ALFALFA-BROME HAY  
USED IN THE NATURAL RATION

|                       | Dry<br>matter<br>% | Crude<br>protein<br>% | Crude<br>fiber<br>% | Ether<br>extract<br>% | Nitrogen-<br>free<br>extract<br>% | Non-<br>protein<br>nitrogen<br>% |
|-----------------------|--------------------|-----------------------|---------------------|-----------------------|-----------------------------------|----------------------------------|
| Corn                  | 87.76              | 9.90                  | 3.13                | 4.36                  | 80.33                             | 0.107                            |
| Alfalfa-<br>brome hay | 89.72              | 14.18                 | 34.38               | 1.84                  | 43.52                             | 0.628                            |

TABLE 2  
COMPOSITION OF THE PURIFIED RATION<sup>1,2</sup>

|                                      |       |                                                                   |       |
|--------------------------------------|-------|-------------------------------------------------------------------|-------|
| Corn starch                          | 42%   | Cellulose <sup>3</sup>                                            | 20%   |
| Glucose                              | 24%   | Lard                                                              | 4%    |
| Hay                                  | 1%    | Urea                                                              | 4%    |
| Mineral mixture                      | 5%    |                                                                   |       |
| CaHPO <sub>4</sub>                   | 11.0% | FeC <sub>6</sub> H <sub>5</sub> O <sub>7</sub> •5H <sub>2</sub> O | 2.5%  |
| CaCO <sub>3</sub>                    | 16.0% | MnSO <sub>4</sub> •4H <sub>2</sub> O                              | 0.7%  |
| K <sub>2</sub> HPO <sub>4</sub>      | 27.0% | KI                                                                | 0.08% |
| MgSO <sub>4</sub> •7H <sub>2</sub> O | 15.0% | ZnCl <sub>2</sub>                                                 | 0.02% |
| Na <sub>2</sub> SO <sub>4</sub>      | 5.0%  | CuSO <sub>4</sub> •5H <sub>2</sub> O                              | 0.03% |
| NaCl                                 | 22.6% | CoSO <sub>4</sub> •7H <sub>2</sub> O                              | 0.07% |

<sup>1</sup> Steers received 400,000 I.U. of vitamin A and 5,000,000 I.U. of vitamin D every two weeks.

<sup>2</sup> Protein equivalent (N x 6.25) = 12.3%.

<sup>3</sup> Solka - Floc, cellulose product, Brown Co., Berlin, N.H.

#### Method of Feeding

The steers received the total ration of four pounds of corn and 15 pounds of hay once daily. The corn was fed first and the hay was placed before the steers by 8 A.M. each day. The animals consumed the corn in about 10 minutes and the hay in about three hours. Water was available in a drinking cup. Crystalline aureomycin was added to the ration for 15 days at 0.5 gram level and then was increased to 1.0 gram for the next

15 days. The aureomycin, when fed, was mixed with the corn before feeding.

In the case of the purified ration, the steers were gradually changed to the new ration over a five-day period. They were given 14 pounds once daily. Steer 714 ate the ration well, while 707 refused to eat it at all. The portion refused was put into the rumen through the fistula opening. After two weeks of this treatment, 707 was removed from the experiment.

Steer 714 went off-feed just prior to the addition of 0.5 gram of aureomycin to the purified ration and did not regain its appetite during the period that aureomycin was included in the ration. The animal was fed through the fistula for the following two weeks.

#### Sampling Procedure

Chemical analysis. The rumen contents were removed through the fistula opening after removing the plastic plug. The solid material was removed by hand, while the liquid portion was removed by use of a beaker. The contents were weighed and thoroughly mixed; after which, approximately a 550-gram representative sample of liquid and solid material was taken for chemical analysis. Approximately one hour was required to perform the above operations, which were completed rapidly so as to prevent the ingesta from becoming too cool. The material that was

taken for chemical analysis was ground in a food grinder and after mixing thoroughly, exactly 500 grams were placed in a brown glass bottle. The samples were frozen and stored in a deep freeze until completion of collections for all of the experiments. The rumen contents that were removed before feeding were designated as the 0-hour samples, while those collected at six and 12 hours after feeding were called 6-hour and 12-hour samples, respectively.

Bacterial and pH determinations. At each collection of rumen material a five milliliter sample of rumen fluid was preserved in 10 milliliters of a 10 per cent formalin solution for total rumen bacterial count. This made a 1:3 dilution. A pH determination was made at each collection of rumen material.

Additional samples were taken for bacterial and pH determinations for each level of aureomycin feeding. The samples were collected at 2-hour intervals for 12 hours for the first three days of each level of aureomycin intake. The first sample was taken before feeding each day. The rumen fluid was drawn into a pipette connected to a large rubber bulb. At each of these collections a pH determination was made and a five-milliliter portion was preserved for total bacterial count. Cultural studies for the presence of rumen streptococci and coliform organisms were made on another portion of fresh rumen fluid. The rumen fluid was transported in a screw cap tube

filled to capacity in order to maintain anaerobic conditions. In addition to the three-day collection periods, samples of the rumen fluid were collected for total bacterial counts and cultural studies before feeding and six hours after feeding on Monday, Wednesday and Friday throughout the period concerned for each level of aureomycin intake.

Bacterial studies were made on the feces from the steers for all levels of aureomycin intake. The fecal samples were collected once per day (before feeding), three times per week throughout the period for each level of aureomycin intake. The fecal material was collected directly from the steer into a sterile Petri dish. Immediately after collection, a 10-gram sample of the fresh fecal material was placed in a sterile 90-milliliter blank and taken directly to the laboratory where the total volume was made up to 100 milliliters. The sample was agitated by shaking in the blank for a short time, then a 10-milliliter portion was preserved in five milliliters of 40 per cent formalin for total bacterial count. This gave a 1:15 dilution. The remaining portion was used for the cultural determinations for the presence of fecal streptococci and coliform organisms in the feces.

Chemical analyses. The samples of rumen contents taken for chemical analysis were dried in a forced-hot air oven at 60°C. The dried samples were ground in a Wiley mill to pass a 20-mesh sieve and stored in brown glass containers until

analyzed. The standard procedures for feed analysis according to the A.O.A.C. (1950) were followed for the determination of moisture, total nitrogen, crude fiber, ether extract and ash.

Non-protein nitrogen in the dried rumen contents was determined by precipitating the protein in a 10-gram sample by mixing with 175 milliliters of 5 per cent trichloroacetic acid in a Waring blender for 10 minutes. The mixture was centrifuged at 2000 r.p.m. The nitrogen in a 50-milliliter aliquot was determined by the Kjeldahl method as outlined in the A.O.A.C. (1950). In the case of the purified ration, a smaller sample had to be used because of the limited amount of the original sample.

The pH of the rumen fluid was determined by the use of the Beckman pH meter.

Bacteriological analyses. The bacteriological analyses of the rumen and fecal material were made in the Dairy Bacteriology laboratory. The microscopic method developed by Bortree et al. (1948) was used for the total count. The stain was prepared by saturating 10 milliliters of ethanol with crystal violet (gentian violet). One milliliter of the ethanol solution was added to 49 milliliters of distilled water, mixed thoroughly and filtered. One milliliter of a 1:15 formalin solution of fecal material was placed in seven milliliters of distilled water to produce a 1:120 dilution. Three milliliters of a 1:3 formalin solution of rumen material were placed in 22 milliliters of distilled water to produce a 1:25 dilution. One milliliter of a 1:120 dilution of fecal material or one millili-

ter of a 1:25 dilution of rumen material and one milliliter of the stain were transferred into eight milliliters of distilled water in a test tube and shaken well. This resulted in a final dilution of 1:250 for the rumen samples and 1:1200 for the fecal samples. The test tube was heated over a low flame until the solution bumped gently. Before cooling, a blood pipette was filled with the bacterial suspension and a drop was placed on a clean Petroff-Hausser counting chamber. The bacteria in 200 small squares were counted for each sample. The number of bacteria present was computed by the following formula:

$$\text{Number of bacteria per milliliter} = \frac{\text{Bacterial count} \times \text{Dilution} \times 20 \times 20 \times 50 \times 1000}{100}$$

100 = average number of small squares counted

20 x 20 = side of small square or 20 millimeter

50 = millimeter depth of material with coverslip on material

1000 = conversion factor to change millimeters to milliliters

The rumen and fecal samples which were collected for cultural studies were taken care of immediately on arrival at the laboratory. One milliliter of the fresh rumen fluid was placed in a sterile 99-milliliter blank to produce a 1:100 dilution. The remaining dilutions necessary for the analysis were made from this dilution. In the case of the fecal samples, one milliliter of the original 1:10 dilution was added to a sterile 99-milliliter blank to yield a 1:1000 dilution. Also, a 1:100 dilution was made by placing 10 milliliters of the 1:10 original dilution in a sterile 90-milliliter blank. Again, the



dilutions necessary for the analysis were made from these dilutions. Lauryl tryptose broth prepared according to the formula of Mallmann and Darby (1941) was used to determine the coliform organisms, while dextrose azide broth was used for determining the presence of rumen and fecal streptococci (Mallmann and Seligmann, 1950). The highest dilution in which turbidity could be observed was used to represent the number of rumen or fecal streptococci present. The highest dilution showing gas formation was used to indicate the number of coliform organisms present.

Amino acid analyses. The samples were ether extracted before analysis for the amino acids. Hydrolyzates for the determination of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine were prepared according to the procedure of Stokes et al. (1945). One gram of the material was dispersed in 25 milliliters of 6 N HCl and autoclaved for eight hours at 15 pounds pressure. The hydrolyzates were cooled, neutralized to pH 6.6-6.8 with 18 N NaOH, made up to 100 milliliters, filtered, covered with a few drops of toluene and stored in the refrigerator until analyzed. The concentration of the stock solution was 10 milligrams per milliliter. In the case of tryptophan, one-half gram of sample was hydrolyzed in 16 milliliters of 4 N NaOH by autoclaving for eight hours at 15 pounds pressure according to the procedure of Kuiken et al. (1947). The hydrolyzate was cooled, neutralized

to pH 7.0 with 12 N HCl, made up to 100 milliliters, filtered with the aid of Super-Cel, covered with a few drops of toluene and stored in the refrigerator until analyzed. This gave a final five milligrams per milliliter concentration for the stock solution. In all cases, the assays were run after the proper dilutions had been made.

The microbiological method was used to determine the amino acids. The composition of the media used for determining the amino acids is given in Table 3. Medium I, Schweigert et al. (1944), was used for Lactobacillus arabinosus 17-5 (8014) to assay for isoleucine, leucine, phenylalanine and valine. Kuiken et al. (1943) included tomato eluate in the media for isoleucine and valine to overcome the lag in growth of L. arabinosus in the tubes containing the lower concentrations of the standard. Medium II, Greenhut et al. (1946), was used for Streptococcus faecalis (9790) to analyze for arginine, histidine and threonine; while Medium III, McMahan and Snell (1944), was used for Leuconostoc mesentroides P-60 (8042) to determine lysine. Medium IV, Krehl et al. (1943), worked best with L. arabinosus for tryptophan; while Medium V, Lyman et al. (1946) was used for L. mesentroides for the determination of methionine.

In the assay of the various amino acids, DL-configurations of isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan and valine were used to prepare standards for these

amino acids, while L-configurations were used for the preparation of the arginine, histidine and lysine standards. A standard curve was determined by adding in triplicate at 0.5 milliliter increments from 0 to five milliliters a known quantity of the amino acid to be studied. The samples to be assayed were diluted and pipetted in duplicate so that the tubes contained 1.0, 2.0 and 3.0 milliliters of the amino acid hydrolyzate. Five milliliters of the basal medium, from which the amino acid to be assayed was omitted, was added to each tube of the standard and samples. The total volume in all of the tubes was brought to 10 milliliters with water. The tubes were capped and sterilized by autoclaving at 15 pounds pressure for 10 minutes. Each tube was inoculated aseptically with one drop of the appropriate organism suspended in 0.9 per cent saline solution. The tubes were incubated 72 hours at 37°C. to permit the development of lactic acid. The lactic acid produced in each tube was titrated electrometrically with 0.1 N NaOH to pH 7.0.

B-vitamin analyses. As in the case of the amino acids, the samples were ether extracted before analysis for the B-vitamins. The hydrolytic procedure outlined by Snell and Strong (1939) was used for riboflavin. One gram of the material was suspended in 50 milliliters of 0.1 N HCl and autoclaved for 30 minutes at 15 pounds pressure. The sample was centrifuged after cooling and 25 milliliters of the supernatant was adjusted to pH 4.6, diluted to 50 milliliters and

filtered. In the case of nicotinic acid, the samples were prepared in the same manner as for riboflavin except that 1.0 N HCl was used to suspend the material (Krehl et al. 1943).

The procedure designed by Buskirk et al. (1942, 1948) which was modified by Skeggs and Wright (1944) was used for the preparation of the material for the pantothenic acid assay. One gram of the material and one gram of mylase-P were dispersed in 10 milliliters of two per cent acetic acid and one milliliter of 1.0 N NaOH (to buffer the reaction at pH 4.2 to 4.5). The mixture was incubated at 37°C. for three hours, after which it was centrifuged, diluted to 100 milliliters and filtered. The concentration of the stock solution for each of the vitamins was 10 milligrams per milliliter.

The B-vitamins were determined microbiologically with Lactobacillus casei (7469) for riboflavin and L. arabinosus for nicotinic acid and pantothenic acid. The media used in these assays are given in Table 4. A standard curve was determined for each B-vitamin. The procedure was similar to that used for the amino acids; however, the increments were varied so that only eight levels were required to make the curve. Also, the procedure for diluting and pipetting the samples, making the volume up to 10 milliliters, sterilizing, inoculating and titrating was the same as that given for the amino acids.

TABLE 3

COMPOSITION OF THE MEDIA USED IN AMINO ACID ASSAY<sup>1</sup>  
(Per 500 milliliters of double-strength medium)

| Composition                                        | I   | II  | III | IV  | V   |
|----------------------------------------------------|-----|-----|-----|-----|-----|
| Casein hydrolyzate (gm)                            | --  | --  | --  | 5.0 | --  |
| H <sub>2</sub> O <sub>2</sub> treated peptone (gm) | --  | --  | --  | --  | 7.5 |
| DL(-)-Alanine (mg)                                 | 200 | 100 | 200 | --  | --  |
| L(/)-Arginine-HCl (mg)                             | 50  | 50  | 100 | --  | --  |
| L-Asparagine (mg)                                  | 200 | 200 | 200 | --  | --  |
| L(-)-Cystine (mg)                                  | 100 | 200 | 200 | 200 | 100 |
| L(/)-Glutamic acid (mg)                            | 400 | 400 | 400 | --  | --  |
| Glycine (mg)                                       | 20  | 20  | 100 | --  | --  |
| L(/)-Histidine·HCl·H <sub>2</sub> O (mg)           | 50  | 50  | 100 | --  | --  |
| DL-Isoleucine (mg)                                 | 200 | 200 | 200 | --  | --  |
| DL-Leucine (mg)                                    | 200 | 200 | 200 | --  | --  |
| L(/)-Lysine·HCl·H <sub>2</sub> O (mg)              | 200 | 200 | 200 | --  | --  |
| DL-Methionine (mg)                                 | 100 | 100 | 200 | --  | --  |
| DL-Phenylalanine (mg)                              | 100 | 100 | 100 | --  | --  |
| L(-)-Proline (mg)                                  | 50  | 50  | 50  | --  | --  |
| DL-Serine (mg)                                     | 50  | 50  | 200 | --  | --  |
| DL-Threonine (mg)                                  | 200 | 200 | 200 | --  | --  |
| DL-Tryptophan (mg)                                 | 50  | 100 | 100 | --  | 100 |
| L(-)-Tyrosine (mg)                                 | 50  | 100 | 100 | --  | 100 |
| DL-Valine (mg)                                     | 200 | 200 | 200 | --  | --  |
| Glucose (gm)                                       | 20  | 20  | 20  | 20  | 20  |

TABLE 3 (concluded)

| Composition                                     | I    | II   | III  | IV  | V      |
|-------------------------------------------------|------|------|------|-----|--------|
| Na acetate (anhyd.) (gm)                        | 20   | --   | 20   | 20  | 12     |
| Na citrate·H <sub>2</sub> O (gm)                | --   | 25   | --   | --  | --     |
| NH <sub>4</sub> Cl (gm)                         | --   | --   | --   | --  | 6      |
| KH <sub>2</sub> PO <sub>4</sub> (mg)            | 500  | --   | 500  | 500 | 500    |
| K <sub>2</sub> HPO <sub>4</sub> (mg)            | 500  | 5000 | 500  | 500 | 500    |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O (mg)       | 200  | 200  | 200  | 200 | 200    |
| FeSO <sub>4</sub> ·7H <sub>2</sub> O (mg)       | 10   | 10   | 10   | 10  | 10     |
| MnSO <sub>4</sub> ·4H <sub>2</sub> O (mg)       | 10   | 10   | 10   | 10  | 10     |
| NaCl (mg)                                       | 10   | 10   | 10   | 10  | 10     |
| Adenine SO <sub>4</sub> ·2H <sub>2</sub> O (mg) | 10   | 10   | 10   | 10  | 10     |
| Guanine HCl·2H <sub>2</sub> O (mg)              | 10   | 10   | 10   | 10  | 10     |
| Uracil (mg)                                     | 10   | 10   | 10   | 10  | 10     |
| Xanthine (mg)                                   | 10   | 10   | 10   | --  | --     |
| Thiamine·HCl (mg)                               | 0.5  | 0.5  | 0.5  | 0.1 | 1.0    |
| Pyridoxine·HCl (mg)                             | 1.0  | 1.0  | 1.0  | 0.1 | 2.0    |
| DL-Ca pantothenate (mg)                         | 0.5  | 0.5  | 0.5  | 0.1 | 2.0    |
| Riboflavin (mg)                                 | 0.5  | 0.5  | 0.5  | 0.2 | 2.0    |
| Nicotinic acid (mg)                             | 1.0  | 1.0  | 1.0  | 0.4 | 2.0    |
| p-Aminobenzoic acid (mg)                        | 0.1  | 0.1  | 0.1  | 0.1 | 0.01   |
| Biotin (ug)                                     | 1.0  | 1.0  | 1.0  | 200 | 5.0    |
| Folic acid (mg)                                 | 0.01 | 0.01 | 0.01 | --  | 0.0015 |
| pH before autoclaving                           | 6.8  | 6.8  | 6.8  | 6.8 | 7.0    |

1 The amino acid to be assayed was omitted from the medium.

TABLE 4

COMPOSITION OF THE MEDIA USED IN THE ASSAY  
FOR SOME OF THE B VITAMINS  
(Per 500 milliliters of double-strength medium)

| Composition                                          | Riboflavin | Nicotinic<br>acid | Pantothenic<br>acid |
|------------------------------------------------------|------------|-------------------|---------------------|
| Peptone (NaOH treated) (gm)                          | 5          | --                | --                  |
| L-cystine (mg)                                       | 100        | 200               | 100                 |
| Yeast supplement (gm)                                | 2.0        | --                | --                  |
| Hydrolyzed casein (gm)                               | --         | 5                 | 5                   |
| DL-Tryptophan (mg)                                   | --         | 200               | 200                 |
| Adenine $\text{SO}_4 \cdot 2\text{H}_2\text{O}$ (mg) | --         | 10                | 5                   |
| Guanine $\text{HCl} \cdot 2\text{H}_2\text{O}$ (mg)  | --         | 10                | 5                   |
| Uracil (mg)                                          | --         | 10                | 5                   |
| Xanthine (mg)                                        | --         | --                | 5                   |
| p-Aminobenzoic acid (mg)                             | --         | 0.1               | 0.1                 |
| Nicotinic acid (mg)                                  | --         | --                | 1.0                 |
| Pyridoxine $\cdot \text{HCl}$ (mg)                   | --         | 0.1               | 2.0                 |
| Riboflavin (mg)                                      | --         | 0.2               | 1.0                 |
| Thiamine $\cdot \text{HCl}$ (mg)                     | --         | 0.1               | 2.0                 |
| Ca pantothenate (mg)                                 | --         | 0.1               | --                  |
| Biotin (ug)                                          | --         | 0.2               | 12.5                |
| Na acetate (gm)                                      | --         | 20                | 20                  |
| Glucose (gm)                                         | 10         | 20                | 20                  |
| Inorganic salts                                      |            |                   |                     |
| Solution A <sup>1</sup> (ml)                         | 5          | 5                 | 5                   |
| Solution B <sup>2</sup> (ml)                         | 5          | 5                 | 5                   |
| pH before autoclaving                                | 6.8        | 6.8               | 6.8                 |

<sup>1</sup> Salts A contain 100 mg  $\text{K}_2\text{HPO}_4$  and 100 mg  $\text{KH}_2\text{PO}_4$  per ml.

<sup>2</sup> Salts B contain 40 mg  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 2.0 mg  $\text{NaCl}$ , 2.0 mg  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and 2.0 mg  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  per ml.

## RESULTS

### Health of the Animals

The steers used in the experiment with the natural ration did not show any signs of digestive disturbances at any time. The addition of aureomycin at 0.5 and 1.0 gram levels did not cause any decrease in appetite in either case. The steers continued to gain in body weight throughout the entire experimental period even though the ration was fed at approximately the maintenance level.

When the purified ration was fed, the steers did not consume it very readily at first. In fact, 707 continued to refuse to eat it for two weeks and was removed from the experiment at that time. Steer 714 received the purified ration for 25 days before the first collection of rumen contents was made. Simultaneously with the feeding of 0.5 gram of aureomycin, 714 began to refuse to eat the ration and was fed through the side for the remainder of the experiment. Approximately one week before the end of the aureomycin feeding period, the steer began to act sluggish and the rumen contents appeared to be separated into two layers, with the top layer consisting mostly of the coarse material while the bottom layer was very watery. The rumen contents were very greasy. Two days before the end of the experimental period the steer had severe diarrhea, a mucus discharge from the nostrils and an increase in body tem-



perature. The paper pulp which was used as the cellulose source in the ration settled to the bottom of the rumen. The steer died two days after the rumen samples were collected for the aureomycin trial. The following conditions were found on autopsy: fat necrosis of the omentum, hemorrhagic pancreatitis, extensive fatty degeneration of the kidneys and liver, petechial and ecchymatic hemorrhages at the base of the heart and on the epicardium, slight degenerative changes in the heart muscle and the heart was somewhat flabby, and the lungs were congested and edematous with beginning of bronchopneumonia. The final diagnosis was toxemia due to degenerative changes of the liver, kidney and heart, while the septicemia present probably originated from the early pneumonia.

Weight and Composition of the Rumen Contents from  
Cattle Fed a Natural and a Purified Ration

The data pertaining to the weights of the rumen contents obtained at the 0-, 6- and 12-hour collections along with the percentages of dry matter, crude protein, crude fiber, ether extract, nitrogen-free extract and non-protein nitrogen are presented in Table 5 for the natural ration and Table 6 for the purified ration. The data are arranged by the hour of collection for each level of aureomycin fed so that comparisons could be made to determine the influence of each level of aureomycin intake on the different ration constituents. As one would expect, due to the fact that the ration fed, espec-

TABLE 5  
INFLUENCE OF AUREOMYCIN ON THE WEIGHT AND COMPOSITION OF THE RUMEN CONTENTS  
FROM CATTLE FED A NATURAL RATION

| Animal no. | Time hr | Aureo- mycin gm | Weight of rumen contents gm | Dry matter % | Crude protein % | Crude fiber % | Ether extract % | NFE % | NPN % | pH   | Bact. count billion |
|------------|---------|-----------------|-----------------------------|--------------|-----------------|---------------|-----------------|-------|-------|------|---------------------|
| 707        | 0       | 0               | 40,860                      | 11.99        | 12.94           | 40.78         | 1.74            | 34.60 | 0.221 | 6.73 | 10,025              |
|            | 0       | 0.5             | 41,768                      | 11.10        | 10.56           | 42.56         | 3.28            | 33.43 | 0.374 | 7.05 | 19,875              |
|            | 0       | 1.0             | 52,210                      | 11.90        | 10.88           | 41.95         | 3.54            | 33.58 | 0.207 | 7.05 | 23,000              |
| 707        | 6       | 0               | 60,382                      | 14.12        | 15.56           | 36.92         | 3.46            | 35.10 | 0.563 | 6.16 | 11,200              |
|            | 6       | 0.5             | 59,020                      | 12.78        | 14.00           | 38.03         | 4.28            | 35.06 | 0.811 | 5.92 | 14,325              |
|            | 6       | 1.0             | 66,057                      | 14.06        | 14.25           | 37.01         | 4.25            | 35.19 | 0.543 | 6.51 | 20,350              |
| 707        | 12      | 0               | 55,842                      | 12.94        | 14.50           | 38.44         | 2.98            | 34.41 | 0.524 | 6.51 | 4,450               |
|            | 12      | 0.5             | 47,216                      | 12.24        | 13.38           | 39.87         | 3.08            | 33.62 | 0.661 | 6.71 | 19,725              |
|            | 12      | 1.0             | 60,609                      | 12.68        | 13.00           | 39.60         | 3.82            | 33.82 | 0.347 | 6.41 | 13,775              |
| 714        | 0       | 0               | 46,308                      | 11.86        | 12.88           | 39.07         | 1.71            | 36.48 | 0.179 | 6.74 | 7,600               |
|            | 0       | 0.5             | 48,351                      | 13.02        | 12.50           | 42.94         | 2.83            | 33.05 | 0.285 | 6.85 | 25,875              |
|            | 0       | 1.0             | 51,302                      | 14.42        | 11.44           | 43.61         | 3.24            | 33.00 | 0.265 | 6.71 | 18,100              |
| 714        | 6       | 0               | 63,333                      | 16.06        | 16.00           | 34.62         | 3.55            | 37.70 | 0.592 | 5.29 | 7,075               |
|            | 6       | 0.5             | 67,873                      | 15.32        | 14.94           | 38.72         | 5.83            | 32.77 | 0.629 | 5.88 | 12,250              |
|            | 6       | 1.0             | 75,818                      | 16.28        | 14.00           | 40.16         | 3.94            | 33.86 | 0.556 | 5.85 | 17,800              |
| 714        | 12      | 0               | 54,026                      | 15.94        | 16.19           | 37.13         | 3.51            | 35.02 | 0.487 | 6.01 | 8,275               |
|            | 12      | 0.5             | 59,474                      | 14.72        | 14.38           | 40.39         | 4.06            | 33.04 | 0.582 | 6.20 | 11,775              |
|            | 12      | 1.0             | 62,879                      | 15.40        | 13.50           | 40.78         | 3.51            | 33.74 | 0.467 | 5.80 | 15,025              |

TABLE 6  
INFLUENCE OF AUREOMYCIN ON THE WEIGHT AND COMPOSITION OF THE RUMEN  
CONTENTS FROM 714 WHEN FED A PURIFIED RATION

| Animal<br>no. | Time<br>hr | Aureo-<br>mycin<br>gm | Weight<br>of rumen<br>contents<br>gm | Dry<br>matter<br>% | Crude<br>protein<br>% | Crude<br>fiber<br>% | Ether<br>extract<br>% | NPN<br>% | pH   | Bact.<br>count<br>billion |
|---------------|------------|-----------------------|--------------------------------------|--------------------|-----------------------|---------------------|-----------------------|----------|------|---------------------------|
| 714           | 0          | 0                     | 33,596                               | 8.48               | 14.75                 | 36.05               | 3.58                  | 0.241    | 6.92 | 13,000                    |
|               | 0          | 0.5                   | 22,019                               | 17.78              | 9.13                  | 31.83               | 6.33                  | 0.654    | 4.07 | 2,475                     |
| 714           | 6          | 0                     | 48,124                               | 11.26              | 15.69                 | 32.52               | 6.88                  | 0.970    | 5.05 | 4,275                     |
|               | 6          | 0.5                   | 50,394                               | 14.60              | 9.50                  | 23.31               | 5.20                  | 1.408    | 4.19 | 1,175                     |
| 714           | 12         | 0                     | 39,725                               | 10.84              | 15.25                 | 38.84               | 7.68                  | 0.757    | 5.12 | 4,025                     |
|               | 12         | 0.5                   | 51,756                               | 11.49              | 12.44                 | 24.16               | 5.35                  | 1.632    | 3.89 | 1.850                     |

ially the natural ration, was high in each constituent considered and that it took the steers several hours to consume the ration, the values obtained for each constituent at the 6-hour collections were higher than the 0- or 12-hour collections. The pH and total bacterial count were taken at each collection of rumen material. However, one must keep in mind that steer 714 was not in normal health when 0.5 gram of aureomycin was added to the purified ration, but the information was included merely to serve as a comparison to a normal condition.

Influence of Aureomycin on the Rate of Removal of Some  
of the Ration Constituents from the Rumen

With the aid of the data presented in Tables 1 and 5, the quantity of each ration constituent present at the 0-, 6- and 12-hour collections for each animal, and the amount of each constituent fed to the animals can be calculated. From the amount present and that fed, calculations were made for the percentage of each constituent which had accumulated in the rumen or which had been removed over a particular 6- or 12-hour period. Tables 7 through 12 show the amounts of dry matter, crude fiber, crude protein, ether extract, nitrogen-free extract and non-protein nitrogen which had accumulated or had been removed from the rumen in 6- and 12-hours in relation to the 0-hour. The rate of disappearance of the ration constituent at the 6- and 12-hour collections was based on the intake of the particular constituent in question.

TABLE 7  
INFLUENCE OF AUREOMYCIN ON THE REMOVAL OF DRY MATTER FROM THE RUMEN  
OF CATTLE FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no. | Aureo-<br>mycin gm | Dry Matter    |        |          | Dry Matter       |            |      | Dry Matter        |            |                  |
|------------|--------------------|---------------|--------|----------|------------------|------------|------|-------------------|------------|------------------|
|            |                    | In rumen gm   | Fed gm | Total gm | In rumen gm      | Removed gm | %    | In rumen gm       | Removed gm | %                |
|            |                    | O-hr. samples |        |          | O-6 hr. samples* |            |      | O-12 hr. samples* |            |                  |
|            |                    |               |        |          |                  |            |      |                   |            | 6-12 hr. samples |
|            | 0                  | 4899          | 7704   | 12603    | 8526             | 4077       | 52.9 | 7226              | 5377       | 69.8             |
|            |                    |               |        |          |                  |            |      |                   |            | 1300             |
|            |                    |               |        |          |                  |            |      |                   |            | 15.2             |
| 707        | 0.5                | 4636          | 7704   | 12340    | 7543             | 4797       | 62.3 | 5779              | 6561       | 85.2             |
|            |                    |               |        |          |                  |            |      |                   |            | 1764             |
|            |                    |               |        |          |                  |            |      |                   |            | 23.4             |
|            | 1.0                | 6213          | 7704   | 13917    | 9288             | 4629       | 60.1 | 7685              | 6232       | 80.9             |
|            |                    |               |        |          |                  |            |      |                   |            | 1603             |
|            |                    |               |        |          |                  |            |      |                   |            | 17.3             |
|            | 0                  | 5492          | 7704   | 13196    | 10171            | 3025       | 39.4 | 8612              | 4584       | 59.5             |
|            |                    |               |        |          |                  |            |      |                   |            | 1559             |
|            |                    |               |        |          |                  |            |      |                   |            | 15.3             |
| 714        | 0.5                | 6295          | 7704   | 13999    | 10398            | 3601       | 46.8 | 8755              | 5244       | 68.1             |
|            |                    |               |        |          |                  |            |      |                   |            | 1693             |
|            |                    |               |        |          |                  |            |      |                   |            | 15.8             |
|            | 1.0                | 7398          | 7704   | 15102    | 12343            | 2759       | 35.8 | 9683              | 5419       | 70.3             |
|            |                    |               |        |          |                  |            |      |                   |            | 2660             |
|            |                    |               |        |          |                  |            |      |                   |            | 21.6             |

\* Based on dry matter intake.

TABLE 8  
INFLUENCE OF AUREOMYCIN ON THE REMOVAL OF CRUDE FIBER FROM THE RUMEN  
OF CATTLE FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no. | Aureo-<br>mycin<br>gm | Crude Fiber          |           |             | Crude Fiber             |               |      | Crude Fiber              |               |      | Crude Fiber             |               |   |
|------------|-----------------------|----------------------|-----------|-------------|-------------------------|---------------|------|--------------------------|---------------|------|-------------------------|---------------|---|
|            |                       | In<br>rumen<br>gm    | Fed<br>gm | Total<br>gm | In<br>rumen<br>gm       | Removed<br>gm | %    | In<br>rumen<br>gm        | Removed<br>gm | %    | In<br>rumen<br>gm       | Removed<br>gm | % |
|            |                       | <u>O-hr. samples</u> |           |             | <u>O-6 hr. samples*</u> |               |      | <u>O-12 hr. samples*</u> |               |      | <u>6-12 hr. samples</u> |               |   |
| 707        | 0                     | 1998                 | 2151      | 4149        | 3148                    | 1001          | 46.5 | 2778                     | 1371          | 63.7 | 370                     | 11.8          |   |
|            | 0.5                   | 1973                 | 2151      | 4124        | 2869                    | 1255          | 58.3 | 2304                     | 1820          | 84.6 | 565                     | 19.7          |   |
|            | 1.0                   | 2606                 | 2151      | 4757        | 3438                    | 1319          | 61.3 | 3043                     | 1714          | 79.7 | 395                     | 11.5          |   |
| 714        | 0                     | 2146                 | 2151      | 4297        | 3521                    | 776           | 36.1 | 3198                     | 1099          | 51.1 | 323                     | 9.2           |   |
|            | 0.5                   | 2703                 | 2151      | 4854        | 4028                    | 826           | 38.4 | 3536                     | 1318          | 61.3 | 492                     | 12.2          |   |
|            | 1.0                   | 3226                 | 2151      | 5377        | 4957                    | 420           | 19.5 | 3949                     | 1428          | 66.4 | 1008                    | 20.3          |   |

\* Based on crude fiber intake.

TABLE 9

INFLUENCE OF AUREOMYCIN ON THE REMOVAL OF CRUDE PROTEIN FROM THE RUMEN  
OF CATTLE FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no. | Aureo-<br>mycin<br>gm | Crude Protein           |      |       | Crude Protein |     |         | Crude Protein |     |         | Crude Protein |      |         |
|------------|-----------------------|-------------------------|------|-------|---------------|-----|---------|---------------|-----|---------|---------------|------|---------|
|            |                       | In rumen                |      | Total | In rumen      |     | Removed | In rumen      |     | Removed | In rumen      |      | Removed |
|            |                       | gm                      | gm   | gm    | gm            | gm  | %       | gm            | gm  | %       | gm            | gm   | %       |
|            |                       | <u>0-hr. samples</u>    |      |       |               |     |         |               |     |         |               |      |         |
|            | 0                     | 634                     | 1024 | 1658  | 1327          | 331 | 32.3    | 1048          | 610 | 59.6    | 279           | 21.0 |         |
| 707        | 0.5                   | 490                     | 1024 | 1514  | 1056          | 458 | 44.7    | 773           | 741 | 72.4    | 283           | 26.8 |         |
|            | 1.0                   | 676                     | 1024 | 1700  | 1324          | 376 | 36.7    | 999           | 701 | 68.5    | 325           | 24.5 |         |
|            |                       | <u>0-6 hr. samples*</u> |      |       |               |     |         |               |     |         |               |      |         |
|            | 0                     | 707                     | 1024 | 1731  | 1627          | 104 | 10.2    | 1394          | 337 | 32.9    | 233           | 14.3 |         |
| 714        | 0.5                   | 787                     | 1024 | 1811  | 1554          | 257 | 25.1    | 1259          | 552 | 53.9    | 295           | 19.0 |         |
|            | 1.0                   | 846                     | 1024 | 1870  | 1728          | 142 | 13.9    | 1307          | 563 | 55.0    | 421           | 24.4 |         |
|            |                       | <u>6-12 hr. samples</u> |      |       |               |     |         |               |     |         |               |      |         |

\* Based on crude protein intake.

TABLE 10  
INFLUENCE OF AUREOMYCIN ON THE AMOUNT OF ETHER EXTRACT ACCUMULATED OR REMOVED  
FROM THE RUMEN OF CATTLE FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no. | Aureo-<br>mycin<br>gm | Ether Extract        |              |             | Ether Extract           |                   | Ether Extract           |                     |      |
|------------|-----------------------|----------------------|--------------|-------------|-------------------------|-------------------|-------------------------|---------------------|------|
|            |                       | In<br>rumen<br>gm    | Intake<br>gm | Total<br>gm | In<br>rumen<br>gm       | Accumulated<br>gm | Removed<br>%            | Ether Extract<br>gm |      |
|            |                       | <u>O-hr. samples</u> |              |             | <u>0-6 hr. samples*</u> |                   | <u>6-12 hr. samples</u> |                     |      |
|            | 0                     | 85.2                 | 161.9        | 247.1       | 295.0                   | 47.9              | 29.6                    | 79.7                | 27.0 |
| 707        | 0.5                   | 152.1                | 161.9        | 314.0       | 322.8                   | 8.8               | 5.4                     | 87.0                | 27.0 |
|            | 1.0                   | 220.0                | 161.9        | 381.9       | 394.7                   | 12.8              | 7.9                     | 101.1               | 25.7 |
|            | 0                     | 93.9                 | 161.9        | 255.8       | 361.1                   | 105.3             | 65.0                    | 58.8                | 16.3 |
| 714        | 0.5                   | 178.1                | 161.9        | 340.0       | 606.2                   | 266.2             | 164.4                   | 250.7               | 41.4 |
|            | 1.0                   | 239.7                | 161.9        | 401.6       | 486.3                   | 84.7              | 52.3                    | 146.4               | 30.1 |

\* Based on ether extract intake.



TABLE 11  
INFLUENCE OF AUREOMYCIN ON THE REMOVAL OF NITROGEN-FREE EXTRACT (NFE) FROM THE RUMEN OF  
CATTLE FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no. | Aureo-<br>mycin gm | NFE           |        |          | NFE              |            |      | NFE               |            |                  |
|------------|--------------------|---------------|--------|----------|------------------|------------|------|-------------------|------------|------------------|
|            |                    | In rumen gm   | Fed gm | Total gm | In rumen gm      | Removed gm | %    | In rumen gm       | Removed gm | %                |
|            |                    | O-hr. samples |        |          | O-6 hr. samples* |            |      | O-12 hr. samples* |            |                  |
|            |                    |               |        |          |                  |            |      |                   |            | 6-12 hr. samples |
|            | 0                  | 1695          | 3940   | 5635     | 2993             | 2642       | 67.1 | 2486              | 3149       | 79.9             |
|            |                    |               |        |          |                  |            |      |                   | 507        | 16.9             |
| 707        | 0.5                | 1550          | 3940   | 5490     | 2645             | 2845       | 72.2 | 1943              | 3547       | 90.0             |
|            |                    |               |        |          |                  |            |      |                   | 702        | 26.5             |
|            | 1.0                | 2086          | 3940   | 6026     | 3268             | 2758       | 70.0 | 2599              | 3427       | 87.0             |
|            |                    |               |        |          |                  |            |      |                   | 669        | 20.5             |
|            | 0                  | 2003          | 3940   | 5943     | 3834             | 2109       | 53.5 | 3016              | 2927       | 74.3             |
|            |                    |               |        |          |                  |            |      |                   | 818        | 21.3             |
| 714        | 0.5                | 2080          | 3940   | 6020     | 3407             | 2613       | 66.3 | 2893              | 3127       | 79.4             |
|            |                    |               |        |          |                  |            |      |                   | 514        | 15.1             |
|            | 1.0                | 2441          | 3940   | 6381     | 4179             | 2202       | 55.9 | 3267              | 3114       | 79.0             |
|            |                    |               |        |          |                  |            |      |                   | 912        | 21.8             |

\* Based on NFE intake.

TABLE 12

INFLUENCE OF AUREOMYCIN ON THE AMOUNT OF NON-PROTEIN NITROGEN (NPN) ACCUMULATED OR REMOVED FROM THE RUMEN OF CATTLE FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no. | Aureo-<br>mycin<br>gm | NPN           |      |       | NPN              |      |      | NPN    |                   |      | NPN   |      |          |
|------------|-----------------------|---------------|------|-------|------------------|------|------|--------|-------------------|------|-------|------|----------|
|            |                       | In            |      |       | Diff.            |      |      | Accum. |                   |      | In    |      |          |
|            |                       | rumen         | Fed  | Total | rumen            | gm   | gm   | %      | Removed           | %    | rumen | gm   | Removed  |
|            |                       | gm            | gm   | gm    | gm               |      |      |        |                   |      | gm    |      | gm       |
|            |                       | O-hr. samples |      |       | O-6 hr. samples* |      |      |        | O-12 hr. samples* |      |       |      | 6-12 hr. |
|            |                       | samples       |      |       | samples*         |      |      |        | samples*          |      |       |      | samples  |
|            | 0                     | 10.8          | 40.1 | 50.9  | 48.0             | 2.9  | --   | 7.2    | 37.9              | 13.0 | 32.4  | 10.1 | 21.0     |
| 707        | 0.5                   | 17.3          | 40.1 | 57.4  | 61.2             | 3.8  | 9.5  | --     | 38.2              | 19.2 | 47.9  | 23.0 | 37.6     |
|            | 1.0                   | 12.9          | 40.1 | 53.0  | 50.4             | 2.6  | 6.5  | --     | 26.7              | 26.3 | 65.6  | 23.7 | 47.0     |
|            | 0                     | 9.8           | 40.1 | 49.9  | 60.2             | 10.3 | 25.7 | --     | 41.9              | 8.0  | 20.0  | 18.3 | 30.4     |
| 714        | 0.5                   | 17.9          | 40.1 | 58.0  | 65.4             | 7.4  | 18.5 | --     | 51.0              | 7.0  | 17.5  | 14.4 | 22.0     |
|            | 1.0                   | 19.6          | 40.1 | 59.7  | 68.6             | 8.9  | 22.2 | --     | 45.2              | 14.5 | 36.2  | 23.4 | 34.1     |

\* Based on NPN intake.

Calculations also were made for the second 6-hour period and are designated as the 6-12 hour samples. In the latter case, the amount present at the 6-hour collection was used as the base to calculate the change between the 6- and the 12-hour collection. In nearly every case, the rate of removal of a ration constituent from the rumen was definitely faster when aureomycin was included in the ration, especially at the 0.5 gram level, than when no aureomycin was fed. Ether extract and non-protein nitrogen are an exception; in that, there was an accumulation during the first 6 hours and the amount present at the 0-hour was much larger when aureomycin was fed.

#### Amino Acid Analyses

Only the 10 essential amino acids were determined in this study. The amino acid composition of the rations fed is presented in Table 13, while Table 14 gives the amino acid composition of the rumen contents from cattle fed the natural ration. Again, the data are arranged by the hour of collection for each level of aureomycin intake. In general, when aureomycin was included in the ration, there was a decline in the percentage of the amino acids present at each collection of the rumen material in comparison to when no aureomycin was fed. The rate of removal of each amino acid from the rumen was calculated for the 6- and 12-hour collections based on the intake

TABLE 13

## AMINO ACID COMPOSITION OF THE RATIONS FED

| Ration               | Arg*<br>% | His<br>% | Isol<br>% | Leu<br>% | Lys<br>% | Met<br>% | Phe<br>% | Thr<br>% | Try<br>% | Val<br>% |
|----------------------|-----------|----------|-----------|----------|----------|----------|----------|----------|----------|----------|
| Corn                 | 0.481     | 0.360    | 0.422     | 0.955    | 0.194    | 0.142    | 0.356    | 0.333    | 0.067    | 0.382    |
| Alfalfa-brome<br>hay | 0.726     | 0.340    | 0.768     | 0.990    | 0.606    | 0.082    | 0.589    | 0.649    | 0.142    | 0.699    |
| Purified             | --        | --       | --        | --       | --       | --       | --       | --       | --       | --       |

\* The first three letters of the amino acid is used as the symbol.

TABLE 14

INFLUENCE OF AUREOMYCIN ON THE AMINO ACID COMPOSITION OF RUMEN CONTENTS FROM  
CATTLE FED A NATURAL RATION

| Animal<br>no. | Time<br>hr | Aureo-<br>mycin<br>gm | Arg<br>% | His<br>% | Isol<br>% | Leu<br>% | Lys<br>% | Met<br>% | Phe<br>% | Thr<br>% | Try<br>% | Val<br>% |
|---------------|------------|-----------------------|----------|----------|-----------|----------|----------|----------|----------|----------|----------|----------|
| 707           | 0          | 0                     | 0.591    | 0.271    | 0.847     | 0.929    | 0.559    | 0.082    | 0.514    | 0.632    | 0.130    | 0.694    |
|               | 0          | 0.5                   | 0.400    | 0.218    | 0.623     | 0.679    | 0.387    | 0.034    | 0.371    | 0.479    | 0.078    | 0.487    |
|               | 0          | 1.0                   | 0.394    | 0.227    | 0.622     | 0.721    | 0.425    | 0.049    | 0.438    | 0.405    | 0.091    | 0.495    |
| 707           | 6          | 0                     | 0.681    | 0.378    | 0.987     | 1.181    | 0.750    | 0.116    | 0.664    | 0.766    | 0.159    | 0.826    |
|               | 6          | 0.5                   | 0.464    | 0.308    | 0.867     | 0.969    | 0.588    | 0.084    | 0.419    | 0.648    | 0.093    | 0.678    |
|               | 6          | 1.0                   | 0.583    | 0.326    | 0.825     | 0.864    | 0.637    | 0.097    | 0.599    | 0.616    | 0.115    | 0.698    |
| 707           | 12         | 0                     | 0.559    | 0.317    | 0.929     | 1.115    | 0.599    | 0.088    | 0.598    | 0.701    | 0.144    | 0.794    |
|               | 12         | 0.5                   | 0.449    | 0.256    | 0.891     | 0.983    | 0.589    | 0.084    | 0.438    | 0.663    | 0.100    | 0.697    |
|               | 12         | 1.0                   | 0.538    | 0.303    | 0.806     | 0.896    | 0.609    | 0.066    | 0.539    | 0.618    | 0.113    | 0.651    |
| 714           | 0          | 0                     | 0.585    | 0.295    | 0.744     | 0.927    | 0.536    | 0.081    | 0.523    | 0.670    | 0.128    | 0.661    |
|               | 0          | 0.5                   | 0.500    | 0.263    | 0.773     | 0.850    | 0.588    | 0.058    | 0.492    | 0.605    | 0.113    | 0.617    |
|               | 0          | 1.0                   | 0.427    | 0.224    | 0.692     | 0.795    | 0.469    | 0.049    | 0.450    | 0.501    | 0.102    | 0.534    |
| 714           | 6          | 0                     | 0.686    | 0.400    | 0.983     | 1.263    | 0.732    | 0.102    | 0.706    | 0.786    | 0.148    | 0.873    |
|               | 6          | 0.5                   | 0.561    | 0.334    | 0.911     | 1.065    | 0.650    | 0.092    | 0.619    | 0.709    | 0.118    | 0.743    |
|               | 6          | 1.0                   | 0.580    | 0.346    | 0.893     | 1.001    | 0.621    | 0.088    | 0.582    | 0.656    | 0.122    | 0.689    |
| 714           | 12         | 0                     | 0.699    | 0.412    | 1.061     | 1.321    | 0.803    | 0.121    | 0.691    | 0.827    | 0.177    | 0.929    |
|               | 12         | 0.5                   | 0.540    | 0.305    | 0.951     | 1.052    | 0.729    | 0.090    | 0.605    | 0.673    | 0.129    | 0.754    |
|               | 12         | 1.0                   | 0.505    | 0.324    | 0.829     | 0.982    | 0.607    | 0.088    | 0.575    | 0.580    | 0.118    | 0.675    |

TABLE 15

INFLUENCE OF AUREOMYCIN ON THE PER CENT OF AMINO ACIDS REMOVED  
FROM THE RUMEN OF CATTLE FED A NATURAL RATION

| Animal no. | Aureo-<br>mycin<br>gm | Time<br>hr | Arg<br>% | His<br>% | Isol<br>% | Leu<br>% | Lys<br>% | Met<br>% | Phe<br>% | Thr<br>% | Try<br>% | Val<br>% |
|------------|-----------------------|------------|----------|----------|-----------|----------|----------|----------|----------|----------|----------|----------|
| 707        | 0                     | 0-6        | 49.9     | 36.4     | 28.8      | 34.8     | 18.5     | 28.0     | 32.6     | 31.7     | 33.9     | 33.2     |
|            | 0                     | 0-12       | 80.4     | 67.7     | 57.3      | 58.6     | 64.5     | 70.7     | 61.4     | 60.8     | 63.3     | 57.1     |
|            | 0                     | 6-12       | 30.5     | 28.9     | 20.3      | 20.0     | 32.2     | 35.4     | 23.7     | 22.4     | 23.5     | 18.5     |
| 707        | 0.5                   | 0-6        | 71.6     | 55.9     | 39.2      | 50.9     | 40.8     | 42.7     | 69.1     | 46.8     | 68.8     | 47.7     |
|            | 0.5                   | 0-12       | 87.3     | 84.2     | 62.3      | 70.1     | 64.1     | 59.8     | 82.6     | 67.9     | 79.8     | 67.5     |
|            | 0.5                   | 6-12       | 26.0     | 36.2     | 21.3      | 22.3     | 23.4     | 22.2     | 19.9     | 21.7     | 17.1     | 21.1     |
| 707        | 1.0                   | 0-6        | 49.1     | 45.5     | 36.7      | 58.2     | 26.8     | 26.8     | 39.1     | 36.3     | 54.1     | 37.6     |
|            | 1.0                   | 0-12       | 71.1     | 69.0     | 61.2      | 71.5     | 54.5     | 74.4     | 69.5     | 55.6     | 72.5     | 64.8     |
|            | 1.0                   | 6-12       | 23.7     | 23.1     | 19.2      | 14.1     | 20.9     | 43.3     | 25.5     | 17.0     | 18.7     | 22.8     |
| 714        | 0                     | 0-6        | 35.1     | 17.5     | 1.5       | 8.4      | 0        | 26.8     | 7.5      | 14.1     | 25.7     | 3.7      |
|            | 0                     | 0-12       | 51.6     | 35.0     | 15.8      | 25.7     | 11.2     | 26.8     | 33.9     | 31.5     | 24.8     | 19.8     |
|            | 0                     | 6-12       | 13.8     | 12.8     | 8.6       | 11.4     | 7.1      | 0        | 17.1     | 10.9     | 0        | 9.9      |
| 714        | 0.5                   | 0-6        | 53.9     | 39.1     | 23.3      | 32.5     | 31.7     | 28.0     | 28.3     | 29.1     | 52.3     | 29.4     |
|            | 0.5                   | 0-12       | 72.8     | 66.0     | 42.2      | 54.4     | 40.2     | 43.8     | 52.8     | 58.6     | 61.5     | 50.1     |
|            | 0.5                   | 6-12       | 18.7     | 23.1     | 11.9      | 16.8     | 5.6      | 17.7     | 17.7     | 20.1     | 8.1      | 14.6     |
| 714        | 1.0                   | 0-6        | 31.2     | 12.1     | 1.7       | 23.5     | 6.3      | 11.0     | 17.4     | 12.5     | 30.3     | 16.5     |
|            | 1.0                   | 0-12       | 70.2     | 50.2     | 51.5      | 57.1     | 46.2     | 40.2     | 51.9     | 62.0     | 64.2     | 52.5     |
|            | 1.0                   | 6-12       | 31.7     | 26.5     | 27.1      | 23.1     | 23.3     | 22.0     | 22.4     | 30.6     | 24.5     | 23.1     |

TABLE 16

INFLUENCE OF AUREOMYCIN ON THE SYNTHESIS OR REMOVAL OF AMINO ACIDS IN OR  
FROM THE RUMEN OF CATTLE FED A PURIFIED RATION

| Animal no. | Aureo-<br>mycin<br>gm | Time<br>hr | Arg<br>% | His<br>% | Isol<br>% | Leu<br>% | Lys<br>% | Met<br>% | Phe<br>% | Thr<br>% | Try<br>% | Val<br>% |
|------------|-----------------------|------------|----------|----------|-----------|----------|----------|----------|----------|----------|----------|----------|
| 714        | 0                     | 0-6        | +30.7    | +36.8    | +52.6     | +52.8    | -36.0    | +41.4    | +55.1    | +61.5    | +34.0    | +52.3    |
|            | 0                     | 0-12       | +22.8    | +27.6    | +37.2     | +41.9    | +19.8    | +32.9    | +35.3    | +42.3    | +24.0    | +36.5    |
|            | 0                     | 6-12       | -6.0     | -6.7     | -10.0     | -7.1     | +87.3    | -6.1     | -12.8    | -11.9    | -7.5     | -10.4    |
| 714        | 0.5                   | 0-6        | +16.2    | +30.6    | +6.4      | +4.1     | -41.4    | -15.6    | -14.8    | +11.9    | -4.0     | +4.4     |
|            | 0.5                   | 0-12       | +26.9    | +38.7    | +10.3     | +17.0    | +21.1    | +6.3     | 0        | +23.0    | +8.0     | +10.9    |
|            | 0.5                   | 6-12       | +9.3     | +6.2     | +3.6      | +12.4    | +106.7   | +25.9    | +17.4    | +9.9     | +12.5    | +6.3     |

of each amino acid; also the rate of removal from six to 12 hours was calculated for each amino acid. The data for the removal of the amino acids are summarized for each time of collection and for each level of aureomycin intake in Table 15. The detailed data for each amino acid in Table 15 are found in Tables 23 through 32 (appendix). The data indicate that the rate of removal of the amino acids was more rapid when 0.5 gram of aureomycin was included in the ration. In the case of the purified ration (Table 16) the data indicate that synthesis of the amino acids does occur in the rumen. The amino acid composition of the rumen contents from the steer fed the purified ration is found in Table 33 (appendix).

#### B-Vitamin Analyses

The rations fed and the rumen contents were assayed for riboflavin, nicotinic acid and pantothenic acid. The B-vitamin composition of the rations fed is given in Table 17.

TABLE 17

#### B-VITAMIN COMPOSITION OF THE RATIONS FED

| Ration            | Riboflavin<br>/gm | Nicotinic<br>acid<br>/gm | Pantothenic<br>acid<br>/gm |
|-------------------|-------------------|--------------------------|----------------------------|
| Corn              | 1.65              | 23.24                    | 2.02                       |
| Alfalfa-brome hay | 6.48              | 25.89                    | 22.44                      |
| Purified          | 0.49              | 0.58                     | 0                          |



Table 18 gives the riboflavin, nicotinic acid and pantothenic acid content of the rumen contents from cattle fed the natural ration at each time of collection for each level of aureomycin fed. As in the case of the amino acids, the amounts of these B-vitamins that were synthesized in or removed from the rumen were calculated. The detailed data for these calculations are presented for riboflavin, nicotinic acid and pantothenic acid in Tables 19, 20 and 21, respectively. The data for the synthesis of the B-vitamins in the case of the purified ration are presented in Table 22. Table 34 (appendix) gives the B-vitamin composition of the rumen contents when the purified ration was fed.

#### pH and Bacteriological Analyses

Samples of rumen fluid were preserved for total bacterial count for three consecutive days at the beginning of each level of aureomycin intake. A pH determination was made at the same time that a sample was collected for the bacteriological analysis. Figure 1 shows graphically the influence of aureomycin on the pH and the total bacterial count of rumen contents from cattle fed the natural ration. Each level of aureomycin intake was offered for a 15-day period. Figure 2 presents the influence of the aureomycin on the total bacterial count of the rumen contents from cattle fed the natural ration for each of these 15-day periods. There was a definite increase in the

total bacterial count when 0.5 gram of aureomycin was included in the ration and not much additional change when the aureomycin was increased to 1.0 gram. Detailed data for these figures are found in Tables 35, 36 and 37 (appendix).

Figures 3 and 4 show the results of the cultural study for the rumen streptococci and the coliform groups present in the rumen of cattle fed the natural ration when aureomycin was included in the daily ration. The average for the first three days of each level of aureomycin intake is shown in Figure 3, while the average for each 15-day period is shown in Figure 4. Detailed data for these figures are presented in Table 38 (appendix).

Samples of fecal material were collected periodically for each level of aureomycin fed while the cattle were on the natural ration. Determinations for the total bacterial count, fecal streptococci and the coliform groups were conducted on these samples. Figure 5 shows the bacterial picture for the total count, while the results of the cultural studies for the fecal streptococci and the coliform groups are shown in Figure 6. Detailed data for these figures are presented in Tables 39 and 40 (appendix).

When steer 714 was fed the purified ration, the same procedure was followed in collecting the samples for pH and bacteriological studies. Figure 7 presents the results for the pH and the total bacterial count when no aureomycin and when 0.5 gram of aureomycin was fed. Detailed data for Figure 7 are presented in Tables 41 and 42 (appendix).

TABLE 18

INFLUENCE OF AUREOMYCIN ON THE B-VITAMIN COMPOSITION OF RUMEN  
CONTENTS FROM CATTLE FED A NATURAL RATION

| Animal<br>no. | Time<br>hr | Aureo-<br>mycin<br>gm | Riboflavin<br>γ/gm | Pantothenic<br>Acid<br>γ/gm | Nicotinic<br>Acid<br>γ/gm |
|---------------|------------|-----------------------|--------------------|-----------------------------|---------------------------|
| 707           | 0          | 0                     | 7.25               | 12.22                       | 11.91                     |
|               | 0          | 0.5                   | 6.57               | 38.99                       | 29.67                     |
|               | 0          | 1.0                   | 4.00               | 11.31                       | 17.54                     |
| 707           | 6          | 0                     | 8.01               | 24.21                       | 51.94                     |
|               | 6          | 0.5                   | 7.21               | 36.29                       | 52.60                     |
|               | 6          | 1.0                   | 5.15               | 26.73                       | 40.58                     |
| 707           | 12         | 0                     | 5.81               | 20.96                       | 41.51                     |
|               | 12         | 0.5                   | 5.43               | 28.12                       | 69.22                     |
|               | 12         | 1.0                   | 3.88               | 18.35                       | 42.63                     |
| 714           | 0          | 0                     | 7.38               | 10.30                       | 10.65                     |
|               | 0          | 0.5                   | 7.80               | 27.03                       | 40.83                     |
|               | 0          | 1.0                   | 4.08               | 14.04                       | 26.72                     |
| 714           | 6          | 0                     | 8.42               | 26.54                       | 46.25                     |
|               | 6          | 0.5                   | 5.85               | 25.07                       | 41.33                     |
|               | 6          | 1.0                   | 4.19               | 22.84                       | 44.17                     |
| 714           | 12         | 0                     | 7.99               | 29.21                       | 65.69                     |
|               | 12         | 0.5                   | 5.36               | 26.50                       | 60.30                     |
|               | 12         | 1.0                   | 3.79               | 22.68                       | 50.92                     |

TABLE 19  
INFLUENCE OF AUREOMYCIN ON THE REMOVAL OF RIBOFLAVIN FROM THE RUMEN OF CATTLE  
FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no.           | Aureo-<br>mycin<br>gm | Riboflavin |              |             | Riboflavin |               |              | Riboflavin |               |              | Riboflavin               |               |              |                         |  |
|----------------------|-----------------------|------------|--------------|-------------|------------|---------------|--------------|------------|---------------|--------------|--------------------------|---------------|--------------|-------------------------|--|
|                      |                       | In<br>mg   | Intake<br>mg | Total<br>mg | In<br>mg   | Removed<br>mg | Removed<br>% | In<br>mg   | Removed<br>mg | Removed<br>% | In<br>mg                 | Removed<br>gm | Removed<br>% |                         |  |
|                      |                       |            |              |             |            |               |              |            |               |              |                          |               |              |                         |  |
| <u>0-hr. samples</u> |                       |            |              |             |            |               |              |            |               |              |                          |               |              |                         |  |
| 707                  | 0                     | 35.5       | 47.1         | 82.6        | 68.3       | 14.3          | 30.4         | 42.0       | 40.6          | 86.2         | <u>0-12 hr. samples*</u> |               |              | <u>6-12 hr. samples</u> |  |
|                      | 0.5                   | 30.5       | 47.1         | 77.6        | 54.4       | 23.2          | 49.3         | 31.4       | 46.2          | 98.1         |                          |               |              |                         |  |
|                      | 1.0                   | 24.9       | 47.1         | 72.0        | 47.8       | 24.2          | 51.4         | 29.8       | 42.2          | 89.6         |                          |               |              |                         |  |
| 714                  | 0                     | 40.5       | 47.1         | 87.6        | 85.6       | 2.0           | 4.2          | 68.8       | 18.8          | 39.9         |                          |               |              |                         |  |
|                      | 0.5                   | 49.1       | 47.1         | 96.2        | 60.8       | 35.4          | 75.2         | 46.9       | 49.3          | 104.7        |                          |               |              |                         |  |
|                      | 1.0                   | 30.2       | 47.1         | 77.3        | 51.7       | 25.6          | 54.4         | 36.7       | 40.6          | 86.2         |                          |               |              |                         |  |

\* Based on riboflavin intake.

TABLE 20

INFLUENCE OF AUREOMYCIN ON THE AMOUNT OF NICOTINIC ACID  
ACCUMULATED OR REMOVED FROM THE RUMEN OF CATTLE FED A  
NATURAL RATION IN 6 AND 12 HOURS

| Animal<br>no. | Aureo-<br>mycin<br>gm | <u>Nicotinic Acid</u> |              |             | <u>Nicotinic Acid</u>   |             |               |                   |
|---------------|-----------------------|-----------------------|--------------|-------------|-------------------------|-------------|---------------|-------------------|
|               |                       | In<br>rumen<br>mg     | Intake<br>mg | Total<br>mg | In<br>rumen<br>mg       | Diff.<br>mg | Accumul.<br>% | Re-<br>moved<br>% |
|               |                       | <u>0-hr. samples</u>  |              |             | <u>0-6 hr. samples*</u> |             |               |                   |
| 707           | 0                     | 58.3                  | 218.5        | 276.8       | 442.8                   | 166.0       | 76.0          |                   |
|               | 0.5                   | 137.6                 | 218.5        | 356.1       | 396.8                   | 40.7        | 18.6          |                   |
|               | 1.0                   | 109.0                 | 218.5        | 327.5       | 376.9                   | 49.4        | 22.6          |                   |
| 714           | 0                     | 58.5                  | 218.5        | 277.0       | 470.4                   | 193.4       | 88.5          |                   |
|               | 0.5                   | 257.0                 | 218.5        | 475.5       | 429.7                   | 45.8        | --            | 21.0              |
|               | 1.0                   | 197.7                 | 218.5        | 416.2       | 545.2                   | 129.0       | 59.0          |                   |

| Animal<br>no. | Aureo-<br>mycin<br>gm | <u>Nicotinic Acid</u>    |                |       | <u>Nicotinic Acid</u>   |               |              |
|---------------|-----------------------|--------------------------|----------------|-------|-------------------------|---------------|--------------|
|               |                       | In<br>rumen<br>mg        | Accumul.<br>mg | %     | Diff.<br>mg             | Accumul.<br>% | Removed<br>% |
|               |                       | <u>0-12 hr. samples*</u> |                |       | <u>6-12 hr. samples</u> |               |              |
| 707           | 0                     | 300.0                    | 23.2           | 10.6  | 142.8                   | --            | 32.2         |
|               | 0.5                   | 400.0                    | 43.9           | 20.1  | 3.2                     | 0.8           | --           |
|               | 1.0                   | 327.6                    | 0              | 0     | 49.3                    | --            | 13.0         |
| 714           | 0                     | 565.7                    | 288.7          | 132.1 | 95.3                    | 20.3          | --           |
|               | 0.5                   | 527.9                    | 52.4           | 24.0  | 98.2                    | 22.9          | --           |
|               | 1.0                   | 493.1                    | 76.9           | 35.2  | 52.1                    | --            | 9.6          |

\* Based on nicotinic acid intake.

TABLE 21

INFLUENCE OF AUREOMYCIN ON THE AMOUNT OF PANTOTHENIC ACID  
ACCUMULATED OR REMOVED FROM THE RUMEN OF CATTLE FED A  
NATURAL RATION IN 6 AND 12 HOURS

| Animal<br>no. | Aureo-<br>mycin<br>gm | Pantothenic Acid  |           |             | Pantothenic Acid  |             |               |                   |
|---------------|-----------------------|-------------------|-----------|-------------|-------------------|-------------|---------------|-------------------|
|               |                       | In<br>rumen<br>mg | Fed<br>mg | Total<br>mg | In<br>rumen<br>mg | Diff.<br>mg | Accumul.<br>% | Re-<br>moved<br>% |
|               |                       | O-hr. samples     |           |             | 0-6 hr. samples*  |             |               |                   |
| 707           | 0                     | 59.9              | 156.5     | 216.4       | 206.4             | 10.0        | --            | 6.4               |
|               | 0.5                   | 180.8             | 156.5     | 337.3       | 273.7             | 63.6        | --            | 40.6              |
|               | 1.0                   | 70.3              | 156.5     | 226.8       | 248.3             | 21.5        | 13.7          | --                |
| 714           | 0                     | 56.6              | 156.5     | 213.1       | 269.9             | 56.8        | 36.3          | --                |
|               | 0.5                   | 170.2             | 156.5     | 326.7       | 260.7             | 66.0        | --            | 42.2              |
|               | 1.0                   | 103.9             | 156.5     | 260.4       | 281.9             | 21.5        | 13.7          | --                |

| Animal<br>no. | Aureo-<br>mycin<br>gm | Pantothenic Acid  |             |               |                   | Pantothenic Acid |      |
|---------------|-----------------------|-------------------|-------------|---------------|-------------------|------------------|------|
|               |                       | In<br>rumen<br>mg | Diff.<br>mg | Accumul.<br>% | Re-<br>moved<br>% | Removed          |      |
|               |                       | 0-12 hr. samples* |             |               |                   | 6-12 hr. samples |      |
| 707           | 0                     | 151.5             | 64.9        | --            | 41.5              | 54.9             | 26.6 |
|               | 0.5                   | 162.5             | 174.8       | --            | 111.7             | 111.2            | 40.6 |
|               | 1.0                   | 141.0             | 85.8        | --            | 54.8              | 107.3            | 43.2 |
| 714           | 0                     | 251.6             | 38.5        | 24.6          | --                | 18.3             | 6.8  |
|               | 0.5                   | 232.0             | 94.7        | --            | 60.5              | 28.7             | 11.0 |
|               | 1.0                   | 219.6             | 40.8        | --            | 26.1              | 62.3             | 22.1 |

\* Based on pantothenic acid intake.

TABLE 22

INFLUENCE OF AUREOMYCIN ON THE SYNTHESIS OR REMOVAL OF  
B-VITAMINS IN OR FROM THE RUMEN OF 714  
FED A PURIFIED RATION

| Animal<br>no. | Aureo-<br>mycin<br>gm | Time<br>hr | Riboflavin<br>% | Nicotinic<br>Acid<br>% | Pantothenic<br>Acid<br>% |
|---------------|-----------------------|------------|-----------------|------------------------|--------------------------|
| 714           | 0                     | 0-6        | +243.8          | +1286.3                | +152.2                   |
|               | 0                     | 0-12       | +220.5          | +1542.4                | +481.1                   |
|               | 0                     | 6-12       | -6.8            | +18.5                  | +206.8                   |
| 714           | 0.5                   | 0-6        | +20.8           | -67.7                  | -63.4                    |
|               | 0.5                   | 0-12       | +28.2           | -35.3                  | +45.6                    |
|               | 0.5                   | 6-12       | +6.1            | +100.5                 | +296.6                   |

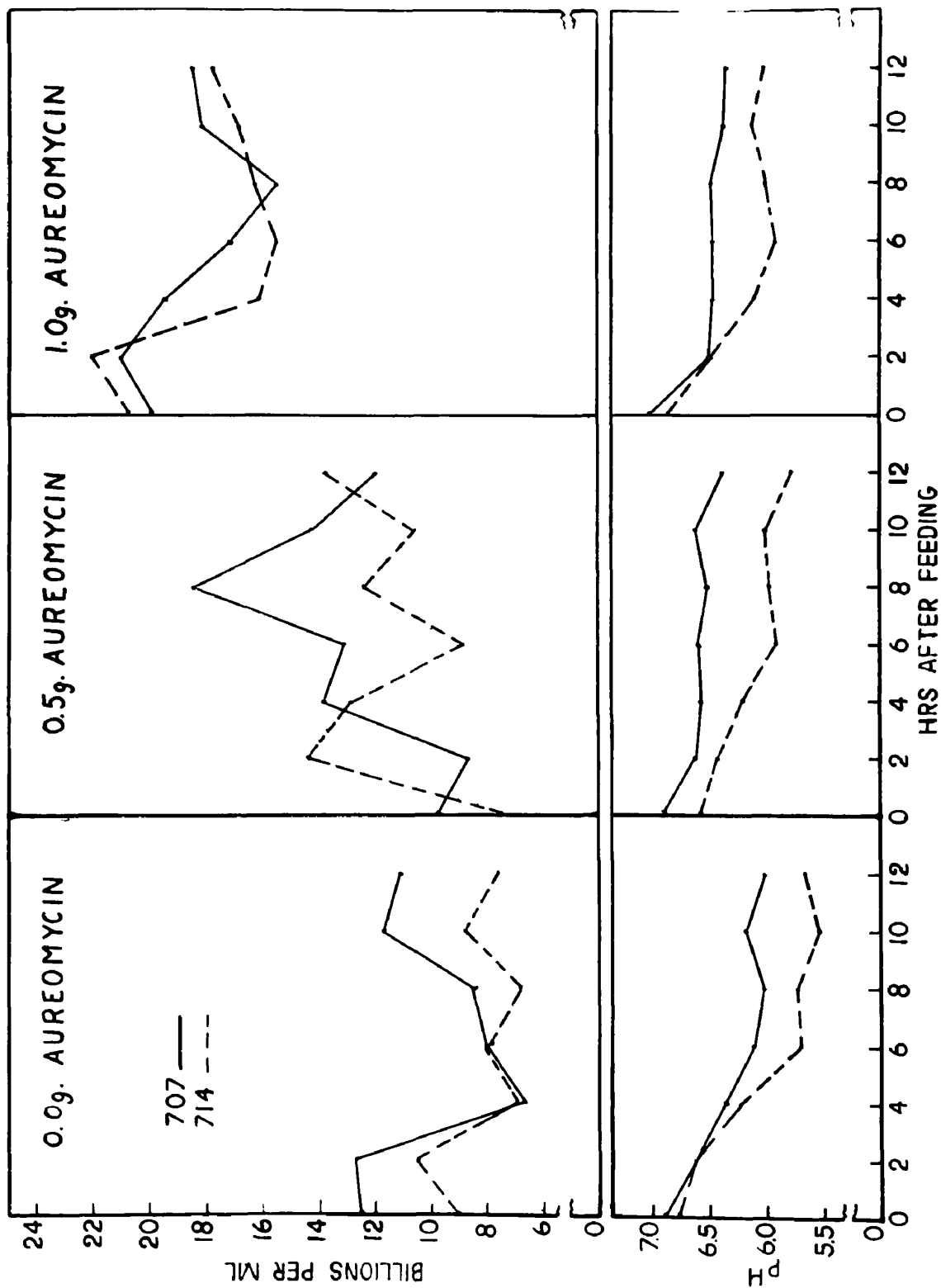


Figure 1. Influence of aureomycin on the pH and total bacterial count of rumen contents from cattle fed a natural ration (av. first 3 days of 15-day period).



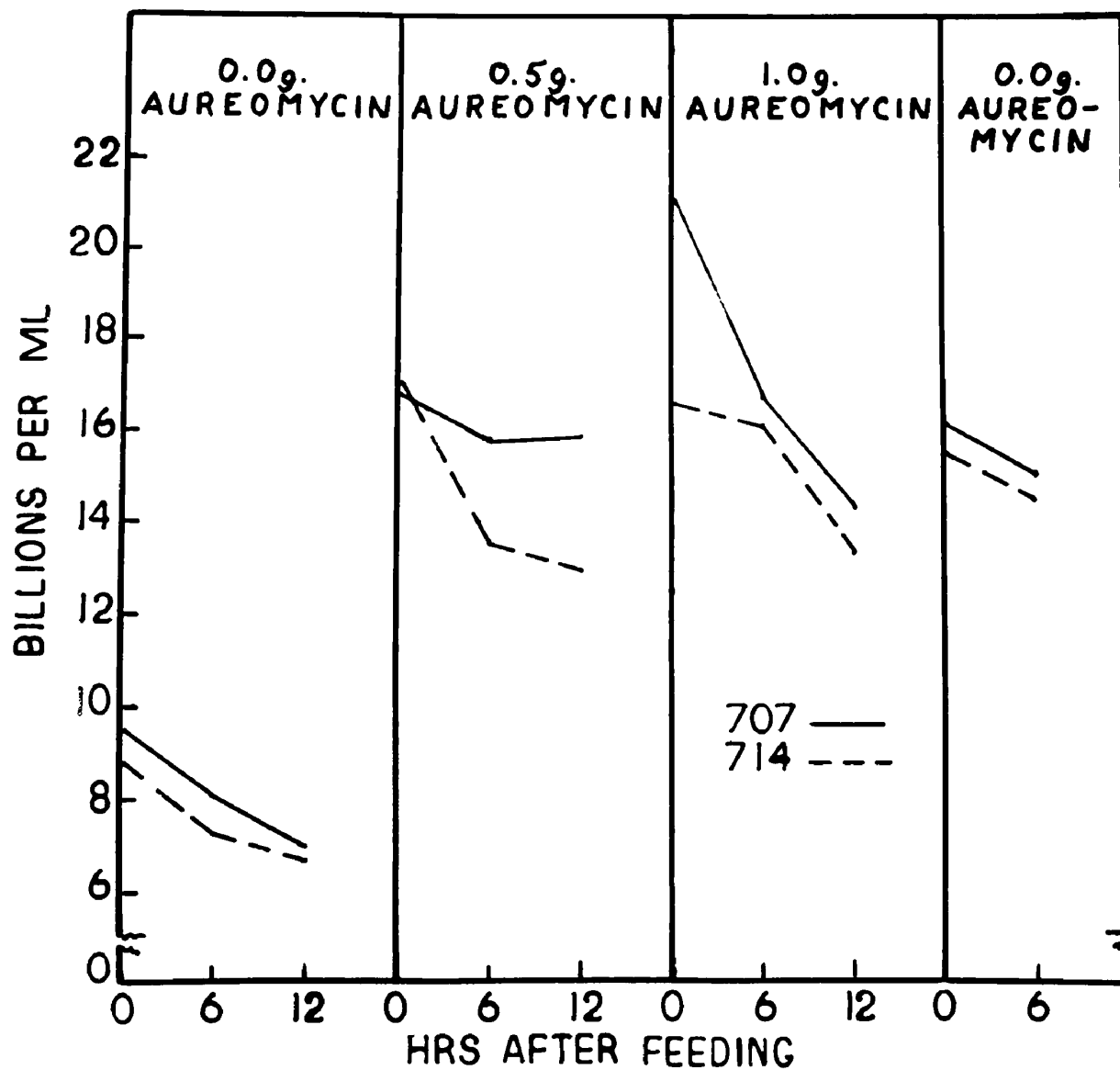


Figure 2. Influence of aureomycin on the total bacterial count of rumen contents from cattle fed a natural ration (av. of 15 days).

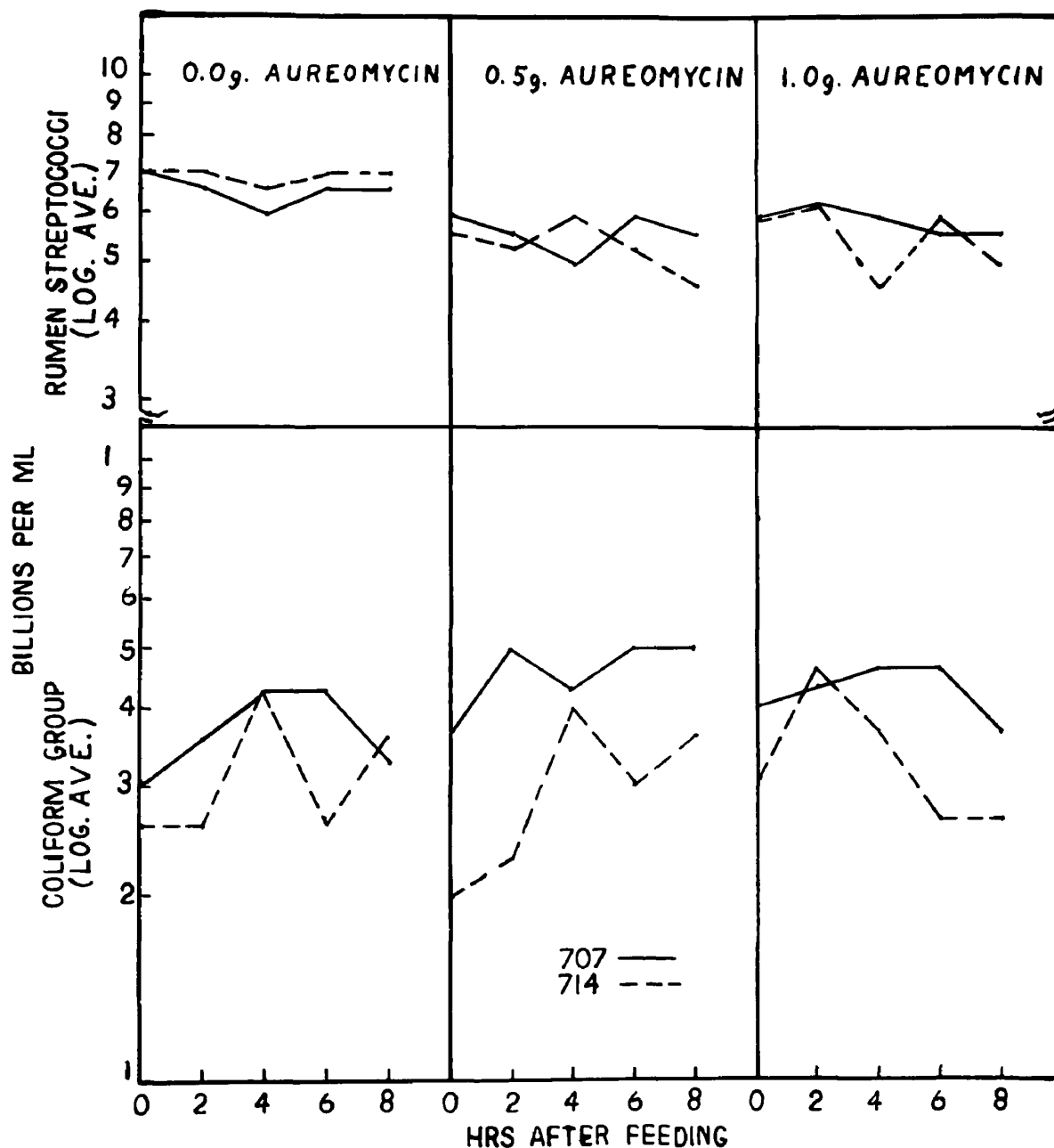


Figure 3. Influence of aureomycin on the rumen streptococci and the coliform group in the rumen of cattle fed a natural ration (av. first 3 days of 15-day period).

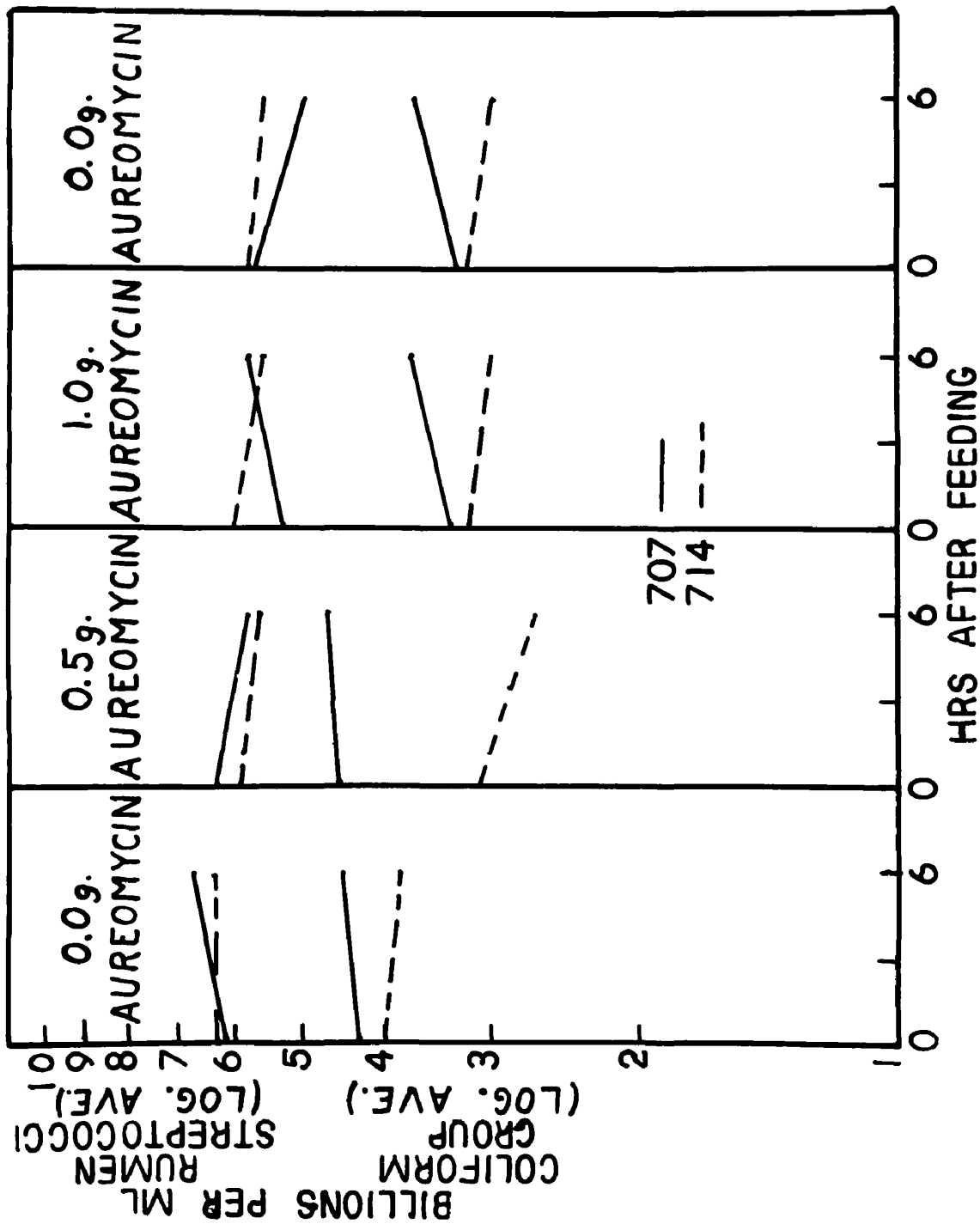


Figure 4. Influence of aureomycin on the rumen streptococci and the coliform group in the rumen of cattle fed a natural ration (av. of 15 days).

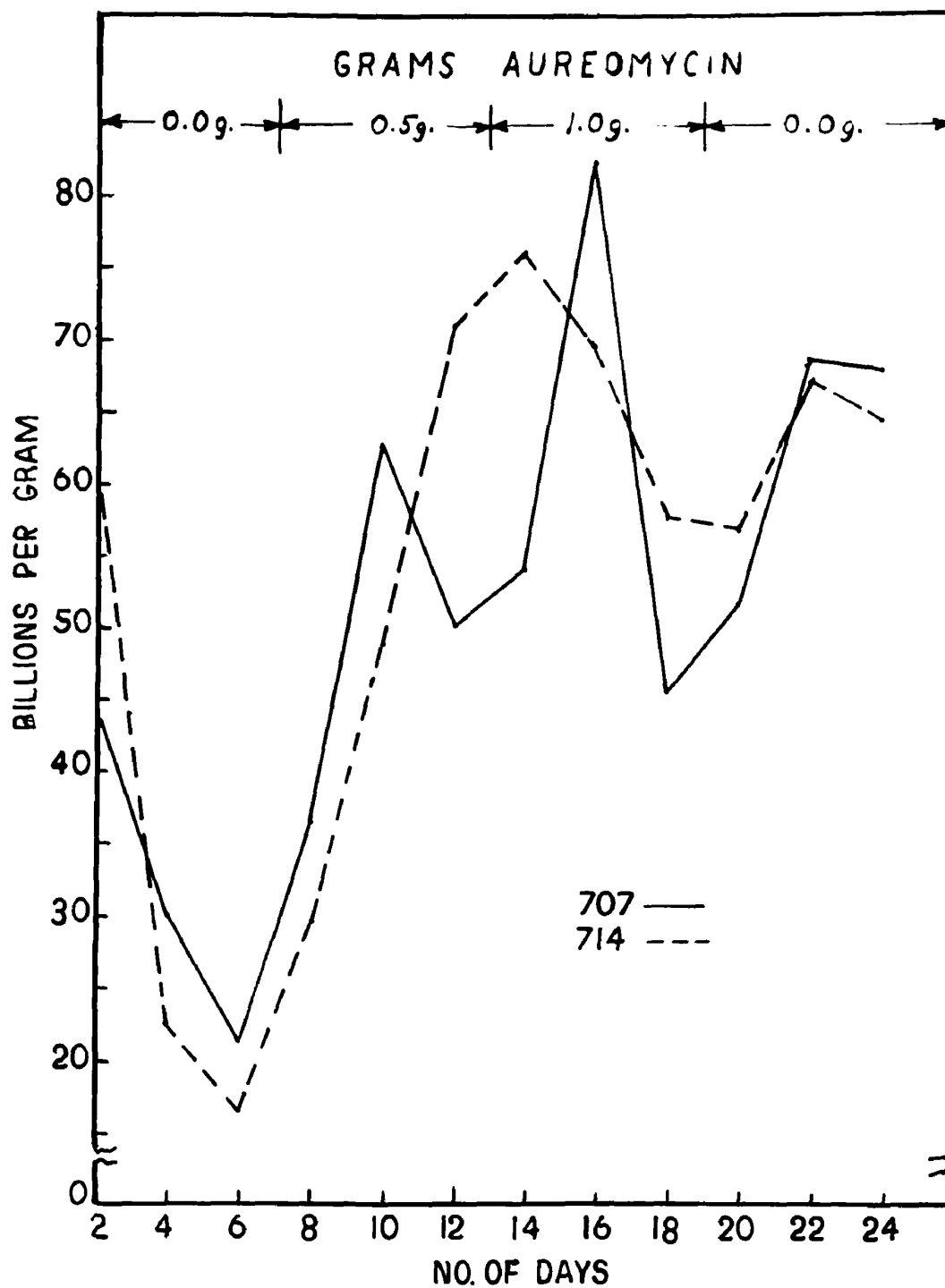


Figure 5. Influence of aureomycin on the total bacterial count of feces from cattle fed a natural ration.

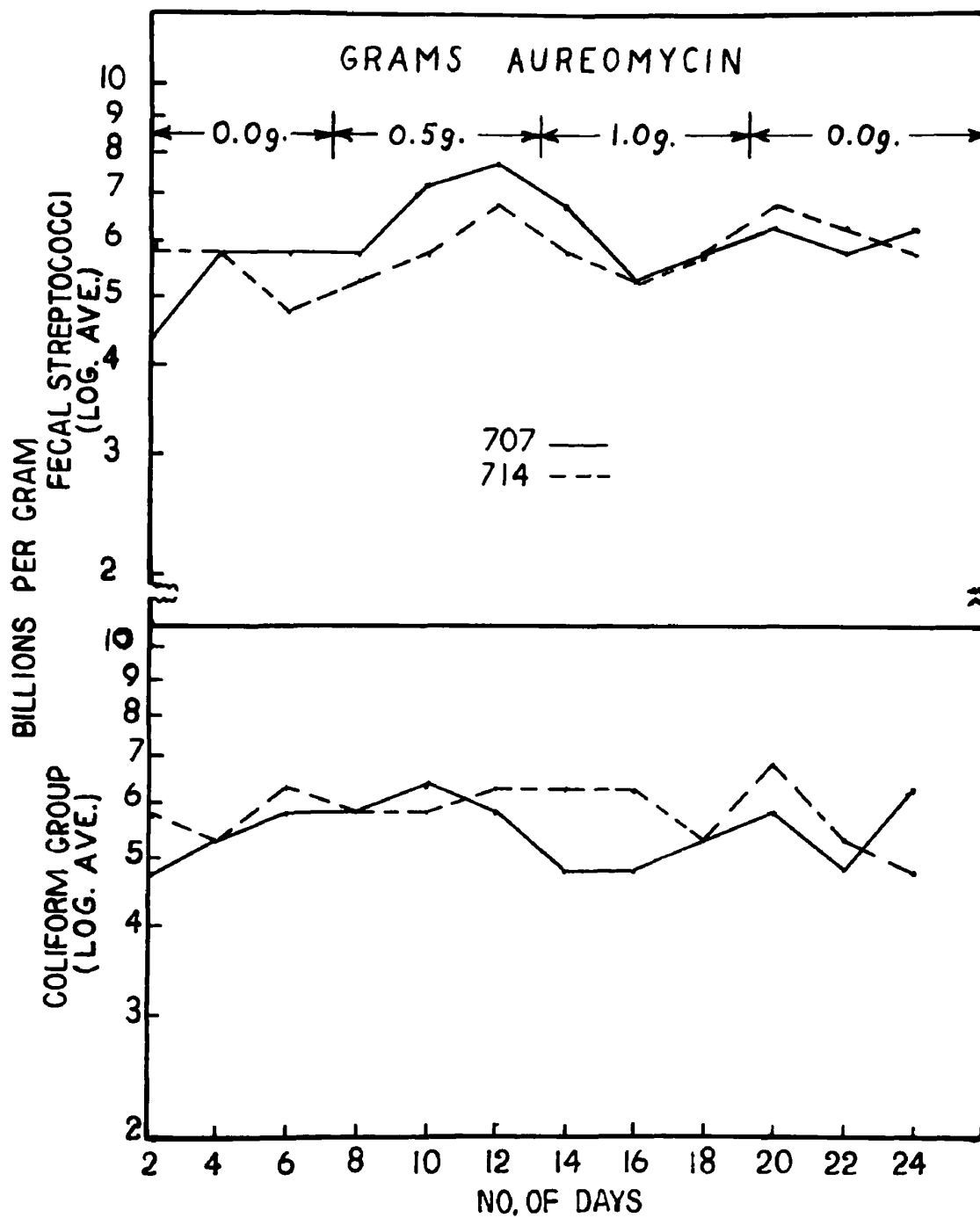


Figure 6. Influence of aureomycin on the fecal streptococci and the coliform group in the feces from cattle fed a natural ration.

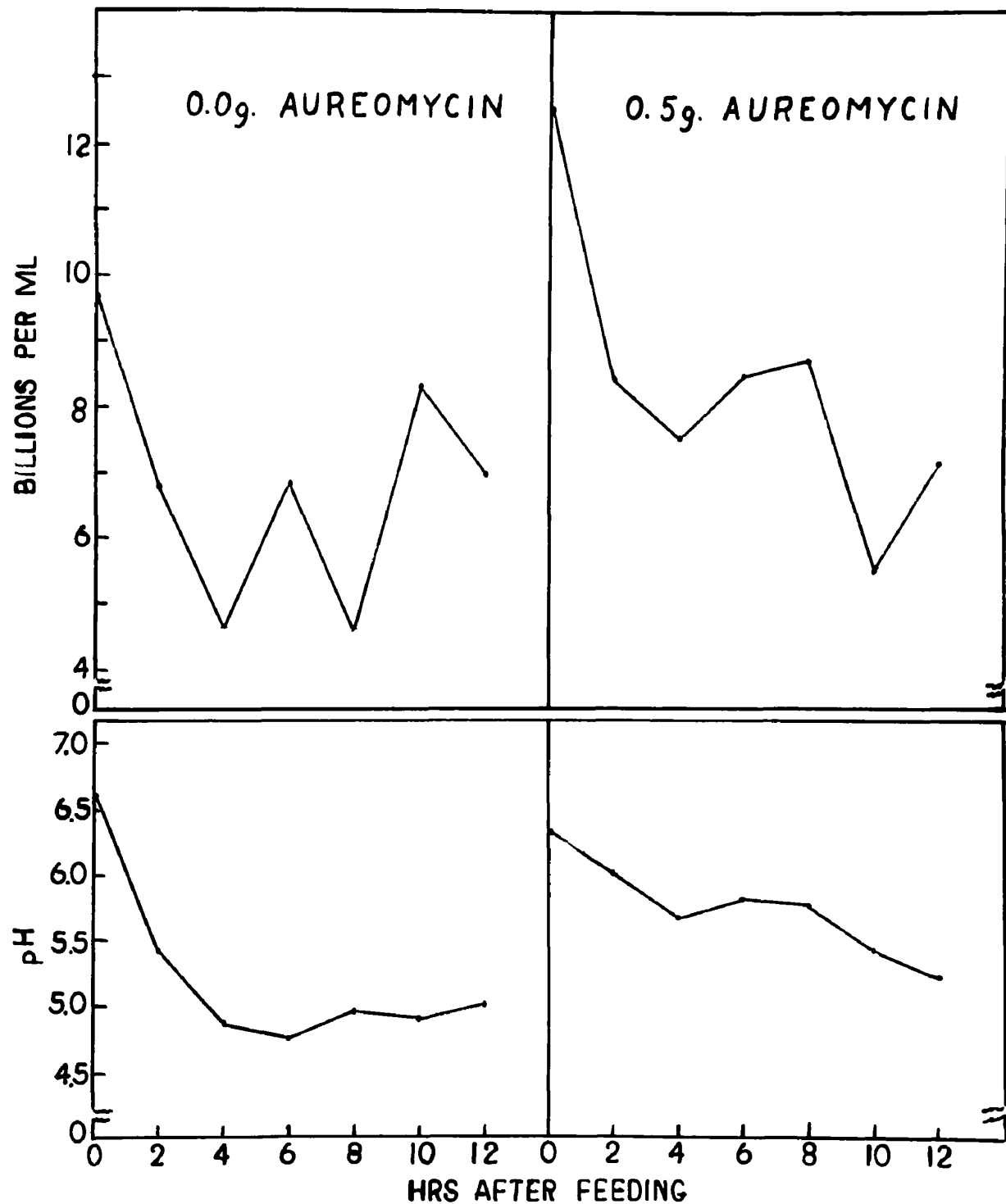


Figure 7. Influence of aureomycin on the pH and total bacterial count of the rumen contents from steer 714 fed a purified ration.

## DISCUSSION

### Health of the Animals

In this investigation, neither of the steers showed any signs of anorexia or diarrhea that had been reported by Bell et al. (1951) with steers and by Colby et al. (1950) with fattening lambs when crystalline aureomycin was included in the ration. Also, the steers used in this study continued to gain in body weight when the aureomycin was fed. Colby et al. observed that the fattening lambs receiving aureomycin lost 0.2 pounds per day while the lambs on the basal ration gained 0.52 pounds per day.

The rations used by both of these investigators contained a high proportion of grain to roughage; in fact, 50 per cent of the total daily ration that Bell et al. used in their digestion trial came from grain. In the study reported here, the daily ration consisted of 79 per cent hay and 21 per cent ground corn. Pounden and Hibbs (1948a, 1948b) reported that grain feeding was responsible for inhibiting the establishment of the varieties of flora which were associated with hay ingestion. Gall et al. (1949c) observed that as the amount of grain in the ration increased, there was a corresponding increase in the number of fast-growing bacteria. Consequently, from the observations of Pounden and Hibbs and Gall et al. as well as those of

Bell et al. and Colby et al., it is possible that the rumen bacteria adapted to a low cellulose-high energy ration were more sensitive to aureomycin; whereas in the present study the bacteria were adapted to a high cellulose-low energy ration and were not as sensitive to aureomycin, at least at the levels fed.

The illness and subsequent death of steer 714 cannot be attributed to a particular cause; however, several investigators have reported toxic effects from urea in the case of cattle and sheep. Hart et al. (1939) slaughtered a steer that had been receiving a ration which contained 4.3 per cent urea and found necrosis in some areas of the liver and badly damaged kidneys. Clark et al. (1951a) observed severe degeneration of the kidney and the liver in sheep dosed with urea. However, they also found that the toxicity of urea was dependent on the activity of the ruminal flora as determined by the basal ration and the presence of available carbohydrate. A greater tolerance for urea was observed when the bacterial flora was conditioned to a high plane of nitrogen metabolism by including casein in the ration. Likewise, the readily available carbohydrate would be mixed with the urea and would help to keep the urea from being converted to ammonia too rapidly which could cause toxic symptoms if the concentration in the blood should become too high. Harris and Mitchell (1941b) fed rations containing up to 3.16 per cent urea, on the dry basis, for 110 days to lambs without any histological evidence of kidney damage. Work et al. (1943) did



not observe any liver or kidney damage in steers fed urea at the rate of 2.29 per cent of the dry matter for 244 days.

#### pH and Bacteriological Analyses

From the data presented in Figure 1, the pH of the rumen contents from 707 for each level of aureomycin intake never went as low as that observed for 714 for the same periods when the animals received the natural ration. In both animals, the pH would decrease after feeding and reach the lowest point in about six or eight hours. However, there was only a slight increase toward alkalinity 12 hours after feeding. This fact is not entirely in agreement with the observations of Kick et al. (1938), Monroe and Perkins (1939), Wegner et al. (1941), Smith (1941) and Myburgh and Quin (1943) who reported that the pH declines for about four hours with a gradual increase toward alkalinity following the decline. A possible explanation for the differences observed in the investigation reported here may be that the animals were fed once daily, whereas the other workers fed their animals twice daily. Consequently, in the present study, the bacteria were conditioned to being fed once daily and performed their fermentation processes at a slower rate than those that were fed twice daily. It is also possible that the cellulose digesting bacteria were still producing acids which would keep the pH down. Roine and Elvehjem (1950) stated that the pH was not determined by the food but by the kind of microflora which developed in the digestive tract.

In the case of the purified ration, the pH was definitely lower when no aureomycin was fed as compared to the period when 0.5 gram of aureomycin was fed as shown in Figure 7. However, steer 714 was off-feed during the aureomycin trial and most likely the bacteria were not as functional as those during the control period. As in the case of the natural ration, the pH increased slightly toward alkalinity at 12-hours after feeding, possibly for the same reasons as mentioned in the discussion of the pH when the natural ration was fed.

There was a definite increase in the total bacterial count of the rumen contents when 0.5 gram of aureomycin was added to the natural ration. The total bacterial count remained at approximately the same level when the aureomycin was increased to 1.0 gram per day, possibly because the bacteria may have developed some resistance to the antibiotic. It is possible that the increased removal of dry matter, crude fiber, crude protein, nitrogen-free extract and the amino acids when 0.5 gram of aureomycin was fed was the result of the activity of the increased bacterial population. Since there were more bacteria to digest the feed, it is logical to assume that they should perform a more complete digestion of the nutrients. Elam et al. (1951a) and Couch et al. (1951) observed an increase in the total number of intestinal microorganisms when penicillin was included in the ration of chicks. Burroughs et al. (1950, 1950d, 1950e, 1951a, 1951b) observed that a complex salt solu-

tion, alfalfa ash extract, autoclaved rumen liquid and an autoclaved water extract of manure were beneficial in aiding rumen microorganisms to digest cellulose. They also observed that an increase in the size and number of bacteria present and an improvement in cellulose digestion occurred when any of these materials were added to a flask in which poor cellulose digestion was found. It is possible that the aureomycin used in the present study either stimulated the bacteria directly or was responsible for the releasing of some factor(s) in the rumen which caused the bacterial population to increase.

As observed in Figures 3 and 4 there was a reduction in the number of rumen streptococci for both animals when aureomycin was included in the ration. In the case of the coliform group, the number present in the rumen of 707 six hours after feeding was always higher than observed at the 0-hour; whereas, in 714, the number was lower at the 6-hour collection. Also, the number of coliform bacteria observed in 707 was consistently higher than in 714. The increase in the coliform group when 0.5 gram of aureomycin was fed may be of some significance because it was at this concentration that the highest removal of the various nutrients from the rumen was observed. Also, in the case of 714, it was interesting to note that the rate of removal of the various nutrients from the rumen was lower than that observed in 707 and that this coincided with a lower number of coliform organisms and total bacterial count than observed in 707. However, over-all, the rate of removal of the

nutrients from the rumen was the highest in both animals when 0.5 gram of aureomycin was fed, although the highest percentage of removal occurred in 707.

Bartley et al. (1951) did not observe any consistent microscopic differences of the rumen microflora between the control and aureomycin-fed calves. Neumann et al. (1951) noted that the total bacterial count was about the same for the control as for the aureomycin-fed heifers, but the types found in the heifers fed aureomycin were much less diverse, which suggested that the normal rumen flora had been disturbed. Neumann et al. also reported some reduction in appetite for a few days after the addition of aureomycin to the ration and then followed by a partial recovery. It was interesting to note that the ration Neumann et al. fed to their beef heifers consisted of a high proportion of grain and that they had some reduction in appetite when aureomycin was added to the ration. Bell et al. (1951) reported more severe results, but they also fed a ration high in grain and fed a higher concentration of aureomycin. As mentioned before, it is possible that the rumen flora adapted to a high proportion of grain may be sensitive to aureomycin, while the flora which developed on the type of ration fed in the present study can withstand a higher concentration of aureomycin. In the present study, the amount of aureomycin fed daily was much higher than that fed by Bell et al. and Neumann et al.

An increase of the total bacterial count of the fecal material was observed when aureomycin was fed to animals receiving the natural ration. The decrease in the total bacterial count just before the aureomycin was fed cannot be explained. The total bacterial count of the feces was about four times higher than that observed for the rumen contents. The rumen contents were higher in moisture which may account for a lower total bacterial count due to a greater dilution. There was not much difference between the number of streptococci in the rumen and fecal material. However, the number of coliform organisms in the feces were higher than that observed in the rumen.

Gall et al. (1951) observed that the types of bacteria in the rumen of animals receiving a purified ration were different from those when the animals were fed casein or urea plus sulphur. In the study reported here, the total bacterial count of the rumen contents when 714 was fed the purified ration was lower than that observed with the natural ration. When 0.5 gram of aureomycin was added to the ration, the total bacterial count remained about the same as the control period. Some change in the types of bacteria must have occurred when the aureomycin was added because there was a definite decrease in the synthesis of the amino acids and B-vitamins. The fact that the animal was off-feed may be closely related to the types of bacteria and their functions under certain conditions.

Passage of Some of the Ration Constituents  
from the Rumen

It is very difficult to measure the rate of passage of the various constituents from the rumen because one is concerned with a moving system. However, the amount of the various constituents present at each collection of rumen contents could be accounted for while the part unaccounted for was assumed to have passed from the rumen either by absorption through the rumen wall or by passing on to the remainder of the digestive tract. It can be observed from Tables 7 through 12 that there are distinct differences in the rate of removal of the various constituents from the rumens of steers 707 and 714. The rate of removal of the different nutrients from the rumen of 714 in six hours was considerably lower than 707. However, in the case of ether extract, there was a greater accumulation in the rumen of 714 than in 707 in six hours. Even though the rate of removal of the nutrients from the rumen of 707 was greater than in 714, the trend in 714 was similar to that in 707. In most cases the rate of removal of the nutrients from the rumen was faster when 0.5 gram of aureomycin was included in the ration. When 1.0 gram of aureomycin was included in the ration, the data for 714 indicated a delayed digestion during the first 6-hour period but an increased rate of removal during the second 6-hour period for dry matter, crude fiber, crude protein and nitrogen-free extract. In the case of 707, when 1.0 gram

of aureomycin was added to the ration, the rate of removal of dry matter, crude fiber, crude protein and nitrogen-free extract from the rumen for both the first and second 6-hour periods was slightly less than when 0.5 gram of aureomycin was fed, but usually a little more than when no aureomycin was fed.

An apparent accumulation of dry matter (Table 7) in the rumen of both 707 and 714, at the 0-hour, resulted when 1.0 gram of aureomycin was fed. This was probably due to the increased crude fiber content of the dry matter as shown in Table 8 and also the weight of the rumen contents was the heavier at this time. A corresponding accumulation of crude protein and nitrogen-free extract is shown in Tables 9 and 11. It is possible that 1.0 gram of aureomycin produced a concentration great enough to have a slight inhibitory effect on the cellulose digesting bacteria; therefore, the crude protein and nitrogen-free extract would be bound by the cellulose so that it could not be utilized by the bacteria or removed from the rumen. Bell et al. (1951) observed that 0.2 gram of aureomycin caused a decrease in the digestibility of dry matter and crude fiber as much as 15 per cent and 50 per cent, respectively, when fed to steers during a digestion trial. Wasserman et al. (1952) using an in vitro technique observed that penicillin stimulated cellulolytic rumen microorganisms at the lower concentrations, neomycin was stimulatory in all concentrations, streptomycin was slightly stimulatory in the lowest concentration and chloromycetin adversely affected the microorganisms.

Several investigators have used animals with rumen fistulas as a means to study the digestion of various nutrients in the rumen. Silver (1935) studied the digestion and absorption of alfalfa hay by removing the rumen contents at the time of feeding and taking "grab" samples at 2-hour intervals thereafter. He compared the percentage composition of the various samples of rumen contents as the period of digestion progressed. Hale et al. (1947) used the lignin ratio technique to study the quantitative digestion in the rumen. This method was useful in studying the significance of rumen digestion as related to the subsequent digestion in the remainder of the digestive tract. The rumen digestion coefficients obtained by Hale et al. for dry matter, crude fiber, crude protein and nitrogen-free extract for the first 6-hour period after feeding were 22.1, 0, 33.3 and 45.1 per cent, respectively. When the same method of calculation that was used in this investigation was applied to the data reported by Hale et al. the values 55.7, 47.1, 59.1 and 66.7 per cent were obtained for dry matter, crude fiber, crude protein and nitrogen-free extract, respectively. Except for crude protein these values are in good agreement with those obtained from 707 when no aureomycin was fed.

In the study reported here approximately 80 per cent of the dry matter had passed from the rumen within 12 hours after feeding. Crude fiber passed out of the rumen at about the same rate as the dry matter while the protein disappeared at a lower



rate and nitrogen-free extract at a slightly higher rate. Hale et al. (1947) observed that the rumen digestion was practically completed 12 to 14 hours after feeding. They suggested that the lignin in the plant material probably imposed a "ceiling" on rumen digestion. Hale et al. (1940) did not observe any differences in the extent of rumen digestion of alfalfa hay when fed at levels varying from 10 to 30 pounds per day. They also noted that there was not much difference in the rumen fill for these varying levels of hay intake.

There was an accumulation of ether extract in the rumen during the first 6-hour period as shown in Table 10. This fact was observed for both 707 and 714; however, 714 showed a greater accumulation for the first 6-hour period and greater passage from the rumen for the second 6-hour period than 707. The amount present in the rumens of both steers at the 0-hour period when aureomycin was fed was considerably higher than when no aureomycin was fed. The accumulation of ether extract was the higher when 1.0 gram of aureomycin was fed. A possible explanation of this accumulation of ether extract at 0-hour may be that there was a delayed synthesis of the fat from carbohydrate in the rumen or a decreased absorption of fat from the rumen. Hale et al. (1940, 1947) also observed a definite increase of the ether extractive substances in the rumen six hours after feeding.

Table 12 shows an accumulation of non-protein nitrogen in the rumen for the first 6-hour period after feeding and a re-

moval for the second 6-hour period. The rate of removal from the rumen was the faster when 1.0 gram of aureomycin was included in the ration.

An increase in the number of bacteria in the rumen was observed when 0.5 gram of aureomycin was fed as shown in Figures 1 and 2. Since the most rapid removal of dry matter, crude fiber, crude protein and nitrogen-free extract occurred when this level of aureomycin was included in the ration, it is possible that the aureomycin stimulated bacterial action in the rumen and also may have caused a change in the wall of the rumen which would facilitate faster absorption. The total bacterial count in the rumen remained approximately the same when 1.0 gram of aureomycin was fed as observed for 0.5 gram of aureomycin. There was a decrease in the amount of nutrients removed from the rumen when 1.0 gram of aureomycin was fed as compared to the 0.5 gram level. It is possible that the increased aureomycin concentration may have inhibited some of the cellulose-digesting bacteria but was not high enough to affect the total bacterial count to any great extent.

#### Amino Acid Analyses

There was a definite decrease in the percentage of the amino acids present at each collection of rumen material as the concentration of aureomycin was increased (Table 14). A possible explanation of this could be that there was a higher percentage of each amino acid removed from the rumen when 0.5

gram of aureomycin was included in the ration as compared to no aureomycin and 1.0 gram of aureomycin (Table 15). Another possible reason could be that since there is an accumulation of dry matter and crude fiber in the rumen at the 0-hour collection, the amino acids present may be diluted by being distributed in a larger volume; therefore when the sample was taken for an assay, the sample contained less of the amino acids in question.

Synthesis of the amino acids could not be observed when the natural ration was fed because the ration itself contained a large quantity of each amino acid. Consequently, only the rate of removal of these amino acids could be determined when the natural ration was fed. No doubt there was some synthesis of the amino acids when the natural ration was fed but there was no method to determine this synthesis in a moving system and especially since the ration already contained such large quantities of the amino acids.

The type of ration may have some influence on the amount of the amino acids that are found in the rumen just as the ration influences the type of bacterial flora present in the rumen. Reed et al. (1949) observed that the bacterial protein obtained from sheep receiving either dry or green feed contained about the same level of cysteine, but the sheep fed the green feed had a higher level of methionine. Block and Stekol (1950) and Block et al. (1951) using radioactive sodium sulfate ( $S^{35}$ )

observed that methionine and cystine were synthesized in the rumen at approximately the same rate and were used by the tissues to make new protein in the quantities needed. McNaught et al. (1951b), separating bacterial cells in a Sharples centrifuge, found that 58 per cent of the bacterial protein was present in the liquid when it was removed from the rumen while the remaining 42 per cent was synthesized during incubation.

Synthesis of the 10 essential amino acids has been shown by Loosli et al. (1949) in sheep and by Agrawala (1950) in steers with a purified ration which had urea as the sole source of nitrogen. Loosli et al. also reported that sheep on a purified ration containing glycine as the only source of nitrogen synthesized the amino acids at a lower level when compared with urea.

In the investigation reported here, a purified ration similar to that used by Loosli et al. (1949) and Agrawala (1950) except that a different cellulose source was fed to steer 714. Data in Table 16 indicate that the amino acids are synthesized in the rumen and these values agree with the lower values reported by Agrawala (1950). He observed an increase in arginine from 52 to 218 per cent, histidine from 35 to 210 per cent, isoleucine from 26 to 190 per cent, leucine from 40 to 178 per cent, lysine from 46 to 192 per cent, methionine from 22 to 260 per cent, phenylalanine from 44 to 194 per cent, threonine from 42 to 150 per cent, tryptophan from 72 to 200 per cent and valine from 26 to 204 per cent in six hours after feeding.

In the investigation reported here, there was an apparent delayed synthesis of lysine in the first 6-hour sample when no aureomycin was fed. Only lysine showed any accumulation in the rumen in the second 6-hour period. When 0.5 gram of aureomycin was included in the ration, only arginine, histidine, isoleucine, leucine, threonine and valine were synthesized in the first 6-hour period. The concentration of lysine, methionine, phenylalanine and tryptophan were lower at six hours after feeding than at the 0-hour. There was an accumulation of all of the amino acids in the second 6-hour period which would indicate that the aureomycin may exert some depressing effect on the bacteria during the first 6-hour period. However, one must keep in mind that steer 714 was off-fed during the aureomycin trial and was fed through the fistula; also, as shown in Table 6, the values for non-protein nitrogen when 0.5 gram of aureomycin was fed were considerably higher than when no aureomycin was fed, which would indicate a delayed utilization of the urea in the ration.

#### B-Vitamin Analyses

Riboflavin. McElroy and Goss (1939, 1940a) observed that the riboflavin content of the rumen ingesta in sheep increased 100-fold over that of the feed. Hunt et al. (1941) found no increase in riboflavin when steers were fed hay alone, but that riboflavin increased when corn was included in the ration.

Teeri et al. (1951b) found from analysis of the feces that cows fed a low quality of late-cut hay had a decreased excretion of riboflavin. Kesler and Knodt (1950, 1951a, 1951c) observed that the riboflavin concentration was highest in the small intestine in the case of calves.

No evidence of synthesis of riboflavin could be detected when the steers were fed the natural ration; even though there must have been some synthesis, there was such a large quantity in the feed which could mask any synthesis. As in the case of the amino acids, only the rate of removal could be determined. The rate of removal of riboflavin from the rumen was the highest when 0.5 gram of aureomycin was fed as observed in Table 19.

Riboflavin synthesis (Table 22) was very evident when the purified ration was fed to 714 without any aureomycin. Agrawala (1950) observed an increase in riboflavin from 162 to 382 per cent. With no aureomycin in the ration, a 243.8 per cent increase in riboflavin was obtained; however, when 0.5 gram of aureomycin was added to the ration a large decrease in riboflavin synthesis occurred. The aureomycin may have had a depressing effect on the rumen bacteria that synthesize riboflavin, also 714 was off-feed at the same time. The combination of these two factors may have been responsible for the results obtained here.

Nicotinic acid. Wegner et al. (1940b) observed a three- to four-fold increase in the concentration of nicotinic acid in the dried rumen contents as compared with the feed. Kesler

and Knodt (1950, 1951a, 1951c) found that nicotinic acid, like riboflavin, was the highest in the small intestine of calves. Lindahl and Pearson (1951) noted that the excretion of nicotinic acid was higher when sheep received a ration containing casein than on a low protein ration.

Synthesis of nicotinic acid was observed in this investigation for both the natural and purified rations. The highest synthesis occurred in the case of the natural ration when no aureomycin was fed. It is possible that the rate of removal of nicotinic acid from the rumen was higher when 0.5 gram of aureomycin was fed just as in the case of the riboflavin, dry matter, crude fiber, crude protein and nitrogen-free extract. Therefore, it appeared that there was a decreased synthesis of this vitamin when aureomycin was included in the ration. The concentration of nicotinic acid at the 0-hour was definitely higher when aureomycin was included in the ration as compared to no aureomycin. This may be due to delayed synthesis of the vitamin by the rumen bacteria or to decreased absorption from the rumen.

There was tremendous synthesis of nicotinic acid in the case of the purified ration when no aureomycin was fed (Table 22). Agrawala (1950) reported a 93.1 to 490.1 per cent increase in the synthesis of nicotinic acid six hours after feeding. A definite decrease in the synthesis of nicotinic acid occurred when aureomycin was added to the ration. A possible explanation

for the decreased synthesis was given in the riboflavin discussion.

Pantothenic acid. Ruminal synthesis of pantothenic acid has been observed by McElroy and Goss (1939) in sheep and in cattle (1941b), and by Wegner et al. (1941c) and Lardinois et al. (1944) in cattle. Teeri et al. (1950, 1951a) observed that the excretion of pantothenic acid from cattle was greater than the dietary intake. Synthesis of pantothenic acid occurred in the rumen when both the natural and purified rations were fed, although not at a very high rate for the natural ration. Accumulation of pantothenic acid occurred during the first 6-hour period when 1.0 gram of aureomycin was fed. The rate of removal of this vitamin was highest when 0.5 gram of aureomycin was included in the ration. The quantity present at the 0-hour was the highest when 0.5 gram of aureomycin was fed which may be as a result of delayed synthesis or due to decreased absorption from the rumen.

Agrawala (1950) observed a 5.4 to 562.2 per cent increase in the pantothenic acid concentration in the rumen contents when the animals received a purified ration. In the investigation reported here, the increase in the pantothenic acid was the highest when no aureomycin was fed. A delayed synthesis resulted when 0.5 gram of aureomycin was added to the ration (Table 22).



## SUMMARY

Two steers, each with a rumen fistula fitted with a plastic plug, were used to investigate the influence of aureomycin on the synthesis and digestion in the rumen when the steers were fed natural and purified rations. The animals were fed a natural ration of 15 pounds of second-cutting alfalfa-brome hay and four pounds of corn once daily throughout the feeding trials. Aureomycin was fed at each level (0, 0.5 and 1.0 gram per day) for 15 days before changing to the next highest level. The period in which the steers received no aureomycin served as the control period for comparison with those in which aureomycin was fed.

The samples of the rumen contents were collected by completely evacuating the rumen before feeding, 0-hour and at 6- and 12-hours after feeding the ration. Determinations for dry matter, crude fiber, crude protein and ether extract as well as nitrogen-free extract were made on both the rumen contents and the ration. Microbiological methods of assay were used for the determination of the 10 essential amino acids, riboflavin, nicotinic acid and pantothenic acid.

Neither of the steers showed any signs of anorexia or diarrhea at any time during aureomycin supplementation when the natural ration was fed. Steer 714 went off-feed just prior to the addition of 0.5 gram of aureomycin to the purified ration and did not regain its appetite during the period that aureomycin was included in the ration.

The pH declined for about six hours after feeding with only a slight increase toward alkalinity at the end of 12 hours. When the aureomycin was included in the natural ration, the pH did not become as acid as that observed when no aureomycin was fed; however, the response was in the same direction in each case.

A definite increase in the total bacterial count of the rumen contents and the feces occurred when aureomycin was included in the ration. The number of rumen streptococci decreased when aureomycin was fed, while the number of coliform organisms in the rumen remained approximately the same in one animal and increased in the other.

The rate of removal of dry matter, crude fiber, crude protein, nitrogen-free extract, non-protein nitrogen, the 10 essential amino acids and riboflavin from the rumen was the highest when 0.5 gram of aureomycin was fed. There was an accumulation of ether extract, nicotinic acid and pantothenic acid in the rumen when aureomycin was included in the ration.

There was an accumulation of dry matter and crude fiber in the rumen at the 0-hour when 1.0 gram of aureomycin was fed which indicated that a slight depression of digestibility of these constituents may have occurred.

The pH and the total bacterial count were approximately the same for the periods when no aureomycin and 0.5 gram of aureomycin was added to the purified ration. The synthesis

of the amino acids was lower when 0.5 gram of aureomycin was included in the purified ration. A definite decrease in the synthesis of riboflavin, nicotinic acid and pantothenic acid also occurred when 0.5 gram of aureomycin was added to the purified ration.

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## APPENDIX

TABLE 23

# INFLUENCE OF AUREOMYCIN ON THE REMOVAL OF ARGININE FROM THE RUMEN OF CATTLE FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no. | Aureo-<br>mycin | Arginine             |      |       | Arginine                |         |      | Arginine                 |         |      | Arginine                |         |   |
|------------|-----------------|----------------------|------|-------|-------------------------|---------|------|--------------------------|---------|------|-------------------------|---------|---|
|            |                 | In rumen             | Fed  | Total | In rumen                | Removed | %    | In rumen                 | Removed | %    | In rumen                | Removed | % |
|            |                 | gm                   | gm   | gm    | gm                      | gm      |      | gm                       | gm      |      | gm                      | gm      |   |
|            |                 | <u>0-hr. samples</u> |      |       | <u>0-6 hr. samples*</u> |         |      | <u>0-12 hr. samples*</u> |         |      | <u>6-12 hr. samples</u> |         |   |
|            | 0               | 29.0                 | 58.1 | 87.1  | 58.1                    | 29.0    | 49.9 | 40.4                     | 46.7    | 80.4 | 17.7                    | 30.5    |   |
| 707        | 0.5             | 18.5                 | 58.1 | 76.6  | 35.0                    | 41.6    | 71.6 | 25.9                     | 50.7    | 87.3 | 9.1                     | 26.0    |   |
|            | 1.0             | 24.5                 | 58.1 | 82.6  | 54.1                    | 28.5    | 49.1 | 41.3                     | 41.3    | 71.1 | 12.8                    | 23.7    |   |
|            | 0               | 32.1                 | 58.1 | 90.2  | 69.8                    | 20.4    | 35.1 | 60.2                     | 30.0    | 51.6 | 9.6                     | 13.8    |   |
| 714        | 0.5             | 31.5                 | 58.1 | 89.6  | 58.3                    | 31.3    | 53.9 | 47.3                     | 42.3    | 72.6 | 11.0                    | 18.7    |   |
|            | 1.0             | 31.6                 | 58.1 | 89.7  | 71.6                    | 18.1    | 31.2 | 48.9                     | 40.0    | 70.2 | 22.7                    | 31.7    |   |

\* Based on arginine intake.

TABLE 24  
INFLUENCE OF AUREOMYCIN ON THE REMOVAL OF HISTIDINE FROM THE RUMEN OF CATTLE  
FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no. | Histidine     |        |          | Histidine        |            |      | Histidine         |            |      | Histidine        |            |      |
|------------|---------------|--------|----------|------------------|------------|------|-------------------|------------|------|------------------|------------|------|
|            | In rumen gm   | Fed gm | Total gm | In rumen gm      | Removed gm | %    | In rumen gm       | Removed gm | %    | In rumen gm      | Removed gm | %    |
|            | O-hr. samples |        |          | O-6 hr. samples* |            |      | O-12 hr. samples* |            |      | 6-12 hr. samples |            |      |
| 0          | 13.3          | 29.7   | 43.0     | 32.2             | 10.8       | 36.4 | 22.9              | 20.1       | 67.7 | 9.3              | 28.9       |      |
| 707        | 0.5           | 10.1   | 29.7     | 39.8             | 23.3       | 16.6 | 55.9              | 14.8       | 25.0 | 84.2             | 8.4        | 36.2 |
|            | 1.0           | 14.1   | 29.7     | 43.8             | 30.3       | 13.5 | 45.5              | 23.3       | 20.5 | 69.0             | 7.0        | 23.1 |
|            | 0             | 16.2   | 29.7     | 45.9             | 40.7       | 5.2  | 17.5              | 35.5       | 10.4 | 35.0             | 5.2        | 12.8 |
| 714        | 0.5           | 16.6   | 29.7     | 46.3             | 34.7       | 11.6 | 39.1              | 26.7       | 19.6 | 66.0             | 8.0        | 23.1 |
|            | 1.0           | 16.6   | 29.7     | 46.3             | 42.7       | 3.6  | 12.1              | 31.4       | 14.9 | 50.2             | 11.3       | 26.5 |

\* Based on histidine intake.

TABLE 25  
INFLUENCE OF AUREOMYCIN ON THE REMOVAL OF ISOLEUCINE FROM THE RUMEN OF CATTLE  
FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no.    | Aureo-<br>mycin<br>gm | Isoleucine        |           |             | Isoleucine        |               |      | Isoleucine        |               |      | Isoleucine        |               |   |
|---------------|-----------------------|-------------------|-----------|-------------|-------------------|---------------|------|-------------------|---------------|------|-------------------|---------------|---|
|               |                       | In<br>rumen<br>gm | Fed<br>gm | Total<br>gm | In<br>rumen<br>gm | Removed<br>gm | %    | In<br>rumen<br>gm | Removed<br>gm | %    | In<br>rumen<br>gm | Removed<br>gm | % |
|               |                       |                   |           |             |                   |               |      |                   |               |      |                   |               |   |
|               |                       |                   |           |             |                   |               |      |                   |               |      |                   |               |   |
| O-hr. samples |                       | 0-6 hr. samples*  |           |             | 0-12 hr. samples* |               |      | 6-12 hr. samples  |               |      |                   |               |   |
| 707           | 0                     | 41.5              | 60.0      | 101.5       | 84.2              | 17.3          | 28.8 | 67.1              | 34.4          | 57.3 | 17.1              | 20.3          |   |
|               | 0.5                   | 28.9              | 60.0      | 88.9        | 65.4              | 23.5          | 39.2 | 51.5              | 37.4          | 62.3 | 13.9              | 21.3          |   |
|               | 1.0                   | 38.6              | 60.0      | 98.6        | 76.6              | 22.0          | 36.7 | 61.9              | 36.7          | 61.2 | 14.7              | 19.2          |   |
| 714           | 0                     | 40.9              | 60.0      | 100.0       | 100.0             | 0.9           | 1.5  | 91.4              | 9.5           | 15.8 | 8.6               | 8.6           |   |
|               | 0.5                   | 48.7              | 60.0      | 108.7       | 94.7              | 14.0          | 23.3 | 83.4              | 25.3          | 42.2 | 11.3              | 11.9          |   |
|               | 1.0                   | 51.2              | 60.0      | 111.2       | 110.2             | 1.0           | 1.7  | 80.3              | 30.9          | 51.5 | 29.9              | 27.1          |   |

\* Based on isoleucine intake.

TABLE 26  
INFLUENCE OF AUREOMYCIN ON THE REMOVAL OF LEUCINE FROM THE RUMEN OF CATTLE  
FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no. | Aureo-<br>mycin<br>gm | Leucine       |      |       | Leucine          |         |      | Leucine                           |         |      | Leucine  |         |    |
|------------|-----------------------|---------------|------|-------|------------------|---------|------|-----------------------------------|---------|------|----------|---------|----|
|            |                       | In rumen      | Fed  | Total | In rumen         | Removed | %    | In rumen                          | Removed | %    | In rumen | Removed | %  |
|            |                       | gm            | gm   | gm    | gm               | gm      | gm   | gm                                | gm      | gm   | gm       | gm      | gm |
|            |                       | O-hr. samples |      |       | O-6 hr. samples* |         |      | O-12 hr. samples*6-12 hr. samples |         |      |          |         |    |
| 707        | 0                     | 45.5          | 84.7 | 130.2 | 100.7            | 29.5    | 34.8 | 80.6                              | 49.6    | 58.6 | 20.1     | 20.0    |    |
|            | 0.5                   | 31.5          | 84.7 | 116.2 | 73.1             | 43.1    | 50.9 | 56.8                              | 59.4    | 70.1 | 16.3     | 22.3    |    |
|            | 1.0                   | 44.8          | 84.7 | 129.5 | 80.2             | 49.3    | 58.2 | 68.9                              | 60.6    | 71.5 | 11.3     | 14.1    |    |
| 714        | 0                     | 50.9          | 84.7 | 135.6 | 128.5            | 7.1     | 8.4  | 113.8                             | 21.8    | 25.7 | 14.7     | 11.4    |    |
|            | 0.5                   | 53.5          | 85.7 | 138.2 | 110.7            | 27.5    | 32.5 | 92.1                              | 46.1    | 54.4 | 18.6     | 16.8    |    |
|            | 1.0                   | 58.8          | 84.7 | 143.5 | 123.6            | 19.9    | 23.5 | 95.1                              | 48.4    | 57.1 | 28.5     | 23.1    |    |

\* Based on leucine intake.

TABLE 27

INFLUENCE OF AUREOMYCIN ON THE REMOVAL OF LYSINE FROM THE RUMEN OF CATTLE  
FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no. | Aureo-<br>mycin<br>gm | Lysine            |           |             | Lysine            |               |        | Lysine            |               |        | Lysine            |               |        |
|------------|-----------------------|-------------------|-----------|-------------|-------------------|---------------|--------|-------------------|---------------|--------|-------------------|---------------|--------|
|            |                       | In<br>rumen<br>gm | Fed<br>gm | Total<br>gm | In<br>rumen<br>gm | Removed<br>gm | %<br>% | In<br>rumen<br>gm | Removed<br>gm | %<br>% | In<br>rumen<br>gm | Removed<br>gm | %<br>% |
|            |                       |                   |           |             |                   |               |        |                   |               |        |                   |               |        |
|            |                       | O-hr. samples     |           |             | O-6 hr. samples*  |               |        | O-12 hr. samples* |               |        | 6-12 hr. samples  |               |        |
|            | 0                     | 27.4              | 44.8      | 72.2        | 63.9              | 8.3           | 18.5   | 43.3              | 28.9          | 64.5   |                   | 20.6          | 32.2   |
| 707        | 0.5                   | 17.9              | 44.8      | 62.7        | 44.4              | 18.3          | 40.8   | 34.0              | 28.7          | 64.1   |                   | 10.4          | 23.4   |
|            | 1.0                   | 26.4              | 44.8      | 71.2        | 59.2              | 12.0          | 26.8   | 46.8              | 24.4          | 54.5   |                   | 12.4          | 20.9   |
|            | 0                     | 29.4              | 44.8      | 74.2        | 74.5              | 0             | 0      | 69.2              | 5.0           | 11.2   |                   | 5.3           | 7.1    |
| 714        | 0.5                   | 37.0              | 44.8      | 81.8        | 67.6              | 14.2          | 31.7   | 63.8              | 18.0          | 40.2   |                   | 3.8           | 5.6    |
|            | 1.0                   | 34.7              | 44.8      | 79.5        | 76.7              | 2.8           | 6.3    | 58.8              | 20.7          | 46.2   |                   | 17.9          | 23.3   |

\* Based on lysine intake.

TABLE 28  
INFLUENCE OF AUREOMYCIN ON THE REMOVAL OF METHIONINE FROM THE RUMEN OF CATTLE  
FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no. | Methionine            |               |     | Methionine |                  |         | Methionine |                   |         | Methionine |                  |         |    |
|------------|-----------------------|---------------|-----|------------|------------------|---------|------------|-------------------|---------|------------|------------------|---------|----|
|            | Aureo-<br>mycin<br>gm | In rumen      | Fed | Total      | In rumen         | Removed | %          | In rumen          | Removed | %          | In rumen         | Removed | %  |
|            |                       | gm            | gm  | gm         | gm               | gm      | gm         | gm                | gm      | gm         | gm               | gm      | gm |
|            |                       | 0-hr. samples |     |            | 0-6 hr. samples* |         |            | 0-12 hr. samples* |         |            | 6-12 hr. samples |         |    |
| 707        | 0                     | 4.0           | 8.2 | 12.2       | 9.9              | 2.3     | 28.0       | 6.4               | 5.8     | 70.7       | 3.5              | 35.4    |    |
|            | 0.5                   | 1.6           | 8.2 | 9.8        | 6.3              | 3.5     | 42.7       | 4.9               | 4.9     | 59.8       | 1.4              | 22.2    |    |
|            | 1.0                   | 3.0           | 8.2 | 11.2       | 9.0              | 2.2     | 26.8       | 5.1               | 6.1     | 74.4       | 3.9              | 43.3    |    |
| 714        | 0                     | 4.4           | 8.2 | 12.6       | 10.4             | 2.2     | 26.8       | 10.4              | 2.2     | 26.8       | 0                | 0       |    |
|            | 0.5                   | 3.7           | 8.2 | 11.9       | 9.6              | 2.3     | 28.0       | 7.9               | 4.0     | 48.8       | 1.7              | 17.7    |    |
|            | 1.0                   | 3.6           | 8.2 | 11.8       | 10.9             | 0.9     | 11.0       | 8.5               | 3.3     | 40.2       | 2.4              | 22.0    |    |

\* Based on methionine intake.

TABLE 29  
INFLUENCE OF AUREOMYCIN ON THE REMOVAL OF PHENYLALANINE FROM THE RUMEN OF CATTLE  
FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no. | Aureo-<br>mycin<br>gm | Phenylalanine        |      |       | Phenylalanine           |         |      | Phenylalanine            |         |      | Phenylalanine           |         |    |
|------------|-----------------------|----------------------|------|-------|-------------------------|---------|------|--------------------------|---------|------|-------------------------|---------|----|
|            |                       | In rumen             | Fed  | Total | In rumen                | Removed | %    | In rumen                 | Removed | %    | In rumen                | Removed | %  |
|            |                       | gm                   | gm   | gm    | gm                      | gm      | gm   | gm                       | gm      | gm   | gm                      | gm      | gm |
|            |                       | <u>0-hr. samples</u> |      |       | <u>0-6 hr. samples*</u> |         |      | <u>0-12 hr. samples*</u> |         |      | <u>6-12 hr. samples</u> |         |    |
|            | 0                     | 25.2                 | 46.6 | 71.8  | 56.6                    | 15.2    | 32.6 | 43.2                     | 28.6    | 61.4 | 13.4                    | 23.7    |    |
| 707        | 0.5                   | 17.2                 | 46.6 | 63.8  | 31.6                    | 32.2    | 69.1 | 25.3                     | 38.5    | 82.6 | 6.3                     | 19.9    |    |
|            | 1.0                   | 27.2                 | 46.6 | 73.8  | 55.6                    | 18.2    | 39.1 | 41.4                     | 32.4    | 69.5 | 14.2                    | 25.5    |    |
|            | 0                     | 28.7                 | 46.6 | 75.3  | 71.8                    | 3.5     | 7.5  | 59.5                     | 15.8    | 33.9 | 12.3                    | 17.1    |    |
| 714        | 0.5                   | 31.0                 | 46.6 | 77.6  | 64.4                    | 13.2    | 28.2 | 53.0                     | 24.6    | 52.8 | 11.4                    | 17.7    |    |
|            | 1.0                   | 33.3                 | 46.6 | 79.9  | 71.8                    | 8.1     | 17.4 | 55.7                     | 24.2    | 51.9 | 16.1                    | 22.4    |    |

\* Based on phenylalanine intake.



TABLE 30

INFLUENCE OF AUREOMYCIN ON THE REMOVAL OF THREONINE FROM THE RUMEN OF CATTLE  
FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no. | Aureo-<br>mycin<br>gm | Threonine         |           |             | Threonine         |               |        | Threonine         |               |        |                  |      |  |
|------------|-----------------------|-------------------|-----------|-------------|-------------------|---------------|--------|-------------------|---------------|--------|------------------|------|--|
|            |                       | In<br>rumen<br>gm | Fed<br>gm | Total<br>gm | In<br>rumen<br>gm | Removed<br>gm | %<br>% | In<br>rumen<br>gm | Removed<br>gm | %<br>% |                  |      |  |
|            |                       |                   |           |             |                   |               |        |                   |               |        |                  |      |  |
|            |                       | 0-hr. samples     |           |             | 0-6 hr. samples*  |               |        | 0-12 hr. samples* |               |        | 6-12 hr. samples |      |  |
| 707        | 0                     | 31.0              | 50.2      | 81.2        | 65.3              | 15.9          | 31.7   | 50.7              | 30.5          | 60.8   | 14.6             | 22.4 |  |
|            | 0.5                   | 22.2              | 50.2      | 72.4        | 48.9              | 23.5          | 46.8   | 38.3              | 34.1          | 67.9   | 10.6             | 21.7 |  |
|            | 1.0                   | 25.2              | 50.2      | 75.4        | 57.2              | 18.2          | 36.3   | 47.5              | 27.9          | 55.6   | 9.7              | 17.0 |  |
| 714        | 0                     | 36.8              | 50.2      | 87.0        | 79.9              | 7.1           | 14.1   | 71.2              | 15.8          | 31.5   | 8.7              | 10.9 |  |
|            | 0.5                   | 38.1              | 50.2      | 88.3        | 73.7              | 14.6          | 29.1   | 58.9              | 29.4          | 58.6   | 14.8             | 20.1 |  |
|            | 1.0                   | 37.1              | 50.2      | 87.3        | 81.0              | 6.3           | 12.5   | 56.2              | 31.1          | 62.0   | 24.8             | 30.6 |  |

\* Based on threonine intake.

TABLE 31

INFLUENCE OF AUREOMYCIN ON THE REMOVAL OF TRYPTOPHAN FROM THE RUMEN OF CATTLE  
FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no. | Aureo-<br>mycin<br>gm | Tryptophan        |           |             | Tryptophan        |               |      | Tryptophan        |               |      | Tryptophan        |               |   |
|------------|-----------------------|-------------------|-----------|-------------|-------------------|---------------|------|-------------------|---------------|------|-------------------|---------------|---|
|            |                       | In<br>rumen<br>gm | Fed<br>gm | Total<br>gm | In<br>rumen<br>gm | Removed<br>gm | %    | In<br>rumen<br>gm | Removed<br>gm | %    | In<br>rumen<br>gm | Removed<br>gm | % |
|            |                       | 0-hr. samples     |           |             | 0-6 hr. samples*  |               |      | 0-12 hr. samples* |               |      | 6-12 hr. samples  |               |   |
|            | 0                     | 6.4               | 10.9      | 17.3        | 13.6              | 3.7           | 33.9 | 10.4              | 6.9           | 63.3 | 3.2               | 23.5          |   |
| 707        | 0.5                   | 3.6               | 10.9      | 14.5        | 7.0               | 7.5           | 68.8 | 5.8               | 8.7           | 79.8 | 1.2               | 17.1          |   |
|            | 1.0                   | 5.7               | 10.9      | 16.6        | 10.7              | 5.9           | 54.1 | 8.7               | 7.9           | 72.5 | 2.0               | 18.7          |   |
|            | 0                     | 7.0               | 10.9      | 17.9        | 15.1              | 2.8           | 25.7 | 15.2              | 2.7           | 24.8 | 0                 | 0             |   |
| 714        | 0.5                   | 7.1               | 10.9      | 18.0        | 12.3              | 5.7           | 52.3 | 11.3              | 6.7           | 61.5 | 1.0               | 8.1           |   |
|            | 1.0                   | 7.5               | 10.9      | 18.4        | 15.1              | 3.3           | 30.3 | 11.4              | 7.0           | 64.2 | 3.7               | 24.5          |   |

\* Based on tryptophan intake.

TABLE 32  
INFLUENCE OF AUREOMYCIN ON THE REMOVAL OF VALINE FROM THE RUMEN OF CATTLE  
FED A NATURAL RATION IN 6 AND 12 HOURS

| Animal no.    | Aureo-<br>mycin<br>gm | Valine            |           |             | Valine            |               |        | Valine            |               |        | Valine            |               |        |
|---------------|-----------------------|-------------------|-----------|-------------|-------------------|---------------|--------|-------------------|---------------|--------|-------------------|---------------|--------|
|               |                       | In<br>rumen<br>gm | Fed<br>gm | Total<br>gm | In<br>rumen<br>gm | Removed<br>gm | %<br>% | In<br>rumen<br>gm | Removed<br>gm | %<br>% | In<br>rumen<br>gm | Removed<br>gm | %<br>% |
|               |                       |                   |           |             |                   |               |        |                   |               |        |                   |               |        |
|               |                       |                   |           |             |                   |               |        |                   |               |        |                   |               |        |
| O-hr. samples |                       | 0-6 hr. samples*  |           |             | 0-12 hr. samples* |               |        | 6-12 hr. samples  |               |        |                   |               |        |
|               | 0                     | 34.0              | 54.5      | 88.5        | 70.4              | 18.1          | 33.2   | 57.4              | 31.1          | 57.1   | 13.0              | 18.5          |        |
| 707           | 0.5                   | 22.6              | 54.5      | 77.1        | 51.1              | 26.0          | 47.7   | 40.3              | 36.8          | 67.5   | 10.8              | 21.1          |        |
|               | 1.0                   | 30.8              | 54.5      | 85.3        | 64.8              | 20.5          | 37.6   | 50.0              | 35.3          | 64.8   | 14.8              | 22.8          |        |
|               | 0                     | 36.3              | 54.5      | 90.8        | 88.8              | 2.0           | 3.7    | 80.0              | 10.8          | 19.8   | 8.8               | 9.9           |        |
| 714           | 0.5                   | 38.8              | 54.5      | 93.3        | 77.3              | 16.0          | 29.4   | 66.0              | 27.3          | 50.1   | 11.3              | 14.6          |        |
|               | 1.0                   | 39.5              | 54.5      | 94.0        | 85.0              | 9.0           | 16.5   | 65.4              | 28.6          | 52.5   | 19.6              | 23.1          |        |

\* Based on valine intake.

TABLE 33

INFLUENCE OF AUREOMYCIN ON THE AMINO ACID COMPOSITION OF RUMEN CONTENTS  
FROM 714 FED A PURIFIED RATION

| Animal<br>no. | Time<br>hr | Aureo-<br>mycin<br>gm | Arg<br>% | His<br>% | Isol<br>% | Leu<br>% | Lys<br>% | Met<br>% | Phe<br>% | Thr<br>% | Try<br>% | Val<br>% |
|---------------|------------|-----------------------|----------|----------|-----------|----------|----------|----------|----------|----------|----------|----------|
| 714           | 0          | 0                     | 0.755    | 0.307    | 0.963     | 0.996    | 0.866    | 0.245    | 0.546    | 0.822    | 0.176    | 0.846    |
|               | 0          | 0.5                   | 0.427    | 0.159    | 0.398     | 0.495    | 0.328    | 0.082    | 0.208    | 0.321    | 0.065    | 0.351    |
| 714           | 6          | 0                     | 0.519    | 0.220    | 0.772     | 0.801    | 0.291    | 0.183    | 0.446    | 0.698    | 0.124    | 0.677    |
|               | 6          | 0.5                   | 0.264    | 0.110    | 0.225     | 0.275    | 0.102    | 0.037    | 0.094    | 0.192    | 0.032    | 0.195    |
| 714           | 12         | 0                     | 0.614    | 0.259    | 0.874     | 0.935    | 0.688    | 0.215    | 0.490    | 0.774    | 0.143    | 0.764    |
|               | 12         | 0.5                   | 0.357    | 0.145    | 0.289     | 0.382    | 0.260    | 0.058    | 0.136    | 0.260    | 0.045    | 0.255    |

TABLE 34

INFLUENCE OF AUREOMYCIN ON THE B-VITAMIN COMPOSITION OF RUMEN  
CONTENTS FROM 714 FED A PURIFIED RATION

| Animal<br>no. | Time<br>hr | Aureo-<br>mycin<br>gm | Riboflavin<br>$\gamma$ /gm | Pantothenic<br>acid<br>$\gamma$ /gm | Nicotinic<br>acid<br>$\gamma$ /gm |
|---------------|------------|-----------------------|----------------------------|-------------------------------------|-----------------------------------|
| 714           | 0          | 0                     | 10.81                      | 23.72                               | 17.47                             |
|               | 0          | 0.5                   | 3.10                       | 2.02                                | 15.53                             |
| 714           | 6          | 0                     | 21.31                      | 31.47                               | 135.83                            |
|               | 6          | 0.5                   | 2.45                       | 0.40                                | 2.81                              |
| 714           | 12         | 0                     | 25.01                      | 91.22                               | 202.53                            |
|               | 12         | 0.5                   | 3.21                       | 1.94                                | 6.98                              |

TABLE 35

INFLUENCE OF AUREOMYCIN ON THE TOTAL RUMEN BACTERIAL COUNT  
FOR 707 WHILE RECEIVING A NATURAL RATION  
(Billions per milliliter)

| Aureo-<br>mycin<br>gm | Days<br>samp-<br>ling | Hours After Feeding |        |        |               |        |        |               |
|-----------------------|-----------------------|---------------------|--------|--------|---------------|--------|--------|---------------|
|                       |                       | 0                   | 2      | 4      | 6             | 8      | 10     | 12            |
| 0                     | 3                     | 12,510              | 12,710 | 6,781  | 8,074         | 8,567  | 11,750 | 11,150        |
| 0                     | 2                     | 9,234               |        |        | 7,028         |        |        | 5,462         |
| 0                     | 1                     | 10,025              |        |        | 11,200        |        |        | 4,450         |
| 0                     | 1                     | <u>6,375</u>        |        |        | <u>6,100</u>  |        |        | --            |
| Av., 15<br>days       |                       | 9,536               |        |        | 8,101         |        |        | 7,021         |
| 0.5                   | 3                     | 9,968               | 8,776  | 13,920 | 13,110        | 18,560 | 14,310 | 12,030        |
| 0.5                   | 3                     | 14,910              |        |        | 15,110        |        |        | --            |
| 0.5                   | 2                     | 18,180              |        |        | 16,230        |        |        | --            |
| 0.5                   | 1                     | 19,875              |        |        | 14,325        |        |        | 19,725        |
| 0.5                   | 2                     | <u>21,090</u>       |        |        | <u>20,080</u> |        |        | --            |
| Av., 15<br>days       |                       | 16,805              |        |        | 15,771        |        |        | 15,878        |
| 1.0                   | 3                     | 19,970              | 21,090 | 19,540 | 17,180        | 15,590 | 18,180 | 18,480        |
| 1.0                   | 2                     | 19,960              |        |        | 15,380        |        |        | --            |
| 1.0                   | 2                     | 20,410              |        |        | 14,220        |        |        | --            |
| 1.0                   | 1                     | 23,000              |        |        | 20,350        |        |        | 13,775        |
| 1.0                   | 1                     | <u>22,950</u>       |        |        | <u>16,686</u> |        |        | <u>14,327</u> |
| Av., 15<br>days       |                       | 21,950              |        |        | 16,686        |        |        | 14,327        |
| 0                     | 2                     | 16,890              |        |        | 16,600        |        |        |               |
| 0                     | 3                     | <u>15,300</u>       |        |        | <u>13,510</u> |        |        |               |
| Av., 10<br>days       |                       | 16,095              |        |        | 15,055        |        |        |               |

TABLE 36

INFLUENCE OF AUREOMYCIN ON THE TOTAL RUMEN BACTERIAL COUNT  
FOR 714 WHILE RECEIVING A NATURAL RATION  
(Billions per milliliter)

| Aureo-<br>mycin<br>gm | Days<br>samp-<br>ling | Hours After Feeding |        |        |               |        |        |               |
|-----------------------|-----------------------|---------------------|--------|--------|---------------|--------|--------|---------------|
|                       |                       | 0                   | 2      | 4      | 6             | 8      | 10     | 12            |
| 0                     | 3                     | 8,869               | 10,570 | 6,814  | 7,958         | 6,803  | 8,831  | 7,663         |
| 0                     | 2                     | 7,600               |        |        | 7,075         |        |        | 8,275         |
| 0                     | 1                     | 9,428               |        |        | 7,925         |        |        | 5,729         |
| 0                     | 1                     | <u>7,776</u>        |        |        | <u>6,275</u>  |        |        | <u>4,825</u>  |
| Av., 15<br>days       |                       | 8,418               |        |        | 7,308         |        |        | 6,623         |
| 0.5                   | 3                     | 6,988               | 14,400 | 12,910 | 8,904         | 12,490 | 10,620 | 13,930        |
| 0.5                   | 3                     | 16,970              |        |        | 14,600        |        |        | --            |
| 0.5                   | 2                     | 24,290              |        |        | 13,340        |        |        | --            |
| 0.5                   | 1                     | 25,875              |        |        | 12,250        |        |        | 11,775        |
| 0.5                   | 2                     | <u>11,250</u>       |        |        | <u>18,750</u> |        |        | <u>--</u>     |
| Av., 15<br>days       |                       | 17,075              |        |        | 13,569        |        |        | 12,853        |
| 1.0                   | 3                     | 20,630              | 22,080 | 16,200 | 15,480        | 16,380 | 16,840 | 17,800        |
| 1.0                   | 2                     | 11,920              |        |        | 14,340        |        |        | --            |
| 1.0                   | 2                     | 14,470              |        |        | 13,380        |        |        | --            |
| 1.0                   | 1                     | 17,775              |        |        | 19,250        |        |        | 7,150         |
| 1.0                   | 1                     | <u>18,100</u>       |        |        | <u>17,800</u> |        |        | <u>15,025</u> |
| Av., 15<br>days       |                       | 16,579              |        |        | 16,050        |        |        | 13,325        |
| 0                     | 2                     | 14,760              |        |        | 14,450        |        |        |               |
| 0                     | 3                     | <u>16,360</u>       |        |        | <u>14,670</u> |        |        |               |
| Av., 10<br>days       |                       | 15,560              |        |        | 14,560        |        |        |               |

TABLE 37

INFLUENCE OF AUREOMYCIN ON THE pH OF RUMEN CONTENTS FROM  
CATTLE RECEIVING A NATURAL RATION  
(Av. first 3 days of 15-day period)

| Animal<br>no. | Aureo-<br>mycin<br>gm | Hours After Feeding |      |      |      |      |      |      |
|---------------|-----------------------|---------------------|------|------|------|------|------|------|
|               |                       | 0                   | 2    | 4    | 6    | 8    | 10   | 12   |
|               | 0                     | 6.90                | 6.61 | 6.35 | 6.21 | 6.12 | 6.28 | 6.08 |
| 707           | 0.5                   | 6.90                | 6.62 | 6.57 | 6.60 | 6.52 | 6.62 | 6.39 |
|               | 1.0                   | 7.07                | 6.50 | 6.46 | 6.46 | 6.48 | 6.36 | 6.35 |
|               | 0                     | 6.78                | 6.61 | 6.24 | 5.70 | 5.73 | 5.54 | 5.66 |
| 714           | 0.5                   | 6.56                | 6.42 | 6.20 | 5.90 | 5.98 | 6.00 | 5.76 |
|               | 1.0                   | 6.88                | 6.48 | 6.10 | 5.91 | 6.00 | 6.13 | 6.03 |



TABLE 38

INFLUENCE OF AUREOMYCIN ON THE RUMEN STREPTOCOCCI AND THE COLIFORM GROUP IN THE RUMEN OF CATTLE FED A NATURAL RATION  
(Values in logarithms)

| Aureo-<br>mycin<br>gm                 | Days<br>samp-<br>ling | 707                 |      |      |      |      | 714  |      |      |      |      |
|---------------------------------------|-----------------------|---------------------|------|------|------|------|------|------|------|------|------|
|                                       |                       | Hours After Feeding |      |      |      |      |      |      |      |      |      |
|                                       |                       | 0                   | 2    | 4    | 6    | 8    | 0    | 2    | 4    | 6    | 8    |
| <u>S t r e p t o c o c c i</u>        |                       |                     |      |      |      |      |      |      |      |      |      |
| 0                                     | 3                     | 6.97                | 6.63 | 5.97 | 6.63 | 6.63 | 6.97 | 6.97 | 6.63 | 6.98 | 6.97 |
| 0                                     | 3                     | 5.97                |      |      | 6.63 |      | 6.80 |      |      | 6.30 |      |
| 0                                     | 1                     | 6.30                |      |      | 7.30 |      | 6.30 |      |      | 6.30 |      |
| 0                                     | 1                     | 6.30                |      |      | 6.30 |      | 6.30 |      |      | 6.30 |      |
| Av., 15 days                          |                       | 6.38                |      |      | 6.72 |      | 6.41 |      |      | 6.47 |      |
| 0.5                                   | 3                     | 5.97                | 5.63 | 4.97 | 5.97 | 5.63 | 5.63 | 5.30 | 5.97 | 5.30 | 4.63 |
| 0.5                                   | 3                     | 7.63                |      |      | 6.30 |      | 6.97 |      |      | 5.97 |      |
| 0.5                                   | 2                     | 6.80                |      |      | 5.30 |      | 5.80 |      |      | 5.30 |      |
| 0.5                                   | 2                     | 4.80                |      |      | 5.80 |      | 5.30 |      |      | 5.80 |      |
| Av., 15 days                          |                       | 6.30                |      |      | 5.84 |      | 5.93 |      |      | 5.59 |      |
| 1.0                                   | 3                     | 5.97                | 6.30 | 5.97 | 5.63 | 5.63 | 5.97 | 6.30 | 4.63 | 5.97 | 4.97 |
| 1.0                                   | 2                     | 5.30                |      |      | 6.30 |      | 6.30 |      |      | 4.80 |      |
| 1.0                                   | 2                     | 5.80                |      |      | 6.30 |      | 5.80 |      |      | 6.30 |      |
| 1.0                                   | 1                     | 4.30                |      |      | 5.30 |      | 6.30 |      |      | 5.30 |      |
| Av., 15 days                          |                       | 5.34                |      |      | 5.88 |      | 6.09 |      |      | 5.59 |      |
| 0                                     | 2                     | 4.80                |      |      | 4.80 |      | 5.80 |      |      | 5.30 |      |
| 0                                     | 3                     | 6.63                |      |      | 5.30 |      | 5.97 |      |      | 5.97 |      |
| Av., 10 days                          |                       | 5.72                |      |      | 5.05 |      | 5.88 |      |      | 5.63 |      |
| <u>C o l i f o r m      G r o u p</u> |                       |                     |      |      |      |      |      |      |      |      |      |
| 0                                     | 3                     | 2.97                | 3.63 | 4.30 | 4.30 | 3.30 | 2.63 | 2.63 | 4.30 | 2.63 | 3.63 |
| 0                                     | 3                     | 4.65                |      |      | 3.97 |      | 3.30 |      |      | 4.30 |      |
| 0                                     | 1                     | 3.30                |      |      | 5.30 |      | 4.30 |      |      | 4.30 |      |
| 0                                     | 1                     | 6.30                |      |      | 4.30 |      | 5.80 |      |      | 4.30 |      |
| Av., 15 days                          |                       | 4.31                |      |      | 4.47 |      | 4.01 |      |      | 3.88 |      |
| 0.5                                   | 3                     | 3.63                | 4.97 | 4.30 | 4.97 | 4.97 | 1.97 | 2.30 | 3.97 | 2.97 | 3.63 |
| 0.5                                   | 3                     | 4.63                |      |      | 4.63 |      | 3.30 |      |      | 3.63 |      |
| 0.5                                   | 2                     | 4.80                |      |      | 5.30 |      | 2.30 |      |      | 1.80 |      |
| 0.5                                   | 2                     | 5.30                |      |      | 4.80 |      | 4.80 |      |      | 2.30 |      |
| Av., 15 days                          |                       | 4.59                |      |      | 4.93 |      | 3.09 |      |      | 2.68 |      |
| 1.0                                   | 3                     | 3.97                | 4.30 | 4.63 | 4.63 | 3.63 | 2.97 | 4.63 | 3.63 | 2.63 |      |
| 1.0                                   | 2                     | 3.30                |      |      | 3.30 |      | 2.80 |      |      | 2.80 |      |
| 1.0                                   | 2                     | 3.80                |      |      | 3.80 |      | 4.80 |      |      | 3.30 |      |
| 1.0                                   | 1                     | 2.30                |      |      | 3.30 |      | 2.30 |      |      | 3.30 |      |
| Av., 15 days                          |                       | 3.34                |      |      | 3.76 |      | 3.22 |      |      | 3.01 |      |
| 0                                     | 2                     | 2.80                |      |      | 3.80 |      | 4.30 |      |      | 3.30 |      |
| 0                                     | 3                     | 3.63                |      |      | 3.63 |      | 2.30 |      |      | 2.63 |      |
| Av., 10 days                          |                       | 3.22                |      |      | 3.72 |      | 3.30 |      |      | 2.97 |      |

TABLE 39

INFLUENCE OF AUREOMYCIN ON THE TOTAL BACTERIAL COUNT OF FECES  
FROM CATTLE FED A NATURAL RATION  
(Billions per milliliter)

| Aureo-<br>mycin<br>gm | Collection<br>No. | Animal |        |
|-----------------------|-------------------|--------|--------|
|                       |                   | 707    | 714    |
| 0                     | 1                 | 44,750 | 61,650 |
| 0                     | 2                 | 30,000 | 22,450 |
| 0                     | 3                 | 21,550 | 16,750 |
| 0.5                   | 4                 | 36,550 | 29,900 |
| 0.5                   | 5                 | 62,800 | 49,000 |
| 0.5                   | 6                 | 50,150 | 71,150 |
| 1.0                   | 7                 | 54,150 | 76,300 |
| 1.0                   | 8                 | 82,400 | 69,450 |
| 1.0                   | 9                 | 45,800 | 57,850 |
| 0                     | 10                | 51,600 | 56,850 |
| 0                     | 11                | 68,750 | 67,300 |
| 0                     | 12                | 68,300 | 64,450 |

TABLE 40

INFLUENCE OF AUREOMYCIN ON THE FECAL STREPTOCOCCI  
AND THE COLIFORM GROUP IN THE FECES  
FROM CATTLE FED A NATURAL RATION  
(Values in logarithms)

| Aureo-<br>mycin<br>gm | Collection<br>No. | Fecal<br>Streptococci |      | Coliform<br>Group |      |
|-----------------------|-------------------|-----------------------|------|-------------------|------|
|                       |                   | 707                   | 714  | 707               | 714  |
| 0                     | 1                 | 4.30                  | 5.80 | 4.80              | 5.80 |
| 0                     | 2                 | 5.80                  | 5.80 | 5.30              | 5.30 |
| 0                     | 3                 | 5.80                  | 4.80 | 5.80              | 6.30 |
| 0.5                   | 4                 | 5.80                  | 5.30 | 5.80              | 5.80 |
| 0.5                   | 5                 | 7.30                  | 5.80 | 6.30              | 5.80 |
| 0.5                   | 6                 | 7.80                  | 6.80 | 5.80              | 6.30 |
| 1.0                   | 7                 | 6.80                  | 5.80 | 4.80              | 6.30 |
| 1.0                   | 8                 | 5.30                  | 5.30 | 4.80              | 6.30 |
| 1.0                   | 9                 | 5.80                  | 5.80 | 5.30              | 5.30 |
| 0                     | 10                | 6.30                  | 6.80 | 5.80              | 6.80 |
| 0                     | 11                | 5.80                  | 6.30 | 4.80              | 5.30 |
| 0                     | 12                | 6.30                  | 5.80 | 6.30              | 4.80 |

TABLE 41

INFLUENCE OF AUREOMYCIN ON THE TOTAL RUMEN BACTERIAL COUNT  
FOR 714 WHILE RECEIVING A PURIFIED RATION  
(Billions per milliliter)

| Animal<br>no. | Aureo-<br>mycin<br>gm | Hours After Feeding |       |       |       |       |       |       |
|---------------|-----------------------|---------------------|-------|-------|-------|-------|-------|-------|
|               |                       | 0                   | 2     | 4     | 6     | 8     | 10    | 12    |
| 714           | 0                     | 9,842               | 6,825 | 4,633 | 6,883 | 4,592 | 8,375 | 6,983 |
| 714           | 0.5                   | 12,750              | 8,475 | 7,567 | 8,542 | 8,775 | 5,525 | 7,175 |

TABLE 42

INFLUENCE OF AUREOMYCIN ON THE pH OF RUMEN CONTENTS FROM  
STEER 714 FED A PURIFIED RATION  
(Av. first 3 days of 15-day period)

| Animal<br>no. | Aureo-<br>mycin<br>gm | Hours After Feeding |      |      |      |      |      |      |
|---------------|-----------------------|---------------------|------|------|------|------|------|------|
|               |                       | 0                   | 2    | 4    | 6    | 8    | 10   | 12   |
| 714           | 0                     | 6.65                | 5.43 | 4.87 | 4.75 | 4.97 | 4.91 | 5.00 |
| 714           | 0.5                   | 6.33                | 5.99 | 5.66 | 5.80 | 5.75 | 5.42 | 5.23 |