The bacteriology of the porcupine caecum
by John LeRoy Johnson

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Bacteriology
Montana State University

Abstract:
The porcupine caecum appears to be similar to the rumen in that it provides a suitable environment for the growth of large numbers of bacteria which rapidly ferment portions of the ingested food.

The principle fermentation products were identified as the short chain volatile fatty acids acetic, propionic and butyric. The acids accumulating in the caecal ingesta consisted of 73 percent acetic, 12 percent propionic and 14 percent butyric. - The average rates at which the acids were produced in the caecal ingesta were 0.110, 0.016 and 0.014 meg, per g,dry weight per hour for acetic, propionic and butyric respectively. It was estimated that a 10 Kg porcupine could obtain from 6 to 22 percent of its maintenance energy requirement from the absorption and utilization of the volatile acids.

Lactic' and. succinic acids appeared not to be important intermediate products in the caecal fermentation.

Approximately twice as much carbon dioxide as methane was produced during in vitro incubation of caecal content.

Naturally occurring nitrogen and carbon sources for the caecal bacteria were sought in the ingesta of the caecum and from the distal end of the small intestine. Substrate amounts, of ammonia were found in the ingesta from both organs. Several amino acids were detected but in less than substrate quantities. Soluble sugars and sugar acids were not detected in the soluble fraction of the ingesta, but arabinose, xylose, glucose and galactose were detected in an acid- hydrolysate of the particulate fraction from the small intestine.

Three hundred twenty-eight strains of caecal bacteria were isolated by randomly picking discrete colonies from 0.5 or 1.0 x 10-8 dilution roll tube cultures. The strains were placed into 43 groups based on morphological and physiological characteristics. Some of the groups were similar to but most could not be identified with the predominant bacterial groups in the bovine rumen.

THE BACTERIOLOGY OF THE PORCUPINE CAECUM

by

John L. Johnson

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Bacteriology

Approved:

Richard H. McBee
Head, Major Department

Richard H. McBee
Chairman, Examining Committee

Louis D. Smith
Dean, Graduate Division

MONTANA STATE COLLEGE
Bozeman, Montana

June, 1964

ACKNOWLEDGMENT

The author takes this opportunity to express his gratitude to Dr. R. H. McBee for his support and helpful advice throughout the course of this investigation. The author is indebted to Dr. N. M. Nelson and Dr. I. K. Mills for their helpful suggestions in the preparation of the manuscript. Technical assistance from Mrs. LaMae Henry, Mrs. Mary Lou Hood and Mrs. Shirley Tonkin is appreciated.

The author is indebted to the National Institutes of Health, Public Health service for financial support through the training program in microbiology 5T1-A1 131-01,02 and 03.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The porcupine caecum appears to be similar to the rumen in that it provides a suitable environment for the growth of large numbers of bacteria which rapidly ferment portions of the ingested food.

The principle fermentation products were identified as the short chain volatile fatty acids acetic, propionic and butyric. The acids accumulating in the caecal ingesta consisted of 73 percent acetic, 12 percent propionic and 14 percent butyric. The average rates at which the acids were produced in the caecal ingesta were 0.110, 0.016 and 0.014 meq per g dry weight per hour for acetic, propionic and butyric respectively. It was estimated that a 10 Kg porcupine could obtain from 6 to 22 percent of its maintenance energy requirement from the absorption and utilization of the volatile acids.

Lactic and succinic acids appeared not to be important intermediate products in the caecal fermentation.

Approximately twice as much carbon dioxide as methane was produced during in vitro incubation of caecal content.

Naturally occurring nitrogen and carbon sources for the caecal bacteria were sought in the ingesta of the caecum and from the distal end of the small intestine. Substrate amounts of ammonia were found in the ingesta from both organs. Several amino acids were detected but in less than substrate quantities. Soluble sugars and sugar acids were not detected in the soluble fraction of the ingesta, but arabinose, xylose, glucose and galactose were detected in an acid hydrolysate of the particulate fraction from the small intestine.

Three hundred twenty-eight strains of caecal bacteria were isolated by randomly picking discrete colonies from 0.5 or 1.0 x 10^{-8} dilution roll tube cultures. The strains were placed into 43 groups based on morphological and physiological characteristics. Some of the groups were similar to but most could not be identified with the predominant bacterial groups in the bovine rumen.

INTRODUCTION

Herbivorous animals are characterized by having an expanded portion or portions in their alimentary tract, which in carnivorous animals are expanded to a lesser degree if at all. The herbivorous animals show wide variations in the anatomy of their digestive system. In ruminants, the distal end of the esophagus is expanded into three compartments: the rumen, which is the largest, the reticulum, and the omasum. These are followed by a relatively small true stomach, the abomasum. Some rodents, for example the hamster and field vole, have a gastric diverticulum and a large caecum. The elephant and the horse have a large caecum and colon. The rabbit and porcupine have only a large caecum.

The expanded portions of the digestive tract provide suitable environments for the growth of anaerobic microorganisms which may aid the host in the digestion of food material. In general the environments are semi-fluid, 60 to 90 percent water, have a constant temperature of 38 to 40 C, and have low oxidation-reduction potentials. In addition, the environment within a particular digestive organ may be influenced by glandular secretions such as saliva, mucin and bile.

Food materials degraded by microorganisms in the digestive tract include mono-, di- and polysaccharides, sugar acids and organic nitrogen compounds. The major products formed during ruminal, caecal, or large intestinal fermentations are the short chain volatile fatty acids, acetic, propionic and butyric, and the gases carbon dioxide and methane. Two common bacterial fermentation acids, lactic and succinic, normally do not accumulate in the ingesta but may be important intermediates in the fermentation of the food material. In addition to the digestion, there is also

synthesis of such materials as vitamins, proteins, and carbohydrates. Some of these are incorporated into the bodies of the microorganisms and may become available to the host only after digestion.

The role of the microflora has been recognized in the degradation of plant material ever since Tappeiner (1884) observed that cellulose disappeared in the rumen of cattle. Baker and Martin (1937a) also credited the microflora of the caecum with the disintegration of pectic substances, hemicelluloses and cellulose in the rabbit. In addition to these early observations, there have been many more on the decomposition of plant material in the rumen, caecum and colon, but only a few of these will be mentioned. The in vitro fermentation of cellulose by rumen bacteria was studied by Woodman and Evans (1938). According to Van der Wath (1948), the only diastases present in the sheep rumen are those secreted by the rumen bacteria. Hale et al. (1940) found 85 percent of the digestible cellulose ingested by a bovine to be digested in the rumen within 14 hours. They found that lignin and true fat were not digested in the rumen. Elsden (1945) observed in vitro fermentation of cellulose, glucose and lactic acid by sheep rumen content. Gray (1947b) obtained data indicating that 70 percent of the digestible cellulose of fodder was degraded in the rumen, 17 percent in the caecum, and 13 percent in the colon. No cellulose digestion occurred in the abomasum or in the small intestine. Baker et al. (1950) studied the breakdown of structural starches in the digestive tracts of ruminant and non-ruminant animals. They demonstrated the bacterial degradation of starch in the rumen, diverticulum, and caecum in ruminants, rodents and swine. Heald (1952) obtained in vitro fermentation of pentoses and uronic acids with

suspensions of sheep rumen bacteria. Doetsch et al. (1953) incubated suspensions of bovine rumen bacteria with cellobiose, maltose, glucose, xylose, pyruvate, lactate, oxalacetate, succinate, fumarate, malate, formate, acetate, propionate, butyrate and β-hydroxy-butyrate. All of these were attacked by rumen bacteria. McBee (1953) found that the addition of cellulose to the hay diet of a sheep increased the rate of cellulose and hemicellulose digestion by the rumen fluid; however, the addition of hemicellulose increased the fermentation rate of only the hemicellulose. Howard (1955) observed a rapid fermentation of purified wheat flour pentosan which consisted of chains of β-1:4-D-xylose units with single L-arabinose units attached along the chain.

The conversion of organic nitrogen compounds in the digestive tract has also been studied but such studies have primarily been limited to the rumen. The degradation of urea has received much attention because of its economic importance as a substitute for protein nitrogen in livestock feed. Pearson and Smith (1943a) observed high levels of urease activity in bovine rumen ingesta. Pearson and Smith (1943b) and Smith and Baker (1944) obtained evidence which indicated that urea may be used as a nitrogen source by rumen microorganisms. Sirotnak et al. (1953) incubated 22 amino acids with suspensions of bovine rumen bacteria. Aspartic acid, glutamic acid, serine, arginine, cysteine, and cystine were found to be dissimilated. MacLeod and Murray (1956) found that a combination of valine, leucine and isoleucine stimulated cellulose digestion. The major products formed when an acid hydrolysate of casein was incubated with sheep rumen ingesta were ammonia, carbon dioxide and volatile fatty acids (El-Shazly, 1952). Feeding

sheep on a diet in which zein contributed 94 percent of the total nitrogen resulted in an incorporation of approximately 40 percent of the zein into microorganism protein (McDonald, 1954). By in vitro experiments, Belasco (1954) compared urea with feed proteins and found urea to be a superior nitrogen source in promoting cellulose digestion by rumen microorganisms. Moore and King (1958) followed the changes in the concentrations of 7 different fractions of nitrogenous materials in the bovine rumen. The maximum changes in concentrations occurred within 1 hour after feeding, regardless of the ration tested, and returned to the prefeeding levels within about 5 hours. Blackburn and Hobson (1960a) studied the proteolytic activity of whole and fractionated sheep rumen contents and found it to be independent of the animals diet. Both the protozoan and bacterial fractions showed proteolytic activity. They also found (1960b) that the proteolytic activity of rumen microorganisms was relatively constant and comparatively high at all times, even before feeding. The metabolic fates of the nitrogenous compounds seem to be dependent upon the general properties of the microorganisms and are not greatly influenced by the diet.

The principal products of carbohydrate fermentation in different parts of the digestive tract have been shown to be remarkably similar, not only in the kinds of acids but in their relative concentrations. Phillipson (1947) credits Tappeiner (1884) for being the first to detect volatile fatty acids as products of cellulose fermentation. Acetic, propionic and butyric acids have been shown to be the main volatile fatty acids in the rumen of sheep (Elsden, 1945). Acetic acid accounted for 55 to 75 percent of the total. Elsden et al. (1946) determined the volatile fatty acids in the

digesta of the sheep, red deer, horse, rabbit, rat and pig. Mixtures of acetic, propionic, and butyric acids were found to be the characteristic fermentation products in the alimentary tracts of all of these species and their average compositions consisted of 67 percent acetic, 19 percent propionic and 14 percent butyric. The in vitro fermentation of cellulose and glucose by rumen contents yielded acetic, propionic and butyric acids (Elsden, 1945). Propionic acid was the major fatty acid in both cases. Very little, if any, butyric acid was formed during the fermentation of cellulose. Gray and Pilgrim (1952) found that when cellulose or hemicellulose was fermented, equal amounts of acetic and propionic acids were produced, with only a small amount of butyric acid. When cellulose was fermented in the presence of casein, a high level of butyric acid was produced. Carroll and Hungate (1954) found that the volatile acids produced during in vitro fermentation rate studies consisted of approximately 62 percent acetic, 22 percent propionic and 16 percent butyric regardless of diet. Balch and Rowland (1957) studied the diurnal variations in the concentrations of volatile fatty acids in the bovine rumen and found that the level of propionic acid was directly proportional to the amounts of readily fermentable carbohydrates in the ration and that the levels of butyric and higher acids were directly proportional to the levels of protein. These results were confirmed by Eusebio et al. (1959), who found that increasing the availability of readily fermentable carbohydrates resulted in an increase of propionic acid at the expense of acetic acid. When linseed-oil meal was included in the diet, the level of butyric acid increased at the expense of the propionic. The metabolic fate of uniformly

C^{14}-labeled glucose introduced into a bovine rumen has been investigated (Otagaki et al., 1963). The specific activity of C^{14} reached a maximum in respired CO_2 at 45 minutes, in the blood volatile fatty acids at 15 minutes and in the plasma glucose at 2 hours after administration of the labeled glucose. Their results indicate that there is little, if any, direct absorption of glucose from the rumen, but that the plasma glucose is synthesized from the absorbed volatile acids.

Pure cultures of numerous rumen bacteria produce lactic and succinic acids as fermentation products (Bryant, 1959). However, the concentrations of these acids in rumen content are usually low. A transitory rise in lactic acid concentration of sheep rumen content was observed when large amounts of glucose or ground grain were placed into the rumen of hay-fed sheep (Hungate et al., 1952). Soluble starch, cellulose, pyruvic acid and glucose were tested for lactic acid production by incubating them in vitro with rumen content (Waldo and Schultz, 1956). They found lactic acid to be produced only from glucose. Jayasuriya and Hungate (1959) did not consider lactate to be an important intermediate in the rumen fermentation of hay-fed steers, but believed that it may have been an intermediate for as much as one-sixth of the total substrate in grain-fed steers. Lactic acid accumulation appeared to be dependent upon the presence of high levels of readily fermentable carbohydrates. In isotope experiments with lactate-2-C^{14}, most of the labeled carbon was recovered in acetic acid. Bruno and Moore (1962) incubated commercial alfalfa meal, heated and unheated alfalfa, glucose and heated starch in vitro with ruminal ingesta and observed that a transitory accumulation of lactic acid occurred with glucose, heated alfalfa and heated

starch. Their results also suggest that the accumulation of lactic acid is dependent upon readily fermentable carbohydrates which either have to be added or else made available by heating. The authors suggested that the lactic acid accumulation might reflect the resistance of the lactic acid fermentation to low pH values. They did not believe that the experiment indicated the importance of lactic acid as an intermediate in the rumen fermentation, since the absence of lactic acid may have reflected either a rapid metabolism or a decreased rate of production.

Low concentrations of succinic acid have been found in sheep rumen content; only a slight increase in concentration occurred after feeding (Sijpesteijn and Elsden, 1952). Suspensions of washed rumen bacteria rapidly converted succinic acid to propionic acid and carbon dioxide. Blackburn and Hungate (1963) calculated velocity constants for the conversion of succinic acid to propionic acid in bovine rumen content. By determining the size of the succinic acid pool in the rumen content and by the use of the conversion velocity constant, they concluded that succinic acid was the major precursor of the propionic acid found in the rumen.

The principal fermentation gases detected from the digestive tract content are carbon dioxide and methane. Pure cultures of carbohydrate-fermenting rumen bacteria, however, have been found to produce CO_2 or CO_2 and H_2 (Bryant, 1959). Smith and Hungate (1958) cite Reiset (1863) for first demonstrating methane formation in the rumen. Tappeiner (1884) detected methane and carbon dioxide as products of cellulose fermentation by rumen bacteria. Lugg (1938) measured the combustible metabolic gases of a sheep and found that about 18 liters of methane were produced per day.

Olson (1940) found $CO_2:CH_4$ ratios of 3.3-4:1 from cows feeding on different kinds of pasture. Only trace amounts of hydrogen were detected. Marstron (1948) obtained $CO_2:CH_4$ ratios of 3.0-3.8:1 during cellulose fermentation with suspensions of washed rumen bacteria. When $CO_2:CH_4$ ratios were measured with respect to time after feeding, they varied from 1:1 following a period of fasting to 3:1 two hours after feeding (Kingwill et al., 1959). McNeill and Jacobson (1955) presented evidence to suggest that their failure to find more than trace amounts of hydrogen in rumen contents was due to extremely active hydrogenase systems. In vitro experiments with a hydrogen gas atmosphere resulted in no carbon dioxide production. The authors suggested that hydrogen gas might be the limiting factor for methane production in a normal rumen. Their observations, however, are not supported by the work of Kingwill et al. (1959) who found that continuous sparging with H_2 showed no effect on the $CO_2:CH_4$ ratios. Carroll and Hungate (1955) incubated rumen content in vitro with C^{14} formic acid. The labeled carbon was recovered primarily as CO_2 indicating hydrogenlyase activity. An estimation of the maximum rate at which formic acid could be produced in the rumen indicated that methanogenic substrates in addition to formic acid were involved in the synthesis of methane. Beijer (1952) incubated various substrates with rumen fluid. Formic acid was converted to methane quantitatively and succinic acid gave a notable rise in methane production. Acetic, propionic and butyric acids did not appear to be fermented. Oppermann et al. (1957) stabilized rumen fluid enrichment cultures which produced methane from formic and acetic acid. Cultures utilizing butyric acid could be established only at 45 C, but cultures utilizing propionic acid were not obtained.

Nelson et al. (1958) obtained enrichment cultures of rumen bacteria which fermented butyric and valeric acids. Butyric acid was completely degraded but propionic accumulated when valeric was fermented.

Methane production was found to be lower in the caecum and large intestine than in the rumen of several African ruminants (Hungate et al., 1959).

The digestive organs, being highly vascularized, enable the fermentation products to become available to the animal by being absorbed into the blood. Barcroft et al. (1944) found the blood draining the rumen of sheep to have higher concentrations of the volatile fatty acids than peripheral blood. Increased levels of acids were not detected in the blood draining the abomasum or the small intestine, but significant amounts were found in blood draining the caecum. Relatively high concentrations of volatile acids were found in the blood draining the caecum and/or colon of the horse, pig and rabbit. The rates at which the sodium salts of acetic, propionic and butyric acids were absorbed from the sheep rumen, appeared in the inverse order of their increasing molecular weights. Danielli et al. (1945) observed that the rate of fatty acid absorption from the rumen was dependent upon pH. At pH 7.5 the salts were lost in the order acetate>propionate> butyrate, whereas at pH 5.8 the order of the absorption rates were butyric > propionic>acetic. It was concluded that the free acids were absorbed at a greater rate than the anions and that different mechanisms were involved. Gray (1947a) studied the rate of acetate and propionate absorption from the rumen of a sheep fitted with a rumen fistula. When alkaline solutions of acetate and propionate were introduced into the rumen, the absorption rates

of the two acids were constant and the ratios remained the same. When the sheep ate during the experiment, propionate appeared to be absorbed at a higher rate. Propionate was also absorbed faster when phosphate buffer, at a concentration similar to that in the saliva, was included in the solution. Lowering the pH of the solution to pH 6.5 caused the propionic acid to be absorbed faster than the acetic. It was concluded that the ratios in which the volatile fatty acids are found in the rumen may not depict the rates of production.

The metabolism of the volatile fatty acids in animal tissue has been studied by many investigators. Phillipson (1947) and Elsden and Phillipson (1948) have reviewed the older literature. The later literature has been reviewed by Barnett and Reid (1961). In general it can be said that the volatile fatty acids are used for both energy and biosynthesis. The amount of energy made available through the oxidation of volatile fatty acids may be calculated from their heats of combustion. Armsby and Moulton (1925) (cited by Carroll and Hungate, 1954) estimated the maintenance energy requirement of a 1000 lb. bovine to be 7,300 Kcal per day. Carroll and Hungate (1954), assuming that the energy requirement of an animal is proportional to the three-fourths power of its weight, as is the basal metabolism rate (Kleiber, 1947), calculated the maintenance requirement of a 500 Kg bovine to be 7,850 Kcal per day. Using a method of zero time fermentation rates, they approximated the amount of volatile acids formed in the bovine rumen. From their calculations, for a 500 Kg cow containing 70 Kg of rumen content, they estimated that during a 24 hour period a hay-fed animal obtained 7,070 Kcal, a grain-fed animal 11,450 Kcal and a grass-fed animal

4,810 Kcal through the oxidation of the volatile acids. The energy obtainable from the volatile acids accounted for 70 percent of the estimated total energy requirements. Emery et al. (1956) came to the conclusion that a cow obtains 3 to 13 percent of its energy from the short chain volatile fatty acids. Stewart et al. (1958) determined that 63 percent of the maintenance energy requirements for steers were supplied by the volatile fermentation acids. Hungate et al. (1959) measured the fermentation rates in the rumen, caecum, and large intestine of zebu, gazelle, eland, suni and camels. The highest fermentation rates occurred in the rumen. Due to the differences in the sizes of the organs, the rumen accounted for 95 to 98 percent of the total fermentation. Hungate et al. (1961) determined the zero time fermentation rates of the individual volatile fatty acids, acetic, propionic, and butyric in bovine rumen content. The microorganisms were credited for contributing 90 percent of the animals carbon requirements. As much as 50 percent of the maintenance energy of the deer may be provided by the rumen fermentation (Short, 1963).

Microorganisms important in the fermentation of ingested food materials have been studied both microscopically and in pure culture. Baker (1933) and Baker and Martin (1937b) studied microscopically the disintegration of vegetable remains in the caecum of the guinea pig. Numerous types of bacteria, thought to be of functional importance, were classified on the basis of their morphology. Protozoa were observed in the caecal content but were found in low numbers and were considered not to be of functional importance. The bacterial population was divided into the iodophilic and aniodophilic bacteria. The iodophilic bacteria contained starch-like

polysaccharides which stained with iodine. Baker and Martin (1937a) investigated the microflora of the rabbit caecum with regard to the disintegration of cell wall substances. Many of the same morphological types of bacteria were found as in the studies on the guinea pig; however, protozoa were not observed in the animals studied. Baker and Martin (1939) studied the caecal microbiology of the horse and found the protozoan population to be far more conspicuous than in either the guinea pig or the rabbit. Baker (1943) divided the rumen population of the ox into three major groups: (1) iodophilic microorganisms; (2) aniodophilic microorganisms; and (3) protozoa.

Few of the bacteria important for the degradation of plant materials in herbivorous digestion were grown in pure culture until Hungate (1947) developed cultural methods for growing cellulose-fermenting bacteria isolated from the rumen. The success of the method can be attributed to strict anaerobiosis and the incorporation of sterile rumen fluid as an enrichment. Bryant and Burkey (1953) and Bryant and Robinson (1961b) modified Hungate's medium so that it was less-selective. The so-called "non-selective" media have been used in studies on the composition of bacterial populations within the rumen.

Since 1947 many of the important rumen bacteria have been isolated and studied in pure culture. The literature has recently been reviewed by Bryant (1959, 1963).

The isolation and study of pure cultures of bacteria from the caeca of nonruminant herbivorous animals have been limited. Barker and Haas (1944) isolated a straight to slightly curved gram positive, anaerobic, rod shaped bacterium from the intestine of a rat. The bacterium produced chiefly

acetic and butyric acids from glucose. It was named Butyribacterium rettgeri. Alexander et al. (1952) isolated cocci similar to Streptococcus bovis and Veillonella gazogenes from the colon of a horse. Hall (1952) isolated a cellulose fermenting coccus, which resembled Ruminococcus flavefaciens, from the caecum of a rabbit.

The purpose of the present investigation was to obtain a better understanding of the caecum and its functional importance to the porcupine digestive system. The yellow haired porcupine (Erethizon dorsatum epixanthum) was selected as the experimental animal because it is a relatively large rodent, thus provides large quantities of caecal material, is readily available in this geographical area, can be collected without special permits, is relatively easy to catch and, after the quills are sheared, is easy to handle. The porcupine is also of interest because its diet consists not only of succulent plants but may also include bark and the cambium layer from conifers and deciduous trees. The nutrition of the porcupine is not complicated by coprophagy as it is in some rodents. The investigation was divided into three major parts: (1) the identification of the major fermentation products, the amounts of them produced and the nutritional value of those products utilized by the porcupine; (2) the identification of carbon and nitrogen sources available to the bacteria of the caecum; and (3) the study of populations of caecal bacteria and the major groups of bacteria in these populations. After some preliminary experiments early in the investigation the assumption was made that the caecal environment is similar to that of the rumen. The culture studies in this investigation were based on that assumption which proved to be incorrect. Therefore, the

studies on the carbon and nitrogen sources were commenced as a result of the culture studies and not prior to them.

MATERIALS AND METHODS

A. Animal Collection

The porcupines were caught by local ranchers, usually in grain fields or hay meadows. Those which have been designated as field animals were used for experiments within 24 hours after capture. Their ingesta consisted of the porcupines natural food in this area. The laboratory porcupines were kept in captivity for several weeks or months before being used in experiments. They were fed commercial rabbit feed containing no antibiotics, Purina Rabbit Chow, WO.

The majority of the experiments required sacrificing the animal. If blood was to be collected from the vessels of the caecal region, the animal was anesthetized with ether, opened with a midline incision and following blood collection the animal was killed by inhalation of chloroform. If it was not necessary to have the animal alive at the time of opening the abdominal cavity, it was first killed by an intramuscular injection of 2 ml of a 40 percent nicotine solution.

B. General Observations

The alimentary tract of each porcupine was removed. The stomach and caecum were weighed, and the lengths of the small and large intestines measured. In some cases the caecum was also weighed after the contents had been removed to determine the actual weight of the fermenting content.

Ingesta were removed from the stomach, caecum, and 3 sections of the large intestine. The sections of the large intestine were designated LI_1 (the first 25 cm of large intestine distal to the caecum), LI_2 (25 cm section

of large intestine distal to LI_1), and LI_3 the remainder of the large intestine containing the fecal pellets in varying states of dehydration.

The percent dry matter in the content of the different organs or parts of an organ was determined by drying samples in an 80 C oven to constant weight.

A glass electrode was used to measure the pH of the caecal content at the base and at the apex of the caecum.

The oxidation-reduction potentials of caecal content were determined with a platinum electrode on samples removed from nonspecific locations in the organ. They were determined at room temperature, in an air atmosphere, on content removed shortly after the animals death. The platinum electrode required about 15 minutes to come to equilibrium.

C. Fermentation Products

The importance of the caecal fermentation to the porcupine can be estimated if one knows what the fermentation products are, the rate at which they are produced, the total amounts produced during a given length of time, and the amounts of these products absorbed and utilized by the animal. The ingesta of the porcupine were quantitatively analyzed for acetic, propionic, butyric, lactic, and succinic acids. The arterial and the venous blood of the caecum was analyzed for total volatile fatty acids.

Portions of the content from the different organs or parts of an organ to be analyzed were mixed 1:1 w/v with 0.2 N H_2SO_4 to inactivate the biological processes. These samples were allowed to stand for at least 24 hours for the equilibration of the soluble components between the liquid

and particulate fractions and after equilibration, they were centrifuged for 20 minutes at 4,000 X g. The particulate fraction was discarded and the supernatant was examined for volatile fatty acids, lactic acid, and succinic acid.

Rates at which volatile fatty acids are produced in the caecum were determined in vitro by the zero time method (Carroll and Hungate, 1954). This procedure permits one to estimate the rate of production of the fermentation products in the animal by plotting their concentrations determined at several successive time intervals of incubation after the removal of the fermenting material from the animal. The slope of the tangent to this curve at zero time approximates the rate of production of the fermentation product in the living animal. Caecal contents were removed from each porcupine shortly after death and placed in a flask gassed with carbon dioxide. A slow stream of CO_2 was run into the flask through a capillary tube during the course of the experiment. The first sample was inactivated at the time the content was placed into the flask and the rest of the samples were removed at 1 hour intervals after the animal's death. The samples were prepared for analysis as described in the preceding paragraph.

Total volatile fatty acids in the alimentary tract contents were determined by steam distillation in the Markham still (Markham, 1942). One to five ml aliquots of the prepared samples, 0.2 ml conc. H_2SO_4, and one drop of 0.1 percent thymol blue solution were placed into the still. More H_2SO_4 was added if the thymol blue was not in the red range (pH 1.2 to 2.8). Condensation of steam into the sample was prevented by heating

the sample prior to distillation by passing steam through the outer jacket of the still for 5 minutes. The amount of distillate collected equaled 15 times the volume of the sample. The distillate was titrated with 0.02 N NaOH to the brom thymol blue end point in the presence of CO_2 free air.

The individual volatile fatty acids were identified and determined quantitatively by gas-liquid chromatography. The acids from 10-20 ml of the acidified and centrifuged caecal content were collected by steam distillation and neutralized with NaOH beyond the thymol blue end point (pH 8.0-9.6) and evaporated to dryness on a steam bath. They were adjusted to the high pH to prevent loss of the volatile acids during drying. The salts of the volatile acids were quantitatively transferred to a 20 ml beaker by rinsing the evaporating dish with 5-7 ml of distilled water added in small portions. The salts were again evaporated to dryness in a near boiling water bath. They were redissolved in 0.2 ml of hot distilled water. The acids were extracted from the aqueous phase with dichloromethane. The beaker was placed into an ice bath and 5 ml of dichloromethane were added; the mixture was allowed to cool for 5 minutes. It was then stirred rapidly with an electric stirrer and conc. H_2SO_4 was added until the thymol blue turned red (pH 1.2-2.8), usually 2 or 3 drops. Two grams of anhydrous Na_2SO_4 were added to take up the water and the stirring was continued for 30 to 45 seconds longer. The stirring blade was then rinsed with 2 ml of dichloromethane, the washing going into the beaker. The beaker was removed from the ice bath and the contents filtered through a medium porosity sintered glass filter into a 10 ml volumetric flask. The beaker

was rinsed several times with small amounts of solvent. The volumetric flask was filled to volume just prior to removing a 30 μl sample for analysis by gas chromatography. This method was found to extract the volatile fatty acids quantitatively from the water solution.

A Beckman GC 2A gas chromatograph was used with a stainless steel column 6 feet long and 1/4 inch in diameter. The column packing material was a modification of that used by Erwin et al. (1961). One hundred ml of Gas Chrom P (60-80 mesh) was first coated with 0.74 g of H_3PO_4 dissolved in 65 ml of dichloromethane. The solvent was evaporated and the H_3PO_4 coated Gas Chrom P was coated with 7.4 g of Tween 80 dissolved in 65 ml of the solvent. The solvent was again evaporated. The packed column was operated at 130 C, with a helium flow of 110 ml per minute. The thermal detector current was set at 300 milliamperes, and the output, attenuated 1, 2, or 5 times, was recorded on a Texas Instruments Servo-Riter integrating recorder.

Standards of reagent grade acetic, propionic and butyric acids were prepared in dichloromethane. The units of area on the chromatographs were linear between the concentrations of 0.003 and 0.100 milliequivalents of acid per milliliter when using a 30 μl sample. An equation for the standard curve of each acid was calculated by the method of least squares.

Lactic acid was determined on diluted digesta using the colorometric Barker and Summerson method as described by Umbreit et al. (1957).

Succinic acid was extracted from digesta with ethyl ether for 16 hours with a Kutscher-Steudel extractor and determined manometrically with pig heart succinic dehydrogenase (Umbreit et al., 1957).

Fermentation gases were collected from caecal content which was removed from a porcupine immediately after death and placed into a flask connected by rubber tubing to a mercury filled gas burette. The flask was incubated in a 39 C water bath and the caecal gases collected for 4 to 6 hours. The gases were separated and quantitatively measured by gas-solid chromatography using a stainless steel column 4 feet long and 1/4 inch in diameter packed with 100-200 mesh silica gel. The column was operated at 40 C, the thermal detector at 150 milliamperes, and the helium flow was set at 55 ml per minute. Each gas sample was introduced into an evacuated 1 cc gas sample loop of the chromatograph through the gas sampling valve. The amount of sample under standard conditions was calculated from the temperature and the pressure at which it was introduced into the gas loop; usually about 100 mm mercury.

Standard curves of carbon dioxide, methane and air were obtained by introducing known samples at various pressures. Cylinder grade CO_2 was used. Methane was obtained in a purified state by distilling condensed natural gas from a vessel immersed in freezing isopropanol. The column gave good separation of H_2, CH_4, air, and CO_2. Nitrogen and oxygen were not separated. The sensitivity of the instrument to H_2 was low and to have been able to have measured it quantitatively one would have had to measure it by difference, but no evidence of H_2 was obtained.

Absorption of volatile fatty acids from the caecum was demonstrated by measuring the differences in their concentrations in blood removed from a large caecal artery and vein. Five ml blood samples were drawn with a syringe and transferred to tubes containing potassium oxalate.

These were refrigerated until analyzed, usually 1 or 2 days.

Volatile fatty acid analyses were made on blood deproteinized with $ZnSO_4$ (McClendon, 1944). Seven ml of the deproteinized blood were placed in a Markham steam still, together with 0.5 ml of 10 N H_2SO_4 and 1 drop of 0.1 percent solution of thymol blue. Seventy ml of the distillate were collected and titrated to the brom thymol blue end point with 0.02 N NaOH while being flushed with CO_2 free air.

Blood samples were also analyzed for urea to determine whether urea was excreted into the caecum. Protein free filtrates of the blood (Folin and Wu, 1938) were incubated with urease and the resulting ammonia determined colorimetrically with Nessler's reagent (Karr, 1924).

D. In Vitro Reactions of Caecal Content

Urease activity in caecal content was determined by the production of ammonia from added urea. Caecal content was mixed 1:1 w/v with a 1 percent aqueous solution of urea. Immediately after mixing, a zero time sample was removed and inactivated by mixing 1:1 v/v with 0.2 N H_2SO_4. The reaction flask was flushed with CO_2, fitted with a bunsen valve and incubated in a 39 C water bath. Ten ml samples were removed at 10 to 20 minute intervals. The inactivated samples were then centrifuged at 4,000 X g for 20 minutes. The supernatants were analyzed for ammonia. One to five ml samples were placed in a Markham still together with one drop of thymol blue and sufficient 10 percent NaOH to turn the mixture alkaline. The distillate was collected with the tip of the condenser immersed in 5 ml of water containing 5 drops of methyl red-methylene blue indicator (McKenzie and

Wallace, 1954). Forty ml of distillate were collected and titrated with 0.02 N H_2SO_4.

Lactic acid dissimilation by caecal bacteria was determined in vitro. Fresh caecal content was placed in a flask and mineral salts dilution solution (Bryant and Robinson, 1961b), containing 1 percent lactic acid, was added 1:1 w/v. The contents of the flask were mixed and a 10 ml zero time sample removed and inactivated with an equal volume of 0.2 N H_2SO_4. These were centrifuged at 4,000 X g for 20 minutes and the supernatants were analyzed for residual lactic acid.

Succinic acid dissimilation by caecal bacteria was determined in a similar manner; the mineral salts solution contained 1 percent succinic acid in place of lactic acid. The inactivated and centrifuged samples were analyzed for residual succinic acid.

E. Nitrogen and Carbon Sources for Caecal Bacteria

Compounds, which might be the natural nitrogen and carbon sources for the caecal bacteria were searched for in content from the caecum and from the distal end of the small intestine. The samples were inactivated by mixing them 1:1 w/v with 0.2 N H_2SO_4, and centrifuged at 4,000 X g for 10 minutes. The supernatant from each sample was analyzed for soluble organic compounds. The particulate fraction of the small intestine content was washed by suspending it in distilled water and recentrifuging. It was then dried in an 80 C oven, ground in a mortar and analyzed for polysaccharides. The particulate fraction of the caecal samples was discarded.

The supernatant samples were analyzed for ammonia and amino acids.

Ammonia was determined by steam distillation and titration. Amino acids were removed from each 10 ml supernatant sample by passing it through a 16 x 30 mm column of Dowex 50 cation exchange resin charged with 4 percent HCl. Amino acids were eluted with 10 ml of 6 N HCl and dried at room temperature under a stream of air. The residue was extracted several times with 1-2 ml volumes of absolute ethanol. The ethanolic extract, a total volume of 5-7 ml, was filtered through sintered glass and dried at room temperature under a stream of air. This second residue was dissolved in 1 ml of water and 2-3 drops of conc. NH_4OH were added to convert the amino acids to the free acids. The samples were then evaporated to dryness on a steam bath. The residue was dissolved in 0.5 ml of distilled water and 5 μl amounts were spotted on thin layer chromatogram plates prepared with a 250 μ layer of plain silica gel H (Research Specialties Company, Richmond, Calif.). The chromatograms were developed in two dimensions with n-butanol-acetic acid-water (2:1:2) for the first direction and isopropanol-NH_4OH (67:33) for the second. The amino acids were detected with ninhydrin.

Supernatant samples from the distal end of the small intestine and from the caecum were analyzed for soluble sugars and sugar acids. Particulate samples from the small intestine were hydrolyzed and analyzed for polysaccharide monomers. The neutral fraction, containing the sugars, from each supernatant sample was obtained by passing the sample through a 16 x 30 mm column of Dowex 50 cation exchange resin and neutralizing the eluate with Dowex 2 anion exchange resin charged with 8 percent $NaHCO_3$. The neutral fraction was decanted from the resin, and the resin was washed

several times with small amounts of distilled water. The washings were added to the neutral fraction which was then dried at room temperature in a stream of air. The residue was dissolved in 0.5 ml distilled water and 5-20 μl amounts were spotted on Whatman No. 1 chromatography paper. The anionic fraction, containing the sugar acids, was eluted from the anion exchange resin with 10 ml of 6 N formic acid. The eluate was evaporated in a vacuum desiccator over NaOH and $CaSO_4$. The residue was dissolved in 0.5 ml of distilled water and 5-20 μl amounts were spotted on Whatman No. 1 paper chromatograms.

One gram amounts of the particulate samples were treated with H_2SO_4 (Block et al., 1958) to hydrolyze cellulose and other polysaccharides. The hydrolysate was neutralized with $BaCO_3$. Excess $BaCO_3$ and $BaSO_4$ were removed by centrifuging at 4,000 X g for 10 minutes. The supernatant was concentrated to 5 ml at room temperature under a stream of air. Five μl amounts were spotted on chromatogram paper.

The sugars and sugar acids were separated by descending paper chromatography. Ethyl acetate-pyridine-water (8:2:1) was used for the separation of sugars and the upper phase of n-butanol-acetic acid-water (4:1:5) was used for the separation of the sugar acids. The spots were developed with $AgNO_3$ solution (Trevelyan, 1950).

F. Caecal Bacteria

Studies on the populations of rumen bacteria have shown that the number of bacteria per gram of rumen content will vary with respect to time after feeding and with location within the rumen (Bryant and Robinson,

1961b, Munch-Peterson and Boundy, 1963). The investigations of Masson (1950), Bauman and Foster (1956), and Hungate et al. (1952) have shown that the composition of the bacterial population of the rumen may vary with changes in the diet; however, Hungate (1957) found no correlation between different rations and differences in the microflora. Culture experiments on the caecal bacteria were designed to determine the diurnal variations in the total numbers and kinds of culturable bacteria, and to compare the compositions of the bacterial populations from different animals.

In order to obtain more than one sample of caecal content from an animal, caecal cannulas were placed in a number of laboratory animals. These were made of plexiglass or teflon and had an inside diameter of one half inch and were closed with a screw cap. They were held in the caecum by a purse-string suture and were brought out through the animals left flank.

The anaerobic culture techniques developed by Hungate (1950) were used for growing caecal bacteria. Anaerobic conditions were continuously maintained in the culture tubes and flasks by displacing the air with oxygen free carbon dioxide, nitrogen, or a mixture of 10 percent carbon dioxide and 90 percent nitrogen. Traces of oxygen were removed from the flushing gases by passing them through a tube of hot reduced copper filings maintained at 520 C in a combustion furnace. The gas or gas mixture was passed into the vessels by means of a Pasteur pipette attached by rubber tubing to the gas source. The reentrance of oxygen was prevented by closing the vessels with rubber stoppers as the pipette was removed. The culture media employed in the study were modifications of those used by

Bryant and Robinson (1961b). The compositions of the various solutions used in the media and the media are given in the Appendix.

Caecal material to be cultured was serially diluted in anaerobic dilution medium and inoculated into roll tubes containing a nonselective medium, which were incubated for one week at 39 C. The colonies were counted under a dissection microscope. Isolated colonies were randomly picked from roll tubes of 0.5 or 1 x 10^{-8} dilution and inoculated into slants of the same medium. Growth from these cultures was used to inoculate differential media designed to detect: (1) fermentation of the carbohydrates glucose, xylose, cellobiose, starch, xylan, and cellulose; (2) gas production from glucose; (3) hydrogen sulfide production; (4) oxygen relationships; (5) gelatin hydrolysis; (6) growth in the absence of rumen fluid; and (7) growth in the absence of carbon dioxide. A weakly buffered glucose medium (pH glucose) was used for determining the terminal pH of fermentation. The results obtained from these media along with cell morphology, gram reaction, and motility were used to group the cultures isolated.

Visible characteristics were recorded for each medium at 1, 3 and 7 days after inoculation, and chemical tests were made at 7 days. Acid production was detected with the use of brom thymol blue, cellulose utilization was detected by visual observation, xylan utilization was quantitatively measured by the orcinol method (Umbreit et al, 1957) and gelatin hydrolysis was determined by liquefaction. Motility was substantiated by diffuse growth in the semi-solid H_2S-Anaerobiosis-Motility medium and by microscopic observation of a wet mount. The H_2S-Anaerobiosis-

Motility medium was not gassed when stab inoculated, which resulted in the oxidation of the upper 8 to 10 mm of medium. The facultatively anaerobic bacteria grew in the oxidized zone as well as in the bottom of the tube.

EXPERIMENTAL

A. General Observations

Since the purpose of the present investigation was to determine the importance of the caecal fermentation to the nutrition of the porcupine, it was important to study normal porcupines, caught in their habitat and eating their natural food. Catching animals not influenced by man and his agricultural crops proved to be difficult. More than half of the porcupines studied were caught while feeding in alfalfa, wheat or barley fields. Porcupines, being nocturnal animals, were easiest and therefore usually caught while feeding between the hours of 7 and 10 P.M. Few collectors were sufficiently interested to deliver the animals within an hour or so of capture at that time of night. Since the time of year, diet and interval between capture and examination were factors which could affect the results, they are recorded in Table I. In some instances data are insufficient to be of any value in interpreting the results.

The determination of the rate at which ingesta leave the caecum was necessary for estimating the amounts of fermentation products absorbed from the ingesta in both the caecum and large intestine. It was assumed that the dry weight loss from the content while passing through the large intestine would be insignificant. The rate of content passage from the caecum through the large intestine was measured by weighing the excreted feces. The feces from 4 laboratory animals were collected twice daily for 3 days and dried to constant weight. The results (Table II) indicate that the amount of ingesta passing through an animal is proportional to its size, being about 10 g per kilogram of body weight per day under those laboratory conditions.

Table I. Field animals used in the investigation.

Animal	Date of Collection	Time of Collection	Hours Time Between Capture and Experiment	Habitat
P_1	2/14/61	12:00 AM	19	stubble field
P_2	2/15/61	8:00 PM	14	alfalfa field
P_3	2/16/61	4:30 PM	4	grass and willows
P_4	2/16/61	8:00 PM	2	alfalfa field
P_5	2/17/61	10:30 AM	3	hawthorne bushes
P_9	2/17/61	4:00 PM	4	-
P_{10}	2/18/61	6:30 PM	2	alfalfa field
P_{14}	2/19/61	-	-	pine trees
P_{15}	2/19/61	-	-	pine trees
P_{16}	2/19/61	-	-	pine trees
P_{17}	2/19/61	-	-	pine trees
P_{18}	2/19/61	-	-	alfalfa and grass
P_{19}	2/19/61	-	-	alfalfa and grass
P_{20}	2/19/61	-	-	grass
P_{22}	7/26/62	10:00 PM	1	-
P_{23}	7/27/62	9:30 PM	1	-
P_{24}	10/ 4/62	8:30 PM	1	wheat field
P_{25}	10/ 4/62	8:30 PM	12	wheat field
P_{26}	10/ 8/62	9:30 PM	2	-
P_{27}	10/ 8/62	9:30 PM	12	-
P_{28}	11/15/62	7:30 PM	15	wheat field
P_{29}	12/10/62	11:00 PM	10	alfalfa field
P_{30}	10/15/63	8:30 PM	2	alfalfa field*
P_{31}	10/16/63	7:00 PM	1	alfalfa field*
P_{32}	10/16/63	7:00 PM	5	alfalfa field*
P_{33}	10/22/63	7:30 PM	2	alfalfa field*
P_{34}	10/22/63	8:30 PM	5	alfalfa field*
P_{35}	1/22/64	-	-	-
P_{36}	1/24/64	-	ca 24	-
P_{37}	1/24/64	-	ca 24	-

* the animals were caught in the same field

Table III lists the weight of each field animal, the total weights of the stomach and caecum and the lengths of the small and large intestines. The weight of the caecal content was determined for animals P_{30} through P_{34} by weighing the empty caecum and subtracting its weight from the total.

Table II. Ingesta passage rate in laboratory porcupines.

Porcupine	Kilograms Live Weight	Grams of Feces Per Day	Grams Feces/Kilogram Animal/Day
A	5.9	45	7.6
B	6.4	64	10
C	5.3	55	10
D	5.1	53	10

Since the caecum is located distal to the small intestine, one might expect to find the amount of ingesta in the caecum to be comparatively more constant with respect to time than the amount of ingesta in the rumen of a ruminant, the stomach acting as a reservoir and passing a rather constant supply of ingesta on to the caecum. In the case of a ruminant, fresh ingesta enter the rumen only during the time that the animal is eating. The data in Table III, however, do not allow one to assume that the amount of content in the caecum is constant since the total weights of the caeca flucuate between 2.8 and 10 percent of the total animal weights. The percent of body weight consisting of caecum does not appear to correlate with the size of the animal. Probably the best estimate for the amount of caecal content present in a porcupine can be obtained by subtracting the average percent empty caecal weight from the average percent full caecal weight which would be 4.4 percent. Elsden et al. (1946) measured the amount of ingesta in the digestive organs of several animals. The ratio of the porcupine caecal content weight to the body weight was greater than what was found for the horse but less than the rabbit and rat. The relative amounts of ingesta in the rumen of the

Table III. Weights and measurements of animals and organs.

Animal	Animal Weight Kg	Stomach Weight g	Stomach Percent Weight	Full Caecum Weight g	Full Caecum Percent Weight	Empty Caecum Weight g	Empty Caecum Percent Weight	Caecal Content Content Weight	Caecal Content Percent Weight	Small Intestine cm length	Large Intestine cm length
P_1	11.4	239	2.1	396	3.5					250	251
P_2	10.5	274	2.6	609	5.8					200	275
P_3	7.7	183	2.4	357	4.6					195	260
P_4	12.3	436	3.5	411	3.3					280	280
P_5	9.5	174	1.8	278	2.9					160	230
P_9	8.2	389	4.7	481	5.9					265	235
P_{10}	7.7	628	8.2	418	5.4					210	230
P_{14}	11.4	408	3.6	709	6.2						
P_{15}	8.2	407	5.0	612	7.5						
P_{16}	10.0	409	4.1	570	5.7						
P_{17}	10.9	434	4.0	460	4.2						
P_{18}	11.8	422	3.6	330	2.8						
P_{19}	10.0	407	4.1	559	5.6						
P_{20}	8.6	216	2.5	380	4.4						
P_{22}	13.6	-	-	-	-						
P_{23}	10.0	321	3.1	712	7.1						
P_{24}	5.5	298	5.4	244	4.4						
P_{25}	4.3	117	2.7	444	10.0						
P_{26}	3.0	140	4.7	300	10.0						
P_{27}	6.4	145	2.3	322	5.0						
P_{28}	10.9	230	2.1	472	4.3						
P_{29}	5.5	290	5.3	430	7.8						
P_{30}	3.4	274	8.1	297	8.7	50	1.5	247	7.3		
P_{31}	9.1	320	3.5	602	6.6	120	1.3	482	5.3		
P_{32}	3.4	172	5.1	323	9.5	62	1.8	261	7.7		
P_{33}	4.5	370	8.2	298	6.6	66	1.5	232	5.2		
P_{34}	5.0	198	4.0	356	7.1	88	1.8	268	5.4		
Average					6.0		1.6		6.2		

sheep and ox and in the caecum plus the colon of the horse were greater than the amount in the porcupine caecum. Hungate et al. (1959) found the weight of the fermenting ingesta in several African ruminants and elephants to vary from 8 to 17 percent of the animals' total weight.

It may also be noted from Table II that the large intestine of the porcupine is about the same length as the small. The long length of the large intestine suggests that it may be an important organ for the absorption of bacterial fermentation products formed in the caecum.

The percent dry weight of content in different organs or parts of an organ are shown in Table IV. The data show that on an average, about 22 percent of the water present in the caecal content is absorbed from the ingesta as it passes through the large intestine. Since this leaves a very dry pellet, it appears that the animal conserves as much of the fermentation products as possible.

The pH values of the caecal content are shown in Table V. The small differences between the pH values of the base and the apex of the caecum indicate that the alkaline ingesta entering the caecum from the ileum are rapidly mixed with the ingesta already present.

The rate of mixing in the porcupine caecum was also indicated by placing $BaSO_4$ in the caecum of a cannulated animal and observing under a fluoroscope its rate of dispersion. The $BaSO_4$ was dispersed throughout the caecum in about 20 minutes. The mixing of the caecal content may be greater in a normal animal since the cannula would restrict the movement of the caecum. In a similar experiment $BaSO_4$ added into the stomach of a rabbit with a stomach cannula was intimately mixed throughout the caecum

Table IV. Percent dry weight of the ingesta in different digestive organs.

Animal	Stomach	Caecum	Large Intestine LI_1	LI_2	LI_3
P_1	12	14	19	18	35
P_2	16	12	11	16	27
P_3	6	14	16	20	39
P_4	21	19	17	20	31
P_5	18	19	17	22	40
P_9	17	15	17	19	37
P_{10}	16	16	16	21	38
P_{14}	21	18	17	18	30
P_{15}	20	20	21	26	46
P_{16}	25	18	18	20	40
P_{17}	18	18	16	21	41
P_{18}	17	18	15	18	43
P_{19}	14	18	19	22	33
P_{20}	7	15	17	20	35
P_{22}	-	19	22	25	36
P_{23}	12	10	12	16	35
P_{24}	13	13	-	16	23
P_{25}	26	13	16	21	29
P_{26}	18	8	9	15	28
P_{27}	6	15	17	23	32
P_{28}	5	12	14	18	28
P_{29}	19	18	19	24	34
P_{30}	11	10	10	13	29
P_{31}	12	15	15	18	32
P_{32}	13	10	-	-	31
P_{33}	15	12	12	15	29
P_{34}	23	11	13	16	27
Average	15	15	16	19	34

in less than a minute after entering from the small intestine.

The caecal content provides an adequately reducing environment for the growth of the anaerobic caecal bacteria as indicated by the low E_h values (Table VI).

B. Fermentation Products

The levels of total volatile fatty acids were determined on the ingesta of the field animals. The concentrations of total volatile acids

Table V. pH values of porcupine caecal content.

Animal	Location in Caecum		
	Base	Apex	Homogenate
P_1	7.20	7.05	-
P_2	7.00	6.80	-
P_3	6.85	6.80	-
P_4	6.63	6.20	-
P_5	6.70	6.16	-
P_9	6.70	6.40	-
P_{10}	6.05	5.70	-
P_{14}	6.25	5.75	-
P_{15}	6.50	6.85	-
P_{16}	5.95	6.00	-
P_{17}	6.50	6.60	-
P_{18}	7.00	6.80	-
P_{19}	6.15	6.75	-
P_{20}	6.80	7.25	-
P_{23}	-	-	6.80
P_{25}	-	-	6.70
P_{27}	-	-	7.10
P_{28}	-	-	7.20
P_{29}	-	-	6.50
P_{30}	-	-	6.70
P_{31}	-	-	6.10
P_{32}	-	-	6.75
P_{33}	-	-	6.65

Table VI. E_h values of caecal content.

Animal	pH	E_h
P_{31}	6.10	-325
P_{32}	6.75	-450
P_{33}	6.65	-445
P_{34}	-	-440

are recorded for 14 animals and the average values are plotted for each organ (Figure 1). The data show that most of the volatile fatty acids present in the ingesta of the porcupine are produced in the caecum. The

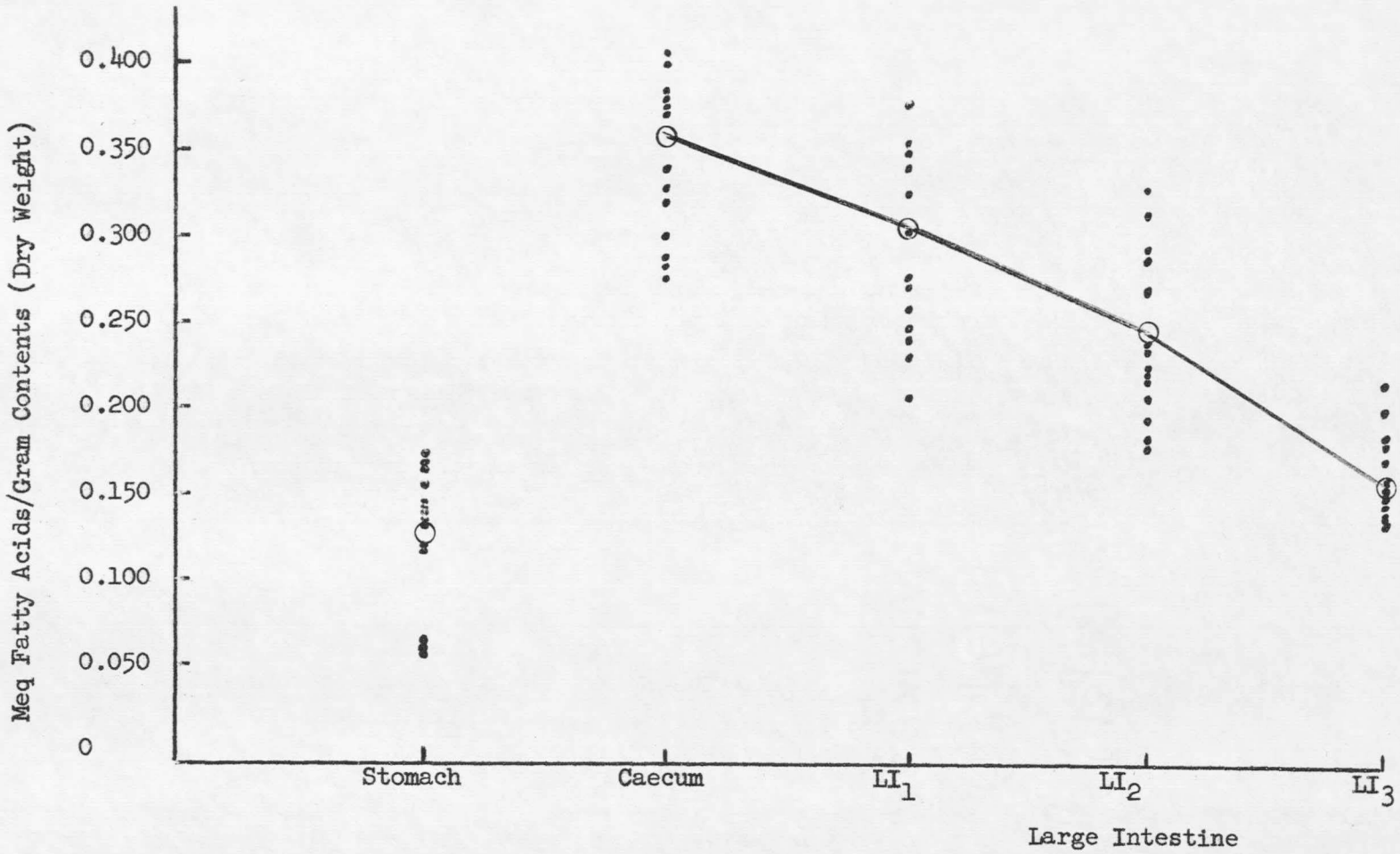

Figure 1. Total volatile fatty acids in the digestive tract contents of 14 porcupines.

levels of acids found in the stomach, caecum, and LI_3 are similar to the amounts found by Elsden et al. (1946) in the stomach, caecum and rectum of the rabbit but a little lower than the levels which they observed in the rat. Subtracting the average concentration of volatile acids in LI_3 from the concentration in the caecum shows that about 54 percent of the acids present in the caecum are absorbed from the ingesta as it passes through the large intestine. Since LI_3 comprises more than half of the large intestine, the 54 percent absorption may be a low figure. This could only be determined by examining freshly excreted fecal pellets. The data show that the volatile fatty acids are absorbed preferentially to water.

The percentages of acetic, propionic and butyric acids in the total acids in the caecum and large intestine are shown in Table VII. The volatile acids in the caecal content consist of approximately 73 percent acetic, 12 percent propionic and 14 percent butyric and the acids in LI_3 ingesta consist of approximately 68 percent acetic, 15 percent propionic and 16 percent butyric. The results indicate that acetic acid is absorbed at a faster relative rate than propionic and butyric; however, the differences are small and may not be significant.

The rate at which the volatile fatty acids were produced in caecal content of six porcupines were determined in vitro by the zero time method (Carroll and Hungate, 1954). Samples were removed from the fermentation vessel at one hour intervals after the time of the animals death. The concentrations of the acids were plotted against time, as shown for animal P_{23} in Figure 2, and curves were drawn to fit the points. The derivative of each curve was estimated at zero time by drawing a line tangent to the curve.

Table VII. Percent* of individual volatile fatty acids in the caecum and large intestine.

Organ	Acid	Animals P_{22}	P_{23}	P_{24}	P_{25}	P_{26}	P_{27}	Average
Caecum	acetic	73.4	74.4	65.7	72.8	80.4	71.1	73.0
	propionic	15.1	9.1	16.9	10.8	8.5	11.6	12.0
	butyric	11.5	16.5	17.4	16.4	11.2	17.3	13.4
LI_1	acetic	73.7	72.5	70.5		81.6		74.6
	propionic	12.2	11.1	13.3		9.0		11.4
	butyric	14.1	16.4	16.1		9.3		14.0
LI_2	acetic	68.2	75.0	71.3				71.5
	propionic	16.4	11.4	13.1				13.6
	butyric	15.4	13.6	15.4				14.8
LI_3	acetic	67.2	75.6	61.6				68.1
	propionic	15.3	11.1	19.9				15.4
	butyric	17.5	13.3	18.5				16.4

* percentages are calculated on the equivalents of acid found

The rates at which the individual volatile fatty acids are produced in the caecum and the concentrations of the acids in the ingesta of the caecum, LI_1 and LI_3 at the time of death of these six animals are shown in Table VIII. The rate at which acetic acid was produced depended upon the length of time between the capture of the animal and the experiment. Experiments were run on animals P_{25} and P_{26} 12 hours after capture, as shown in Table 1; however, the rates at which propionic and butyric acids were produced were affected little, if at all, by the time lag. The concentrations of the volatile acids in the caecal ingesta, but not in LI_3 contents, were affected by the rate of acid production. The rates of absorption from the caeca were not as rapid as the rates of production, but in the large intestine the rates of production were decreased and the

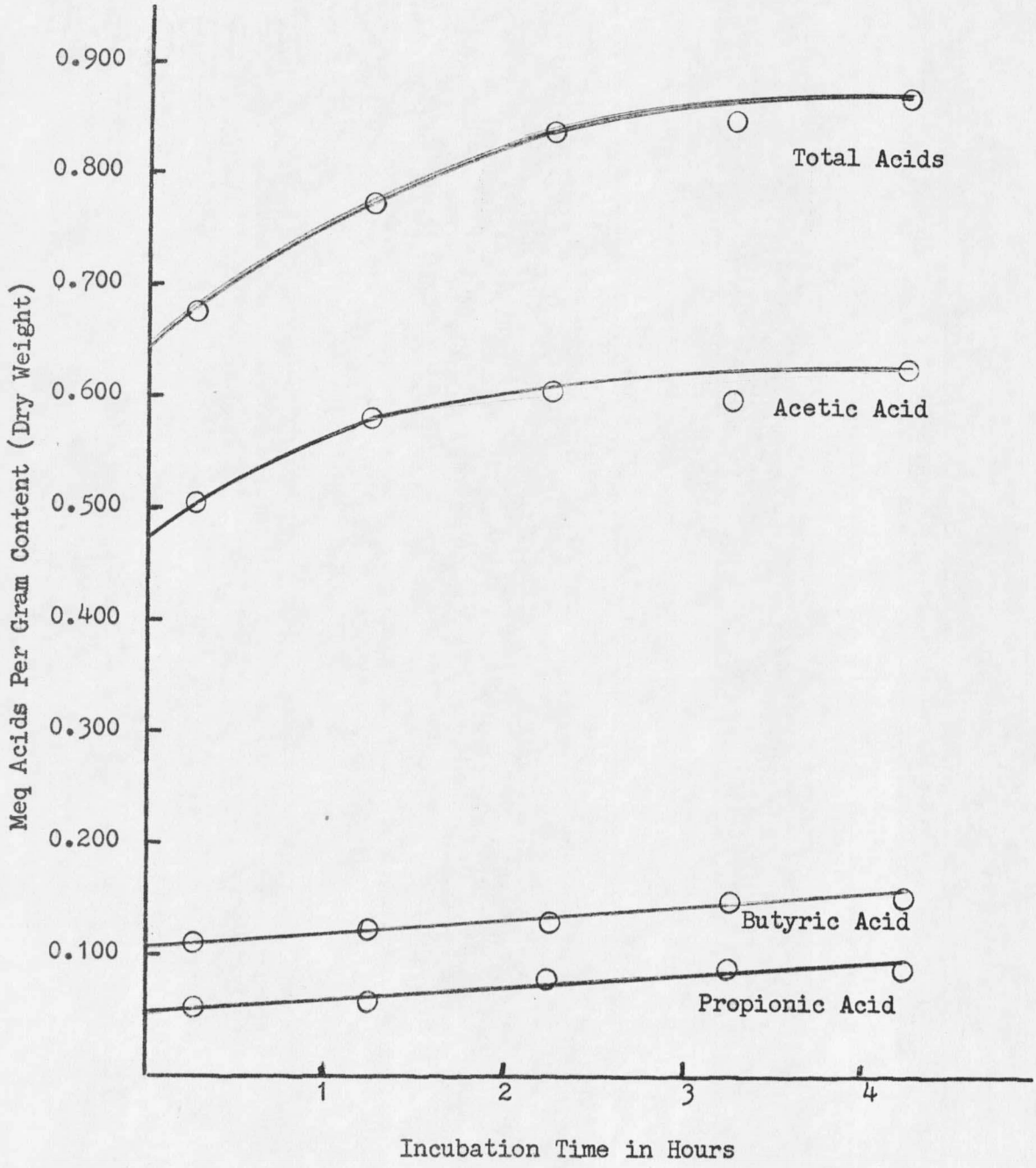

Figure 2. Fermentation rates in the porcupine caecum, P_{23}.

Table VIII. Individual volatile acids in porcupine ingesta.

	Production Rate Per Hour In Caecum			Concentration at Time of Death								
				Caecum			LI_1			LI_3		
		Propionic			Propionic			Propionic			Propionic	
Animal	Acetic		Butyric	Acetic		Butyric	Acetic		Butyric	Acetic		Butyric
P_{22}	0.075*	0.015	0.015	0.360	0.075	0.055	0.310	0.051	0.059	0.155	0.035	0.040
P_{23}	0.100	0.015	0.016	0.475	0.055	0.101	0.375	0.057	0.085	0.085	0.013	0.015
P_{24}	0.210	0.015	0.015	0.375	0.110	0.110	-	-	-	0.156	0.050	0.047
P_{25}	0.030	0.020	0.010	0.320	0.045	0.070	-	-	-	-	-	-
P_{26}	0.225	0.010	0.025	1.04	0.101	0.104	0.729	0.080	0.083	-	-	-
P_{27}	0.045	0.020	0.005	0.270	0.040	0.065	-	-	-	-	-	-
Average	0.11	0.016	0.014									

* recorded as meq per gram dry weight of ingesta

volatile fatty acids were absorbed to consistently low levels. The ratios of the volatile fatty acids appeared to remain relatively constant during the in vitro zero time fermentation rate studies (Table IX) indicating that the volatile acids had been absorbed from the caecum in approximately the same ratios as they were produced.

Table IX. Percent of caecal volatile fatty acids during in vitro fermentation rate studies.

Incubation Time	Acid	Animal					
		P_{22}	P_{23}	P_{24}	P_{25}	P_{26}	P_{27}
< 1/2 hr	acetic	73.4	74.4	65.7	72.8	80.4	71.1
	propionic	15.1	9.1	16.9	10.8	8.5	11.6
	butyric	11.5	16.5	17.4	16.4	11.2	17.3
1 hr	acetic	73.7	74.8	71.3	70.0	80.0	71.6
	propionic	15.4	8.6	13.3	13.6	8.5	12.9
	butyric	10.9	16.6	15.4	16.4	11.1	15.5
2 hr	acetic	73.4	72.1	71.4	70.0	79.6	72.2
	propionic	14.5	11.2	14.5	12.7	8.5	12.8
	butyric	12.1	16.7	14.1	17.3	11.7	14.9
3 hr	acetic	70.7	69.0	68.0	69.8	75.0	72.1
	propionic	15.7	12.6	16.9	13.4	11.5	13.3
	butyric	13.6	18.4	15.2	16.8	13.7	14.6
4 hr	acetic	70.6	70.9		71.3	77.0	
	propionic	15.4	10.5		12.7	8.4	
	butyric	14.0	18.6		16.0	14.2	

To compare the rate of absorption from the caecum with the rate from the rumen the concentrations of volatile fatty acids were determined in the arterial and the venous blood of the caecum of 13 porcupines. The acid concentrations and the differences in concentration between the venous and arterial blood (V-A) are shown in Table X. The average of the (V-A) values

Table X. Volatile fatty acids in arterial and venous blood from the porcupine caecum.

Animal	Meq per liter		
	Arterial	Venous	(V-A)
P_1	2.02	4.27	2.25
P_2	3.77	4.08	0.31
P_3	2.83	5.06	2.23
P_4	3.62	7.83	4.21
P_5	5.84	6.41	0.57
P_9	4.38	6.56	2.18
P_{10}	5.17	11.38	6.21
P_{14}	4.45	5.97	1.52
P_{16}	4.12	2.18	-1.94
P_{17}	1.59	3.71	2.12
P_{18}	5.06	7.63	2.57
P_{19}	5.25	6.74	1.49
P_{20}	3.92	6.37	2.45
Average	4.00	6.01	2.01

from the porcupines was about 20 percent lower than the maximum differences between the portal and carotid blood (P-C) of sheep (Schambye and Phillipson, 1949; and Schambye, 1951). The maximum (V-A) values obtained from the porcupines, however, were greater than the maximum (P-C) values determined from sheep. The data show that there is active absorption of volatile fatty acids from the caecum and the quantity per volume of blood may be at least as great as that found in the rumen fermentation.

The amounts of lactic acid found in the ingesta of 16 field animals are shown in Table XI. In general the stomach content contained the greatest amounts, but even they were low. The average milliequivalent amounts of lactic acid were about half the average of the volatile fatty acids in the stomach content, and in the caecal content about one twenty-fifth. The generally lower concentrations of lactic acid in the caecal

Table XI. Lactic acid in porcupine ingesta.

Animal	Stomach	Caecum	Large Intestine		
			LI_1	LI_2	LI_3
P_1	0.016*	0.042	0.034	0.026	0.023
P_2	0.046	0.027	0.058	0.038	0.029
P_3	0.101	0.019	0.040	0.050	0.022
P_4	0.022	0.015	0.028	0.024	0.031
P_5	0.052	0.021	0.043	0.026	0.023
P_9	0.082	0.010	0.032	0.054	0.025
P_{10}	0.012	0.002	0.030	0.055	0.002
P_{14}	0.060	0.009	0.017	0.021	0.007
P_{15}	0.031	0.006	0.018	0.019	0.006
P_{16}	0.017	0.006	0.020	0.020	0.010
P_{17}	0.075	0.012	0.017	0.017	0.012
P_{18}	0.147	0.006	0.013	0.018	0.008
P_{19}	0.017	0.006	0.021	0.021	0.017
P_{20}	0.048	0.010	0.025	0.036	-
P_{22}	-	0.008	0.005	0.004	0.005
P_{23}	0.020	0.003	0.014	0.012	0.006
Average	0.050	0.013	0.026	0.028	0.015

* meq per gram dry weight

content could reflect ingestion and absorption of lactic acid with little in vivo production, or ingestion plus in vivo production of lactic acid coupled with a rapid conversion of it to other compounds. The often higher concentrations of lactic acid in the large intestine indicate that lactic acid is produced more rapidly than it is converted to other compounds, or, if lactic acid is not converted to other compounds, production is greater than absorption. There is no evidence that lactic acid absorption is a significant source of energy to the animal. This was confirmed in two experiments in which acid was determined during in vitro incubation (Table XII). The data indicate that if lactic acid is produced, it is converted to other compounds as fast as it is formed.

Table XII. Lactic acid in caecal content during in vitro incubation.

Animal	Incubation Time				
	< 1/2 hr	1 hr	2 hr	3 hr	4 hr
P_{22}	0.008*	0.007	0.005	0.004	0.005
P_{23}	0.003	0.003	0.003	0.002	0.002

* meq per gram dry weight

The amount of succinic acid was determined on the stomach content of one animal and on the caecal content of two. The stomach content of P_{26} contained 0.0054 meq of succinic acid per gram dry weight of ingesta. The caecal ingesta of P_{25} and P_{26} contained 0.0036 and 0.0032 meq per gram dry weight respectively. During in vitro incubation of caecal ingesta from animal P_{26}, the concentration of succinic dropped from 0.0032 to 0.0021 meq per gram dry weight in one hour; however, after 4 hours incubation the succinic acid had risen to 0.0095 meq per gram dry weight of ingesta. This preliminary investigation indicates that the concentration of succinic acid is very low in the ingesta. The amounts of acid found with in vitro incubation of caecal content is not indicative as to whether it is converted to other compounds or absorbed during in vivo conditions. Rumen studies have shown that succinic acid produced by the bacteria is converted to propionic acid. This also may be the situation in the caecum.

Fermentation gases, produced during the in vitro fermentation of caecal content, were collected from 3 field animals. Carbon dioxide and methane were the only fermentation gases detected by gas-solid chromatography. Methane was found in the gases from P_{35} only in trace amounts; however, in animals P_{36} and P_{37} the CO_2:CH_4 ratios were 2.2:1 and 1.5:1

respectively. The porcupines used in the experiments were in captivity approximately 24 hours prior to the experiment and, as a result, the rate of fermentation was probably low. The substrates for methanogenesis were not determined.

C. In Vitro Reactions of Caecal Content

The importance of lactic and succinic acids as intermediates in the metabolism of the caecal microflora was determined by incubating them in vitro with caecal content.

The rates at which lactic acid is converted were determined in the caecal ingesta from 2 laboratory and 4 field animals. The amounts of acid remaining after intervals of in vitro incubation are shown in Table XIII. The data indicate that there is dissimilation but do not allow one to estimate the rate when limited by the concentration of substrate.

Table XIII. Lactic acid dissimilation in caecal content.

Animal	Incubation Time			
	0	1/2 hr	1 hr	1 1/2 hr
2/8	3.3*	2.4	0.3	0.3
10/15	9.8	9.4	8.2	7.2
P_{30}	11.4	9.6	9.4	9.4
P_{31}	10.6	10.6	10.4	10.6
P_{33}	11.0	10.8	10.0	9.6
P_{34}	12.4	10.6	10.6	10.6

* mg lactic acid per ml reaction mixture

The dissimilation rates for succinic acid were determined in the caecal content of 2 field animals. The results are shown in Table XIV. The data indicate that there is some dissimilation; however, the results are not consistent and it may be that there is not an active succinate-fermenting flora in the caecum.

Table XIV. Succinic acid dissimilation by porcupine caecal content.

Animal	Incubation Time			
	0	1/2 hr	1 hr	1 1/2 hr
P_{30}	0.79*	0.79	0.68	0.71
P_{31}	1.10	0.75	0.85	0.78

* mg succinic acid per ml of reaction mixture

D. Nitrogen and Carbon Sources for Caecal Bacteria

A preliminary study was conducted to determine what nitrogen and carbon sources are available for bacteria growing in the caecum. Unfortunately this was done after most of the culture work was completed and the knowledge gained could not be applied to this investigation.

The caecal content has a high urease activity as evidenced by Figure 3; however, the blood urea nitrogen does not decrease when the blood passes through the capillary bed of the caecum, (Table XV). The apparent increase of urea nitrogen in the venous blood of the caecum indicates that urea is being absorbed from the caecum, a phenomenon which cannot be explained with the present data.

Material from the caecum and the distal end of the small intestine was analyzed for ammonia. The average level of ammonia nitrogen in the

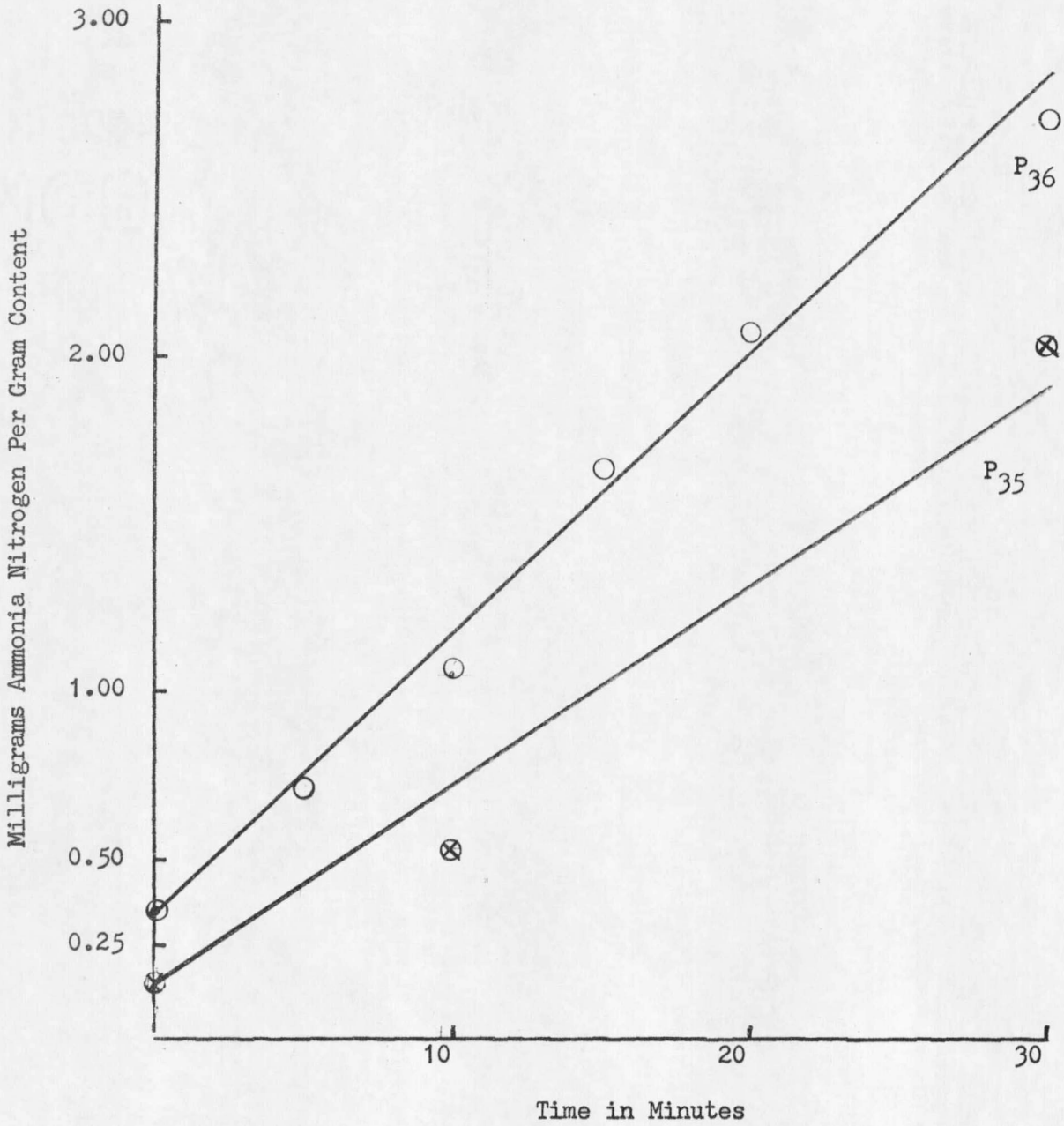

Figure 3. Urease activity in caecal content.

Table XV. Blood urea nitrogen.

Animal	Arterial	Venous
P_3	11.7*	12.5
P_4	7.6	8.0
P_5	5.1	10.9
P_9	10.5	10.8
P_{10}	2.9	3.0
P_{14}	5.8	7.3
P_{16}	3.3	2.6
P_{17}	6.8	7.8
P_{18}	13.8	14.2
P_{19}	8.5	8.2
P_{20}	7.9	9.8

* mg per 100 ml blood

caecal content of 8 porcupines (Table XVI) was as great as or greater than the amount of ammonia nitrogen added to culture media used in growing the caecal bacteria and many of the predominant rumen bacteria.

Table XVI. Ammonia nitrogen in caecal content.

Animal	Ammonia Nitrogen
P_{30}	0.092*
P_{31}	0.004
P_{32}	0.22
P_{33}	0.15
P_{34}	0.16
P_{35}	0.072
P_{36}	0.17
P_{37}	0.25
Average	0.14

* mg NH_3 nitrogen per gram content, wet weight

Ammonia concentrations in the ingesta of the distal end of the small intestine and caecum were compared to determine whether it was formed prior to or after the ingesta entered the caecum. The ingesta of the

small intestine of P_{35} contained 0.083 mg ammonia nitrogen per gram wet weight and the caecal ingesta 0.072 mg. Animal P_{36} contained 0.10 and 0.17 mg ammonia nitrogen per gram wet weight ingesta in the small intestine and caecum respectively. The results show that there is free ammonia in the small intestinal ingesta but demonstration of the production of it in the caecal ingesta was inconclusive.

An estimation of the importance that free amino acids may have in the nutrition of the caecal bacteria was made by analyzing the supernatant samples of the ingesta from the distal end of the small intestine and the caecum of animals P_{35} and P_{36}. Using two dimensional thin layer chromatography amino acid spots were detected which appeared to be tyrosine, valine, lysine, phenylalanine, alanine, serine, glycine, and asparagine when compared with known amino acids. The amounts of the amino acids in the small intestinal and caecal ingesta were not determined quantitatively; however, the spots obtained from 5 μl of sample (equivalent to a 10 fold concentration of the amounts found in the ingesta) were lighter than those obtained from 5 μl amounts of 16 μM/ml standards. It is probable that amino acids do not exist in the ingesta in substrate amounts, but they may be important as bacterial growth factors.

Soluble sugars and sugar acids were qualitatively sought in the supernatant fraction of the small intestinal and caecal content. No sugars or sugar acids were detected when 20 μl amounts of samples, concentrated 10 fold, were placed on the chromatograms. Well defined spots were obtained when 50 μg amounts of the sugar standards were placed on the paper chromatograms. Sugars detected following the acid hydrolysis

of the particulate fraction of the small intestinal content included arabinose, xylose, glucose and galactose.

E. Caecal Bacteria

Microscopic counts of the bacteria in the porcupine caecal content show that it supports large populations of bacteria,(Table XVII). The purposes of the culturing experiments were to: (1) determine fluctuations in the total numbers of viable bacteria between animals and in the same animal; (2) identify some of the predominant groups of bacteria present in the bacterial populations of the caecum; and (3) determine changes in the composition of predominant groups of caecal bacteria with respect to time of day and animal.

Table XVII. Total microscopic counts of porcupine caecal contents.

Animal	Bacteria per gram $X\ 10^8$
P_1	130
P_2	100
P_3	169
P_4	134
P_5	158
P_9	120
P_{10}	299
P_{14}	142
P_{15}	172
P_{16}	138
P_{17}	127
P_{18}	217
P_{19}	75
P_{20}	71

Roll tube media containing porcupine caecal fluid, bovine rumen fluid or elk rumen fluid as enrichment sources (Bryant and Robinson, 1961b) and

a semi-synthetic medium (Bryant and Robinson, 1961a) were compared for their ability to grow caecal bacteria. The total numbers of culturable bacteria per gram of fresh caecal content, obtained in two experiments (Table XVIII), show that the caecal and rumen enrichments are of about equal quality for growing the caecal bacteria, but that the semi-synthetic medium does not appear to grow as many of the bacteria. On the basis of these experiments, and because it was the most readily available, bovine rumen fluid was used in the media for most of the culture studies.

Table XVIII. Total numbers of culturable bacteria comparing media and media enrichments.

Enrichment	Experiment 1 Bacteria per Gram*	Experiment 2 Bacteria per Gram*
Porcupine caecal fluid	6.1×10^9	1.5×10^{10}
Bovine rumen fluid	7.4×10^9	1.4×10^{10}
Elk rumen fluid	6.4×10^9	0.96×10^{10}
Synthetic medium	1.4×10^9	0.74×10^{10}

* average of 5 roll tubes

The effects of different concentrations of a medium enrichment were determined for porcupine caecal fluid. Media were prepared containing 10, 20, 30 and 40 percent caecal fluid and inoculated with diluted caecal content. The results (Table XIX) show that there was little difference in the numbers of bacteria grown at the different levels of caecal fluid, although the 10 percent level was found to be slightly inferior to the

Table XIX. Effect of culture media containing different concentrations of porcupine caecal fluid enrichment on the numbers of culturable bacteria.

Percent Caecal Fluid	Bacteria Per Gram Contents
10	1.1×10^9
20	1.5×10^9
30	1.4×10^9
40	1.4×10^9

others. A microscopic count of the inoculum showed it to contain 11×10^9 bacteria per gram. The viable bacteria count of 1.4×10^9 bacteria per gram indicates that about 13 percent of the bacteria present in the caecum grew.

Diurnal changes in the numbers of bacteria in the caecal ingesta and in the rate of caecal fermentation would influence the nutritional importance of the caecum. An experiment was designed to measure fluctuations in the number of culturable bacteria, the moisture content of the ingesta and the pH of the caecal material at approximately 4-hour intervals during a 24-hour period. A cannulated laboratory animal, having free access to feed and water at all times, was used in the experiment. The moisture content of the ingesta was relatively constant during the experiment, (Table XX) indicating that there were no large additions of only liquids or solids to the caecum. There were no abrupt changes in the caecal fermentation rate as indicated by the gradual pH changes. The numbers of culturable bacteria fluctuated by a factor of 2 or 3 and did not

correlate with the pH or the moisture content of the ingesta. It should be mentioned that removing samples from the caecum every 4 hours resulted in a very frightened porcupine. This may have affected its food consumption or caused other effects during the course of the experiment.

Table XX. Changes in the viable bacteria count, moisture content and pH of the caecal material in a cannulated porcupine with respect to time of day.

Time	Bacteria* Per Gram	Percent Moisture	pH
1:30 AM	7.8×10^9	85.4	-
6:00 AM	3.6×10^9	83.6	6.49
10:00 AM	11×10^9	84.0	6.42
2:00 PM	13×10^9	86.7	6.65
7:00 PM	4.6×10^9	89.6	6.61
10:00 PM	11×10^9	84.6	6.72

* average counts using 10 roll tubes for each

Therefore another experiment which involved removing only one sample from the animal per day and designed for measuring fluctuations in the bacterial populations of the caecal content was performed. Samples of ingesta were collected at different times of day, on different days, and the order of sample collection was randomized. The results show only about a 30 percent fluctuation in the numbers of culturable bacteria, (Table XXI). The two 8:00 A.M. samples, taken on different days, show a 23 percent variation in the number of culturable bacteria. The results indicate no diurnal patterns in the numbers of anaerobic bacteria in the caecum, at least under laboratory conditions.

Table XXI. Viable bacteria counts at different times of day.

Time	Bacteria* Per Gram
4:00 AM	1.9×10^{10}
8:00 AM	1.7×10^{10}
	2.2×10^{10}**
12:00 PM	1.5×10^{10}
	1.6×10^{10}**
4:00 PM	1.9×10^{10}
8:00 PM	2.1×10^{10}
10:00 PM	1.7×10^{10}
12:00 AM	1.9×10^{10}

* counts obtained from average of 5 roll tubes
**duplicate experiments run on different days

Total viable bacteria counts were made on the ingesta of 5 field porcupines. The results show that the total number of culturable bacteria per gram of caecal content are about the same in the field as in the laboratory animals (Table XXII).

Table XXII. Viable bacteria counts on caecal content from field animals.

Animal	Bacteria* Per Gram
P_{30}	8.2×10^{9}
P_{31}	1.4×10^{10}
P_{32}	1.3×10^{10}
P_{33}	7.8×10^{9}
P_{34}	1.2×10^{10}

* counts obtained from average of 10 roll tubes

The kinds of bacteria found in the porcupine caecum and an estimation of their relative numbers were determined by examining cultures derived from 600 colonies picked from cultures made on 10 different caecal samples. Isolated colonies from the 0.5 and 1.0 x 10^{-8} dilution roll tubes were picked into slants of the same medium. Each slant culture was designated a strain and given a strain code number. The slant cultures were used to inoculate the 12 differential media used to determine physiological characteristics. The source of the inoculum and the time and date on which it was collected is listed for each group of bacterial strains isolated from laboratory and field animals (Table XXIII).

Table XXIII. Source of inoculum, time and date of inoculation and the bacterial strains isolated.

Sample Code	Inoculum Animal	Inoculum Date	Inoculum Time	Strains Isolated
A	laboratory	2/12/63	-	P_{4001} through P_{4056}
B	laboratory	2/25/63	-	P_{4057} through P_{4181}
C	laboratory	6/ 8/63	12:00 PM	P_{4222} through P_{4271}
D	laboratory	6/11/63	8:00 PM	P_{4272} through P_{4341}
E	laboratory	6/13/63	4:00 AM	P_{4342} through P_{4421}
F	field P_{30}	10/15/63	-	P_{5001} through P_{5025}
G	field P_{31}	10/16/63	-	P_{6001} through P_{6050}
H	field P_{32}	10/16/63	-	P_{7001} through P_{7050}
I	field P_{33}	10/29/63	-	P_{8001} through P_{8050}
J	field P_{34}	10/29/63	-	P_{9001} through P_{9050}

Inocula from many of the colonies did not grow when picked into the slant tubes and some cultures were contaminated as shown by microscopic examination. As a result a total of 328 strains (217 from the laboratory animals and 111 from the field porcupines) were used in the study. The bacteria were grouped on the basis of cell shape and size, motility, gram reaction and physiological characteristics. The physiological characteristics were at times difficult to determine. The results obtained in the xylan, pH glucose and gelatin media were valid as far as positive reactions; however, there may have been false negative reactions. The utilization of xylose, cellobiose and starch was recorded as positive if there was growth in the carbohydrate containing medium and not in the fermentation blank medium. Acid production was determined by adding a drop of brom thymol blue indicator at the end of a 1-week incubation period. The tubes showing growth gave only a weak reaction if any at all. Cellulose digestion was measured on a 4+ visual scale, 4+ being complete utilization with 1+ resulting only in discoloring and clumping of the cellulose suspension. A survey of the numbers of bacterial strains found in the major morphological and physiological groups is given in Table XXIV. A considerably higher percentage of cocci was isolated from the field than from the laboratory animals, and the percentage of straight and curved rods was lower in the field animals. The cellulose-digesting strains were rated only 1+ and 2+, and because of this there may have been a considerable number of strains recorded falsely as being positive. The number of cellulose-utilizing bacteria was particularly low in the field animals which indicates that cellulose digestion may not be of major importance

Table XXIV. Morphology and physiological characteristics of caecal bacteria isolated from laboratory and field animals.

Morphology or Characteristic	Laboratory Animals		Field Animals	
	Number	Percent*	Number	Percent*
cocci	58	27	44	40
curved rods	75	34	35	32
straight rods	84	39	32	29
cellulose digesters 1+	36		2	
2+	14		5	
Total	50	23	7	6.3
xylan digesters	10	5	13	12
starch utilizers	15	6.9	11	10
gelatin liquefiers	41	19	6	5.4
gas producing	50	23	4	3.6

* a total of 217 laboratory animal strains and 111 field animal strains

in the caecum or that the medium was capable of growing only a small number of the predominant cellulose digesting bacteria. Xylan utilizing bacteria constituted 5 and 12 percent of the strains isolated from the laboratory and field animals, respectively. The percentages of starch utilizing strains in the laboratory and field animals were 6.9 and 10 percent, respectively. These two observations may indicate that starch and xylan are of more importance to the wild than to the laboratory animals. The high percentage of gelatin-liquefying strains isolated from the laboratory animals may have been due to the leakage of body fluids into the caecum around the cannula since this zone was filled with fibrin and leucocytes. This possibility is supported by the fact that 21 of the

gelatin-hydrolyzing strains were in the P_{4001} to P_{4056} series obtained from a single caecal sample. The same 21 strains account for about half of the gas-producing cultures isolated from the laboratory animals. The low number of gas-producing strains isolated from the field animals indicate that the normal caecal flora of the porcupine may produce little fermentation gas.

Placing the isolated cultures into groups was difficult because of their extreme heterogeneity. The 328 strains used in the study were separated into 43 groups, many of which were quite heterogeneous. Part of the heterogeneity may have been due to the generally poor growth on all of the differential media, which may have resulted in many false negative results. The groups were formed on the basis of cell shape and size and on the basis of the physiological reactions.

The cocci were placed into 11 groups (Table XXV). The diameters of the cells were recorded as small 0.2-0.3μ, medium 0.4-0.6μ and large >0.6μ.

The straight rod-shaped bacteria were the most heterogeneous, being separated into 24 groups (Table XXVI). The width of the cells were recorded as narrow <0.5μ, medium 0.5-0.8μ or wide >0.8μ. The lengths were recorded as short < 4μ, medium 4-6μ and long >6μ.

The curved rod-shaped bacteria were placed into 9 groups (Table XXVII). The size relations were the same as those used for the straight rod-shaped bacteria.

The cultures in each group were generally isolated from several caecal samples (Tables XXVIII, XXIX and XXX). Occasionally, however, the

strains in a group were isolated predominantly from a single sample of caecal content, for example, groups 3 and 7 of the cocci and group 3 from the curved rods. Such results probably reflect an undefined abnormal situation in the animal.

The groups of caecal bacteria were difficult to identify with the predominant groups of rumen bacteria (Bryant, 1959, 1963). Group 2 of the cocci appeared to be similar to Peptostreptococcus elsdenii. The majority of the straight rod-shaped bacteria were morphologically similar to Bacteroides sp., but could not be physiologically identified with described species found in the rumen. Group 3 of the curved rods was similar to Butyrivibrio fibrisolvens.

Table XXV. Groups of the cocci.

Group Code	No. Strains	Morphology	M	GR	pH Glucose G	pH Glucose pH	Gas	X	C	S	X'	C'	No R	No CO_2	H_2S	OR	Gel
1	5	Large, s and pr	-	+	T	5.9-6.2	-	-	-	-	-	-	±	±	-	a	-
2	5	Large, pr	-	±	T	5.4-6.3	4+	-	-	-	-	-	-	-	-	a	-
3	48	Average, pr and ch	-	-	T	6.0-6.4	-	-	-	-	-	∓	-	-	-	a	-
4	11	Average, s pr and ch	-	±	∓T	6.6	-	-	-	-	-	-	-	∓	-	a	-
5	6	Average, pr and ch	-	±	T	4.0-6.0	-	-	±	±	-	∓	±	±	-	fa	-
6	4	Average, s to ch	-	-	T	4.3-5.0	±	∓	∓	-	-	-	-	-	-	a	-
7	14	Small, mostly s, pr and ch	-	-	T	4.9-5.2	-	-	-	-	-	-	+	+	-	a	-
8	6	Small, s pr and ch	-	∓	-	-	-	-	∓	-	-	-	-	-	-	a	-
9	1	Small, s pr and ch	-	+	T	4.0	-	-	+	+	-	+	+	+	-	fa	-
10	1	Very small, pr and ch	-	-	T	4.6	4+	-	-	-	-	-	-	-	-	a	-
11	1	Very small, s pr and gr	-	-	T	6.4	-	+	-	-	-	-	+	+	-	a	+

s, pr, ch, gr = singles, pairs, chains, groups
M, GR = motility, gram reaction
G, pH = type of growth, final pH of glucose medium
gas = gas production from glucose (scale to 4+)
X, C, S = xylose, cellobiose, starch
Gel = gelatin hydrolysis
±, ∓ = more positive, more negative
a, fa = anaerobic, facultatively anaerobic

X', C' = xylan, cellulose
No R = medium containing no rumen fluid
No CO_2 = medium containing no CO_2
H_2S = hydrogen sulfide
OR = oxygen relationships
+, - = positive reaction or growth, negative reaction or no growth

Table XXVI. Groups of straight rods.

Group Code	No. Strains	Morphology	M	GR	pH Glucose G	pH Glucose pH	Gas	X	C	S	X'	C'	No R	No CO_2	H_2S	OR	Gel
1	26	*Coccoid to long, wide, s	-	∓	T	4.7-5.2	∓	+	+	±	±	∓	+	+	±	a	∓
2	1	Coccoid to long, wide, ch	+	+	-	-	-	-	-	-	-	-	+	+	-	a	-
3	1	Short to long, wide, s pr ch	-	-	T	6.2	-	-	+	-	-	-	+	+	-	a	-
4	2	Short to medium, wide, s ch	-	-	T	5.4-5.6	-	-	-	-	-	-	-	-	-	a	-
5	2	Short to medium, wide, s, oval or elliptical shaped	-	-	T	5.8-6.3	-	-	±	-	-	-	+	+	-	a	-
6	8	Short to medium, wide, s ch	-	-	-	-	-	∓	∓	-	-	-	∓	∓	-	a	∓
7	4	Medium to long, wide, s ch	-	-	-	-	-	-	-	-	-	-	-	-	-	a	-
8	23	Short to medium, medium, s ch	±	-	-	-	-	∓	∓	-	-	-	±	±	-	a	-
9	11	Short to medium, medium, s pr ch	-	-	-	-	-	-	-	-	-	-	∓	∓	-	a	-
10	2	Short, medium, s	+	-	T	5.3	4+	±	+	+	±	-	+	+	-	a	±
11	1	Coccoid to short, medium, s	+	-	T	4.4	-	-	-	-	-	-	+	+	-	a	-
12	2	Short to medium, medium, s	±	-	-	-	-	-	-	-	-	-	±	±	+	a	-
13	1	Short to medium, medium, ch	-	+	-	-	-	-	-	-	+	-	-	-	+	a	-
14	2	Coccoid to short, medium, s pr ch	-	-	T	6.4	-	+	+	-	-	-	-	-	-	a	+
15	1	Medium, medium, ch	-	-	F	5.3	-	+	+	-	-	+	-	-	-	a	-

Table XXVI. Cont.

Group Code	No. Strains	Morphology	M	GR	pH Glucose G	pH Glucose pH	Gas	X	C	S	X'	C'	No R	No CO_2	H_2S	OR	Gel
16	3	Medium, medium, s	-	-	T	5.3-5.7	-	-	±	±	-	-	±	±	∓	a	±
17	4	Medium, medium, s	-	+	T	3.8-4.0	-	-	-	-	-	-	-	-	-	a	-
18	1	Medium, medium, s	+	-	T	5.4	-	-	+	-	-	-	-	-	-	a	-
19	4	Long, medium, s	-	-	-	-	-	-	-	-	∓	-	-	-	-	a	-
20	5	Long, medium, s ch	-	-	T	6.0-6.6	-	±	±	-	-	-	-	-	+	a	∓
21	5	Long, medium, s	+	-	-	-	-	-	+	+	-	-	±	+	-	a	-
22	2	Long, medium, s	-	+	T	6.0-6.4	-	±	±	±	-	±	±	+	±	a	-
23	5	Long, medium, ch	±	-	-	-	-	-	-	-	-	-	+	+	-	a	-

s, pr, ch = singles, pairs, chains
M, GR = motility, gram reaction
G, pH = type of growth, final pH of glucose medium
T, F = turbid growth, flocculent growth
gas = gas production from glucose (scale to 4+)
X, C, S = xylose, cellobiose, starch
X', C' = xylan, cellulose
Gel = gelatin hydrolysis
No R = medium containing no rumen fluid
No CO_2 = medium containing no CO_2
H_2S = hydrogen sulfide production
OR = oxygen relationships

* length and width (e.g. group 1 = coccoid to long length and wide width, and group 15 = medium length and medium width)
+ = positive reaction or growth
- = negative reaction or no growth
± = more positive than negative
∓ = more negative than positive
a = anaerobic

Table XXVII. Groups of curved rods.

Group Code	No. Strains	Morphology	M	GR	pH Glucose G	pH Glucose pH	Gas	X	C	S	X'	C'	No R	No CO_2	H_2S	OR	Gel
1	7	Small, s	+	-	-	-	-	-	-	-	-	-	-	-	-	a	-
2	20	*Medium, medium, s, few ch	±	-	T	6.2-6.6	-	∓	∓	-	∓	-	±	∓	-	a	-
3	25	Medium, medium, s, few pr	+	-	T	4.9-5.3	4+	-	±	-	-	±	+	+	-	a	+
4	11	Medium, medium, s	+	-	T	5.6-6.2	-	∓	±	-	∓	∓	±	+	-	a	-
5	2	Long, medium, s pr	±	-	T	5.3-5.5	-	-	+	±	-	-	±	+	-	a	-
6	3	Medium, medium, s	-	±	-	-	-	∓	∓	∓	-	-	+	+	-	a	-
7	13	Medium to long, medium, ch	+	-	-	-	-	∓	±	∓	-	-	±	±	-	a	-
8	22	Medium to long, medium, s ch	∓	-	-	-	-	-	-	-	-	-	-	-	-	a	-
9	7	Long, medium, s ch	±	-	T	4.5-5.2	-	-	∓	∓	-	-	+	+	-	a	-

s, pr, ch = singles, pairs, chains
M, GR = motility, gram reaction
G, pH = type of growth, final pH of glucose medium
T = turbid growth
gas = gas production from glucose (scale to 4+)
X, C, S = xylose, cellobiose, starch
X', C' = xylan, cellulose
Gel = gelatin hydrolysis
No R = medium containing no rumen fluid
No CO_2 = medium containing no CO_2
H_2S = hydrogen sulfide production
OR = oxygen relationships

* = length and width
+ = positive reaction or growth
- = negative reaction or growth
± = more positive than negative
∓ = more negative than positive
a = anaerobic

Table XXVIII. Distribution of strains in the groups of cocci among the isolation series.

Group Code	No. Strains	A	B	C	D	E	F	G	H	I	J
1	5			1			1	1	1	1	
2	5	2	1							2	
3	48		38	2	1	2		2		1	2
4	11				1			3	5		2
5	6			3					1	1	1
6	4	1	1	1		1					
7	14								12	2	
8	6							4			2
9	1			1							
10	1	1									

Table XXIX. Distribution of strains in the groups of straight rods among the isolation series.

Group Code	No. Strains	A	B	C	D	E	F	G	H	I	J
1	26	4	8	4	3	2	1	1		2	1
2	1						1				
3	1						1				
4	2		2								
5	2				1				1		
6	8				6				1		1
7	4		1			1	1	1			
8	23		2	7	3	1	5		1	1	3
9	11		1	2	3		2	1	1		1
10	2				1	1					
11	1					1					
12	2				1	1					
13	1								1		
14	2				1			1			
15	1					1					
16	3		1		1	1					
17	4		3		1						
18	1	1									
19	4	2		1						1	
20	5			2		1	2				
21	5			1	2	2					

Table XXIX. Cont.

Group Code	No. Strains	A	B	C	D	E	F	G	H	I	J
22	2			1	1						
23	5	3		1		1					

Table XXX. Distribution of strains in the groups of curved rods among the isolation series.

Group Code	No. Strains	A	B	C	D	E	F	G	H	I	J
1	7						6	1			
2	20		3	2	2	4		5	1	2	1
3	25	21	1		3						
4	11			1				5	1	2	2
5	2					2					
6	3	1				2					
7	13			1	3	4		1		4	
8	22	2	9	2	5	1			1	2	
9	7	1	1	1		3			1		

DISCUSSION

Although the anatomy of the digestive tract is variable in different herbivorous animals, the principal fermentation products produced by the ruminal, caecal and colonal fermentations are similar in kinds and ratios (Elsden et al., 1946; and Hungate et al., 1959). The nutritional importance of the rumen fermentation has been established for the bovine (Carroll and Hungate, 1954; Hungate et al., 1961; and Stewart, 1958) and the deer (Short, 1963). One of the primary objectives of this investigation was to determine the contribution of the caecal fermentation to the nutrition of the porcupine. It was found that the volatile fatty acids in the caecal ingesta consisted of 73 percent acetic, 12 percent propionic and 13 percent butyric. These ratios are similar to those found in the bovine rumen when the animal is on a high roughage diet. This is not surprising when one considers that the substrates available to the caecal bacteria have been predigested by the animal, leaving only the equivalent of roughage. Thus most of the readily digestible carbohydrates and proteins have been removed from the food prior to fermentation by the caecal flora. Rumen studies have shown that high levels of proteins in the feed increase the butyric acid ratio to those of propionic and acetic, whereas high levels of readily fermentable carbohydrates increase the concentrations of propionic acid (Balch and Rowland, 1957; and Eusebio et al., 1959). The generally low levels of propionic and butyric acids in the caecal ingesta indicate that the amounts of protein and carbohydrates readily fermentable in the caecum are low. The removal of free sugars and sugar acids from the ingesta prior to their entering the caecum has been substantiated in the present investigation by the use of paper chromatography. Soluble

sugars and sugar acids were not detectable in the ingesta from the caecum and from the distal end of the small intestine.

The rates of fermentation were determined in the caecal content from 6 field animals. The data show that keeping an animal without feed for 12 hours before experimentation results in a lower fermentation rate. This means that if a maximum fermentation rate is to be maintained in the caecum, the porcupine will have to eat at less than 12 hour intervals. Porcupines, being nocturnal, probably eat during much of the night; this could not be determined because few animals were caught later than 10:00 P.M. It would have been interesting to have measured the fermentation rates in the caecal ingesta of animals caught during the day, but the difficulty of finding animals during daylight made it infeasible.

The average production rates of the volatile fatty acids in the caecal ingesta of the six animals were acetic 0.110, propionic 0.016 and butyric 0.014 meq per gram dry weight of ingesta per hour (Table VIII). It may be argued that the rates are high because the experiments on 4 of the animals were run within an hour or two of capture and the experiments run on only 2 animals 12 hours after capture. If one assumes that the porcupine is going to eat during most of the night, a low fermentation rate may exist for only about 8 hours out of the 24 and the above fermentation rate averages would then be in line. After knowing the fermentation rate per unit amount of caecal content, one can calculate the total production per unit time if the total amount of ingesta in the caecum is known. The total caecum weight was determined on 20 porcupines and the empty caecal weight on 6 (Table III). The average percent total animal weight consisting

of caecal ingesta was 4.4 percent. Therefore, on an average a 10 Kg porcupine would contain about 440 g of caecal content. The same animal would contain about 66 g of ingesta by dry weight since the caecal content contains an average of 15 percent dry matter (Table IV).

The average amounts of volatile fatty acids produced in the caecal ingesta during a 24 hour period would be 175 meq acetic, 25.3 meq propionic and 22.2 meq butyric. One hundred and forty-nine meq of acetic, 21.0 meq of propionic and 17.4 meq of butyric acids would be absorbed from the caecum per day because for a 10 Kg animal approximately 100 g of ingesta leaves the caecum during this period of time, taking with it approximately 35.9 meq of volatile fatty acids consisting of 26 meq of acetic, 4.3 meq of propionic and 4.8 meq of butyric (Figure 1, Table VII). Of the total acids leaving the caecum approximately 54 percent of them are absorbed (Figure 1) and from the percentages of the individual acids present in the ingesta of LI_3 (Table VII), it was calculated that 15 meq acetic, 1.8 meq propionic and 2.2 meq butyric were absorbed from the large intestine per 24 hours. The total amounts of acids absorbed by the animal consisted of 164 meq acetic, 22.8 meq propionic and 19.6 meq butyric.

From the heats of combustion, it was calculated that the absorbed acids would be equivalent to 53 Kcal of energy. If one makes the same assumption as Carroll and Hungate (1954), that the maintenance energy requirement is proportional to the three-fourths power of the body weight, as Kleiber (1947) has shown for the metabolic rate of animals from the size of the mouse to that of an elephant and by using Carroll and Hungate's maintenance energy requirement estimation of 7,850 Kcal for a

500 Kg bovine, the maintenance energy requirement of a 10 Kg porcupine would be 435 Kcal per day. The products utilized from the caecal fermentation would thus provide about 12 percent of the maintenance energy requirement. The same type of calculation using the maximum fermentation rates and acid concentrations in the caecal content, animal P_{26} (Table VIII), and the average values for absorption from the large intestine (Table VII), the animal would obtain 96 Kcal of energy from the caecal fermentation. This would equal about 22 percent of the porcupine's maintenance energy requirement. Calculations using the minimum fermentation rate values, P_{25}, show that about 5.5 percent of the maintenance energy requirement is provided by the caecal fermentation products. The maintenance energy requirement per unit of body weight is high for the porcupine because of its small size. The maintenance energy requirements per Kg body weight per day for a 10 Kg porcupine would be 43.5 Kcal, and for a 5 Kg porcupine 52.5 Kcal as compared to 15.7 Kcal for a 500 Kg bovine.

The caecal fermentation products appear to be functionally important only in large porcupines and then only when the fermentation is proceeding at an average or greater than average rate. The caecal fermentation may be of considerable functional importance under starvation conditions during periods of poorest diet. Relative to other rodents, survival pressures appear to be limited on the porcupine since they are able to maintain their populations by having only one young per year. There probably has not been a very great evolutionary selection for animals having large caeca.

Hungate et al. (1957) have suggested that small ruminant animals may obtain higher fermentation rates in the rumen by increased food consumption. This would cause an increased turnover rate and the rumen flora would be maintained in a continuous logarithmic growth phase, thus producing greater amounts of fermentation products.

Studies on the individual volatile acids of the rumen have indicated that the absorption rates of different acids are not the same and that the concentrations of the individual acids may not depict the rates at which they are produced (Barcroft et al., 1944; Danielli et al., 1945; and Gray, 1947). If the rates of absorption are dependent not only upon concentration but also upon acid species, one would expect the ratios of the acids to change during the course of an in vitro fermentation. Measurements of the ratios of the individual acids at different times during in vitro fermentation rate experiments show little change in the ratios, indicating that the absorption rates of the acids are dependent primarily upon the concentration of the acids in the ingesta. This was also substantiated by measuring the ratios of the acids in the ingesta in the 3 segments of the large intestine. The absorption again appears to be dependent only upon concentration; however, in this case a comparatively constant pH would mask any dependence of rate on pH.

About 84 percent of the volatile fatty acids produced in the caecum are absorbed into the blood directly from the caecum. Fifty-four percent of the volatile acids present in the ingesta which entered the large intestine were absorbed as the content passed along the intestine (Figure 1). Therefore, of the volatile acids absorbed by the animal, about 90

percent were absorbed from the caecum and 10 percent from the large intestine. The levels of the volatile acids in the venous and the arterial blood of the caecum also indicated a high rate of absorption from the caecum.

In summary, the nutritional value of the caecal fermentation appears to be of less importance to the porcupine than the rumen fermentation is to the ruminant. However, the caecum must be important to the rodent because presumably it would not carry around an organ consisting of as much as 10 percent of the animals' total weight if it were not.

Low levels of lactic and succinic acid were found in the caecal ingesta of all of the porcupines; this is also characteristic of rumen ingesta. In vitro incubation of caecal ingesta show little change in the concentrations of these acids eliminating absorption as their fate if they were produced. In vitro incubation of caecal content with substrate amounts of either lactic or succinic acid showed some acid conversion to other compounds. The rates of dissimilation were low with high levels of substrate so when the amount of substrate is limiting, the rate of conversion may be too low to account for much of the volatile acid production in the caecum. The amount of data was too limited to allow any definite statements as to the importance of lactic and succinic acids as intermediates in the bacterial degradation of the caecal ingesta.

The ratios of the fermentation gases produced during the caecal fermentation are similar to the ratios obtained during in vitro fermentation of caecal ingesta from several African ruminants (Hungate et al., 1959).

Ammonia appears to be the most important source of nitrogen for the caecal bacteria (Table XVI). Several amino acids were also detected in the material entering the caecum, but an estimation of their concentrations suggested that their amounts were inadequate to serve as important nitrogen sources. Although there was high urease activity in the caecal content (Figure 3), the levels of blood urea nitrogen failed to drop when the blood passed through the capillary bed of the caecum. On the contrary, the levels of blood urea nitrogen were consistently higher in the venous blood of the caecum, indicating absorption of urea from the caecum. This is difficult to explain because of the presence of the high urease activity in the caecal ingesta. Contradictory results were obtained from the experiments to determine whether the ammonia found in the caecal content was produced only during the tryptic digestion in the small intestine or also by bacteria in the caecal ingesta.

Free sugars and sugar acids are not natural substrates of the caecal bacteria since these compounds could not be detected in the material entering the caecum or in the caecal contents. The sugars arabinose, xylose, galactose, and glucose were detected in the acid hydrolysate of the particulate fraction of the small intestine contents. Gluconic, galacturonic, glucuronic acids and gluconic lactone were not detected in the acid hydrolysate. The results show that the free sugars, sugar acids and starches are removed from the ingesta by the time they reach the caecum, leaving only polysaccharides, polyuronides, etc., not digestible by the animal as substrates for the caecal bacteria. This was in part substantiated by the fact that 12 percent of the bacterial strains

isolated from field animals were capable of utilizing xylan.

Total numbers of culturable bacteria in the caecal ingesta remained at a comparatively constant level at different times of day indicating that the fermentation was proceeding at a relatively constant rate throughout the day.

The experiments comparing media and media enrichments showed that media containing rumen fluid could grow as great a number of caecal bacteria as media containing caecal fluid enrichment. As a consequence, it was assumed that the environments of the rumen and caecum were similar. The assumption was found to be incorrect when bacteria from only 328 colonies out of nearly 600 which were picked from roll tubes into the slants grew, a fatality of approximately 40 percent. The marginal growth of a large number of the strains probably resulted in many false negative results in the differential media. In some cases false positive results may have been reported, particularly in regard to cellulose utilization where a large number of cultures were recorded as 1+. The growth reactions in xylose, cellobiose and starch media were recorded as positive if growth appeared in the test medium and not in the carbohydrate blank medium. In some cases actual fermentation of the carbohydrate may not have occurred.

Studies of the morphology and physiology of 328 strains have shown that there are many kinds of bacteria present in the caecum. Over 90 percent of the isolates gave a negative gram reaction. Thirty-one percent of the isolates were cocci, 34 percent curved rods and 35 percent straight rods. The normal caecal flora appears to be composed of primarily non-gas producing bacteria, as evidenced by the fact that only 3.6 percent of the

strains isolated from 5 field animals produced gas. This may be advantageous to a rodent since gas escape would be a greater problem from the caecum than from a rumen. Only 6.3 percent of the cultures isolated from field animals were cellulolytic. Whether this small percentage is real or just the reflection of inadequate culture media is not known. Morphologically many of the strains appeared similar to the bacteria found in the bovine rumen. Out of the 11 groups of cocci isolated from caecal content, only group 2 appeared physiologically similar to a common rumen bacterium, Peptostreptococcus elsdenii. Many of the straight rod-shaped bacteria were morphologically similar to species of Bacteriodes, but none of the 23 groups could be identified with described species. Group 3 of the curved rods appears to be similar to Butyrivibrio fibrisolvens (Bryant and Small, 1956); the other 8 groups were not identified. The results of the present investigation show that the bacterial populations of the caecum are very heterogeneous and in general the isolated strains do not fit into the taxonomic groups most commonly isolated from the rumen.

SUMMARY

1. The amounts of short chain volatile fatty acids acetic, propionic and butyric were measured in the ingesta from the caecum, stomach and three sections of the large intestine of the porcupine. The rates at which the acids were produced were determined in vitro on the caecal content from six field porcupines. The caloric values of the absorbed fatty acids were estimated to supply from 6 to 22 percent of the total maintenance energy requirement of the animal.

2. Lactic and succinic acids were found in low concentrations in the porcupine's ingesta. In vitro incubation of caecal content did not result in an increase of the acids, discounting absorption as a fate of either of these acids which may be produced. Incubation of fresh caecal content with substrate amounts of the acids resulted in dissimilation of both acids but at low rates. Lactic and succinic acids were believed not to be important intermediate products in the bacterial fermentation.

3. The fermentation gases were measured by gas-solid chromatography. Carbon dioxide and methane were detected in about 2:1 ratios during in vitro incubation of caecal content.

4. Naturally occurring carbon and nitrogen sources for the growth of caecal bacteria were sought in the ingesta from the caecum and the distal end of the small intestine. Soluble sugars and sugar acids were not detected in the soluble fraction of the ingesta from either organ. Acid hydrolysis of the particulate fraction of the ingesta from the small intestine yielded arabinose, xylose, galactose and glucose. The caecal content showed high urease activity, but the levels of urea in the arterial and the venous blood of the caecum indicated that there was no absorption

of urea into the caecum. The levels of ammonia in the caecal ingesta were high enough to account for ammonia as a nitrogen source for the bacteria. Several amino acids were detected in the ingesta from the distal end of the small intestine and the caecum but the amounts appeared to make them unimportant as sources of nitrogen; however, they may function as growth factors.

5. The caecal and ruminal environments are similar in many respects; both have low oxidation-reduction potentials suitable for the growth of anaerobic bacteria; both have a constant temperature of 38 to 40 C; and the ingesta from both organs contain about the same amount of moisture. The major secretions entering the caecum are those from the stomach, small intestine, liver and pancreas, whereas only large quantities of saliva enter the rumen. The caecal bacteria have access to the ingesta only after the porcupine has removed the readily digestible materials. All of the bacteria growing in the caecum must have been able to survive the acid conditions of the stomach and escaped the digestive enzymes of the stomach and small intestine.

6. Total numbers of culturable caecal bacteria were determined by the roll tube method. There did not appear to be any diurnal variations in the numbers of culturable bacteria in the caecal content of a cannulated laboratory porcupine. The numbers randomly fluctuated about 30 percent when samples were removed from the animal at different times of day on different days. Three hundred twenty-eight strains of caecal bacteria were isolated from cannulated laboratory porcupines and 5 field porcupines. The strains were placed into 43 groups based on morphological

and physiological characteristics. Many of the groups were heterogeneous and when homogeneous groups were obtained, the strains were usually isolated from the same inoculum sample. The groups appeared to be morphologically similar to those found in the rumen but showed many physiological differences.

7. It was concluded that the environment of the porcupine caecum is superficially similar to that of the rumen. The differences are not well characterized but they are reflected in the fact that the caecal flora is quite different from the rumen flora. The contribution of the caecal fermentation to the nutrition of the porcupine is limited because of the porcupine's high energy requirement per unit weight of animal.

APPENDIX

Preparation of Solutions and Culture Media

The solutions used for the preparation of various media were prepared in the following ways. Mineral salts solution 1 contained 0.6 percent K_2HPO_4. Mineral salts solution 2 contained the following percentages: KH_2PO_4, 0.6; $(NH_4)_2SO_4$, 1.2; NaCl, 1.2; $MgSO_4$, 0.12 and $CaCl_2$, 0.12. The stock resazurin solution was 0.1 percent. A 0.1 M phosphate buffer was prepared at pH 6.8. Glucose, Na_2CO_3 and L-cysteine•HCl were prepared in 10, 8 and 2.5 percent solutions respectively. The boiling flask containing each solution was heated in a boiling water bath and the air displaced by flushing the flask with CO_2. The flask was stoppered and autoclaved for 20 minutes at 18 lbs pressure. The solutions were dispensed into sterile rubber stoppered culture tubes under an atmosphere of CO_2. A 2.5 percent solution of L-cysteine•HCl was neutralized to pH 11 with NaOH, followed by the addition of 2.5 percent $Na_2S \cdot 9H_2O$. The solution was sterilized in the manner just described except that N_2 was used in place of CO_2. A three sugar (3S) solution containing 2.5 percent of each xylose, glucose and cellobiose was filter sterilized with a Seitz filter, and tubed under a nitrogen atmosphere. Five percent solutions of xylose and cellobiose were prepared in the same manner as the 3S solution. A 2 percent cellulose suspension was prepared by grinding Whatman No. 1 filter paper with distilled water in a ball mill for 72 hours.

Bovine rumen fluid was collected from abattoir animals. Porcupine caecal content, collected from field animals, was diluted 1:1 w/v with distilled water. The ruminal and caecal fluids were clarified by centrifuging under an atmosphere of CO_2 in capped centrifuge tubes at 30,000 X g

for 20 minutes in a Serval refrigerated centrifuge. The clarified ruminal and caecal fluids were refrigerated under an atmosphere of CO_2 in rubber stoppered bottles until used.

Dry materials weighed out and added to media before autoclaving included glucose, soluble starch, xylan, gelatin, ferric ammonium citrate, agar (BBL), trypticase (BBL), yeast extract (BBL) and L-cysteine·HCl.

Following are the compositions of the media used in the culturing experiments.

Dilution Medium

Component	Percent
Salt sol. 1	7.5
Salt sol. 2	7.5
Resazurin sol.	0.1
Distilled water	79.0
Autoclave	
Na_2S-cysteine sol.	1.0
Na_2CO_3 sol.	5.0
Gas atmosphere	
CO_2	100

Roll Tube Medium
Slant Medium*

Component	Percent
Salt sol. 1	3.75
Salt sol. 2	3.75
Resazurin sol.	0.1
Distilled water	45.5
Soluble starch	0.05
Agar	2.0
Rumen fluid	40.0
Autoclave	
Na_2S-cysteine sol.	1.0
Na_2CO_3 sol.	5.0
3S sugars	1.0
Gas atmosphere	
CO_2	100

* contained 7.5 percent of each salt sol. 1 and 2 and 36 percent distilled water

Starch Medium

Component	Percent
Salt sol. 1	7.5
Salt sol. 2	7.5
Trypticase	0.5
Soluble starch	0.1
Resazurin sol.	0.1
Distilled water	62.0
Rumen fluid	20.0
Autoclave	
Cysteine·HCl sol.	2.0
Na_2CO_3 sol.	0.75
Gas atmosphere	
CO_2	10
N_2	90

Carbohydrate Medium

Component	Percent
Salt sol. 1	7.5
Salt sol. 2	7.5
Trypticase	0.5
Resazurin sol.	0.1
Distilled water	60.0
Rumen fluid	20.0
Autoclave	
Cysteine·HCl sol.	2.0
Na_2CO_3 sol.	0.75
Sugar sol.	2.0
Gas atmosphere	
CO_2	10
N_2	90

The carbohydrate blank medium was prepared as above with the omission of the sugar solution.

Gelatin Medium

Component	Percent
Salt sol. 1	7.5
Salt sol. 2	7.5
Trypticase	0.5
Resazurin sol.	0.1
Soluble starch	0.05
Distilled water	56.0
Gelatin	5.0
Rumen fluid	20.0
Autoclave	
Cysteine·HCl sol.	2.0
Na_2CO_3 sol.	5.0
3S sugar sol.	2.0
Gas atmosphere	
CO_2	100

Xylan Medium

Component	Percent
Salt sol. 1	7.5
Salt sol. 2	7.5
Resazurin sol.	0.1
Xylan	0.1
Distilled water	58.0
Rumen fluid	20.0
Autoclave	
Cysteine·HCl sol.	2.0
Na_2CO_3 sol.	5.0
Xylose sol.	0.5
Gas atmosphere	
CO_2	100

Cellulose Medium

Component	Percent
Salt sol. 1	7.5
Salt sol. 2	7.5
Resazurin sol.	0.1
Cellulose susp.	2.5
Distilled water	54.5
Rumen fluid	20.0
Autoclave	
Cysteine•HCl sol.	2.0
Na_2CO_3 sol.	5.0
Cellobiose sol.	1.0
Gas atmosphere	
CO_2	100

No Rumen Fluid Medium

Component	Percent
Salt sol. 1	7.5
Salt sol. 2	7.5
Yeast extract	1.0
Trypticase	1.0
Soluble starch	0.05
Resazurin sol.	0.1
Distilled water	75.0
Autoclave	
Cysteine•HCl sol.	2.0
Na_2CO_3 sol.	5.0
3S sol.	2.0
Gas atmosphere	
CO_2	100

H_2S-Anaerobiosis-Motility Medium

Component	Percent
Salt sol. 1	7.5
Salt sol. 2	7.5
Resazurin sol.	0.1
Ferric ammonium citrate	0.05
Trypticase	0.5
Distilled water	57.0
Rumen fluid	20.0
Agar	0.5
Autoclave	
Cysteine•HCl sol.	2.0
Na_2CO_3 sol.	5.0
3S sol.	2.0
Gas atmosphere	
CO_2	100

No Carbonate Medium

Component	Percent
Salt sol. 1	7.5
Salt sol. 2	7.5
Resazurin sol.	0.1
Trypticase	0.5
Soluble starch	0.05
Buffer	50.0
Rumen fluid	30.0
Autoclave	
Cysteine•HCl sol.	2.0
3S sol.	2.0
Gas atmosphere	
N_2	100

pH Glucose Medium

Component	Percent
Salt sol. 1	7.5
Salt sol. 2	7.5
Trypticase	0.5
Resazurin sol.	0.1
Distilled water	53.0
Rumen fluid	20.0
Autoclave	
Glucose sol.	10.0
Cysteine•HCl	2.0
Na_2CO_3 sol.	0.75
Gas atmosphere	
CO_2	10
N_2	90

Gas From Glucose Medium

Component	Percent
Salt sol. 1	7.5
Salt sol. 2	7.5
Resazurin sol.	0.1
Trypticase	1.0
Glucose	1.0
Cysteine•HCl	0.05
Distilled water	65.0
Rumen fluid	20.0
Agar	2.0
Gas atmosphere-air	

Adjust to pH 6.8 before adding agar, melt agar, tube in 8 ml amounts and autoclave 20 minutes.

The nonsterile components of each medium were placed into a boiling flask and heated in a boiling water bath. The gas or gas mixture for the particular medium was passed into the flask, and when the medium was near boiling, the flask was stoppered and autoclaved at 18 lbs pressure for 20 minutes. The previously sterilized components of each medium were added after autoclaving. Anaerobic methods were not used for preparing the gas from glucose medium. The pH of each medium was 6.8 with the exception of the pH glucose medium which was 6.6.

The dilution fluid and the roll tube media were dispensed in 9 ml amounts, under a CO_2 atmosphere, into 16 x 150 and 18 x 150 mm tubes respectively. The differential and slant media were dispensed in 6 ml amounts into 16 x 150 mm tubes under the gas or gas mixture used for the particular medium. The gas from glucose medium was dispensed in 8 ml amounts into 16 x 150 mm culture tubes prior to sterilizing. They were

cotton plugged, inoculated as shake tubes, and overlaid with 2 ml of sterile 2 percent agar.

LITERATURE CITED

Alexander, F., M. J. D. Macpherson, and A. E. Oxford. 1952. Fermentative activities of some members of the normal coccal flora of the horse's large intestine. J. Comp. Pathol. 62:252-259.

Armsby, H. P. and C. R. Moulton. 1925. The animal as a converter of matter and energy. Chem. Catalog Co., New York.

Baker, F. 1933. Studies in the microbiology of organisms associated with the disintegration of vegetable remains etc. Zentr. Bakteriol. Parasitenk. Abt. II. 88:17-44

Baker, F. 1943. Direct microscopic observations upon the rumen population of the ox. I. Qualitative characteristics of the rumen population. Ann. Appl. Biol. 30:230-239.

Baker, F. and R. Martin. 1937a. Some observations of the iodophile microflora of the caecum of the rabbit: with special regard to the disintegration of cell wall substances. Zentr. Bakteriol. Parasitenk. Abt. II. 96:18-35.

Baker, F. and R. Martin. 1937b. Observations upon the disintegration of cell-wall substances in the caecum of the guinea-pig. Zentr. Bakteriol. Parasitenk. Abt. II. 97:201-222.

Baker, F. and R. Martin. 1939. Studies in the microbiology of the horse. Zentr. Bakteriol. Parasitenk. Abt. II. 99:400-424.

Baker, F., H. Nasr, F. Morrice, and J. Bruce. 1950. Bacterial breakdown of structural starches and starch products in the digestive tract of ruminant and non-rumen mammals. J. Pathol. Bacteriol. 62:617-638.

Balch, D. A. and S. J. Rowland. 1957. Volatile fatty acids and lactic acid in the rumen of dairy cows receiving a variety of diets. Brit. J. Nutrit. 11:288-298.

Barcroft, J., R. A. McAnally and A. T. Phillipson. 1944. Absorption of volatile acids from the alimentary tract of the sheep and other animals. J. Exptl. Biol. 20:120-129.

Barker, H. A. and V. Haas. 1944. Butyribacterium, a new genus of gram-positive, non-sporulating anaerobic bacteria of intestinal origin. J. Bacteriol. 47:301-305.

Barnett, A. J. G. and R. L. Reid. 1961. Reactions in the rumen. Edward Arnold (Publishers), Ltd. London.

Bauman, H. E. and E. M. Foster. 1956. Characteristics of organisms isolated from the rumen of cows fed high and low roughage rations. J. Bacteriol. 71:333-338.

Beijer, W. H. 1952. Methane fermentation in the rumen of cattle. Nature. 170:576-577.

Belasco, I. J. 1954. Comparison of urea and protein meals as nitrogen sources for rumen microorganisms: Urea utilization and cellulose digestion. J. Animal Sci. 13:739-747.

Blackburn, T. H. and P. N. Hobson. 1960a. Proteolysis in the sheep rumen by whole and fractionated rumen contents. J. Gen. Microbiol. 22:272-281.

Blackburn, T. H. and P. N. Hobson. 1960b. Breakdown of protein and proteolytic activity in the sheep rumen at different times after feeding. J. Gen. Microbiol. 22:290-294.

Blackburn, T. H. and R. E. Hungate. 1963. Succinic acid turnover and propionate production in the bovine rumen. Appl. Microbiol. 11:132-135.

Block, R. F., E. L. Durrum and G. Zweig. 1958. Paper chromatography and paper electrophoresis. Academic Press Inc., New York.

Bryant, M. P. 1959. Bacterial species of the rumen. Bact. Revs. 23:125-153.

Bryant, M. P. 1963. Symposium on microbial digestion in ruminants: Identification of groups of anaerobic bacteria active in the rumen. J. Animal Sci. 22:801-813.

Bryant, M. P. and L. S. Burkey. 1953. Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. J. Dairy Sci. 36:205-217.

Bryant, M. P. and I. M. Robinson. 1961a. Studies on the nitrogen requirements of some ruminal cellulolytic bacteria. Appl. Microbiol. 9:96-103.

Bryant, M. P. and I. M. Robinson. 1961b. An improved nonselective culture medium for ruminal bacteria and its use in determining diurnal variation in numbers of bacteria in the rumen. J. Dairy Sci. 44:1446-1456.

Bryant, M. P. and N. Small. 1956. The anaerobic monotrichous butyric acid-producing curved rod-shaped bacterium of the rumen. J. Bacteriol. 72:16-21.

Bruno, C. F. and W. E. C. Moore. 1962. Fate of lactic acid in rumen ingesta. J. Dairy Sci. 45:109-115.

Carroll, E. J. and R. E. Hungate. 1954. The magnitude of the microbial fermentation in the bovine rumen. Appl. Microbiol. 2:204-214.

Carroll, E. J. and R. E. Hungate. 1955. Formate dissimilation and methane production in bovine rumen contents. Arch. Biochem. Biophys. 56:525-536.

Danielli, J. F., M. W. S. Hitchcock, R. A. Marshall and A. T. Phillipson. 1945. The mechanism of absorption from the rumen as exemplified by the behaviour of acetic, propionic and butyric acids. J. Exptl. Biol. 22:75-84.

Doetsch, R. N., R. Q. Robinson, R. E. Brown and J. C. Shaw. 1953. Catabolic reactions of mixed suspensions of bovine rumen bacteria. J. Dairy Sci. 36:825-831.

Elsden, S. R. 1945. The fermentation of carbohydrates in the rumen of sheep. J. Exptl. Biol. 22:51-62.

Elsden, S. R., M. W. S. Hitchcock, R. A. Marshall and A. T. Phillipson. 1946. Volatile acid in the digesta of ruminants and other animals. J. Exptl. Biol. 22:191-202.

Elsden, S. R. and A. T. Phillipson. 1948. Ruminant digestion. Ann. Rev. Biochem. 17:705-726.

Emery, R. S., C. K. Smith, and C. F. Huffman. 1956. The amounts of short chain acids formed during rumen fermentation. J. Animal Sci. 15:854-862.

Erwin, E. S., G. J. Marco and E. M. Emery. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768-1770.

Eusebio, A. N., J. C. Shaw, E. C. Leffel, S. Lakshmanan and R. N. Doetsch. 1959. Effect on rumen volatile fatty acids and rumen microbial dissimilation of glucose-C^{14} of corn meal when fed exclusively and in combination with hay or certain additives. J. Dairy Sci. 42:692-697.

Folin, O. and H. Wu. 1919. A system of blood analysis. J. Biol. Chem. 38:81-110.

Gray, F. V. 1947a. The absorption of volatile fatty acids from the rumen. J. Exptl. Biol. 24:1-10.

Gray, F. V. 1947b. The digestion of cellulose by sheep. J. Exptl. Biol. 24:15-19.

Gray, F. V. and A. F. Pilgrim. 1952. Origins of the volatile fatty acids in the rumen. Nature. 170:375-376.

Hale, E. B., C. W. Duncan and D. F. Huffman. 1940. Rumen digestion in the bovine with some observations on the digestibility of alfalfa hay. J. Dairy Sci. 23:953-967.

Hall, E. R. 1952. Investigations on the microbiology of cellulose utilization in domestic rabbits. J. Gen. Microbiol. 7:350-357.

Heald, P. J. 1952. The fermentation of pentoses and uronic acids by bacteria from the rumen contents of sheep. Biochem. J. 50:503-508.

Howard, B. H. 1955. Ruminal fermentation of pentosan. Biochem. J. 60:i.

Hungate, R. E. 1947. Studies on cellulose fermentation. III. The culture and isolation of cellulose-decomposing bacteria from the rumen of cattle. J. Bacteriol. 53:631-645.

Hungate, R. E. 1950. The anaerobic mesophilic cellulolytic bacteria. Bacteriol. Revs. 14:1-49.

Hungate, R. E. 1957. Microorganisms in the rumen of cattle fed a constant ration. Can. J. Microbiol. 3:289-311.

Hungate, R. E., R. W. Dougherty, M. P. Bryant and R. M. Cello. 1952. Microbiological and physiological changes associated with acute indigestion in sheep. Cornell Vet. 42:423-449.

Hungate, R. E., R. A. Mah and M. Simesen. 1961. Rates of production of individual volatile fatty acids in the rumen of lactating cows. Appl. Microbiol. 9:554-561.

Hungate, R. E., G. D. Phillips, A. McGregor, D. P. Hungate, and H. K. Buechner. 1959. Microbial fermentation in certain mammals. Science. 130:1192-1194.

Jayasuriya, G. C. N. and R. E. Hungate. 1959. Lactate conversions in the bovine rumen. Arch. Biochem. Biophys. 82:274-287.

Karr, W. G. 1924. A method for the determination of blood urea nitrogen. J. Lab. Clin. Med. 9:329-333.

Kingwill, R. G., R. A. Oppermann, W. O. Nelson and R. E. Brown. 1959. Factors affecting the composition of rumen gas. J. Dairy Sci. 42:912-913.

Kleiber, M. 1947. Body size and metabolic rate. Physiol. Revs. 27:511-541.

Lugg, J. W. H. 1938. Identification and measurement of the combustible gases that occur in the gaseous metabolic products of sheep. J. Agr. Sci. 28:688-694.

Markham, R. 1942. A steam apparatus suitable for micro-Kjeldahl analysis. Biochem. J. 36:790-791.

Marstron, H. R. 1948. The fermentation of cellulose in vitro by organisms from the rumen of sheep. Biochem. J. 42:564-574.

Masson, Marjorie. 1950. Microscopic studies of the alimentary micro-organisms of the sheep. Brit. J. Nutrit. 4:viii-ix.

MacLeod, R. A. and J. F. Murray. 1956. Some factors affecting cellulose digestion by rumen microorganisms in vitro. J. Nutrit. 60:245-259.

McBee, R. H. 1953. Manometric method for the evaluation of microbial activity of the rumen with application to utilization of cellulose and hemicelluloses. Appl. Bacteriol. 1:106-110.

McClendon, J. F. 1944. Microdetermination of volatile fatty acids in blood. J. Biol. Chem. 154:357-360.

McDonald, I. W. 1954. The extent of conversion of food protein to microbial protein in the rumen of the sheep. Biochem. J. 56:120-125.

McKenzie, H. A. and H. S. Wallace. 1954. The Kjeldahl determination of nitrogen: A critical study of digestion conditions-temperature, catalyst and oxidizing agent. Australian J. Chem. 7:55-70.

McNeill, J. J. and D. R. Jacobson. 1955. Studies on the methane and hydrogen metabolism of bovine rumen bacteria. J. Dairy Sci. 38:608.

Moore, W. E. C. and K. S. King. 1958. Determination of the intraruminal distribution of soluble nitrogen. J. Dairy Sci. 41:1451-1455.

Munch-Peterson, E. and C. A. P. Boundy. 1963. Bacterial content in samples from different sites in the rumen of sheep and cows as determined by two culture media. Appl. Microbiol. 11:190-195.

Nelson, W. O., R. A. Oppermann, and R. E. Brown. 1958. In vitro studies on methanogenic rumen bacteria. II. Fermentation of butyric and valeric acid. J. Dairy Sci. 41:545-551.

Olson, T. M. 1940. Bloat in dairy cattle. J. Dairy Sci. 23:343-353.

Oppermann, R. A., W. O. Nelson, and R. E. Brown. 1957. In vitro studies on methanogenic rumen bacteria. J. Dairy Sci. 40:779-788.

Otagaki, K. K., A. L. Black, J. C. Bartley, M. Kleiber and B. O. Eggum. 1963. Metabolism of uniformly labeled glucose-C^{14} introduced into the rumen of a lactating cow. I. Transfer of C^{14} to respired air, volatile fatty acids, and major milk constituents. J. Dairy Sci. 46:690-695.

Pearson, R. M. and J. A. B. Smith. 1943a. The utilization of urea in the bovine rumen. 2. The conversion of urea to ammonia. Biochem. J. 37:148-153.

Pearson, R. M. and J. A. B. Smith. 1943b. The utilization of urea in the bovine rumen. 3. The synthesis and breakdown of protein in rumen ingesta. Biochem. J. 37:153-164.

Phillipson, A. T. 1947. The role of the microflora of the alimentary tract of herbivora with special reference to ruminants. 3. Fermentation in the alimentary tract and the metabolism of the derived fatty acids. Nutrit. Absts. Revs. 17:12-18.

Reiset, J. 1863. Recherches chimiques sur la respiration des animaux d'une ferme. Compt. Rend. Acad. Sci., Paris. 56:740-747.

Schambye, P. and A. T. Phillipson. 1949. Volatile fatty acids in the portal blood of sheep. Nature. 164:1094-1095.

Schambye, P. 1951. Volatile acids and glucose in portal blood of sheep. Nord. Vet. Med. 3:555-574.

El-Shazly, K. 1952. Degradation of protein in the rumen of sheep. 2. The action of rumen micro-organisms on amino-acids. Biochem. J. 51:647-653.

Short, H. L. 1963. Rumen fermentations and energy relationships in white-tailed deer. J. Wildl. Mgmt. 27:184-195.

Sijpesteijn, A. K. and S. R. Elsden. 1952. The metabolism of succinic acid in the rumen of the sheep. Biochem. J. 52:41-45.

Sirotnak, F. M., R. N. Doetsch, R. E. Brown and J. C. Shaw. 1953. Amino acid metabolism in bovine rumen bacteria. J. Dairy Sci. 36:1117-1123.

Smith, J. A. B. and F. Baker. 1944. The utilization of urea in the bovine rumen. 4. The isolation of the synthesized material and the correlation between protein synthesis and microbial activities. Biochem. J. 38:496-505.

Smith, P. H. and R. E. Hungate. 1958. Isolation and characterization of Methanobacterium ruminantium n. sp. J. Bacteriol. 75:713-718.

Stewart, W. E., D. G. Stewart and L. H. Schultz. 1958. Rates of volatile fatty acid production in the bovine rumen. J. Animal Sci. 17:723-736.

Tappeiner, H. 1884. Untersuchungen über die Gärung der Cellulose insbesondere über deren Lösung im Darmkanale. Ztschr. Biol. 20:53-134.

Trevelyan, W. E., D. P. Procter and J. S. Harrison. 1950. Detection of sugars on paper chromatograms. Nature. 166:444-445.

Umbreit, W. W., R. H. Burris and J. F. Stauffer. 1957. Manometric techniques. Burgess Publishing Co., Minneapolis, Minn.

Waldo, D. R. and L. H. Schultz. 1956. Lactic acid production in the rumen. J. Dairy Sci. 39:1453-1460.

Wath, J. G. Van der. 1948. Studies on the alimentary tract of merino sheep in South Africa. XI. Digestion and synthesis of starch by ruminal bacteria. Onderstepoort J. Vet. Sci. Animal Ind. 23:367-383.

Woodman, H. E. and R. E. Evans. 1938. The mechanism of cellulose digestion in the ruminant organism. IV. Further observations from in vitro studies of the behavior of rumen bacteria and their bearing on the problem of the nutritive value of cellulose. J. Agric. Sci. 28:43-63.