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URIC ACID-UTILIZING ANAEROBIC BACTERIA OF THE CHICKEN CECUM

By

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ABSTRACT

URIC ACID-UTILIZING ANAEROBIC
BACTERIA OF THE CHICKEN CECUM

By

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Liquid scintillation counting of chicken cecal samples following the introduction of ^{14}C-8-uric acid into the cloaca of adult White Leghorn males confirmed the presence of a reverse peristalsis of the lower gut. Non-volatile, ^{14}C-labeled uric acid metabolites resulting from in vivo fermentation by cecal bacteria were not absorbed from the ceca in significant amounts; neither was it possible to partition these products into either lipid or protein fractions.

The anaerobic bacteria of the ceca were isolated and cultivated in an anaerobic glove box, and enumerated using drop-plate techniques. The majority of the cecal anaerobes required 48-72 hours of incubation in the glove box: isolates utilizing uric acid required up to 120 hours of incubation for complete visualization. A maximum of 10^{11} anaerobic bacteria/gm. (wet weight) were recovered from the cecal samples of adult White Leghorn males. These samples consisted of the whole organ and its contents. An average of 53% of the total cecal bacteria were able to utilize uric acid.

The surface-grown colonies obtained from the drop-plates allowed

for the convenient isolation and identification of the cecal microflora. Strains that utilized uric acid were isolated into pure culture and identified using Gram-reaction and morphology, seven carbohydrate fermentation tests, two biochemical tests and gas chromatographic analysis for acid metabolic products. Approximately 96-98% of the cecal anaerobes utilizing uric acid were Gram-positive. These particular isolates (numbering 410) were classified into 20 groups representing strains of four genera: Propionibacterium, Lactobacillus, Peptostreptococcus and Eubacterium.

The numbers of cecal anaerobes utilizing uric acid were not increased by a low level of antibiotics in either in vivo or in vitro studies. Additionally, as determined by cecal uric acid levels, these low levels of dietary antibiotics failed to stimulate the activity of bacteria that utilized uric acid. The results of several experiments indicated that the mode of action of antibiotics in promoting the growth of chickens would not be mediated or otherwise explained by an altered cecal microflora capable of utilizing uric acid. In an in vitro study, the antibiotics used at low levels tended to reduce the numbers of anaerobes that could utilize uric acid.

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INTRODUCTION

The ceca of the chicken are a pair of blind sacs located at the junction of the small and large intestine. In the adult chicken the average cecum is 16-18 cm. long; the fresh weight of the contents ranges from 2 to 8 gm. The contents were found to contain 20% solids, 0.2 to 1.0% volatile fatty acids (up to C_6), a total nitrogen content of 1.5% and a total bacterial count on the order of 10^{10}/gm. fresh weight as determined by Gram stain (Shrimpton, 1966).

Through the use of radiographic techniques, Akester et al. (1967) observed that a reverse peristalsis of the cloaca was responsible for a backflow of urine into the ceca but not into the ileum.

The actual function(s) of a chicken's cecum has not been determined. In fact, cecectomized chickens are essentially indistinguishable from their normal complete counterparts in weight gain, adult body weight, disease resistance and several other criteria (Beattie and Shrimpton, 1958). Based on the review of studies on cecal function, Barnes (1972) summarized the suggested functions of the avian cecum to be:

1. Site of digestion of cellulose by bacteria
2. Site of digestion of protein and carbohydrate
3. Site of microbial synthesis and absorption of vitamins
4. Site of absorption of non-protein nitrogen
5. Site of absorption of water

An additional function, suggested by the presence of a reverse peristalsis of the lower gut, would be the metabolism and reutilization of

excretory products (in particular, the uric acid in the urine) diverted into the ceca. The purpose of this dissertation was to investigate the breakdown of uric acid and possible reutilization by the anaerobic bacteria of the ceca in the attempt to ascribe a possible function of the avian ceca. The cecal microflora of the chicken has been shown to contain large numbers of anaerobic bacteria (ranging from 10^8 to 10^{10} per gram wet weight) that can utilize uric acid (Barnes and Impey, 1974: Barnes et al., 1972: Mead and Adams, 1975). The bacteria having the possibility of utilizing uric acid in various avian species include strains of the following genera: Bacteriodes, Fusobacterium, Clostridium, Peptostreptococcus, Eubacterium and anaerobic Streptococcus.

The anaerobic decomposition of uric acid by bacteria has been investigated by several workers. Uric acid has been metabolized by several Clostridium spp. to yield the following breakdown products: ammonia, acetic acid, carbon dioxide, glycine and formate (Liebert, 1909; Karlsson and Barker, 1949; Barker and Beck, 1942; Barker, 1961).

Barnes and Impey (1972) developed a uric acid agar growth medium for the purpose of demonstrating the breakdown of this substrate by cecal isolates.

The bacterial flora of the chicken ceca has not been completely identified by workers using either the roll tube or traditional anaerobic jar techniques. The development of anaerobic glove box techniques in this laboratory may result in the isolation and identification of a greater proportion of the cecal flora.

The identification of the cecal anaerobes to the species level has been accomplished using the Gram stain, carbohydrate fermentation

patterns, biochemical tests and gas chromatographic (GC) analysis of alcohols and volatile and non-volatile acid metabolites produced by the cultures. The use of the gas chromatograph in the identification of anaerobes has become almost indispensible. Several anaerobic laboratory manuals have described complete GC procedures for analysis of the volatile and non-volatile fatty acids produced by broth cultures of the bacteria. However, improvements in the current GC methodologies can result in a more rapid and accurate measurement of these bacterial products.

The role of uric acid in the nutrition of the chicken has been investigated by several workers. Wiseman et al. (1956) proposed that chicks grow more rapidly when conditions of diet and antibiotic supplementation encourage the increase in numbers and activity of those bacteria which remove a potentially toxic substance, uric acid, from the intestinal tract. Uric acid in combination with antibiotics was included in the diet of poultry by Bare et al. (1964a). Tests revealed an increased degradation of uric acid in the tract of the "uric acid-antibiotic" fed chicks. From the results of this study it was theorized that the dietary antibiotics served to counteract the growth depressing effects of uric acid by stimulating those bacteria capable of removing this substrate from the intestinal tract.

Possibly the numbers and activity of the cecal anaerobes that can utilize uric acid can also be enhanced by antibiotic supplementation in the diet. However, there is presently no datum available to confirm or refute this theory. Further investigation into the effects of dietary antibiotics, utilized at growth promoting levels, could

provide useful information concerning the mode of action of antibiotics in growth promotion. The mode of action of antibiotics in stimulating growth of the chicken has been suggested in the literature but is a poorly understood phenomenon. Popular theories about this "mode of action" can be briefly summarized as: 1. Removal of bacteria that are in competition with the host for the ingested nutrients and/or emit harmful toxins, and 2. A thinning of the intestinal wall which permits a greater rate of absorption of nutrients. This dissertation is, in part, the investigation of an additional theory; viz, that low levels of antibiotics (growth promoting) serve to alter the numbers or activity of the cecal bacteria that can utilize uric acid. These changes of the microflora may result in a decreased level of uric acid in the intestinal tract and a concurrent increase in uric acid metabolites. These metabolites, such as formate and acetate and possibly others, may be nutritionally beneficial to the chicken. In the absence of a direct cecal absorption of the uric acid metabolites, the chicken may benefit from the activity of the bacteria that attack uric acid in an indirect manner, such as coprophagy or the feeding of dried cage layer excreta (anaphage).

LITERATURE REVIEW

Anatomy and Physiology of the Avian Ceca

The ceca of birds are situated at the junction of the small and large intestine. In some species of birds, they are a large, prominent pair of blind sacs; in other species of birds they may be single, rudimentary or absent (Sturkie, 1976). In a study by Leopold (1953), the seed-eating species of birds such as the quail, partridge, pheasant and wild turkey were found to possess individual cecum approximately 17 cm. long while the browsing species like the grouse had cecum 44 cm. long. In the adult chicken, the average cecum is 16-18 cm. long; the fresh weight of the contents ranges from 2 to 8 gm. The contents were found to contain 20% solids, 0.2 to 1.0% volatile fatty acids (up to C_6), a total nitrogen content of 1.5% and a total bacterial count on the order of 10^{10}/gm fresh weight as determined by Gram stain (Shrimpton, 1966). Guarding the entrance of the cecum into the intestine are two muscular, ileocecal valves. Histologically, the ceca are similar to the intestine except that the villi are not as long. In the owl the ceca are quite glandular and exhibit a high degree of secretory activity, in the passerine species they are present as a lymph-epithelial type (Ziswiler and Farner, 1972).

Olson and Mann (1935) observed that the pH of the cecal contents is lower than that of the contents of either the preceeding or succeeding portions of the intestine. Only fluids were found to enter the ceca with passage of ingesta from mouth to ceca occurring in 1.5 to 3.5 hours. Upon feeding carmine, a water soluble dye, to chickens,

it was concluded that the cecum evacuates itself in 120 hours.

Duke et al. (1968) studied the passage rate of a turkey breeder ration through the ring-necked pheasant. These workers reported that the average minimum passage time was one to two hours. The average maximum passage time for ingesta not receiving cecal digestion was 8.5 hours. For material receiving cecal digestion the average maximum passage time was 35 hours. Jayne-Williams and Fuller (1971) reported that the ceca of the chicken are evacuated every 6 to 8 hours. Brown (1922) observed that only liquid enters the ceca and suggested that the material entering the ceca was essentially of the nature of an overflow from the intestinal contents. He postulated that the peristaltic movements of the ceca themselves were responsible for filtering out solids during cecal filling. The accumulation of solid material in the ceca created a tension in the cecal wall and stimulated the ceca to empty themselves. A majority of the intestinal contents do not enter the ceca.

Through the use of a cecal fistula, Beattie and Shrimpton (1958) observed that the pH of cecal contents in vivo ranged between 6.7 and 8.5. They observed a pH gradient in the ceca of 12-week-old chickens with the cecal tips being more alkaline than the cecal duct. Due to the presence of this pH gradient they suspected that the cecal contents are not homogeneous and thus may contain several distinct micropopulations. Additionally, it seemed quite improbable that all of the cecal contents are removed by every evacuation.

Function of the Avian Cecum

The function of the avian cecum has not been conclusively determined. Thornburn and Willcox (1965a) conducted digestibility trials with normal and cecectomised birds to study the digestion of the crude fiber complex. Results from the same bird were compared before and after cecectomy and the cecectomised birds were also compared with normal intact birds of the same age. The trials showed a reduction in fecal dry matter after cecectomy indicating that the ceca are active in the absorption of water from the ingesta. It was observed that fecal dry matter content seemed to be more a characteristic of the bird than of the food it eats. There was also a reduction in the overall digestibility of dry matter in the food after cecectomy, and also in that of crude fiber. However, the digestibility of crude fiber was observed to be dependent on the food being eaten and on its crude fiber content. Cellulose digestibility in a given bird was lowered by cecectomy, an observation that was not consistent between different birds, indicating that cellulose is digested by the cecal bacteria. No effect was found on pentosan and starch digestion.

Thornburn and Willcox (1965b) studied the digestion of the crude fiber complex in the ceca using a cecal fistula to introduce food materials. They determined that the extent to which digestion occurs in the cecum, as evidenced by weight loss of a substrate from a permeable bag placed in the cecum, apparently depends both on the nature of the material and on the length of time it spends in the cecum. A slight digestion of bran was observed with very little, if any, digestion of hay. No clear conclusions could be drawn from the chemical analysis

of bran and hay after their incubation in the cecum. Inman and Ringer (1973) observed that ingested cellulose did not enter the ceca of chukar partridge, ruffed grouse or bobwhite quail in the same concentration as was found in the non-cecal excreta. In their opinion the cecal aperature was of sufficient size to allow for the passage of Solka Floc (cellulose) particles into the ceca without difficulty, hence, they concluded that there was no crude fiber digestion occurring in the ceca. Masson (1954) found potato starch granules in ceca following their inclusion into the diet; this starch had not undergone significant degradation in the upper intestine.

In a study on water absorption in the chicken, Sperber (1960) observed that the adult chicken produces approximately 23 ml. of urine per hour but does not excrete this same amount. Under the assumption that the large intestine and coprodeum cannot account for a sufficient amount of water reabsorption he suggested that the ceca might be responsible for some water reabsorption.

The suggested functions of the avian cecum were summarized by Barnes in 1972 to be:

1. Site of digestion of cellulose by bacteria
2. Site of digestion of protein and carbohydrate
3. Site of microbial synthesis and absorption of vitamins
4. Site of absorption of non-protein nitrogen
5. Site of absorption of water.

Barnes (1972) concluded that the major function of the cecum could be the metabolism and reutilization of the excretory products diverted

into the ceca with the possible reabsorption of water and any of the vitamins, volatile fatty acids or amino acids synthesized by the micro-organisms.

Effects of Cecectomy

Sunde et al. (1950) removed the ceca from laying hens and observed increased levels of biotin in their eggs as well as an increase in hatchability. They concluded that there is a competition between the hen and the cecal microflora for the biotin present in the lower gut. However, cecectomized hens were observed to have slightly lower hen-day egg production than normal birds.

Tortuero et al. (1975) observed elevated levels of serum cholesterol as well as increased levels of egg yolk cholesterol in cecectomized layers.

Beattie and Shrimpton (1958) reported that cecectomized chickens grew as well as normal chickens and at 12 weeks of age they tended to be heavier than normal birds.

Metabolism of the Cecal Flora

Beattie and Shrimpton (1958) measured the gaseous metabolities of the cecal microflora using a cecal fistula. A total production of about 100 ul/hr. per cecum of methane and acidic gases calculated as CO_2 was observed. Small amounts of hydrogen sulphide and alkaline gas calculated as ammonia were also detected.

The incubation of cecal contents by Bell and Bird (1966) was

reported to result in an increase in urea, pointing to its production by the cecal flora. Additionally, the levels of volatile fatty acids and ammonia in the ceca always exceeded the amounts formed in the large intestine. These workers concluded that the cecum was not the source of the small amounts of urea normally found in the fowl's blood and suggested that the intestinal bacteria could be a probable source of the blood urea.

Shrimpton (1954) fed day-old chicks an all vegetable ration deficient in vitamin B_{12} in a study of the absorption of this vitamin by the ceca. In this experiment some of the birds less than one month old died from a deficiency of vitamin B_{12} in spite of the production of this vitamin by their cecal microflora. After one month of age the birds on this diet that had survived were able to absorb sufficient vitamin B_{12} to allow for growth but with onset of lay their eggs contained levels of the vitamin that were inadequate for normal growth and body maintenance of their offspring. When potato starch was substituted for the corn starch in the all vegetable ration there was a significant increase in the vitamin B_{12} status of the laying hens. There was no significant difference in the concentration of vitamin B_{12} in the cecal contents of birds receiving either type of starch. Shrimpton (1954) proposed that the increased levels of B_{12} in birds fed the potato starch diet were due to an increased availability of the vitamin to the bird. The mechanism for the increased availability of the vitamin remains to be determined. However, he observed that the pH of the cecal contents was not a factor in any absorptive mechanism operating in the cecum of the hen.

From studies on the influence of diet on cecal metabolism, Shrimpton (1966) proposed that metabolites arising from the cecal microflora could be absorbed by the bird. Shrimpton (1954) and Jackson et al. (1955) suggested that there was a negligible amount of absorption occurring in young birds and that significant net absorption of nutrients from the ceca may not occur until the birds are 6 weeks old. Jayne-Williams and Fuller (1971) reported that vitamins synthesized in the lower gut of chickens are not generally absorbed from this site and the animal may only benefit from these nutrients through coprophagy.

Reverse Peristalsis of the Lower Intestine of the Bird

Polin et al. (1967) reported that a backflow of urine from cloaca to ceca can account for the high cecal concentrations of amprolium, thiamine and their end-products of metabolism in the chicken. Their observation was based upon the fact that the concentrations of these substances in the cecal contents were considerably reduced in the chicken with the artificial anus.

In a study of the hydropenic chicken, Koike and McFarland (1966) reported that the absence of a functional sphincter between the cloaca and rectum allows for a urine reflux. An apparent sphincter between the small intestine and rectum prevented the passage of a contrast media (Hypaque) into the small intestine, although there was passage into the ceca. Mead and Adams (1975) reported that uric acid enters the cecum via a back-flow of urine from the cloaca. Bell and Bird (1966) were unable to detect uric acid in the cecal contents and

reported that solid materials from the ileum and colon do not enter the ceca. Additionally, the retroperistaltic movement of cloacal urine was reported to be the source of intestinal urea and the presence of urea in cecal contents points to its possible production by the cecal flora.

Akester et al. (1967), using radiographic techniques, observed the backflow of Barium Sulphate, which had been placed in the coprodeum, up the intestine into the colon and ceca but not the ileum.

Recovery and Identification of the Cecal Flora of the Chicken

A variety of experiments have been conducted in an attempt to accurately typify the cecal microflora of the chicken. This area of research has been hampered in the past by the slow development and adaptation of satisfactory anaerobic bacteriological methods as well as the apparently vast number of genera, species and in some cases, subspecies of obligately anaerobic bacteria present in the avian ceca.

Barnes and Impey (1971) have described their recommended isolation procedure for the chicken cecal flora.

Using an ethyl violet azide agar medium under strictly anaerobic conditions, Barnes and Goldberg (1962) observed as many as 10^8/gm. of a group of obligately anaerobic, gram-negative, bacteria ("Bacteroides") in the chicken ceca. Cecal samples were obtained as cecal droppings from older birds while cecal samples from younger birds were obtained directly from freshly killed chicks.

Barnes and Impey (1968) isolated 4 groups of gram-negative non-sporing anaerobic rods (family Bacteroidaceae) from the ceca of

chickens, turkeys and ducks. Two of these groups belonged to the genus Sphaerophorus but had characteristics different from normal strains. Another group belonged to the Bacteroides genus and resembled B. fragilis. A last group differed from Bacteroides and Sphaerophorus and was present at 10^9/gm. of cecal contents and was not assigned to a genus. It was noted that no single selective medium could be used for the isolation of all these organisms. Comparisons of direct microscopic counts with total viable counts showed that there were bacteria present which were not being isolated by the methods used.

In a study with 14 week-old chicks, Pensack and Hutanen (1961) reported that, in the ceca, obligate anaerobes were approximately 10 times as numerous as facultative organisms. Clostridia welchii was present at only 10^5/gm. as opposed to 10^{10} for other anaerobes.

Mead and Adams (1975) studied the changes in the cecal flora of chicks aged from approximately 3 hours to 14 days. Using strict anaerobic methods, they observed that the cecal microflora became predominantly strictly anaerobic from the 4th day of age. Direct microscopic counts varied between 3×10^{10} and 2.3×10^{11} throughout the experiment. By the end of the experiment it was determined that only about 10% of the total microscopic count could be isolated.

Using the Hungate roll tube technique, Barnes et al. (1972) were able to isolate more than 20% of the total anaerobic bacteria present in the ceca of chicks at 2, 3, 4 and 6.5 weeks of age. There was no significant difference in the total numbers of bacteria present in the ceca between 2 and 6.5 weeks of age (about 10^{11}/gm.. on direct microscopic count).

Timms (1968) observed greater numbers of bacteria in the ceca than in the rest of the intestine in chickens aged 18 days, 7 weeks and 5 months. Bacteroides spp. were confined to the ceca of each age group and were present in a similar concentration to that of the lactobacilli. The anaerobes enumerated included Cl. welchii at approximately 1 x $10^{2.7}$ in all ages and bacteroides at approximately 8.7 x 10 in all ages.

Under optimal conditions, Barnes and Impey (1970) found it possible to isolate more than 25% of the total flora in the chicken and turkey ceca. In 5 weeks old chickens they reported that the gram-negative non-sporulating anaerobes (Bacteroidaceae) and the gram-positive non-sporulating rods and Bifidobacterium were present in almost equal proportions and formed about 80% of the flora isolated. The rest of the flora was Peptostreptococcus spp. (15%) together with a number of organisms including curved rods which have not been characterized. In this experiment 5 distinct groups of gram-negative anaerobes (Bacteroidaceae) were isolated from chicken and turkey ceca together with 3 groups of gram-positive non-sporing rods and Bifidobacterium and 4 groups of Peptostreptococcus.

Salinitro et al. (1973) examined the cecal contents of 5 weeks old broiler chickens and observed direct microscopic counts of 2.6 x 10^{10} to 7.0 x 10^{10} bacteria/gm.

In broiler chickens 5 weeks old, 77% of 325 bacterial strains randomly isolated from cecal digesta were found to be obligate anaerobes (Salinitro et al., 1974a). Of these obligate anaerobes, 6.75% were pleomorphic gram-negative cocci, approximately 2% were Peptostreptococcus, 47% were gram-positive rods, P. acnes and Eubacterium

spp., 24% were gram-negative rods (B. clostridiiformis, B. hypermegas, B. fragilis) and 20% were spore-forming rods (Clostridium spp.). Gram-positive facultative cocci and E. coli constituted 17.5% of the bacterial strains isolated from the ceca of chickens.

Salinitro et al. (1974b) reported that the total microscopic count in the ceca of 5 weeks old chicks varied from 3.83×10^{10} to 7.64×10^{10} per gram. Using a rumen fluid medium (M98-5), about 60% of the direct count was recovered while the use of Medium 10 recovered only about 45%. At least 11 groups of bacteria were isolated from high dilutions (10^{-9}) of cecal material. Data on morphology and physiological and fermentation characteristics of 90% of the 298 isolated strains indicated that these bacteria represented species of anaerobic gram-negative cocci, facultative cocci, Streptococcus, Peptostreptococcus, Propionibacterium, Eubacterium, Bacteroides and Clostridium.

A previously unidentified gram-negative, strictly anaerobic pleomorphic, budding-like organism, present in the ceca of chickens in high number (0.1×10^{10} to 1.34×10^{10}/gm.) was identified as Gemmiger formicilis by Salinitro et al. (1976). This organism was found in the luminal contents of the ceca and constituted, on the average, 6% of the total isolates recovered.

Dilution of Cecal Samples

Anaerobes from the chicken ceca have been recovered when dilutions of cecal contents were prepared in anaerobic dilution solution (ADS) of Bryant and Burkey (1953) or RCM hemin broth (reinforced clostridial medium) by Barnes and Impey (1970; 1972; 1974) and

Barnes et al. (1972). Serial dilutions of cecal contents have also been prepared in ADS by Salinitro (1974a; 1974b) and Mead and Adams (1975). Holdeman and Moore (1973) recommended a solution of salts and gelatin containing resazurin for the preparation of dilution blanks.

Growth Media for Cecal Anaerobes

Anaerobic bacteria of the chicken ceca, obtained from cecal droppings or directly from the bird, have been isolated on RCM broth and agar containing a variety of dyes and antibiotics, Ethyl violet-azide agar (EVA), Basal medium of Beerens (1953-1954), Beerens basal medium with glucose and phosphate (BGP) and BGP plus gelatin (Barnes and Goldberg, 1962). Barnes and Impey (1968) isolated the anaerobes in chicken, turkey and duck cecal contents using RCM, VL medium (modified from Beerens et al. (1963), Basal medium (VL medium without glucose) and BGP.

For the isolation of anaerobic bacteria in the ceca of chickens and turkeys, Barnes and Impey (1970; 1971) used the Medium 10 (M10) of Caldwell and Bryant (1966) in roll tubes. The following media were used when traditional anaerobic techniques (anaerobic jar) were used: VL medium, VLBM (VL medium plus 5% laked blood and 0.5 ug./ml. menadione), VL1f medium (VL medium plus 5% liver and 10% fecal extracts), VL-hemin broth or agar (VL medium plus 1 ug./ml. hemin), VLBM kanamycin agar (VLBM plus 100 ug./ml. kanamycin sulfate), RCM and RCM hemin broth or agar (RCM plus 1 ug./ml. hemin).

A total growth medium for all organisms isolated from the chicken cecum by Barnes and Impey (1972) was BGPhlf agar which consisted of

BGP agar plus 1 ug./ml. hemin, 5% liver extract and 5% fecal extract.

Salinitro et al. (1974a; 1974b) used roll tubes of the following media for the cultivation of cecal anaerobes from 5 weeks old broiler chicks: Medium 98-5 (Bryant and Robinson, 1961); a modified medium 98-5 which differed from Medium 98-5 in that glycerol, trypticase, and hemin are included and mineral solution S2 is substituted for mineral solutions 1 and 2, Medium 10 and supplemented Medium 10 (SM-10). The growth of many isolates was enhanced by rumen fluid, yeast extract and cecal extract additions to basal media. These studies indicated that some of the numerically superior chicken anaerobes can be isolated and cultured when media and methods that have been developed for ruminal bacteria are employed.

Data on growth requirements of anaerobic strains indicated that many could be cultured in a simple medium consisting of an energy source, minerals, reducing agent, trypticase and/or yeast extract or vitamin mixture. Yeast extract or vitamin mixture is highly stimulatory and its effect as a medium suggests that chicken ceca anaerobes may have specific vitamin requirements for growth. Volatile Fatty Acids (VFAs) may not be required for growth by most cecal anaerobes (Salinitro et al., 1974a).

Medium 10 in roll tubes was reported to be inferior to VLBM (Barnes and Impey, 1970) agar, used in traditional petri plate techniques, in the ability to isolate the maximum possible percentages of cecal bacteria in chickens and turkeys. These results indicated that the growth medium rather than the Hungate roll tube technique was at fault. The addition of liver and chicken fecal extracts to the Medium 10 resulted in improved counts.

Anaerobic Decomposition of Uric Acid

The anaerobic decomposition of uric acid by bacteria has been investigated by several workers. It was observed that *Clostridium acidiurici* and *Clostridium cyclindrosporum* converted uric acid into ammonia, acetic acid and carbon dioxide (Liebert, 1909; Karlsson and Barker, 1949; Barker and Beck, 1942). Allantoin was found to not be an intermediate in the anaerobic process. Barker (1961) reported that several *Clostridium spp.* would decompose uric acid into xanthine and in later steps into glycine, formate, carbon dioxide, ammonia and acetate. The anaerobic utilization of uric acid by some Group D streptococci was detected on a uric acid agar slant containing a phenol red indicator (Mead, 1974). The breakdown products were reported to be ammonia, carbon dioxide, and acetic acid. The breakdown of uric acid by aerobic bacteria of chicken (Rouf and Lomprey, 1968) has been shown to be accomplished by uricase (Wiseman and Wright, 1965).

Evidence for the active breakdown of uric acid in the cecum comes from the high pH, the presence of ammonia and the unaccountable presence of urea (Barnes, 1972; Bell and Bird, 1966).

Detection of Uric Acid Breakdown by the Cecal Anaerobes

A uric acid agar medium originally described by Schefferle (1965) was employed by Barnes and Impey (1972) to determine the breakdown of this substrate by the cecal anaerobes.

This medium consisted of Basal-hlf agar (BGP without glucose or phosphate) incorporating uric acid at 0.1% w/v in a lower layer and

1.0% w/v in an upper layer. Uricase activity was indicated by clear zones which surrounded the colonies and which remained after flooding the plates with 1 N HCl.

In the investigation of uric acid decomposing anaerobes in the avian cecum, Barnes and Impey (1974) used a supplemented uric acid agar (SUA) and a uric acid agar (SUA without liver and chicken fecal extracts) in the anaerobic jar.

Mead and Adams (1975) used Supplemented Medium 10 containing 0.2% uric acid (uric acid-SM-10) in their study of the cecal flora of the chicken from day-old to 14 days of age.

Numbers and Types of Cecal Anaerobes that Utilize Uric Acid

The cecal microflora of the chicken has been shown to contain large numbers of anaerobic bacteria (ranging from 10^8 to 10^{10} per gram wet weight of cecal contents) that can utilize uric acid (Barnes and Impey, 1974; Barnes *et al*. 1972; Mead and Adams, 1975). None of the isolates studied by these workers exhibited an absolute requirement for uric acid; additionally it was not determined if the breakdown of uric acid by the cecal microflora contributed in any way to the nutrition of the bird.

In an extensive study of the cecal microflora of the chicken, turkey, duck, pheasant and guinea fowl, anaerobic bacteria capable of decomposing uric acid were found at levels between 5.4×10^8 and 1.8×10^{10} per gram of cecal material (wet weight). The isolates that utilized uric acid included strains of: *Bacteroides clostridiiformis* var. clostridiiformis, *Fusobacterium plauti*, *Clostridium malenominatum*,

Peptostreptococcus productus and several unknowns: 3 Bacteroides spp., 2 Fusobacterium spp., 2 Peptostreptococcus spp. and 1 anaerobic Streptococcus sp. (Barnes and Impey, 1974).

In 2 to 6.5 weeks old chickens, all cecal samples contained 10^7 to 10^9 anaerobes per gram and sometimes 10^{10}/gm. of uric acid utilizers (Barnes et al., 1972). This property was shared by at least one group of Peptostreptococcus, 4 groups of gram-negative, non-sporing anaerobes, 3 groups of gram-variable or gram-positive non-sporing rods and several species of Clostridium. The Clostridium were never present as a major portion of the cecal anaerobes.

An average of 1×10^9 bacteria/gm. that utilized uric acid was observed in the cecal contents of chickens, pheasants and ducks (Barnes, 1972). Subsequent microscopic and bacteriological examination of cecal contents from each species of bird indicated major differences in the genera of the anaerobic bacteria present.

Comparison of Anaerobic Methodologies

Several studies have been conducted to measure the success of the various anaerobic methodologies in the isolation and cultivation of anaerobes. Barnes and Impey (1970) reported that the Hungate method permitted the isolation of 28 to 33% of the anaerobes present in the chicken ceca while only 6-30% were recovered using the anaerobic jar.

It was reported by Leach et al. (1971) that the use of roll tubes in comparison to agar plates in a glove box was at a serious disadvantage. It was found to be more difficult to distinguish and count different colonies in roll tubes and also more difficult and time

consuming to pick single colonies from the roll tube as opposed to agar plates.

Aranki et al. (1969) and Aranki and Freter (1972) have described a flexible vinyl chamber that matched or excelled the roll tube method with respect to all parameters tested including the redox potential obtainable in the media, oxygen concentration in the gas phase and efficiency in isolating anaerobic bacteria from the mouse ceca. Comparative studies had indicated that the conventional anaerobic jar method was inadequate for the isolation of strict anaerobes from human gingival specimens and from the mouse cecum due to the exposure of specimens and media to air during plating on the open laboratory bench. The Hungate method requires a specially trained technician because of the considerable manual dexterity required for the simultaneous handling of rubber stoppers, gas outlets and tubes during the transfer of cultures or media. Glove box techniques require no special training beyond routine bacteriological methods.

Problems Associated with the Isolation and Identification of Intestinal Anaerobes

Some of the problems associated with the isolation and identification of intestinal anaerobes have been summarized by Moore and Holdeman (1974):

1. Direct microscopic count has been less reproducible than the cultural counts because the material examined is from diverse locations.

2. Correction to dry weight value is essential for comparison of different specimens. Differences in moisture content alone may account for a 3-fold difference in reported microscopic or cultural counts among samples when counts are on a wet weight basis. It is important to compare direct counts with cultural counts even though both counts are subject to error.
3. The manner of preparing dilutions may be a major source of error in cultural counts; it is suggested that erroneous culture counts arise from growth in dilution fluids during sample preparation. They recommended the addition of gelatin to a solution of reduced mineral salts that was used as a dilution blank. The gelatin will help poise the oxidation-reduction potential and chelate metals that may occur as contaminants in the salts solution.
4. Simple mixing by pipet is not sufficient to make an even suspension of organisms and the transfer of "enriched material" from the bottom of succeeding dilution blanks is a probable cause of error. Adequate mixing of each dilution is accomplished by shaking each tube a distance of one foot 25 times in 2 seconds. The best growth medium for the recovery of intestinal anaerobes is reported to be the RGCA (Rumen Fluid-Glucose-Cellobiose-Agar) of Bryant and Robinson (1961): a slow-growth medium containing small amounts of nutrients, particularly carbohydrates.

In many cases correct speciation of intestinal anaerobes can be accomplished using carbohydrate fermentation patterns, biochemical tests and gas chromatographic analysis of volatile and non-volatile

acid metabolites produced.

Fermentation and Biochemical Testing of Anaerobes

Holland et al. (1977b) have described a simple rapid procedure for the presumptive identification of clinical isolates. Three different batteries of test media were used for bacterial identification. The standard identification procedure or long form (LF) consisted of 10 to 15 ml. volumes of 20 to 30 test media in 16 by 150 mm. tubes (Holland et al., 1977a) and was used in making comparisons with rapid procedures. The abbreviated or "short-form" (SF) batteries for fermentation media were prepared by adding filter-sterilized carbohydrate (CHO) into either 3 ml. volumes of thioglycollate medium without glucose or indicator or 0.5 ml. volumes of peptone-yeast extract (PY). Fermentation reactions were read by adding a few drops of dilute bromothymol blue to the tubes after incubation. Fermentation was indicated by a color change from blue to yellow. Blue-green and green colors were considered negative. Results of all the SF media were read in 18-48 hours. The average incubation time was 39 hours. The 3 ml. SF media, in comparison to the standard tests, correctly identified 98% and 83% of the 325 isolates tested to genus and species levels, respectively. The three SF procedures identified comparable percentages of 53 isolates studied.

Rapid fermentation and biochemical testing of anaerobic bacteria were reported by Schreckenberger and Blazevic (1974; 1976). Rapid fermentation tests for several CHO were prepared by adding 1.0 gm. amounts to 100 ml. of a buffer-salt solution containing bromothymol

blue, without heating. The media were adjusted to pH 7.2, dispensed in 0.5 ml. aliquots and frozen at -20°C until used. All rapid fermentation tests were performed by scraping up a loopful of growth from the surface of an agar plate and inoculating directly into the substrate that had been preheated to 35-37°C. All tests were incubated aerobically at this temperature and read after 4 hours of incubation. Questionable reactions were compared with the fermentation base medium which had been inoculated with the same organism but contained no CHO. An overall correlation of 89% was achieved between the rapid tests and the Virginia Polytechnic Institute method in the testing of 112 strains of anaerobes. Preparation and storage of rapid biochemical test media for nitrate and indole were similar to those for the CHO media. Tryptone broth at 1% concentration (Difco) and Nitrate broth at 0.9% (Difco) in 0.5 ml. amounts were inoculated and incubated as before and the tests read in four hours. Kovac's reagent was used to detect indole production in the tryptone broth and 0.8% sulfanilic acid in 5N acetic acid with 0.5% alphanaphthylamine in 5N acetic acid were used to detect the reduction of nitrate. The tests for nitrate and indole and starch (also described in this paper) showed a 95% or greater correlation when compared to the standard biochemical tests (Holdeman and Moore, 1973).

Fay and Barry (1974a; 1974b) have developed methods for determining carbohydrate fermentation and indole production in clinically significant gram-negative, non-sporulating anaerobes. In their report, fermentation of carbohydrates (CHO) at 0.6% concentration was detected rapidly in 1 ml. volumes of either thioglycollate medium without

dextrose or indicator or in a peptone-yeast base broth. The pH of the CHO mediums was determined with a pH meter equipped with a semi-micro combination electrode following incubation in GAS PAK jars. These workers determined that the lag time before acid products will accumulate was significantly reduced when broth volumes of 1 ml. were used as opposed to either 5.0 or 8.5 ml. amounts. Optimal production of indole was observed in 1-2 days in 1 ml. amounts of thioglycollate broth without glucose but with 0.02% tryptophan. Prolonged incubation was required more frequently with conventional methods of indole testing.

Gas Chromatographic Analysis of Anaerobic Bacteria Broth Cultures

The use of the gas chromatograph in the identification of anaerobes has become almost indispensible. Moore et al. (1966) reported that the fermentation characteristics of 20 species of clostridia have shown that the relative proportions and concentrations of the acids and alcohols produced by these cultures may be very useful in their characterization and identification. Chromatographic patterns are highly reproducible among strains of the same species.

Complete chromatographic procedures have been outlined in the Anaerobe Laboratory Manual 2nd Ed. (Holdeman and Moore, 1973); Laboratory Methods in Anaerobic Bacteriology CDC Laboratory Manual (Dowell and Hawkins, 1974) and the Wadsworth Anaerobic Bacteriology Manual 2nd Ed. by Sutter et al. (1975). The volatile fatty acids detected include: formate, acetate, propionate, isobutyrate, n-butyrate, isovalerate, n-valerate, isocaproic, n-hexanoic (n-caproic) and

heptanoic. The non-volatile fatty acids include: lactic, pyruvic, succinic, oxaloacetic, oxalic, methyl malonic, malonic and fumaric.

The alcohols detected are: ethanol, propanol, isobutanol, butanol, isopentanol and pentanol. To detect alcohol and volatile acid products, a peptone-yeast extract-glucose (PYG) culture is acidified and extracted with ether and the ether extract is chromatographed. To detect non-volatile acid products, the PYG culture is acidified, methylated, extracted with chloroform and the chloroform extract is chromatographed (Anaerobe Laboratory Manual).

Column packings used have been Resoflex, Poropak and Chromosorb G. Using the CDC extraction procedure, Hauser and Zabransky (1975) evaluated a new packing material for anaerobic work. Their use of SP-1220 (Supelco Inc., 1975) demonstrated several advantages: total elution of volatile fatty acids examined was decreased to 12 minutes and gave excellent separation of propionic and isobutyric acids as well as demonstrating the presence of formic acid which was not seen previously when Resoflex was used.

For optimum detection of all metabolic products, the Wadsworth Anaerobic Bacteriology Manual recommends the use of the flame ionization detector for assaying volatile fatty acids and volatile organic compounds and the thermal conductivity detector for assaying methyl derivatives, formic acid and hydrogen gas. Rogosa and Love (1968) accomplished the direct quantitation of lower fatty acid homologues, methanol and ethyl alcohol in aqueous bacterial fermentation media. A hydrogen flame detector and a column packing of Polypack-2, uncoated, 80-120 mesh (Hewlett-Packard) were employed.

The VPI (Virginia Polytechnic Institute) chromatography techniques outlined in the Anaerobe Laboratory Manual have been used successfully for the analysis of chicken cecal isolates (Moore et al., 1969).

Bricknell and Finegold (1972) reported that the VPI extraction procedure can result in a selective extraction of fatty acids and alcohols by the solvent: a portion of the C_2 and C_3 compounds remain in the aqueous phase which is not chromatographed. A flame ionization procedure which permits direct injection of aqueous supernatant culture is a preferable method since it eliminates the need for the time consuming solvent extraction and yields an accurate representation of the components in the sample (Wadsworth Manual).

Salinitro et al. (1974a; 1974b) have used the gas chromatographic techniques of Lambert and Moss (1972) to identify the fermentation products of glucose by anaerobic bacteria of the chicken ceca.

Enumeration of Anaerobic Bacteria on Agar Plates

Several methods have been devised for the enumeration of viable anaerobes. Watt (1972) used the spread plate method to count clinical isolates. In this method, 0.02 ml. inocula taken from serial dilutions was spread onto the surface of agar plates with sterile glass spreaders. The dilution chosen for determining surface viable counts was that giving 100-700 discrete colonies per plate. The drop-plate method for counting bacteria was described by Miles and Misra (1938). In this method, serial dilutions of organisms are inoculated onto partially dried agar plates using dropping pipettes giving 50 drops of broth/ml. The agar plates had been sufficiently dried to absorb

a 0.02 ml. drop of broth culture, dropped from a height of 2.5 cm., in about 15-20 minutes. Drop size measured 1.5 to 2.0 cm. in diameter. Counts were made in drop areas containing the largest number of colonies without signs of confluence or gross dimunation in colony size due to overcrowding. A possible drawback to the drop-plate is that the organism may divide before the drop is absorbed by the agar and over-estimating the count at the moment of sampling may occur (Meynell and Meynell, 1965). Accordingly, it was suggested that with rapidly dividing cultures, the drop-plate technique is less reliable than the pour-plate or overlay methods.

Role of Uric Acid in Avian Nutrition

The role of uric acid in the nutrition of the chicken has received considerable attention in the literature. Wiseman et al. (1956) proposed that chicks grow more rapidly when conditions of diet and antibiotic supplementation encourage an increase in numbers and activity of those bacteria which remove a potentially toxic substance, uric acid, from the intestinal tract.

Several workers have included uric acid in combination with antibiotics, in the diet of poultry. Bare et al. (1964a) fed a diet containing 2% uric acid to one day old male chicks. At 4 weeks of age these birds showed a significant weight depression. Chicks fed the uric acid diet supplemented with 11 mg./kg. bacitracin and 44 mg./kg. procaine penicillin G did not exhibit a growth depression. These workers proposed that uric acid depresses growth by acting as an irritant and thus interfering with the absorption of nutrients from the

intestinal tract or the uric acid inhibits the microbial biosynthesis of known vitamins or other nutrients essential to the host. Tests revealed an increased degradation of uric acid in the tract of the "uric acid-antibiotic" fed chicks. This increased uricolytic activity was attributed to the increased number of Aerobacter spp. observed in the excreta of the birds on this dietary regime; a concurrent reduction in the number of lactobacilli was also noted. From the results of this study, Bare et al. (1964a) theorized that the dietary antibiotics served to counteract the growth depressing effects of uric acid by stimulating those bacteria capable of removing this substrate from the intestinal tract, by providing an additional source of vitamins and by removing those bacterial species competing with the host for the vitamins available.

Lee and Blair (1972) observed that body weight gains for broiler chicks fed a 3.43% uric acid diet and a basal diet were significantly less than for those birds receiving the semi-purified basal diet plus several crystalline amino acids (individually) or a diet containing 20.09% dried autoclaved poultry manure. Additionally, birds receiving the uric acid diet displayed a lower feed conversion efficiency than birds receiving any of the other diets tested. Feed intake of chicks receiving the uric acid diet was not significantly lower than that of chicks receiving the other diets.

McNab et al. (1974), using colostomized hens, studied the digestibility of uric acid present in dried poultry manure. The true digestibility coefficient of uric acid, calculated by a regression analysis of absorbed versus ingested nutrients, was observed to be

91.2%. Despite this high value, they concluded that it was unlikely that this nitrogen is retained or utilized by the bird.

Martindale (1975) fed dried poultry manure (DPM) to colostomized hens and reported that urate excretion was greater during DPM feeding by approximately the amount consumed. Doses of 10.6 uCi. of ^{14}C-urate introduced into the crop were quantitatively excreted into the urine within 24 hours. In this study, he suggested that dietary urate is completely absorbed in the gut and is not utilized in the body. Martindale (1975) and Lee and Blair (1972) were in agreement with the claim that urate is not available as a source of non-protein nitrogen for the chick and any improvement of growth due to feeding DPM was due to the non-urate nitrogen and metabolizable energy content of the DPM.

Measurement of Uric Acid Levels in Avian Feces and Cecal Contents

The levels of uric acid in avian excreta have been measured by Pudelkiewicz *et al*. (1968) using differential spectrophotometry in conjunction with an enzymatic digestion of very dilute solutions of the excreta. These workers obtained excreta with 3 levels of uric acid by varying the nitrogen content of a semi-purified basal diet by adding either corn gluten feed or corn gluten meal at the expense of the whole diet. The excreta obtained from the birds fed the basal diet (#1), 45% corn gluten feed-basal diet (#2) and 45% corn gluten meal-basal diet (#3) contained 10.53, 5.66 and 16.02% nitrogen, respectively. The excreta obtained from the birds fed diets 1, 2 and 3 contained approximately 200, 88 and 335 mg. uric acid per gram, respectively.

Using the uricase digestion method of Dubbs et al. (1956) to determine urates, McNabb et al. (1975) reported that avian urine contains 55 to 72% uric acid, 11 to 21% ammonia and 2 to 11% urea. No significant changes in the proportions of these nitrogenous compounds could be attributed to dietary protein intake or changes in water availability.

Bell and Bird (1966) were unable to detect urate in the cecal contents of adult chickens using the murexide test. In this experiment it was not practical to employ UV spectrophotometry in conjunction with uricase as the solutions under examination showed extremely large absorption around 290-295 mu.

Effects of Antibiotics on Cecal Anaerobes

The effect of protein level and penicillin on growth and cecal flora of the chick was studied by Anderson et al. (1952). A high level of protein (26%) tended to depress growth and feed efficiency and these effects were reversed when penicillin was included in the diet. The addition of penicillin G Potassium at 10 ppm. to the different protein-level diets resulted in a decreased pH of the cecal contents and increased numbers of anaerobes and microaerophiles. Accordingly, the penicillin was believed to stimulate the growth of aciduric bacteria (lactobacilli). In a later study, Anderson et al. (1953) found that penicillin reduced the numbers of cecal anaerobes. However, the numbers of cecal anaerobes were increased when a combination of penicillin at 10 ppm. and coliform bacteria (atypical strain of E. coli) were fed to the birds.

Gordon et al. (1957-58) noted that penicillin increased the numbers of total anaerobes and decreased the numbers of total aerobes in the intestine of the chicken. Qualitative and quantitative bacterial counts performed on the cecal contents of birds receiving either procaine penicillin at 50 mg./kg. diet or the antibiotic-free diet showed no conspicious differences with the possible exception of the streptococci.

In a study of the cecal flora of penicillin-fed chicks reared in old or new quarters, Lev et al. (1957) observed that the antibiotic eliminated Clostridium welchii. However, in other experiments, these workers found that feeding the antibiotic did not reduce the numbers of Cl. welchii but a marked impairment of toxigenicity, which is equivalent (for the host) to the elimination of the organism was noted.

Role of Antibiotics in Stimulating the Growth of the Chicken

The significant increases in weight gain and feed efficiency resulting from the administration of antibiotics (such as penicillin and the tetracyclenes) to growing chicks was discovered approximately 30 years ago. Since that time numerous studies have been conducted in an attempt to elucidate the mode of action of antibiotics in promoting growth. Coates (1962) and McGinnis (1956) have presented general discussions on the question of antibiotic action and have mentioned that such factors as the age of the bird, environment, nutrition and the presence of disease will alter the response of the chicken to antibiotics. For the most part, the antibiotics exert their effects by altering the microflora of the chicken.

The relation of the environment to antibiotic growth stimulation has been investigated by Hill et al. (1953) and Lillie et al. (1953). These workers reported that a greater antibiotic response to penicillin occurred when the birds had been raised in an "old" environment (quarters previously used for rearing chickens) as opposed to a "new" environment. The growth response to the antibiotics in the old quarters was of a greater magnitude and occurred at an earlier age than the response observed in the new quarters. Bacteriological examination of the cecal microflora of birds by Hill et al. (1953) revealed striking differences between the two types of environments and the manner in which these bacterial types responded to penicillin. These workers agreed with Coates et al. (1952) that at least part of the growth stimulus obtained from penicillin can be attributed to the suppression of a subclinical infection by the antibiotic. Huhtanen and Pensack (1964) reported that penicillin will reverse the growth depression, observed in young chicks, caused by a malabsorption syndrome involving Streptococcus faecalis.

Lev et al. (1957) theorized that antibiotics may stimulate growth by eliminating organisms which produce substances that irritate, thicken and hence decrease permeability of the gut with the consequent impairment of the absorption of nutrients. Lev and his co-workers concluded that the biochemical function of the gut is a more important factor than population trends or the presence or absence of a particular organism in explaining the mode of action of antibiotics. Lev and Forbes (1959) concluded that penicillin at 45 mg./kg. diet stimulated the growth rate of chicks by either eliminating Clostridium welchii from the gut or else decreasing the toxigenicity of the bacteria by

decreasing lecithinase production.

In a discussion of the mode of antibiotics in poultry, Combs (1956) made the following points: the site of action of an antibiotic is the intestinal tract and only the bacteria are affected; the level of antibiotic administered is quite important in relation to the environment of the bird. He noted that published observations on changes in the types and numbers of bacteria in the intestinal tract of chicks and poults are variable and inconclusive.

Combs (1956) originally proposed a list of three possible modes of action of antibiotics in growth promotion. Visek (1964) summarized the proposed mechanisms of growth promotion:

1. Suppression of bacteria responsible for clinical infections usually too mild to be recognized.
2. Production of growth-depressing toxins by bacteria is reduced or eliminated.
3. The antimicrobial agent leads to the synthesis of microbial vitamins which are available to the host or, the destruction of essential nutrients is inhibited.
4. The wall of the intestinal tract becomes thinner thereby increasing the efficiency of absorption of nutrients.

An additional point to be considered in the action of antibiotics is the stimulation of production of as yet unidentified growth stimulating factors (Combs, 1956). Coates et al. (1952) concluded that it is very unlikely that a single mode of action can explain all of the results reported in the literature.

MATERIALS AND METHODS

I. ^{14}C-8-Uric Acid-Glycerol Experiments

A series of experiments were conducted to study the movement of ^{14}C-8-uric acid through the lower digestive tract and circulatory system of adult White Leghorn males. The birds used in these experiments were housed in individual cages measuring 20 cm. x 40 cm. x 45 cm. and received a layer-breeder ration (appendix). The birds were sacrificed by injecting sodium phenobarbitol (320 mg./ml.) at 1 ml./kg. body weight.

1. Introduction into the Cloaca

Two replicates of this experiment were performed and the data combined for statistical analysis. Six experimental birds and a control bird were used in each replicate. To enhance reverse peristalsis of the intestine, the birds had been removed from feed and water 10 hours previous to use (Koike and McFarland, 1966). A blunted 18 g. x 3.75 cm. needle was used to introduce 100 ul. of ^{14}C-8-uric acid glycerol (^{14}C dose) into the cloaca of each bird. These ^{14}C doses contained 100,000 dpm for the first experimental replicate and 78,000 for the second. The control birds received 100 ml. of glycerol; the tissues obtained from these birds were analyzed by liquid scintillation counting to obtain background counts. Approximately 30 minutes prior to the introduction of the ^{14}C doses, each bird had received 0.2 ml

of glycerol into the cloaca. This measure normally induced immediate defecation and thereby prevented the rapid loss of the ^{14}C doses introduced subsequently. In addition, a large band of adhesive tape was used to cover the vent area in order to reduce the loss of the ^{14}C in the feces.

The experimental birds in each replicate were divided into 3 pairs; with a pair of birds being sacrificed at 1 (1H), 2 (2H) and 4 (4H) hours following ^{14}C dosing and their tissues removed for analysis by liquid scintillation counting. Blood samples from the 1H birds were obtained just prior to the sacrifice of the bird. Accordingly, blood samples from the 2H birds were obtained at one hour and two hours following ^{14}C dosing and in the 4H birds at 2 hours and 4 hours. Tissue removed from each bird included both cecum, not to include the ileo-cecal junction; large intestine, to include the ileo-cecal junction and: liver samples in the first experimental replicate, kidney samples in the second replicate. Fecal samples were obtained from a pan beneath each bird and/or from inside the adhesive tape wrapping. Blood samples were allowed to clot overnight and the serum was removed for digestion and counting. The remainder of the tissue and fecal material samples were diluted 1:3 (w/w) in distilled water and homogenized for 1 minute. A 0.5 ml. aliquot of each homogenate or blood serum was placed into a liquid scintillation vial containing 1.5 ml. of Unisol Tissue Solubilizer (Isolab). The vial was tightly capped and tissue digestion proceeded for a minimum of 24 hours at room temperature. At this time, 0.75 ml. of water-free methanol was mixed with the contents of each vial and 12 ml. of Unisol-Compliment (Isolab)

was added. After mixing, a fairly clear scintillation counting cocktail resulted. Color quenching encountered in the analysis of the fecal samples was partially rectified by the addition of several drops of hydrogen peroxide. Each tissue sample was counted on three occasions, an internal standard was then added to each vial and the sample recounted. The scintillation counter and operating conditions are described below:

Scintillation Counter: Nuclear-Chicago 720 Series Liquid scintillation system equipped with a Nuclear-Chicago Model 6725 control module. Tissue samples were counted at 20°C. The individual modules were adjusted to the following settings:
scaler module: minutes: 10; Scaler A: 40K; Scaler B: 40K; Scaler C: 1M, all scalers were set at a low count reject of 0; program calculator: list; program start: auto recycle; program stop: time
timer module: program: auto; repeat count: 1; operation: program
analyzer module: power switch: gate; gate H.V.: 13; Data H.V.: 10; Level 2: 9; Level 3: 0; Level 4: 9; Level 5: 9; data attenuator: 1; Channel A: L3-L4; Channel B: L3-infinity; Channel C: L1-infinity.

2. Injection into the Brachial Vein

Six experimental birds and a control bird were used in this experiment. The birds were anaesthetized with ethyl ether, an incision was made in the abdomen and the ceca of each bird were ligated approximately 1 cm. from the ileo-cecal junction. Each experimental bird received an injection of 100 ul. of ^{14}C-8-uric acid-glycerol containing 108,000

dpm, into the brachial vein. The birds were removed from the ether and allowed to revive. As described previously, blood samples were obtained and the following tissues removed for analysis: ceca, kidney, large intestine and feces.

3. Injection into the Ligated Cecum

Six experimental birds and a control bird were anaesthetized and their ceca ligated in the manner described previously. The experimental birds received a 100 ul. injection of ^{14}C-8-uric acid-glycerol (approximately 110,000 dpm) per cecum. The birds were removed from the ether and allowed to revive. As described previously, blood samples were obtained and the following tissues removed for analysis: ceca, large intestine and kidney.

II. Identification of ^{14}C-8-Uric Acid Metabolites

In this experiment, 12 adult White Leghorn males were utilized: the birds were divided into 3 groups with 3 experimental and one control bird per group. The ceca of all the birds were ligated in the manner described previously. The experimental birds each received a 100 ul. injection of ^{14}C-8-uric acid-glycerol (approximately 100,000 dpm) per cecum. The ceca of all the birds were removed 4 hours after the injection, diluted 1:3 in water and homogenized. Three different extraction procedures discussed below were devised in the attempt to partition the uric acid metabolites into lipid or protein fractions. The ceca of one group of birds were extracted by one of the 3

procedures investigated. Scintillation counting of the various extracts obtained in the following procedures utilized 1.5 ml. of Unisol (Isolab), 0.75 ml. of water-free methanol and 10 ml. of Unisol-Compliment (Isolab) as a complete scintillation cocktail.

Procedure A (Modification of Davidson and Thomas, 1969)

Step

A1 Extract 10 ml. of the cecal homogenate with 40 ml. of a methanol-chloroform-water solution (2:1:8) for 30 seconds in a Vertis blender jar. Place the contents of the jar into a plastic centrifuge tube with a tight fitting lid.

A2 Centrifuge at 2000 rpm for 20 minutes.

A3 Remove chloroform layer with a pipette and place a 0.5 ml. sample into a scintillation vial for counting.

A4 Remove methanol-water layer and count a 0.5 ml. sample.

A5 Place the tissue residue into a blender jar containing 20 ml. of methanol-water (1:1) and homogenize. Remove this homogenate to a centrifuge tube for centrifugation. Use a pipette to draw off the supernatant; count a 0.5 ml. sample.

A6 Add 20 ml. of chloroform-methanol (1:2) to the tissue residue and homogenize and centrifuge as described previously. Remove the supernatant and count a 0.5 ml. sample.

A7 Extract tissue residue with 40 ml. of phenol-acetic acid-water (PAW) (1:1:1), homogenize, centrifuge and count sample of supernatant as described previously.

A8 Repeat PAW extraction as described above.

Procedure B

Step

B1 Place diluted ceca into a Vertis blender jar and homogenize for 20 seconds. Remove homogenate to a plastic centrifuge tube, centrifuge at 2000 rpm for 20 minutes and pour off supernatant into a 16 x 125 mm screw top tube. Obtain 0.5 ml. sample of the supernatant for counting.

B2 Place measured amount of supernatant into a 16 x 125 mm. screw top tube and dilute with ether (1:5 ether:water). Seal tube, shake vigorously for 5 minutes on two occasions, centrifuge briefly, remove ether layer with a pasteur pipette. Place this ether layer into a sealed 16 x 100 mm. tube.

B3 Repeat ether extraction as described in step B2 and combine both ether extracts.

B4 Backwash the ether extracts 1:1 with distilled water, centrifuge and remove the water layer, count a 0.25 ml. sample of this layer.

B5 Dry down the ether extract under a hood, add 1.5 ml. of Unisol (Isolab) to the residue and place into a scintillation vial for counting.

B6 Add ammonium sulfate (AS) solution to measured amount of the tissue residue in a blender jar to achieve a 30% solution of the AS. Homogenize, centrifuge, pour off supernatant, count a 0.25 ml. sample of the supernatant and a 0.25 gm. sample of the tissue residue.

B7 Repeat AS extraction of tissue residue, adjusting to a 50% solution of the AS. Count samples as described in step B6.

B8 Add a 15% trichloroacetic acid (TCA) solution to the 30% AS extract in a 1:3 dilution. Place in blender jar, homogenize, remove to centrifuge tube and centrifuge. Wash the TCA precipitate with water 2-3 times and count a 0.25 gm. sample.

B9 Add the TCA to a measured amount of the original water layer (step B1) and proceed as described in step B8.

Procedure C

Step

C1 Homogenize the ceca (diluted 1:3 with water) in a Vertis blender jar. Dilute the homogenate 1:1 with chloroform and rehomogenize. Place this mixture into a plastic centrifuge tube and centrifuge briefly at 2800 rpm. Remove the chloroform layer with a pipette and place a 0.5 ml. sample into a scintillation vial for counting. Also obtain a 0.25 ml. sample of the water layer for counting.

C2 Dilute the water layer obtained above 1:5 with ethyl ether. Place into a sealed 16 x 125 mm. screw top tube and shake vigorously for 5 minutes on two occasions. Centrifuge this tube briefly at 1000 rpm. Remove the ether layer with a pipette and place into a sealed test tube.

C3 Perform a second ether extraction on the water layer and combine the ether extracts. Backwash the ether extracts with distilled water (1:1) and count a 0.25 ml. sample of this water layer.

C4 Place the ether extracts into a 16 x 100 tube and dry down under hood. Add 1.5 ml. of Unisol (Isolab) and place into a scintillation vial for counting.

C5 Dilute the original water layer (step C1) with a 0.5% Lithium Carbonate solution (1:1), homogenize, centrifuge and count 0.25 ml. of the supernatant.

C6 Repeat the Lithium Carbonate extraction of the water layer two more times as described above, removing 0.25 ml. samples of each supernatant for counting.

III. Growth Medium Determination Experiments

The growth media investigated in these experiments included: Medium 10, Supplemented Uric Acid agar (SUA) (Barnes and Impey, 1974), VLB medium, VLS medium (Beerens and Fievez, 1971), VL-liver extract-fecal extract agar (VL1f) (Barnes and Impey, 1970), Brain-Heart Infusion agar plus 10% fecal extract and 5% liver extract (BHI1f), BGP-hemin-liver extract-fecal extract agar (BGPh1f), BGPh1f-uric acid agar (BGPh1f-UA), Basal-hemin-liver extract-fecal extract-uric acid agar (Basal-h1f-UA), and Basal-hemin-uric acid agar (Basal-hemin-UA) (Barnes and Impey, 1972). These media were prepared on the bench top and placed into an anaerobic glove box (Aranki et al., 1969; Aranki and Freter, 1972). All growth media were stored in the glove box 24-48 hours prior to use. The liver and fecal extracts included in several of the growth media had been prepared in adequate amounts to last for the entire series of studies.

Cecal samples utilized in these experiments were obtained from

White Leghorn males ranging in age from 14 to 18 weeks. The birds were housed in a Jamesway growing battery and received a 17.8% protein layer-breeder ration. The food and water were supplied ad libitum. The birds were destroyed by an overdose of sodium phenobarbitol injected directly into the heart (1 ml./kg. body weight of 320 mg./ml. concentration). The cecal samples obtained from these birds were anaerobically processed in the following manner:

Preparation of cecal samples: The ceca were aseptically removed from the bird and placed into a sterile, tared aluminum dish. The dish was immediately weighed and placed into a sterile Waring blender jar and a 20 cm. square piece of sterilized aluminum foil was placed over the top of the jar. Based on the net weight of the ceca, VPI (Virginia Polytechnic Institute) dilution blank (Holdeman and Moore, 1973) was measured out in a sterilized graduated cylinder to achieve a 10-fold dilution of the ceca (w/v) and added to the jar. A gas cannula consisting of a blunted 18 g. x 15 cm. hypodermic needle connected to a CO_2 gas cylinder was hooked over the edge of the jar. The CO_2 was allowed to flow for approximately 15 seconds at a rate of approximately 500 ml./min. The VPI dilution blank had been previously reduced in the glove box and was maintained in a reduced state outside of the chamber by using the CO_2 gas cannula. Following the CO_2 gassing of the ceca and VPI dilution blank, the aluminum foil was crimped down along the lip of the jar. A sponge-padded block of wood was placed over the top of the jar and the Waring blender switched on for 20-30 seconds. Approximately 10 minutes were required to remove, weigh, dilute, gas and homogenize the ceca of 4 birds. These 4 blender jars were then

transferred into the glove box. Inside the glove box, serial dilutions of 10^{-3} to 10^{-10} were made of each homogenate using a Drummond pipet-aid.

Inoculation of agar plates: The cecal bacteria were enumerated using the drop-plate technique of Miles and Misra (1938). A Pasteur pipette that delivered approximately 1/40 ml. per drop was used. Serial dilutions of each homogenate ranging from 10^{-6} to 10^{-10} were inoculated onto a single agar plate. The inocula was completely absorbed onto the agar within 10 minutes and covered an area approximately 5 mm. in diameter. The plates were inverted and placed into a sealed plastic bag for incubation inside the glove box.

Enumeration of the anaerobes utilizing uric acid was accomplished by counting those colonies surrounded by a clear zone in the agar. Testing of the uric acid growth media for "true" uric acid breakdown is described by Mead and Adams (1975) and Barnes and Impey (1972). The total number of colonies present in each dilution was counted with the aid of a hand-held magnifying glass (2X-3X).

Overgrowth of the agar plates by motile organisms was controlled by partially drying the agar media before use and by using 2% agar concentrations in each type of growth medium.

In Experiment 1, six different growth media were evaluated: Medium 10; VLB; BHIlf; BGPhlf; VLS and VLlf. Cecal samples obtained from 2 birds, designated Al and Bl, were drop-plate inoculated in duplicate. Colony counts were obtained at 24, 48, 72 and 96 hours of incubation and expressed on a wet-weight basis.

In Experiment 2, five growth media were evaluated: VLlf; Medium 10;

VLB; BGPhlf; and BHIlf. Cecal samples obtained from 2 birds, designated A2 and B2, were drop-plate inoculated in triplicate. Colony counts were obtained at 24, 48, 96 and 120 hours of incubation and expressed on a wet-weight basis.

In Experiments 3 and 4, BGPhlf-UA agar and SUA agar were evaluated. In Experiment 3, the cecal samples obtained from 2 birds, designated as A3 and B3, were drop-plate inoculated in duplicate. At 105 hours of incubation, colonies utilizing uric acid and the total number of colonies were counted. These counts were expressed on a wet-weight basis. In Experiment 4, cecal samples obtained from 2 birds, designated as A4 and B4 were drop-plate inoculated in triplicate. Colonies utilizing uric acid and the total number of colonies were counted at 24, 48, 72, 96 and 120 hours of incubation and the counts expressed on a wet-weight basis.

In Experiment 5, three different growth media were evaluated: BGPhlf-UA, Basal-hlf-UA and Basal-hemin-UA, The cecal samples obtained from 2 birds, designated as A5 and B5, were drop-plate inoculated in duplicate. Colonies utilizing uric acid and the total number of colonies were counted at 24, 48, 72, 96 and 120 hours of incubation. These counts were expressed on a wet-weight basis.

IV. Identification of Uric Acid-Utilizing Anaerobic Bacteria

In this study, 12 separate experiments were conducted in an attempt to identify those numerically significant anaerobic bacteria, present in the ceca of White Leghorn males aged 6 or 10 weeks, that were capable of utilizing uric acid. A total of 14 birds were used in each experiment with 10 of the birds receiving an antibiotic-supplemented layer-breeder ration (17.8% protein) for a period of 7 days. The remaining 4 birds, serving as controls, received an antibiotic-free layer-breeder ration for the 7-day period. The birds were housed in pairs, in cages measuring 20 cm. x 40 cm. x 45 cm., and received food and water ad libitum. The individual antibiotics were included in the ration at a growth-promoting level (Feed Additive Compendium, 1975). The following chart lists the antibiotics used and antibiotic concentration.

Antibiotic	Concentration
BMD*	11.0 ppm.
Aureomycin	11.0 ppm.
Flavomycin	2.2 ppm.
Neo-Terra	6.6 ppm.
3-Nitro (Roxarsone)	27.5 ppm.
Lincomycin	4.4 ppm.

*Bacitracin Methylene Disalicylate.

At the termination of each experiment the birds were killed by an overdose of sodium phenobarbitol and their ceca removed aseptically. The cecal samples were anaerobically prepared and drop-plate inoculated, in triplicate, in the manner described previously, onto plates of Basal-hlf-uric acid agar. To obtain bacterial counts corrected to a dry weight basis, each cecal homogenate was poured into a tared petri dish and the net weight of the contents was measured before and after drying in a 70^{0}C oven. The plates were incubated for 7 days at 37°C in the glove box. At the end of the incubation period, counts of colonies utilizing uric acid and the total colony counts were obtained. Isolated colonies demonstrating uric acid breakdown were picked to fresh plates of Basal-hlf-uric acid agar to obtain pure cultures. The pure cultures were subcultured to test for true uric acid breakdown and oxygen tolerance and to obtain 18-24 hour Gram stains. Isolates that failed to demonstrate true uric acid breakdown or that grew aerobically were discarded. The remaining isolates were inoculated onto slants of BGP (Beerens-glucose-phosphate) agar and into 8 ml. portions of PYG (Peptone-yeast extract-glucose) broth. These media were prepared in 16 x 125 mm. screw-top tubes. The growth media were incubated for 48-72 hours or until adequate growth was obtained. At this time, the BGP cultures were placed into a refrigerator at 4°C and the PYG cultures were frozen at -20°C. The BGP slant cultures or, on occasion, the PYG broth cultures were used to inoculate PY (Peptone-yeast extract) broth. The PY cultures were used to inoculate carbohydrate fermentation and biochemical testing media. The PYG broth cultures were thawed and analyzed, by gas chromatography,

for volatile fatty acids, ethanol and non-volatile fatty acids.

Carbohydrate fermentation and biochemical testing procedures

The choice of those particular biochemical and fermentation tests utilized in this study for the identification of Gram-positive isolates utilizing uric acid was dictated by several factors:

1. Reliability of results.
2. Minimal requirement for identification necessitated by the large number of isolates obtained.
3. The desire to make direct comparisons with other workers who have identified chicken cecal anaerobes.

The procedure employed for the identification of the chicken's cecal anaerobes was similar to the "short form" method of Holland et al. (1977b) with some modifications. The carbohydrate fermentation and biochemical tests were conducted using 0.75 ml. volumes of the test media in 16 x 125 mm. screw-top tubes that were stored, inoculated and incubated in the anaerobic glove box. All of the test media were reduced in the glove box for 48 hours prior to use. The test media were inoculated with 2 drops of a 24-48 hr. PY broth culture and tightly sealed. The fermentation test media were reduced and stored for up to one month before use. On occasion, the test media were inoculated with a single drop of a 24-48 hr, PYG broth culture. Prior testing with the PYG broth culture inoculum had indicated that the acidic nature of the inoculum derived from those isolates capable of fermentating the glucose in the PYG broth did not have an effect upon

the ultimate pH of the test media. The carbohydrate (CHO) test media were prepared as 1% solutions in PY broth (soluble starch at 0.5%). The CHO test media were adjusted to pH 7.1, dispensed in 0.75 ml. volumes and autoclaved for 15 minutes at 121°C. The soluble starch solution required heating in order to dissolve completely all of the soluble starch prior to pH adjustment and autoclaving. The following CHO test media were used for the identification of the cecal anaerobes: cellobiose, fructose, lactose, mannose, sucrose, and soluble starch. Peptone-yeast extract broth at pH 7.1 was used as a fermentation base test medium. A lactic acid test medium was prepared as a 0.9% solution of 85% lactic acid in PY broth adjusted to pH 7.5 and autoclaved for 15 min. at 121°C.

Acid production in the CHO and lactic acid test media was determined by adding 1 drop of a 1% Bromothymol Blue Solution (BTB) to each tube following an incubation period of 48-72 hours. A yellow color indicated a positive test: blue-green and green indicated a negative test. A drop of BTB was also added to the inoculated tube of fermentation base. Questionable tests were compared to the fermentation base in order to determine accurate interpretation of these tests.

Indole production testing was conducted in 1% tryptone broth (Difco) (Schrekenberger and Blazevic, 1974) that had been dispensed in 0.75 ml. volumes and autoclaved for 15 min. at 121°C. The reduced tubes of tryptone broth were inoculated and incubated as described above. Indole production was determined by adding 2 drops of Kovac's reagent to each tube: a positive test was indicated by the development of a deep pink surface layer. The uninoculated tryptone broth tubes were stored in the glove box for up to one month prior to use.

The test for nitrate reduction was conducted in a 0.9% nitrate broth (Difco) (Schreckenberger and Blazevic, 1974) prepared and inoculated in the same manner as the tryptone broth and incubated for 48-72 hours. To test for nitrate reduction, a drop each of reagent A (0.8% sulfanilic acid in 5N acetic acid) and reagent B (0.5% alpha-naphthylamine in 5N acetic acid) were added to each tube. A positive test was indicated by the development of a pinkish-red color. A few milligrams of zinc powder were added to all negatives to test for false negatives.

Gas Chromatography procedures:

Two different column packings and sample preparation procedures were used to detect the volatile fatty acid (VFA) and ethanol products and the non-volatile fatty acid (n-VFA) products of the PYG broth cultures of the cecal isolates. A Hewlett-Packard (HP) model 5730 programmable gas chromatograph with a flame ionization detector and equipped with a HP 3380A Integrator-Recorder and a HP 7671A automatic sampler were used. The automatic sampler was equipped with a Hamilton Model 701-N 10 ul. syringe. A HP 19001A glass column, configuration 5, 180 cm. long with 1.25 mm. O.D. and 2 mm I.D. was used in this instrument.

VFA analysis

Column packing: Supelco 3% Carbowax 20M/0.5% H_3PO_4 on 60/80 Carbopack B (Carbowax). This column was conditioned according to the Supelco recommendations. Operating conditions for the chromatograph

were as follows: Nitrogen carrier gas flow: 30 ml./min., oven temperature programmed at 115°C for 4 min., temperature increase from 155° to 190°C at 4°/min. and 190°C held for 8 minutes. The injector and detector blocks were each set at 200°C. The injection port septum was changed every 50 samples. Reconditioning of the Carbowax column was accomplished by injecting 3 ul. portions of water several times with the column temperature at 220°C.

VFA analysis of PYG cultures was accomplished in 21 minutes. The automatic sampler was adjusted to inject 1 ul. samples of the broth cultures onto the Carbowax column every 23 minutes. The syringe wash cycle was set at 5. The integrator-recorder was adjusted to the following settings: stop timer: 20 min., area reject: 1000, slope sensitivity: 0.3 mV./min., attenuation: 4 and chart speed: 0.5 cm./min.

VFA sample preparation

PYG broth cultures were removed from the freezer and thawed at room temperature, vortexed briefly and centrifuged for 25 min. at 1500 rpm. One ml. of each culture supernatant was aseptically removed and placed in a Hewlett-Packard No. 5080-8712 size A reference vial. The sample was acidified with 200 µl. of 9N H_2SO_4. The vials were sealed with teflon-lined aluminum caps (Hewlett-Packard No. 5080-8713) and shaken several times to allow for thorough mixing.

VFA standard

Individual VFAs were dissolved in double-distilled water and titrated against 0.041N KOH to determine their concentrations. The

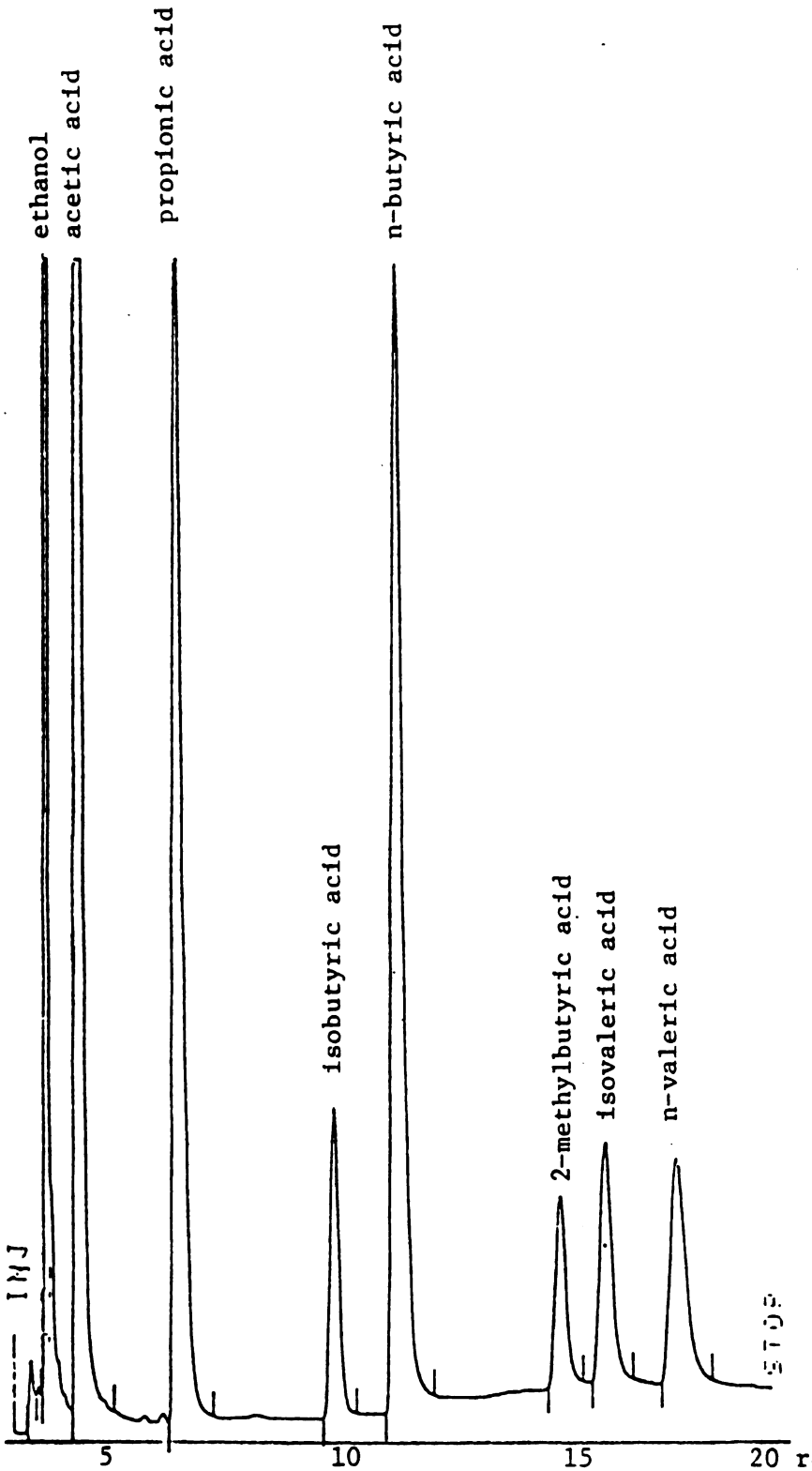

Figure 1. Volatile Fatty Acid Chromatogram.

of the broth culture extracts onto the SP-1000 column every 19 minutes. The integrator-recorder was adjusted to the following settings: stop timer: 15 min., area reject: 1000, slope sensitivity: 0.3 mV./min., attenuation: 4, chart speed: 0.5 cm./min.

n-VFA sample preparation

PYG broth cultures of the cecal isolates were removed from the freezer and thawed at room temperature, acidified to pH 2 or below with several drops of 50% H_2SO_4 and vortexed briefly. The preparation and extraction of the broth cultures for the n-VFA analysis followed the procedure of Holdeman and Moore (1973) in the Anaerobe Laboratory Manual, 2nd. Ed. The chloroform layer of each culture was placed in a HP reference vial as described previously.

n-VFA standard

The individual fatty acids were dissolved in 0.1M H_2SO_4 at approximately 50 meq. concentrations (designated as a stock solution). The n-VFA standard was mixed in the following manner:

lactic acid stock solution	0 -	10 ml.
pyruvic acid stock solution	-	10 ml.
succinic acid stock solution	-	10 ml.
0.1M H_2SO_4	-	70 ml.

A 1 ml. aliquot of this mixture was acidified, methylated and extracted in the manner described for the PYG broth cultures. A 0.2 ml. portion

of the chloroform extract was added to a HP reference vial containing 0.8 ml. of H_2O and sealed.

The retention time of each component in this standard was observed to decrease slightly during the course of the analysis and is expressed in minutes over a range representing initial and final values observed:

pyrivuc acid A	3.84-3.07
pyruvic acid B	7.85-6.82
lactic acid	5.81-4.94
succinic acid	14.06-13.86

Lactic and succinic acids each exhibited a single peak on the chromatogram; however pyruvic acid obtained from several different sources always exhibited 2 peaks. Due to the fact that PYG broth cultures containing pyruvic acid also exhibited 2 peaks, a decision was made that the methylation and extraction procedure used in the sample preparation uniformly resulted in two pyruvic acid peaks.

A typical chromatogram of the n-VFA standard is illustrated in Figure 2.

V. Antibiotic-Agar Plate Experiment

The purpose of this experiment was to observe the Gram reaction and cellular morphology of anaerobic cecal bacteria that had been isolated on Basal-hlf-uric acid agar containing one of several antibiotics. The following list indicates the 10 different growth media, which included 8 different antibiotic-supplemented and 2 types of

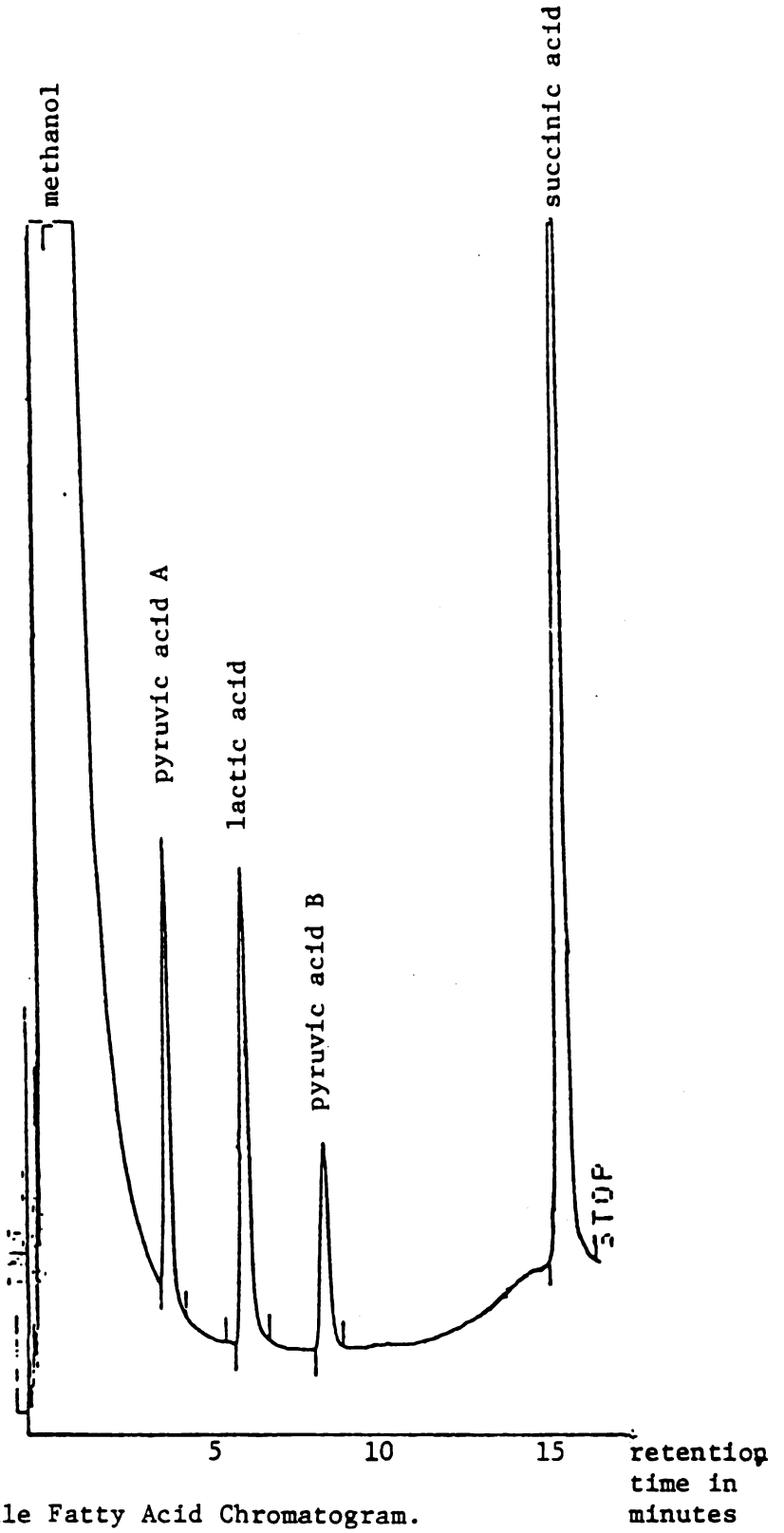

Figure 2. Non-volatile Fatty Acid Chromatogram.

control media, that were utilized in this study. The antibiotics were added to the agar growth media (Basal-hlf-uric acid agar) in concentrations which approximated growth promoting levels in poultry rations.

Antibiotic supplementation to Basal-hlf-uric acid agar

Antibiotic/growth medium	Concentration in agar
Aureomycin	11.0 ppm.
Tylosin	22.0 ppm.
BMD*	11.0 ppm.
3-Nitro	27.5 ppm.
Flavomycin	2.2 ppm.
Lincomycin	4.4 ppm.
Neo-Terra	6.6 ppm.
Furazolidone	11.0 ppm.
Basal-hlf-uric acid agar	0.0
Basal-hlf-agar	0.0

*Bacitracin Methylene Disalicylate.

A Wiley mill, 0.5 mm screen opening, was used to grind the antibiotic premixes prior to their inclusion into the agar. The antibiotics were added, as aqueous solutions or suspensions, to the agar medium that had been previously autoclaved and cooled to 45°C. The

Basal-hlf-uric acid agar plates were poured as a bilayer containing 0.01% uric acid in the lower level and 0.1% uric acid in the upper level. The antibiotic concentration was identical in both layers. In order to allow for proper mixing and maximum dispersion of the antibiotics in the agar plates, the molten agar was initially mixed using a magnetic stirring bar and then swirled frequently during pouring. Inspection of the antibiotic-agar plates indicated that the non-soluble antibiotics were evenly dispersed throughout the agar. The control growth media of the present experiment was the Basal-hlf agar with and without uric acid for the purpose of observing any possible selective action of this supplement.

The freshly poured plates were placed in the anaerobic glove box for 72 hours prior to use to allow for complete reduction, partial drying and growth of any bacterial contaminants. The procedure for antibiotic addition to the growth medium did not result in the concomitant introduction of significant numbers of bacterial contaminants.

Cecal samples:

The ceca were aseptically removed from 3 adult White Leghorn females and anaerobically processed, diluted and drop-plate inoculated, in duplicate, as described previously.

At the end of an incubation period of 6 days, bacterial counts were obtained for each plate. At least one colony of each colonial morphology displayed was transfered onto a fresh plate of the Basal-hlf-UA agar. These "transfer" plates consisted of a single layer medium containing 0.1% uric acid. Following 24 hours of incubation,

the "transfer" plates were removed from the glove box, uric acid utilization was noted and a Gram stain of each isolate was prepared using the Kopeloff modification. The bacterial isolates were classified on the basis of Gram reaction and cellular morphology: rods, cocci and coccal-bacillus (c-b). The bacterial counts were expressed on a dry weight basis.

VI. Uric Acid Quantitation Experiments

A series of experiments were conducted to measure the amounts of uric acid present in the cecal contents and intestinal feces of White Leghorn male chickens at 6, 10 and 24 weeks of age. An attempt was made to alter the cecal uric acid levels in birds 10 weeks old by adding low levels of antibiotics to their diet. The purpose of the first three experiments was to obtain average values for uric acid levels in the cecal contents of chickens of various ages. Uric acid levels were measured in birds aged 6 and 10 weeks for the purpose of comparing these values with the types and numbers of cecal anaerobes utilizing uric acid that were isolated in previous experiments. Uric acid levels were obtained from the birds 24 weeks old in order to permit comparison between the three ages as well as with published values. Fecal samples were analyzed for uric acid content as a check on the accuracy and validity of the uric acid extraction and quantitation procedures employed in this study. With the exception of the birds that received the antibiotics, the same diet was administered to all of the birds included in these studies.

At the time of cecal and fecal sample collection, all of the birds used in these experiments had been receiving a 17.8% protein, layer-breeder ration for a period of 2 weeks. The use of a uniform diet was intended to reduce the variation in cecal and fecal uric acid levels between the different age groups. Accordingly, comparisons between the various ages were more valid. At the end of the experiment, all of the birds appeared to be in good health and presented no gross visceral abnormalities when their ceca were removed.

The antibiotic-supplemented diets were fed to the birds for a period of 7 days. The assumption was made that any influence of the antibiotics on the cecal flora utilizing uric acid would be manifest after this period by an alteration in the level of uric acid detected in the cecal samples.

Uric acid extraction and quantitation procedure:

The method used to extract and quantitate the uric acid present in either cecal feces or intestinal feces follows the procedure of Pudelkiewicz et al. (1968) with minor modifications. These modifications were as follows: All of the fecal or cecal sample solutions were diluted with the 0.1M glycine buffer rather than distilled water. The extract-uricase mixtures were transferred into the cell of the spectrophotometer using a Gilson GME automatic transferrator. Initial extinction readings on test samples could be obtained within 10 seconds of mixing. Spectrophotometer: Hitachi, Perkin-Elmer, Coleman 139 UV-Vis. Selector switch was set at X1.

Uricase Reagent: Uricase Type IV (Sigma) diluted in the glycine buffer to allow for a decrease in the extinction coefficient within a range of .001 to .002 absorbance units/min. measured over a 30 minute time period.

Uric acid standards: A uric acid standard solution was obtained (Sigma) containing 0.05 mg. uric acid/ml. Standard solutions were prepared daily in glycine buffer with 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 ug. uric acid/ml.

Collection of cecal and fecal samples: The chickens used in these experiments were sacrificed by an overdose injection of sodium phenobarbitol. The ceca were removed from the bird and the cecal contents were expressed into an aluminum weight dish.

Intestinal feces were obtained from dropping pans placed beneath the cages of the chickens. Care was taken to avoid including cecal droppings in these samples.

The cecal and fecal samples were dried at 50°C for 24 hours prior to uric acid extraction. Duplicate analysis was performed on each sample in order to determine its uric acid content.

Chickens 6 weeks old:

Cecal and fecal samples were obtained from 20 birds. These birds were reared in a Petersime growing battery and received a 17.8% protein, layer-breeder ration starting at 4 weeks of age.

Chickens 10 weeks old:

Cecal and fecal samples were obtained from 19 birds. These birds were individually housed in cages measuring 20 cm. x 40 cm. x 45 cm. and received the layer-breeder ration starting at 6 weeks of age.

Chickens 24 weeks old:

Cecal and fecal samples were obtained from 15 birds. These birds were housed in cages as described above and received the layer-breeder ration starting at 16 weeks of age.

Antibiotic-fed Chickens:

In this study, 70 birds aged 10 weeks were used. The birds were divided into groups of 10 and were housed in a Petersime growing battery. Six of the groups received a layer-breeder ration that had been supplemented with a single antibiotic at a growth promoting level (Feed Additive Compendium, 1975) for a period of 7 days. The remaining group of 10 birds served as a control and received an antibiotic-free ration. The antibiotics used and their concentrations in the diet are listed below:

Antibiotic	Concentration
BMD*	11.0 ppm.
Aureomycin	11.0 ppm.
Flavomycin	2.2 ppm.
Neo-Terra	6.6 ppm.
3-Nitro	27.5 ppm.
Lincomycin	4.4 ppm.

*Bacitracin Methylene Disalicylate

These birds were sacrificed and cecal samples obtained in the manner described previously. Each of the samples was analyzed, in duplicate, to determine uric acid content.

RESULTS

I. ^{14}C-8-Uric Acid-Glycerol Experiments

Complete data from these experiments are listed in the Appendix (Tables 36-38).

1. Introduction into the Cloaca

The results from this experiment are summarized in Table 1. An average of 76% of the ^{14}C added to each bird was recovered with the total recovery ranging from 37 to 111%. Cecal samples contained 11% of the total ^{14}C dose on the average, with recovery in this tissue ranging from 5-25%. The average large intestinal sample contained 9% of the ^{14}C dose. The range was 1-56% for this tissue. Fecal samples accounted for 9-79% of the total ^{14}C dose added to each bird with the average sample containing 39%. Liver and kidney samples and blood samples obtained 4 hours post-introduction contained background levels only. Blood samples obtained 1 and 2 hours following introduction of the ^{14}C dose into the cloaca contained very small amounts of the ^{14}C dose on the average at 0.9 and 0.7%, respectively.

2. Injection into the Brachial Vein

The results from this experiment are summarized in Table 2. Total recovery of the ^{14}C injected ranged from 63-108% and averaged 82%. Cecal and kidney samples contained no ^{14}C above background levels.

Table 1. Summary Table: Recovery of ^{14}C Observed in Various Tissues of Adult White Leghorn Males Following Introduction of ^{14}C-8-Uric Acid-Glycerol into the Cloaca.*

Tissue	No. of Samples Analyzed	Percent Recovery of Total ^{14}C Introduced**	
		Range	Mean ± S. D.
ceca	12	5-25	11 ± 7
large intestine	12	1-56	9 ± 14
feces	12	9-79	39 ± 27
liver	6	0	0
kidney	6	0	0
Blood Samples			
1 hr. post-introduction	8	0-3.6	0.9 ± 0.7
2 hrs. post-introduction	8	0-0.3	0.7 ± 0.5
4 hrs. post-introduction	4	0	0
Total recovery per bird		37-111	76 ± 24

* Data obtained from 12 birds with duplicate analysis of tissues.

**Data converted to log values for statistical analysis.

Table 2. Summary Table: Recovery of ^{14}C Observed in Various Tissues of Adult White Leghorn Males Following Injection of ^{14}C-8-Uric Acid-Glycerol into the Brachial Vein*.

Tissue	No. of Samples Analyzed	Percent Recovery of Total ^{14}C Injected**	
		Range	Mean ± S.D.
ceca	6	0	0
kidneys	6	0	0
large intestine	6	0-11	4.3 ± 5.0
feces	6	50-98	71 ± 22
Blood Samples			
1 hr. post-injection	4	2-4	2.6 ± 0.9
2 hrs. post-injection	4	0-2	1.4 ± 0.6
4 hrs. post-injection	2	0	0
Total recovery per bird		63-108	82 ± 21

* Data obtained from 6 birds with duplicate analysis of tissues.

**Data converted to log values for statistical analysis.

A range of 0-11% of the ^{14}C dose was recovered in the large intestinal samples with an average recovery of 4.3%. An average of 71% of the total ^{14}C dose was recovered in the fecal samples. Recovery in the feces ranged from 50-98%. Blood samples obtained at 1 hour post-injection contained an average of 2.6% of the total dose and blood samples obtained at 2 hours averaged approximately one-half of the 1 hour samples: 1.4%. At 4 hours following ^{14}C injection blood samples contained only background counts of the ^{14}C.

3. Injection into the Ligated Ceca

The results from this experiment are summarized in Table 3. Total recovery of the ^{14}C injected per bird averaged 44% and ranged from 29-74%. Approximately 41% of the ^{14}C injected was recovered in the average cecal sample. Recovery of ^{14}C in the cecal samples ranged from 29-73%. On the average, the large intestinal samples and blood samples obtained 1 and 2 hours post injection contained very small amounts of the ^{14}C dose: 0.2%, 0.3% and 0.6%, respectively. Blood samples obtained 4 hours post injection and kidney samples contained only background levels of the ^{14}C.

II. Identification of ^{14}C-8-Uric Acid Metabolites

In general, none of the extraction procedures investigated provided clear, consistent or otherwise satisfactory information that was useful in partitioning ^{14}C-8-uric acid metabolites into either lipid or protein fractions.

Table 3. Summary Table: Recovery of ^{14}C Observed in Various Tissues of Adult White Leghorn Males Following Injection of ^{14}C-8-Uric Acid-Glycerol into the Ligated Ceca.*

Tissue	No. of Samples Analyzed	Percent Recovery of Total ^{14}C Injected**	
		Range	Mean ± S.D.
ceca	6	29-73	41 ± 16
large intestine	6	0-1	.2 ± 0.1
kidney	6	0	0
Blood Samples			
1 hr. post-injection	4	0-1.2	0.3 ± 0.5
2 hrs. post-injection	4	0.4-1.0	0.6 ± 0.2
4 hrs. post-injection	2	0	0
Total recovery per bird		29-74	44 ± 18

* Data obtained from 6 birds with duplicate analysis of tissues.

**Data converted to log values for statistical analysis.

III. Growth Medium Determination Experiments

The analysis of variance for Experiment 1 is presented in Table 4. The mean square values and degrees of freedom for all the interactions were pooled for the calculation of the error mean square. Accordingly: differences in counts among the media and among the incubation times were highly significant ($P \leq .01$) and the differences in counts between the birds were significant ($P \leq .05$).

The counts of cecal anaerobes observed per bird in Experiment 1 are shown in Table 5. The average difference in counts obtained from bird B1 over bird A1 was approximately Log 0.013.

The counts of cecal anaerobes observed over time for each growth medium in Experiment 1 are shown in Table 6. The Duncan's multiple range test was used to rank the mean numbers of bacteria recovered on the various growth media from the highest counts to the lowest counts. Accordingly: of those media tested, BGPhlf agar, in particular, and BHIlf agar were each capable of supporting the growth of the highest number of bacteria. The remaining growth media investigated were able to support decreasing numbers of cecal bacteria in the following order: VLlf, VLB, VLS and Medium 10 with Medium 10 supporting the lowest number of bacteria of those media investigated. Statistical analysis of the mean number of bacteria observed for each incubation time using the Duncan's multiple range test indicated that the bacterial counts increased through 24 to 72 hours of incubation but did not increase significantly from 72 to 96 hours of incubation (Table 6).

The analysis of variance for experiment 2 is presented in Table 7. The mean square values and degrees of freedom for all the

Table 4. Analysis of Variance for Experiment 1.

Source of Var.	d.f.	M.S.
Media	5	8.7147**
Birds	1	.1266*
Time	3	2.5348**
Error	38	.0243

* Significant at $P \leq .05$.

**Significant at $P \leq .01$.

Table 5. Counts of Cecal Anaerobes Observed per Bird in Experiment 1.

Incubation Time (hours)	Log Counts/gm. Wet Weight	
	Bird A1	Bird B1
24	9.014±1.234*	9.219± .801
48	9.795± .986	9.831±1.001
72	9.960± .956	10.096±1.094
96	10.124± .956	10.161±1.113
Mean	9.723± .492	9.826± .367

*Average of 6 values ±S.D.

Table 6. Counts of Cecal Anaerobes Observed Over Time for each Growth Medium in Experiment 1.

Growth Medium	Log Counts/gm. Wet Weight Incubation Time (Hours) 24	48	72	96	Mean**
BGPhlf	9.916± .225*	10.622±.214	10.840±.315	10.865±.302	10.485 a
BHIlf	9.836± .184	10.358±.222	10.552±.073	10.555±.094	10.325 abc
VLlf	9.511± .154	10.265±.248	10.639±.191	10.639±.191	10.263 bc
VLB	9.642±..389	10.159±.229	10.396±.255	10.491±1.93	10.172 c
VLS	8.872± .316	9.741±.130	9.940±.228	10.139±.173	9.673 d
Medium 10	7.253±1.109	7.797±.142	8.003±.424	8.078±.404	7.850 e
Mean**	9.172 a	9.824 b	10.062 c	10.128 c	

* Average of 6 values ±S.D.

**Values with the same letter are not significantly different.

Table 7. Analysis of Variance for Experiment 2.

Source of Var.	d.f.	M.S.
Media	4	2.1292*
Bird	1	15.6333**
Time	3	.8536
Error	31	.3304

* Significant at $P \leq .05$.

**Significant at $P \leq .01$.

interactions were pooled to calculate the error mean square. Accordingly: differences in counts among the media were significant and differences in counts between the birds were highly significant.

The counts of cecal anaerobes observed per bird in Experiment 2 are shown in Table 8. The average differences in bacterial counts obtained from bird A2 over bird B2 were approximately Log 0.345.

The counts of cecal anaerobes observed over time for each growth medium in Experiment 2 are shown in Table 9. The Duncan's multiple range test was used to rank the mean numbers of bacteria recovered on the various growth media from the highest counts to the lowest counts. In this experiment, BGPhlf, BHIlf and VLB agars were each capable of supporting the growth of the highest numbers of bacteria of those media investigated. The VLlf agar supported the growth of fewer bacteria than the above mentioned media. Medium 10 supported the growth of the lowest number of bacteria compared to the other media investigated in Experiment 2.

The analysis of variance for the uric acid counts observed in Experiment 3 is presented in Table 10. Accordingly, differences in counts between the media or the birds were not statistically significant.

The analysis of variance for the total bacterial counts observed in Experiment 3 is shown in Table 11. Accordingly: differences in counts between the media and the birds were not statistically significant.

The analysis of variance for the ratios of uric acid counts to total counts in Experiment 3 is presented in Table 12. Accordingly,

Table 8. Counts of Cecal Anaerobes Observed per Bird in Experiment 2.

Incubation Time (hours)	Log counts/gm. Wet Weight	
	Bird A2	Bird B2
24	9.862±.611*	8.817±.484
48	10.322±.857	9.028±.446
72	10.506±.912	9.159±.268
120	10.675±.978	9.382±.299
Mean	10.342 .349	9.097 .237

*Average of 6 values ±S.D.

Table 9. Counts of Cecal Anaerobes Observed Over Time for each Growth Medium in Experiment 2.

Growth Medium	Log counts/gm. Wet Weight Incubation Time (Hours) 24	48	96	120	Mean**
BGPhlf	9.389±1.089*	9.999±1.447	10.082±1.356	10.719±1.079	10.047 a
BHIlf	9.897± .585	10.036± .700	10.094± .760	10.126± .814	9.995 a
VLB	9.448±1.003	9.970±1.103	10.239±1.351	10.323±1.339	10.038 a
VLlf	9.398± .472	9.668± .651	9.798± .668	9.989± .622	9.713 b
Medium 10	8.569± .442	8.702± .518	8.999± .710	9.076± .656	8.836 c
Mean	9.340	9.675	9.843	10.028	

* Average of 6 values ±S.D.

**Values with same letter are not significantly different.

Table 10. Analysis of Variance for Uric Acid Counts in Experiment 3.

Source of Var.	d.f.	M.S.
Media	1	1.2073
Birds	1	.5748
Error	1	.3045

Table 11. Analysis of Variance for Total Counts in Experiment 3.

Source of Var.	d.f.	M.S.
Media	1	.7194
Birds	1	.0513
Error	1	.3626

Table 12. Analysis of Variance for Uric Acid: Total Counts in Experiment 3.

Source of Var.	d.f.	M.S.
Media	1	.0019
Birds	1	.0004
Error	1	.1614

the differences in counts between the media or the birds were not statistically significant.

The counts of total cecal anaerobes and anaerobes utilizing uric acid observed for each growth medium in Experiment 3 are shown in Table 13. These results indicate that, on the average, approximately one third of the number of cecal anaerobes recovered were able to utilize uric acid. The ability of the BGPhlf-UA agar to support the growth of greater numbers of cecal anaerobes than the SUA agar was not statistically significant (Table 13).

The counts of total cecal anaerobes and anaerobes utilizing uric acid (uric acid counts) observed per bird in Experiment 3 are shown in Table 14. These results indicate that an average of one third of the bacteria isolated from Birds A3 and B3 were able to utilize uric acid. However, approximately 3 times as many uric acid counts were observed in the cecal samples of Bird B3 compared to bird A3.

The analysis of variance for Experiment 4 is presented in Table 15. The mean square values and degrees of freedom for all the interactions were combined to calculate the error mean square. Accordingly: differences in counts between the birds investigated and among the various incubation times were significant.

The counts of cecal anaerobes utilizing uric acid observed for each growth medium in Experiment 4 are shown in Table 16. Statistical analysis of the mean number of uric acid bacteria observed for each incubation time using the Duncan's multiple range test indicated that these counts increased from 24 to 48 hours of incubation, however, the maximum uric acid counts were obtained within 72 hours of

Table 13. Counts of Total Cecal Anaerobes and Anaerobes Utilizing Uric Acid Observed for Each Growth Medium in Experiment 3.

Growth Medium	Log Counts/gm. Wet Weight		Percent of Total
	Uric Acid Counts	Total Counts	
SUA	9.797±.768*	10.339±.544	29
BGPhlf-UA	10.896±.192	11.258±.244	45
Mean	10.347±.777	10.798±.649	35

*Average of 4 values ±S.D.

Table 14. Counts of Total Cecal Anaerobes and Anaerobes Utilizing Uric Acid Observed per Bird in Experiment 3.

Bird	Log Counts/gm. Wet Weight		Percent of Total
	Uric Acid Counts	Total Counts	
A3	9.967±.958*	10.649±.870	21
B3	10.725±.359	10.947±.322	60
Mean	10.346±.536	10.798±.211	36

*Average of 4 values ±S.D.

Table 15. Analysis of Variance for Experiment 4.

Source of Var.	d.f.	M.S.
Media	1	.2696
Birds	1	2.1639*
Time	4	2.7460*
Error	13	.1608

*Significant at $P \leq .05$

Table 16. Counts of Cecal Anaerobes Utilizing Uric Acid Observed for each Growth Medium in Experiment 4.

Incubation Time (hours)	Log Counts/gm. Wet Weight Growth Medium BGPh1f-UA	SUA	Mean**
24	9.494±.740*	8.740±1.297	9.112 a
48	10.596±.115	10.483±.417	10.535 b
72	10.783±.162	10.882±.368	10.833 c
96	11.002±.316	10.981±.306	10.992 c
120	11.404±.454	11.031±.264	11.218 c
Mean	10.656±.716	10.387±1.133	

* Average of 4 values ±S.D.

**Values with the same letter are not significantly different.

incubation.

The counts of cecal anaerobes utilizing uric acid observed per bird in Experiment 4 are shown in Table 17. These results indicate a marked similarity in the number of uric acid bacteria observed for either bird at each incubation time. Additionally, the average number of uric acid bacteria obtained from birds A4 and B4 were quite similar; differing by log 0.103 counts.

The analysis of variance for uric acid counts in experiment 5 is shown in Table 18. The mean square values and degrees of freedom for all interactions were pooled for the calculation of the error mean square. Accordingly, differences in counts among the media, incubation times and between the birds were highly significant.

The analysis of variance for total counts in Experiment 5 is presented in Table 19. The mean square values and degrees of freedom for all interactions were pooled for the calculation of the error mean square. Accordingly, differences in counts among the media were highly significant and differences in counts among incubation times were significant.

The analysis of variance for the ratio of uric acid to total counts in Experiment 5 is presented in Table 20. The mean square values and degrees of freedom for all interactions were pooled for the calculation of the error mean square. Accordingly, difference in the ratio of uric acid to total counts were not statistically significant with respect to media used, incubation time and bird investigated.

The counts of total cecal anaerobes and anaerobes utilizing uric acid for each growth medium in Experiment 5 are shown in Table 21.

Table 17. Counts of Cecal Anaerobes Utilizing Uric Acid Observed per Bird in Experiment 4.

Incubation time (hours)	Log Counts/gm. Wet Weight	
	Bird A4	Bird B4
24	9.377±1.076*	8.857±1.136
48	10.517± .335	10.562± .284
72	10.904± .159	10.761± .359
96	10.968± .126	11.115± .380
120	11.214± .422	11.165± .372
Mean	10.596± .867	10.493± .948

*Average of 4 values ±S.D.

Table 18. Analysis of Variance for Uric Acid Counts in Experiment 5.

Source of Var.	d.f.	M.S.
Media	2	2.0805**
Birds	1	.9763**
Time	4	.8070**
Error	22	.1025

** Significant at $P \leq .01$.

Table 19. Analysis of Variance for Total Counts in Experiment 5.

Source of Var.	d.f.	M.S.
Media	2	1.6996**
Birds	1	.5227
Time	4	.5424*
Error	22	.1299

**Significant at $P \leq .01$

* Significant at $P \leq .05$

Table 20. Analysis of Variance for Uric Acid: Total Counts in Experiment 5.

Source of Var.	d.f.	M.S.
Media	2	.0002
Birds	1	.0010
Time	4	.0007
Error	22	.0015

Table 21. Counts of Total Cecal Anaerobes and Anaerobes Utilizing Uric Acid Observed for each Growth Medium in Experiment 5.

Growth Medium	Log Counts/gm. Wet Weight		Percent of Total
	Mean** Uric Acid Counts	Mean** Total Counts	
BGPhlf-UA	10.683±.498*a	10.956±.337 c	53
Basal-hlf-UA	10.890±.312 a	11.127±.209 c	58
Basal-hemin-UA	10.017±.513 b	10.342±.563 d	47
Mean	10.530±.456	10.808±.413	53

* Average of 10 values ±S.D.

**Values with the same letter are not significantly different.

Statistical analysis, using Duncan's multiple range test, of mean uric acid counts and mean total counts for each growth medium investigated in Experiment 5 indicated that BGPhlf-UA and Basal-hlf-UA agars were superior to Basal-hemin-UA agar in the recovery of uric acid bacteria and in recovering the highest total number of bacteria. In this experiment, approximately one-half of the bacteria isolated were able to utilize uric acid.

The counts of total cecal anaerobes and anaerobes utilizing uric acid observed over time in Experiment 5 are shown in Table 22. The statistical analysis of these data using the Duncan's multiple range test indicated that maximum counts of uric acid bacteria were observed within 48-72 hours of incubation and that maximum total bacterial counts were observed within 48 hours of incubation. Total bacterial counts did not increase significantly beyond 48 hours of incubation.

IV. Identification of Uric Acid-Utilizing Anaerobic Bacteria

In this study, 410 Gram-positive, obligately anaerobic, cecal isolates utilizing uric acid were obtained from chickens fed the control and antibiotic supplemented diets. A very small percentage (1-2%) of the uric acid-utilizing bacteria isolated were Gram-negative and attempts were not made to identify these few isolates. The Gram-positive cecal isolates obtained were composed of four genera: Propionibacterium, Lactobacillus, Peptostreptococcus and Eubacterium divided into 20 different groups (Table 23). Groups 1, 13, 20, 23 and 24 had been previously identified and discussed by Salinitro et al.

Table 22. Counts of Total Cecal Anaerobes and Anaerobes Utilizing Uric Acid Observed Over Time in Experiment 5.

Incubation Time (hours)	Log Counts/gm. Wet Weight	
	Mean** Uric Acid Counts	Mean** Total Counts
24	9.984±.625*a	10.311±.655 d
48	10.353±.599 b	10.801±.424 e
72	10.625±.584 c	10.871±.429 e
96	10.717±.479 c	10.943±.370 e
120	10.803±.381 c	11.112±.394 e

* Average of 6 values ±S.D.

**Values with the same letter are not significantly different.

Table 23. Identification of Bacterial Groups Utilizing Uric Acid That were Isolated from Chicken Ceca.

Group No.	Designation	Reference
1	*Propionibacterium acnes* Group IV a	Salinitro *et al*. 1974a
2	*P. acnes*	VPI Manual*
5	*Lactobacillus spp.*	VPI Manual
6	*L. acidophilus*	VPI Manual
8	*Peptostreptococcus spp.*- unidentified	
10	*Peptostreptococcus* Group J	Barnes and Impey, 1974 Group NE 1/71
11	*P. intermedius*	VPI Manual
12	*Peptostreptococcus* Group K	Barnes and Impey, 1974 Group NE 3/250
13	*Peptostreptococcus* Group III	Salinitro *et al*. 1974a
14	*P. productus* Group Ha	Barnes and Impey, 1974
16	*Eubacterium* Group I	Barnes and Impey, 1974 Group 3/191
17	*Eubacterium* Group 2	Barnes and Impey, 1974 Group 3/198
18	*E. tortuosum*	VPI Manual
19	*E. ventriosum*	VPI Manual
20	*Eubacterium* Group 5	Salinitro *et al*. 1974a Group IVc
21	*E. limosum*	VPI Manual
22	*Eubacterium* Group 3	Barnes and Impey, 1974 Group NE 3/197

Table 23. Continued.

Group No.	Designation	Reference
23	Eubacterium Group 4	Salinitro et al. 1974a Group IVb
24	Eubacterium Group 6	Salinitro et al. 1974a Group IVd
26	Eubacterium spp. unidentified	

*Anaerobe Laboratory Manual, 2nd Edition, Virginia Polytechnic Institute and State University. Eds. L. V. Holdeman and W. E. C. Moore, 1973.

(1974a). Barnes and Impey (1974) had previously identified and discussed Groups 10, 12, 14, 16 and 17. The remaining cecal isolates were identified by referring to the VPI Anaerobe Manual (Holdeman and Moore, 1973). Groups 8 and 26 were composed of unidentified species of Peptostreptococcus and Eubacterium, respectively. Uric acid-utilizing isolates were placed into the "unidentified" groups (8 and 26) on the basis of Gram morphology and gas chromatographic analysis. The complete identity of these particular isolates was not determined due to difficult interpretation or extreme variation of the biochemical and carbohydrate test results, loss of viable BGP agar slant cultures and, in some cases, lack of sufficient types and numbers of biochemical and carbohydrate fermentation tests. In many cases, reevaluation of an isolate, difficult to classify with existing tests, would result in a complete identification of that isolate. In the present study GC analysis of the PYG cultures was a valuable aid in the interpretation of the Gram morphology of the organisms. The Gram morphology of the cecal isolates obtained from the agar plates was consistently less diverse than that obtained from either PY or PYG broth cultures. For this reason, cellular sketches included in the VPI manual required some experienced interpretation in order to be useful. The reference strains for the 20 groups of cecal anaerobes utilizing uric acid isolated in this study are listed in Table 24.

Due to the great diversity of bacterial groups encountered in this study and also to the sampling method utilized, no trends, groupings or significant differences in the cecal population could be assigned to differences in age or antibiotic supplementation.

Table 24. Reference Strains for Chicken Cecal Anaerobic Isolates.

Group	1[1]	2	5	6	8
Gram Reaction	+	+	+	+	+
aerobic growth	-	-	-	-	-
cell morphology[2]	extremely pleomorphic rods-some palisading	pleomorphic rods	rods	large rods	cocci to coccal-bacillus, some chains
products[3]	PROPIONIC acetic butyric lactic succinic	PROPIONIC acetic	LACTIC acetic succinic	LACTIC acetic succinic	ETHANOL ACETIC some: lactic succinic pyruvic
cellobiose	- [+4]	- [+]	- w	+	±
fructose	- [+]	+	+	+ [-]	+
lactose	- [5]	-	+	+	+ [-]
mannose	+ [6]	+ [-9]	± w	+	- [+]
sucrose	- w[7]	-	+	+	±
starch pH	- w	±	-	+	- [+]
lactate	+	+	-	-	- [+]
indol	± [8]	+ [-]	+ [-]	-	- [+]
nitrate	±	+ [-]	+ [-]	-	- [+]
uric acid	+	+	+	+	+

Table 24. Continued.

Group	10	11	12	13	14
Gram Reaction	+	+	+	+	+
Aerobic growth	-	-	-	-	-
Cell Morphology	cocci in chains	cocci in chains	elongated cocci-pointed ends	cocci	coccal-bacillus, singles, short tangled chains
Products	ACETIC ETHANOL	LACTIC ACETIC SUCCINIC	ACETIC ethanol latic	LACTIC ACETIC propionic	LACTIC SUCCINIC acetic
cellobiose	-	+	+	+	+
fructose		+			+
lactose	+	+	+	+	+
mannose		+		+	+
sucrose				+	
starch pH	-	±	-	-	- ^{w10}
lactate	-	-	-		-
indol	-	- $^{+}$	-	+	-
nitrate	-	-	-	-	-
uric acid	+	+	+	+	+

Table 24. Continued.

Group	16	17	18	19	20
Gram reaction	+	+	+	+	+
aerobic growth	-	-	-	-	-
Cell Morphology	rods pointed ends twisted chains	short rods few pairs long forms and spindles	long rods long wavy chains	coccal-bacillus shorts chains, pairs	short rods, slender, small to medium size, in chains
Products	LACTIC SUCCINIC	SUCCINIC acetic propionic	LACTIC BUTYRIC SUCCINIC acetic	LACTIC SUCCINIC	LACTIC butyric
cellobiose	-	+	± d[11]	±	-
fructose			- w	+ w	+ w
lactose	+	+	± d	+ -	-
mannose			-	+ w	-
sucrose			+ w	±	+
starch pH	-	-	-	- +	-
lactate	-	+	-	-	- +
indol	-	-	-	-	-
nitrate		-	±	-	-
uric acid	+	+	+	+	+

Table 24. Continued.

Group	21	22	23	24	26
Gram reaction	+	+	+	+	+
Aerobic Growth	-	-	-	-	-
Cell Morphology	long pleomorphic rods may be short chains	slender rods, singles pairs, short chains cells at right angles	rods-oval shaped medium to large in size singles, pairs chains	rods medium to large in size long chains	rods medium to large in size
Products	LACTIC ACETIC BUTYRIC succinic propionic	ETHANOL acetic lactic	LACTIC BUTYRIC ACETIC	ACETIC SUCCINIC	SUCCINIC ETHANOL ACETIC LACTIC some: n-butyric pyruvic i-valeric
cellobiose	- w	-	-	-	-
fructose	+		+	+	±
lactose	- w	-	+ w	+	+
mannose	- +w		- w	-	±
sucrose	- w		-	+	+ -
starch pH	± w	-	-	-	±
lactate	+	-	-	-	±
indol	-	-	-	-	-
nitrate	-	-	-	-	- +
uric acid	+	+	+	+	+

Table 24. Continued.

1. Group identity listed in Table 23.
2. As determined for PY, PYG broth cultures and in some cases, for agar plate colonies.
3. Acids listed in capital letters represent a primary major product of the isolate, acids listed in small case letters represent secondary products of the isolates.
4. Most cultures negative, occasional positive culture
5. All cultures negative.
6. All cultures positive.
7. Most cultures negative, occasional weakly positive culture.
8. Variable reactions.
9. Most cultures positive, occasional negative culture.
10. Most cultures positive, occasional weakly negative culture.
11. Delayed reaction.

Overall, the 20 groups of bacteria isolated in this study from drop-plate replicates of individual cecal samples were present in numbers averaging 4.5×10^{11} bacteria/gm. dry weight. The counts ranged from 1.5×10^{10} to 5.12×10^{12} bacteria/gm. dry weight.

The distribution of the 20 bacterial groups obtained from the cecal samples of birds fed the control diet versus, collectively, those of birds fed the antibiotic supplemented diets are listed in Table 25. In general, most of the bacterial groups were present in similar percentages of the total number of isolates obtained from either the control diet or antibiotic diet fed chickens. The actual in vivo distribution of the various bacterial groups utilizing uric acid in birds of either 6 or 10 weeks of age, receiving either a control diet or an antibiotic supplemented diet could not be determined in this experiment.

V. Antibiotic Agar Plate Experiment

In this study, 741 individual colonies or representative colonial types from 24 hr. cultures were examined for Gram reaction and cellular morphology. The total number of colonies that were observed on the 6 agar plates for each type of growth medium, the percent of the total colony count observed per growth medium and also a numerical and percentage listing of the different types of colonies recovered on each type of medium are shown in Table 26. The occasional Gram-negative coccal-bacillus recovered in this study was grouped with the Gram-negative cocci counts.

The selective activity of several of the antibiotics used in this

Table 25. Distribution of Cecal Isolates Utilizing Uric Acid in Chickens Fed Either a Control Diet or an Antibiotic Supplemented Diet.

Group No.	Group Designation	Number of Cecal Isolates	
		Control	Antibiotic-fed
1	*Propionibacterium acnes* Group IVa	2 (2)*	11 (4)
2	*P. acnes*	4 (3)	9 (3)
5	*Lactobacillus spp.*	7 (5)	5 (2)
6	*L. acidophilus*	0 (0)	1 (1)
8	*Peptostreptococcus spp.* (unidentified)	8 (6)	17 (6)
10	*Peptostreptococcus* Group J	4 (3)	6 (2)
11	*P. intermedius*	5 (4)	7 (7)
12	*Peptostreptococcus* Group K	11 (9)	38 (12)
13	*Peptostreptococcus* Group III	0 (0)	6 (2)
14	*P. productus* Group Ha	7 (5)	12 (4)
116	*Eubacterium* Group 1	8 (7)	27 (9)
17	*Eubacterium* Group 2	6 (5)	12 (4)
18	*E. tortuosum*	2 (2)	12 (4)
19	*E. ventriosum*	14 (12)	22 (8)
20	*Eubacterium* Group 5	3 (3)	2 (1)
21	*E. limosum*	3 (3)	10 (3)
22	*Eubacterium* Group 3	10 (8)	21 (7)
23	*Eubacterium* Group 4	5 (4)	6 (2)

Table 25. Continued.

Group No.	Group Designation	Number of Cecal Isolates	
		Control	Antibiotic-fed
24	Eubacterium Group 6	7 (5)	22 (7)
26	Eubacterium spp. (unidentified)	17 (15)	41 (16)
	TOTALS =	123	287

*Indicates the percentage of the total number of isolates obtained from either the control diet or antibiotic-diet fed chickens.

Table 26. Numbers and Types of Bacterial Colonies Observed per Growth Medium in the Antibiotic-Agar Plate Experiment.

Growth Medium	Total Number of Colonies	% of Total Colony Count	Gram Reaction and Cellular Morphology					Summary	
			Rods		Cocci		Coccal-bacillus	%	%
			Gram+	Gram-	Gram+	Gram-	Gram+	Gram+	Gram-
Furazolidone	51	7	36(70)*	0	4(8)	5(10)	6(12)	78	22
Tylosin	47	6	18(38)	22(47)	3(6)	0	4(9)	53	47
Lincomycin	78	11	2(2)	38(49)	31(40)	7(9)	0	42	58
Neo-Terra	56	8	10(18)	6(11)	16(28)	11(20)	13(23)	69	31
BMD**	127	17	20(16)	81(64)	6(5)	15(11)	5(4)	25	75
Aureomycin	55	7	19(35)	19(35)	1(2)	8(14)	8(14)	51	49
Flavomycin	106	14	49(46)	12(11)	26(25)	5(5)	14(13)	84	16
3-Nitro	53	7	7(13)	15(28)	12(23)	8(15)	11(21)	57	43
Basal-hlf-UA	79	11	15(19)	19(24)	17(22)	9(11)	19(24)	65	35
Basal-hlf	89	12	29(35)	21(23)	21(23)	11(11)	7(8)	66	34
Total	741	100							
Control Summary	168	23	44(26)	40(24)	38(23)	20(12)	26(15)	64	36

* Percent of total per growth medium shown in parenthesis. **Bacitracin Methylene Disalicylate.

study was demonstrated by comparing the control plate summary (Table 26) to the various antibiotic agar plates. Furazolidone effectively reduced the numbers of Gram-negative (G-) bacterial colonies recovered. Tylosin, Lincomycin and BMD appeared to be active against the Gram-positive (G+) bacterial colonies. Flavomycin, a supposedly selective agent against G+ bacteria, was more effective in reducing the numbers of G- colonies. The broad spectrum antibiotics, Neo-Terra and Aureomycin, resulted in G+:G- colony count ratios that were fairly similar to the control plate summary. A selective agent against G+ bacteria, 3-Nitro, appeared to eliminate G+ rods but stimulated the growth of G+ coccal-bacillus. Approximately 23% of the total colonies examined were obtained on the control plates (Basal-hlf-UA and Basal-hlf). The comparison of G+ to G- colony counts obtained on the Basal-hlf-UA and the Basal-hlf agars indicated that the uric acid was not a selective agent against particular bacterial groups (G+ or G-).

Comparisons among the various media listed in Table 26 illustrate the ability of the various antibiotics to reduce bacterial numbers in relation to the control plate summary. In this experiment, approximately one-third of the control plate bacteria recovered were Gram-negative and two-thirds were Gram-positive. The similarity in bacterial counts obtained for each control plate medium again demonstrated that the uric acid did not exert a selective influence on the recovery of cecal anaerobic bacteria.

The total number of colonies utilizing uric acid that were observed on the six agar plates of each type of growth medium, the percent of total uric acid-utilizing colony counts observed per growth medium and also the numerical and percentage listing of the different

colonial types utilizing uric acid recovered on each growth medium are listed in Table 27. The uric acid-utilizing colony counts listed for the Basal-hlf agar were obtained on the Basal-hlf-UA "transfer" plates used for the 24-hour Gram stains. The non-selective nature of the uric acid was demonstrated by the fact that none of the colonies obtained on the Basal-hlf agar was lost upon transfer to the uric acid medium. The Basal-hlf-UA and Basal-hlf agars recovered 50% of the total number of uric acid-utilizing colonies observed in this experiment. Greater numbers of uric acid-utilizing colonies were recovered from the control plates than from any of the antibiotic-agar plates. There were no uric acid-utilizing colonies observed on the Furazolidone, Lincomycin, Neo-Terra and 3-Nitro agar plates. Approximately 14% of the total colonies examined were able to utilize uric acid and of these colonies 96% were G+ and 4% were G- (Table 27).

The total bacterial counts, expressed on a dry weight basis, that were obtained on the various growth media used in the antibiotic-agar plate experiment, are listed in Table 28. Additionally, the percent of the total bacteria recovered per growth media and numerical and percentage listing of bacterial types per growth medium are presented. The conversion of the colony counts to actual bacterial counts allows for a more meaningful expression of the percent of total bacteria recovered for each type of growth medium. An average of 36% of the total bacteria recovered in this experiment were obtained from the control plate agars (Table 28) which represented an average of 11% of the total colonies observed (Table 26). The fact that total bacterial counts were lower in every antibiotic growth medium studied in relation to

Table 27. Numbers and Types of Bacterial Colonies Utilizing Uric Acid Observed per Growth Medium in the Antibiotic-Agar Plate Experiment.

Growth Medium	Total Number of Colonies	% of Total Colony Count	Gram Reaction and Cellular Morphology					Summary	
			Rods		Cocci		Coccal-bacillus	%	%
			Gram+	Gram-	Gram+	Gram-	Gram+	Gram+	Gram-
Furazolidone	0								
Tylosin	6	6	5 (83)*	0	0	0	1(17)	100	0
Lincomycin	0								
Neo-Terra	0								
BMD***	23	21	13(57)	1(4)	6(26)	0	3(13)	96	4
Aureomycin	6	6	3(50)	0	1(17)	0	2(33)	100	0
Flavomycin	18	17	5(28)	0	7(39)	0	6(33)	100	0
3-Nitro	0								
Basal-hlf-UA	29	27	8(28)	2(7)	7(24)	0	12(41)	93	7
Basal-hlf	24**	23	13(54)	1(4)	7(29)	0	3(13)	96	4
Total	106	100	47(45)	4(4)	28(26)	0	26(25)	96	4

Colonies utilizing uric acid: 96% Gram-positive; 4% Gram-negative
Colonies utilizing uric acid = 14% of total colonies

*Percent of total per growth medium is shown in parenthesis; **Counts obtained after 24 hours of incubation on Basal-hlf-UA "transfer" plate; ***Bacitracin Methylene Disalicylate.

Table 28. Numbers and Types of Bacteria Observed per Growth Medium in the Antibiotic-Agar Plate Experiment.

Growth Medium	Total Number of Bacteria	Percent of Total	Gram Reaction and Cellular Morphology				
			Rods		Cocci		Coccal-bacillus
			Gram+	Gram-	Gram+	Gram-	Gram+
Nitrofur-azolidone	$6.2x10^{10}$	0.21	$4.6x10^{10}$ (74)*	0	$3.6x10^{9}$ (6)	$5.0x10^{9}$ (8)	$7.4x10^{9}$ (12)
Tylosin	$6.6x10^{11}$	2.28	$2.1x10^{11}$ (32)	$3.6x10^{11}$ (55)	$3.9x10^{10}$ (6)	0	$5.1x10^{10}$ (7)
Linco-mycin	$2.8x10^{11}$	0.97	$2.2x10^{9}$ (1)	$1.7x10^{11}$ (61)	$3.9x10^{10}$ (14)	$6.8x10^{10}$ (24)	0
Neo Terra	$1.4x10^{12}$	4.84	$4.2x10^{11}$ (30)	$3.6x10^{11}$ (26)	$3.0x10^{11}$ (22)	$8.0x10^{10}$ (6)	$2.3x10^{11}$ (16)
BMD**	$1.8x10^{11}$	6.22	$3.7x10^{11}$ (21)	$1.1x10^{10}$ (62)	$7.5x10^{10}$ (4)	$1.6x10^{11}$ (9)	$7.1x10^{10}$ (4)
Aureo-mycin	$7.0x10^{11}$	2.42	$2.5x10^{11}$ (35)	$2.1x10^{11}$ (30)	$1.4x10^{10}$ (2)	$1.2x10^{11}$ (17)	$1.1x10^{11}$ (16)
Flavo-mycin	$2.5x10^{12}$	8.64	$1.2x10^{12}$ (47)	$2.7x10^{11}$ (11)	$4.3x10^{11}$ (17)	$7.3x10^{10}$ (3)	$5.7x10^{11}$ (22)
3-Nitro	$6.3x10^{11}$	2.18	$8.1x10^{10}$ (13)	$2.0x10^{11}$ (32)	$1.4x10^{11}$ (22)	$8.1x10^{10}$ (13)	$1.3x10^{11}$ (20)
Basal-hlf-UA	$9.9x10^{12}$	34.22	$1.9x10^{12}$ (19)	$2.5x10^{12}$ (25)	$2.1x10^{12}$ (21)	$1.1x10^{12}$ (11)	$2.3x10^{12}$ (24)
Basal-hlf	$1.1x10^{13}$	38.02	$3.0x10^{12}$ (28)	$2.5x10^{12}$ (23)	$3.3x10^{12}$ (31)	$7.3x10^{11}$ (7)	$1.2x10^{12}$ (11)

*Percent of total per growth medium shown in parenthesis. **Bacitracin Methylene Disalicylate.

the average of the control plates indicated that each antibiotic was effective in reducing total bacterial counts.

The numbers of uric acid-utilizing bacteria recovered per growth medium in the antibiotic-agar plate experiment are listed in Table 29. These numbers are expressed as a percent of the total bacteria recovered that utilized uric acid. In addition, the numbers of the various bacterial types and percentages of total per growth medium are listed. Approximately one-half of the total number of bacteria utilizing uric acid observed in this study were recovered on the six plates of the Basal-hlf-UA agar. About 22% of the total bacteria recovered in this study, representing 14% of the total colony counts, were able to utilize uric acid. Overall 96% of the uric acid-utilizing bacteria were Gram-positive and 4% were Gram-negative.

VI. Uric Acid Quantitation Experiments

The average levels of uric acid in mg% detected in the feces and cecal contents of White Leghorn chickens 6, 10 and 24 weeks of ages are listed in Table 30. Statistical analysis (Table 31) indicated that cecal uric acid levels were similar in the 3 age groups with an average of approximately 2 mg%.

Statistical analysis of the uric acid levels detected in the fecal samples obtained from birds of the various ages indicated a significant difference (Table 32). The Duncan's multiple range test was used to rank the average fecal uric acid levels observed. Accordingly, fecal samples obtained from birds aged 6 and 10 weeks contained similar

Table 29. Numbers and Types of Bacteria Utilizing Uric Acid Observed per Growth Medium in the Antibiotic-Agar Plate Experiment.

Growth Medium	Total Number of Bacteria	% of Total Colony Total	Gram Reaction and Cellular Morphology				
			Rods		Cocci		Coccal-bacillus
			Gram+	Gram-	Gram+	Gram-	Gram+
Furazolidone	0						
Tylosin	$7.0x10^{10}$	1	$5.6x10^{10}$ (80)*	0	0	0	$1.4x10^{10}$ (20)
Lincomycin	0						
Neo-Terra	0						
BMD***	$1.5x10^{11}$	2	$1.7x10^{10}$ (11)	$1.1x10^{10}$ (8)	$7.5x10^{10}$ (50)	0	$4.7x10^{10}$ (31)
Aureomycin	$7.3x10^{10}$	1	$3.3x10^{10}$ (45)	0	$1.4x10^{10}$ (19)	0	$2.6x10^{10}$ (36)
Flavomycin	$5.8x10^{11}$	8	$1.7x10^{11}$ (30)	0	$8.0x10^{10}$ (13)	0	$3.3x10^{11}$ (57)
3-Nitro	0						
Basal-hlf-UA	$3.6x10^{12}$	50	$8.5x10^{11}$ (24)	$1.1x10^{11}$ (4)	$1.3x10^{12}$ (36)	0	$1.3x10^{12}$ (36)
Basal-hlf	$2.7x10^{12}$**	38	$1.0x10^{12}$ (38)	$1.2x10^{11}$ (4)	$1.2x10^{12}$ (45)	0	$3.5x10^{11}$ (13)
Controls only	$6.3x10^{12}$	88	$1.9x10^{12}$ (30)	$2.3x10^{11}$ (4)	$2.5x10^{12}$ (39)	0 (27)	$1.7x10^{12}$
Uric Acid-Utilizing bacteria in control media: 96% Gram-positive; 4% Gram-negative							

*Percent of total per growth medium is shown in parenthesis; **Counts obtained after 24 hours of incubation on Basal-hlf-UA "transfer" plate; ***Bacitracin Methylene Disalicylate.

Table 30. Uric Acid Levels (Dry Weight) in the Feces and Cecal Contents of White Leghorns Receiving a Layer-Breeder Ration.

Samples	Bird Age In Weeks	Number of Birds	Uric Acid Levels-mg%		
			Mean*	S.D.	Range
feces	6	20	99.0 a	19.0	72-128
cecal contents	6	20	1.6	0.7	.3-3.1
feces	10	19	107.0 a	20.0	70-150
cecal contents	10	19	2.1	0.9	.4-3.9
feces	24	15	157.0 b	49.0	74-251
cecal contents	24	15	2.0	1.6	.4-6.1

*Values followed by the same letter are not significantly different ($P \leq .05$).

Table 31. Analysis of Variance for Uric Acid Levels in the Cecal Contents of Chickens of Various Ages.

Source of Var.	d.f.	M.S.
Age	2	1.36
Error	51	1.15

Table 32. Analysis of Variance for Uric Acid Levels in the Feces of Chickens of Various Ages.

Source of Var.	d.f.	M.S.
Age	2	16374.3**
Error	51	945.8

**Significant at $P \leq .01$.

levels of uric acid (average of 103 mg.%) and the birds aged 24 weeks had significantly higher ($P \leq .05$) fecal uric acid levels at 157 mg%.

The average levels of uric acid in mg% detected in the cecal contents of White Leghorns 10 weeks of age receiving an antibiotic supplemented ration are listed in Table 33. Statistical analysis of this data (Table 34) indicated that there was no significant difference in cecal uric acid levels among groups at the 5% level. The average uric acid level detected was approximately 1.9 mg%.

Table 33. Uric Acid Levels (Dry Weight) in the Cecal Contents of 10-weeks-old White Leghorns Receiving Antibiotics.

Group	Antibiotic	Dietary Level	Number of Birds	Uric acid Levels-mg%		
				Mean	S.D.	Range
1	Lincomycin	4.4 ppm.	10	2.1	1.6	.7-4.8
2	3-Nitro	27.5 ppm.	10	2.0	1.3	.4-4.6
3	BMD*	11.0 ppm.	10	1.9	0.9	.5-3.4
4	Neo-Terra	6.6 ppm.	10	2.1	1.7	.8-6.3
5	Aureomycin	11.0 ppm.	10	1.8	1.4	.4-4.9
6	Flavomycin	2.2 ppm.	10	1.8	0.9	.7-3.5
7	Control	----	10	1.8	1.4	.5-4.1

*Bacitracin Methylene Disalicylate

Table 34. Analysis of Variance for Uric Acid Levels in the Cecal Contents of Antibiotic-Fed Chickens.

Source of Var.	d.f.	M.S.
Antibiotic	6	0.19
Error	63	1.75

DISCUSSION

I. ^{14}C-8-Uric Acid-Glycerol Experiments

1. Introduction into the Cloaca

Apparently at least small portions of ^{14}C-8-uric acid placed into the cloaca were transported into the ceca by a reverse peristalsis of the lower intestine. This cloacal "backwash" is therefore partly responsible for the presence of uric acid in the ceca. A second possible source of cecal uric acid could be the direct absorption of uric acid from the blood into the ceca. This point was investigated in a subsequent experiment. The presence of only small amounts of ^{14}C in the blood samples would seem to exclude the possibility of the transport of ^{14}C from the cloaca to the ceca via the circulatory system. On occasion a somewhat greater than 100% recovery of the total ^{14}C introduced to an individual bird was recovered. This observation was due to incorrect estimation of the ^{14}C dpm in the ^{14}C doses or in the internal standards.

2. Injection into the Brachial Vein

The direct injection of the ^{14}C into the circulatory system of the chicken resulted in the rapid and complete clearance of the labeled uric acid from the blood within 2-4 hours. The source of the ^{14}C in the large intestinal and fecal samples could be due to the following route: blood-kidney-urine-cloacal backwash up the intestine. The

absence of ^{14}C in the cecal samples of birds with ligated ceca indicated the inability of this organ to absorb directly the ^{14}C-8-uric acid or labeled metabolites from the blood. Accordingly, the circulatory system of the chicken would not appear to supply the ceca with uric acid in a direct manner. As noted above, incorrect estimation of the dpm in the ^{14}C-8-uric acid injections or internal standards resulted, on occasion, in slightly greater than 100% recovery of the ^{14}C injected.

3. Injection into the Ligated Ceca

On the average less than half of the ^{14}C injected per bird was recovered. This observation could have been due to either the incorrect estimation of ^{14}C dpm in the ^{14}C injections or internal standards or: conversion of the ^{14}C-8-uric acid by the cecal flora into a chemical form that could not be recovered and quantitated by the scintillation procedures employed. The average cecal sample contained most of the ^{14}C recovered. This observation indicated that the ^{14}C-uric acid and presumably, its labeled metabolites, were not absorbed from the ceca. The presence of small amounts of ^{14}C in the large intestinal samples was not expected. A possible source of the ^{14}C in these samples could have been due to the following route: ceca-blood-kidney-urine-cloacal backwash to the large intestine. However, due to the low amounts of ^{14}C recovered in the blood samples the above route would not readily appear to account for the presence of the ^{14}C in the large intestine unless appreciable accumulation of ^{14}C had occurred

in the cloaca. The small amounts of ^{14}C recovered in the blood samples may be explained in two ways: some absorption of the ^{14}C-uric acid or its metabolites may have occurred at the level of the ceca or the blood ^{14}C may simply be an artifact of the injection procedure.

III. Growth Medium Determination Experiments

The numbers of cecal bacteria obtained from 2 birds of the same age, sex and strain, housed and fed under similar conditions would be expected to be similar. A very likely cause of dissimilar bacterial counts between such birds might be due to cecal emptying by the bird immediately prior to obtaining the cecal sample. Another possible cause of dissimilar bacterial counts could be the mixing of intestinal and cecal contents during violent death throes. To avoid differences in cecal counts due to the above mentioned reasons, the birds used in these experiments were handled carefully and sacrificed by an overdose injection of sodium phenobarbitol. Birds sacrificed in this manner expired in a quiet manner within 5 minutes following the injection of the barbituate and did not defecate.

The significant differences in bacterial counts obtained from bird A1 and bird B1 may be explained by individual variations or may be due to the cecal sampling procedure employed or to the nature of the cecal sample (whole organ plus contents). In spite of the differences in anaerobic counts observed per bird in Experiment 1, the differences among the ability of the various growth media to support growth of

cecal anaerobes were significant.

In Experiment 1, the best growth media (supporting growth of the highest number of bacteria) were BGPhlf, a medium utilized by Barnes and Impey (1971) as a total growth medium for cecal anaerobes, and BHIlf, a medium developed in this laboratory. Comparisons of BHIlf agar to BHI agar for the recovery of cecal anaerobes was not made. Such a comparison would have allowed for a more precise appraisal of the value of the fecal and liver extracts added to agar media used to cultivate chicken anaerobes. The results of Experiment 5 indicated that the fecal and liver extract supplements were essential ingredients for the recovery of maximum numbers of cecal anaerobes.

The poorest growth medium (supporting growth of the lowest number of bacteria) investigated in Experiment 1 was Medium 10. The ability of this medium, utilized in roll tubes, to support growth of high numbers of cecal anaerobes was reported by Salinitro, et al. (1974a). Barnes and Impey (1970) reported that Medium 10 required fecal and liver extract supplements in order to yield a high recovery of cecal anaerobes. Colony counts of the cecal anaerobes were lower if chicken cecal extract was substituted for rumen fluid in Medium 98-5 (Salinitro et al. (1974a).

Counts of cecal anaerobes observed in Experiment 1 did not significantly increase beyond 72 hours of incubation in the glove box. The non-significant increase in counts between 72 and 96 hours of incubation may have been due to the presence of an occasional slow-growing isolate or may have simply reflected the inability of the experimenter to perceive certain small colonies until they had achieved

sufficient size for visualization. A hand-held magnifying glass (2X-3X) was used to inspect the agar plates during colony counting in an effort to obtain counts that were as accurate as possible.

The results of Experiment 2 indicated that bacterial counts of cecal anaerobes did not increase significantly beyond 24 hours of incubation. This observation contrasted sharply with the results of Experiment 1 where bacterial counts were observed to increase up to 72 hours of incubation. The differences in counts observed between bird A2 and B2 were more apparent than the differences observed between bird A1 and B1.

The results of Experiment 2 indicated, as was observed in Experiment 1, that the best total growth media investigated for cecal anaerobes included BGPhlf and BHIlf agars. In Experiment 2 VLB agar was capable of supporting approximately the same number of cecal anaerobes as the above mentioned media.

The consistently poorest growth medium investigated in Experiments 1 and 2 was Medium 10. A slightly modified preparation procedure for the Medium 10 used in Experiment 2 as compared to Experiment 1 resulted in the recovery of higher average counts in Experiment 2. However, Medium 10 would not be recommended for use in cultivating cecal anaerobes in the glove box in spite of the successes for this medium reported by other workers.

Due to the results of Experiments 1 and 2, BGPhlf agar was determined to be an excellent growth medium for cecal anaerobes using the glove box procedures. Additional positive aspects of this medium include the ease and simplicity of preparation and adaptability of

the growth medium for the incorporation of a wide number of substrates (Barnes and Impey, 1971). Bacterial counts obtained using BHIlf, VLB and VLlf agars indicate their suitability for the cultivation of cecal anaerobes. In particular, BHIlf agar, due to the great simplicity in preparation in comparison to the other growth media investigated, would be a most convenient non-specific growth medium for cecal anaerobes.

The results of Experiment 3 indicated that SUA and BGPhlf-UA agars were able to recover approximately equal total numbers of bacteria and equal numbers of bacteria that utilized uric acid. Based on this information, the results of Table 12 were expected: no significant differences in the proportion of uric acid counts for either media were observed. In this experiment, approximately 35% of the total number of cecal bacteria that were isolated from White Leghorn males aged 14 to 18 weeks were able to utilize uric acid. Barnes and Impey (1972) reported that approximately 50% of the total cecal flora of chickens aged 6.5 weeks utilized uric acid. There are no data in the literature that would indicate the proportion of anaerobic bacteria utilizing uric acid, in the cecal flora of older chickens. The different proportions of uric acid bacteria in the cecal samples of birds A3 and B3 indicated that the cecal floras of birds may differ qualitatively (ability to utilize uric acid) while their total bacterial counts may not differ significantly.

The results of Experiment 4 indicated that the uric acid bacteria required approximately the same period of incubation (48-72 hours) to achieve the highest numbers, as was observed for the total cecal

flora counts. However, in order to maximize the recovery of uric acid bacteria from the cecal samples analyzed in later studies, an incubation period of at least 5 days (120 hours) to a maximum of 7 days (168 hours) was employed. This prolonged incubation period resulted in the more extensive clearing of the uric acid growth media by colonies utilizing uric acid and hence simplified the process of isolating pure cultures of these bacteria.

The results of Experiment 5 indicated that all three media investigated supported the growth of similar percentages of uric acid bacteria (approximately 53%). This observation contrasts to earlier findings (Experiment 3) but is in excellent agreement with the observations of Barnes and Impey (1972). As was indicated earlier, the significant reduction in counts when liver and fecal extracts were omitted from an agar medium point to the requirement of these supplements by the cecal bacteria. The results of Experiment 5 compare favorably with the results of previous experiments: maximum bacterial counts are observed within 48 hours of incubation and maximum uric acid counts are observed within 48-72 hours of incubation.

IV. Identification of Uric Acid-Utilizing Anaerobic Bacteria

A most important observation of this experiment, in comparison to the reports of other workers, is that 98-99% of the cecal anaerobes utilizing uric acid were Gram-positive. Barnes and Impey (1971) reported that two groups of Gram-negative bacteria (Bacteroidaceae) attacked uric acid. Barnes (1972) reported that uric acid breakdown

was demonstrated by several species of Gram-negative rods and one species of Gram-negative coccal-bacillus that had been isolated from the cecal samples of chickens aged 6.5 weeks. In addition, approximately one-half of the cecal flora of these birds were able to attack uric acid.

The inability to isolate, in this study, significant numbers of Gram-negative cecal bacteria capable of utilizing uric acid led to the suspicion that either the basic diet used for the antibiotic supplemented rations or the Basal-hlf-uric acid agar used to cultivate the bacteria was selective for Gram-positive bacteria. A subsequent study (Antibiotic-Agar Plate Experiment) investigated these possibilities.

The apparent inability of the dietary antibiotics, used at low levels, to alter the cecal microflora that could utilize uric acid was in agreement with the observation that these dietary antibiotics failed to alter the level of uric acid in the cecal contents of chickens aged 10 weeks (Uric Acid Quantitation Experiment 4). These in vivo studies indicated that the low levels (growth promoting levels) of dietary antibiotics were not able to stimulate the numbers (growth) or activity of the cecal anaerobes utilizing uric acid.

Due to the design of this study, the accurate enumeration of the cecal isolates could not be accomplished. Based on the information obtained from this study a more accurate estimation of the numerical distribution or significance of particular cecal isolates could be determined in the following manner: Use of increased drop-plate replicates per serial cecal dilution (20-30 agar plates) in combination with the use of pooled cecal samples.

Carbohydrate Fermentation and Biochemical Testing Procedures

Due to the great number of anaerobic cecal isolates obtained in this study, a rapid method of media preparation, inoculation and testing was devised. A minimal number of CHO and biochemical test media were employed in order to aid in the identification of the cecal isolates. A more complete characterization of the isolates was not attempted due to the confines of time and expense involved. The individual test media were chosen on the basis of their ability to provide precise, accurate and reproducible results for chicken cecal isolates. For this reason, separate test media for indole production and nitrate reduction were employed rather than the combination indole-nitrate medium used by Holland et al. (1977b).

Holdeman and Moore (1975) reported that, while catalase production may help indicate that a particular isolate is a Propionibacterium, approximately one third of all these species are catalase negative. Additionally, they reported that the catalase test cannot be used to differentiate between Peptococcus and Peptostreptococcus species because catalase production is variable among strains of the same species in those groups. For the above reasons, the catalase test was not performed on the Gram-positive bacteria isolated from the chicken's ceca. A judgement was made that the time, effort, and expense of performing the catalase test, using glove box procedures, would overshadow the actual value of this test in contributing information for the identification of the isolates.

The final pH of the lactic acid test medium, equilibrated to the glove box atmosphere, was 7.0-7.1. Sterilization of the various CHO

media in the autoclave for a period of 15 minutes did not result in the destruction (caramelization) of the sugars and also allowed for a simple and rapid preparation of the test media.

The choice of 0.75 ml. volumes of the test media represents a compromise between the desire to economize and the potential need to buffer the small amounts of acid present in the PYG broth cultures that were occasionally used as an inoculum source for the test media.

Gas Chromatography Procedures

Quantitation of the bacterial metabolic end products was not performed in this study. Generally, knowledge of the relative proportions of VFAs and n-VFAs produced by a bacterial culture offers little assistance to the microbiologist attempting to identify it. However, quantitation, if desired, would have been a simple matter because the chromatograph was equipped with an integrator.

The only alcohol tested for was ethanol. Previous workers (Holdeman and Moore, 1973) had indicated that this is the sole alcohol produced by Gram-positive cecal isolates.

Aqueous broth cultures were chromatographed directly for VFA analysis, thus eliminating the possibility of fatty acid partitioning that may have occurred using the extraction procedure of Holdeman and Moore (1973) in the VPI manual. Additionally, the use of $MgSO_4$, which according to the VPI manual can be detrimental to the life span of a precision glass syringe, was circumvented.

The automatic sampler presented several advantages over the use of manual injection:

1. The GC (gas chromatograph) could be operated 24 hours a day. Overall, this resulted in a great savings of carrier and detector gases as compared to the manual GC.
2. The sample size was completely standardized, a point that would be most important if quantitation was desired.
3. Great savings of the time and effort of the experimenter were realized.
4. Completion of the analysis of a whole group of samples in a shorter period of time resulted in column packing conditions and performance that were nearly identical for the first to the last sample.

The only possible disadvantage of the automatic sampler was the periodic breakage of the expensive micro-syringes. Frequent monitoring and syringe maintenance (cleaning) were required to avoid time consuming delays.

The choice of which particular VFAs and non-VFAs to include in the standard solutions was dictated by the VPI manual. The only acids tested for were those that had been shown to be produced by Gram-positive cecal isolates. The concentration of the non-VFAs used in the standard solutions was also dictated by the VPI manual. However, for reasons of convenience, the VFA standard utilized in this study had been obtained from laboratory co-workers who were interested in the VFA production of rumen bacteria. The concentration of the VFAs in this standard was suitable for the present experiment.

The VPI manual (Holdeman and Moore, 1973) along with the CDC manual (Dowell and Hawkins, 1974) and the Wadsworth manual (Sutter

et al., 1975) have indicated that isothermal operation of the gas chromatograph is sufficient for the separation of the VFAs and the non-VFAs. The use of temperature programming in the present experiment resulted in reduced separation times while permitting complete separation of the individual acids. Savings of operator time and effort as well as carrier and detector gases were realized with the reduction of separation times.

The use of the 1 ul. sample sizes and routine, periodic temperature and solvent reconditioning of either column packing resulted in nearly identical performances of either column from the start to the finish of the analysis of over 400 bacterial samples. In fact, after more than 1,000 VFA samples had been run through the Carbowax column, this material was actually performing at a more satisfactory level than was initially observed. Accordingly, the life span of a well poured and well maintained Carbowax column could not be determined in this study. Likewise, the SP-1000 column also improved with age and after more than 500 samples showed no signs of breakdown or other problems.

Preliminary experimentation using the non-VFA standards indicated that overnight methylation at room temperature resulted in a higher concentration of the various methyl derivatives than was observed when these acids were methylated at 55°C for one hour.

V. Antibiotic-Agar Plate Experiment

From the results of this study it would appear that the normal cecal flora of the adult female White Leghorn is composed of 23% G+

(Gram-positive) rods, 24% G- (Gram-negative) rods, 26% G+ cocci, 9% G- cocci and 18% G+ c-b (coccal-bacilli). Salinitro et al. (1974a) identified 90% of 298 random isolates obtained from the ceca of broiler chickens 5 weeks old. These isolates consisted of 32% G+ rods, 14% G- rods, 26% G+ cocci, 7% G- cocci, 10% G+ spore-forming rods and the 11% remaining were classified as miscellaneous rods. Overall, these workers observed 21-32% G- bacteria and 68-79% G+ bacteria. These results compare favorably with the observation made in the adult chickens of 33% G- and 67% G+ bacteria. In an additional study, Salinitro et al. (1974b) reported that the cecal anaerobes of broiler chicks 5 weeks of age were 31% G- and 69% G+.

Barnes and Impey (1970) observed slightly different percentages of G+ and G- bacteria in the ceca of chickens 5 weeks old with 55-69% G+ and 40-45% G- bacteria.

Based on the comparison of the ratio of G+ to G- bacteria recovered in this experiment with published values, it would appear that the growth medium and isolation procedures employed in this laboratory are satisfactory for the study of cecal anaerobes.

Barnes and Impey (1972) reported that the G- anaerobes of the chicken ceca are more resistant to Zinc Bacitracin than the G+, but with some exceptions. The inclusion of BMD in the agar growth medium of the cecal anaerobes was also observed to reduce G+ bacterial counts.

Barnes (1972) reported that one-half of the cecal flora of chickens 6.5 weeks old were able to decompose uric acid and included G- rods, G- c-b, G+ non-sporulating rods and G+ cocci or c-b. Of the total number of bacteria isolated in this experiment, only 22% utilized uric

acid and none of the G- cocci isolated were able to utilize uric acid.

The great percentage (96%) of G+ uric acid-utilizing bacteria observed in this experiment is in excellent agreement with previous results where 98% of the uric acid-utilizing bacteria were G+.

The various antibiotics were included into the agar growth media for the purpose of investigating a possible mode of action for antibiotics in growth promotion. The proposed theory for the mode of action of antibiotics is as follows: The low levels of antibiotics may stimulate the growth or activity of cecal bacteria that utilize uric acid; the chicken may benefit from the removal of a toxic substance from the gut or from the useful uric acid metabolites, such as amino acids, produced by the bacteria. In addition, the chicken might obtain these metabolites directly, as in the occurrence of cecal absorption, or indirectly, as in the practice of coprophagy or the feeding of anaphage (dried cage layer excreta) as a poultry diet supplement.

However, the results of this study indicated that antibiotics used at low levels do not stimulate the growth of cecal bacteria that utilize uric acid _in vitro_. This evidence suggests that growth promoting levels of antibiotics would not stimulate the weight gain or growth rate of growing chickens by enhancing uric acid breakdown _in vivo_ by cecal anaerobes.

VI. Uric Acid Quantitation Experiments

From the results of these experiments, it would appear that the uric acid extraction and quantitation procedures employed were able to recover higher levels of fecal uric acid than had been reported by previous workers. Pudelkiewicz *et al.* (1968) fed White Plymouth Rock males a diet containing 25.1% crude protein and observed 87 mg. uric acid/gm. of feces. In comparison, White Leghorn males at 6 or 10 weeks of age had higher fecal uric acid levels when fed a lower level of protein than that used by Pudelkiewicz and co-workers. Bose and Ghosh (1945) also employed a uricase method for measuring uric acid and observed a range of 43-107 mg. uric acid/gm. in the excreta of 10 birds. The age and diet of these birds were not reported.

The presence of approximately 2 mg.% of uric acid observed in the cecal contents of White Leghorns contrasts with the observations of Bell and Bird (1966). These workers had detected no urate in the cecal contents of the hen using the murexide test for uric acid.

The differences in fecal uric acid levels observed between the younger White Leghorns (6 and 10 weeks old) and the older birds (24 weeks old) may have been due to several factors:

1. Normal physiological differences between sexually mature birds (24 weeks old) and the sexually immature birds (6 and 10 weeks old).
2. Differences in the rate of nitrogen turnover between the younger growing birds and the older birds might affect the numbers of

bacteria, present in the gut, that could remove uric acid from the feces. Accordingly, a high rate of nitrogen turnover might stimulate the activity of these uric acid bacteria and thereby result in lower levels of uric acid in the excreta.

3. The calcium level of the diet (3.27%) may have resulted in nephron damage or visceral urate deposition in the younger birds which could result in a lower excretion rate of uric acid. Shane and Young (1967) concluded that a high Ca:P ratio in pullet rearing rations would contribute to nephrosis and renal failure. These workers observed a 17% mortality associated with nephrosis in White Leghorn pullets that had been fed a 3% Ca and 0.3% P diet from 8 to 20 weeks of age and then subsequently fed a ration with 5% Ca. While no gross visceral abnormalities were observed in the young White Leghorns fed a high Calcium diet, histological changes in the kidneys could have been present.

The inability of the antibiotics used at low levels to decrease the cecal uric acid levels of White Leghorns corresponds well with the results of other experiments conducted in this laboratory. These same antibiotics, used in both *in vitro* and *in vivo* studies, did not increase the numbers of uric acid-utilizing bacteria that were recovered from the cecal samples of birds 6 or 10 weeks of age.

An interaction of dietary calcium with serum antibiotic levels has been reported by Price *et al.* (1959) and Slinger *et al.* (1961). Such an interaction may have accounted for the inability of the low levels of antibiotics to alter the activity or numbers of cecal anaerobes as was observed in *in vivo* studies conducted in this laboratory.

However, it does not seem likely that the in vivo activity of all the antimicrobial agents used in this study would be reduced due to a dietary calcium interaction. (T. S. Chang, pers. comm.).

SUMMARY

I. ^{14}C-8-Uric Acid-Glycerol Experiments

At least small amounts of the ^{14}C-uric acid placed into the cloaca of adult White Leghorn males are transported into the ceca by a reverse peristalsis of the lower intestine. This cloacal "backwash" is therefore partly responsible for the presence of uric acid in the ceca. Non-volatile ^{14}C-labeled uric acid metabolites were not absorbed from the ceca into the circulatory system in significant amounts.

II. Identification of ^{14}C-8-Uric Acid Metabolites

In general, none of the extraction procedures investigated provided clear, consistent or otherwise satisfactory information that was useful in partitioning ^{14}C-8-uric acid metabolites into either lipid or protein fractions.

III. Growth Medium Determination Experiments

BGPhlf and BHIlf agars were excellent overall growth media for cecal anaerobes. Up to 10^{11} bacteria/gm. (wet weight) could be recovered using these media. Satisfactory growth media included VLlf, VLB and VLS agars. Medium 10 was determined to be a poor growth medium for cecal anaerobes. Bacterial counts generally increased from 24 to 72 hours of incubation but did not increase significantly from 72 to

120 hours of incubation.

High numbers of cecal anaerobes utilizing uric acid (uric acid bacteria) could be recovered on Basal-hlf-UA, BGPhlf-UA and SUA agars. Significantly fewer uric acid bacteria were isolated on Basal-hemin-UA agar, presumably due to the requirement for liver and fecal extracts by these bacteria. The uric acid bacteria required 48 to 72 hours of incubation; however, for optimum visualization of these colonies, at least 120 hours of incubation were required. Approximately one-half of the cecal bacteria isolated on the optimal growth media were able to utilize uric acid.

Comparisons between birds in each experiment usually indicated that significant differences in bacterial counts existed between individuals of similar strain, age and sex, housed and fed under identical conditions.

IV. Identification of Uric Acid-Utilizing Anaerobic Bacteria

Contrary to the results of previous workers, it was observed that the overwhelming majority (98%) of the cecal bacteria utilizing uric acid were Gram-positive. The Gram-positive bacteria identified (410 isolates) were classified into 20 groups representing strains of four genera: Propionibacterium, Lactobacillus, Peptostreptococcus and Eubacterium.

An accurate estimation of the numerical distribution or significance of particular cecal isolates was not accomplished due to the design of this experiment.

The use of dietary antibiotics at low levels did not appear to alter the numbers of the uric acid-utilizing anaerobes that were present in the ceca of White Leghorns 6 or 10 weeks of age.

V. Antibiotic-Agar Plate Experiment

A total of 741 individual colonies or representative colonial types were isolated on Basal-hlf-UA agar plates containing various antibiotics and on two types of control media. All of the antibiotics utilized in this study were capable of reducing bacterial numbers and/or bacterial groups *in vitro* when compared to the control plates. None of the antibiotics were selective for the uric acid bacteria.

The majority (96%) of the cecal isolates utilizing uric acid were Gram-positive.

The Basal-hlf-uric acid agar was a non-selective growth medium and supported the growth of virtually every bacterial group reported to be present in the avian cecum.

The cecal flora of the adult White Leghorn hen was observed to be composed of 23% G+ (Gram-positive) rods, 24% G- (Gram-negative) rods, 26% G+ cocci, 9% G- cocci and 18% G+ coccal-bacilli.

VI. Uric Acid Quantitation Experiments

The uric acid levels in the feces of White Leghorn males fed a 17.8% protein, layer-breeder ration were similar at 6 and 10 weeks of age (average of 103 mg.%) but increased significantly by the time

the birds reached 24 weeks of age to approximately 157 mg.%.

The level of uric acid in the cecal contents remained constant in birds aged 6 to 24 weeks at approximately 2 mg.%.

Growth-promoting levels of antibiotics added to the ration had no apparent stimulatory effect upon the uric acid-utilizing bacteria in the ceca of White Leghorns at 10 weeks of age. Statistical analysis did not detect significant differences in cecal uric acid levels of these birds.

APPENDIX

Table 35. CLB-72 Layer-Breeder Ration Composition and Analysis.

Ingredient	% of Diet
Corn	67.60
49% Soybean meal	15.15
17% Alfalfa meal	2.00
50% Meat and Bone Meal	2.50
60% Fish Meal	2.00
Whey, dried	2.00
Fat, AV	0.50
Salt	0.25
Limestone, ground	6.75
Dicalcium phosphate	0.75
Premix*	0.50
Calculated Analysis	
crude protein, %	17.80
fat, %	3.88
fiber, %	3.48
calcium, %	3.27
Phosphorus, %	
total	0.554
available	0.396
Metabolizable Energy	
kcal/kg.	2924.0

*Available from Dawes Laboratories, Inc. 450 State Street, Chicago Heights, IL. 60411.

Table 36. Recovery of ^{14}C Observed in Various Tissues of Adult White Leghorn Males Following Introduction of ^{14}C-8-Uric Acid-Glycerol into the Cloaca.*

Tissue	Bird	dpm Introduced Per Bird	Average Recovered dpm. per Sample	Percent Recovery	Log. Value of Percent	Average Recovery Per Tissue Type ±S.D.
ceca	4HA1	100000	11100	11.1	1.0453	
	4HB1	"	5000	5.0	.6989	
	4HA1	"	5810	5.8	.7634	
	2HB1	"	10000	10.0	1.000	
	1HA1	"	13260	13.6	1.1335	
	1HB1	"	7220	7.2	.8573	
	4HA2	78000	13728	17.6	1.2455	
	4HB2	"	5536	7.1	.8512	
	2HA2	"	17472	22.4	1.3502	
	2HB2	"	17236	22.1	1.3443	
	1HA2	"	4695	6.1	.7795	
	1HB2	"	25103	25.1	1.3996	11 ± 7
large intes.	4HA1	100000	1600	1.6	.2041	
	4HB1	"	18100	18.1	1.2576	
	2HA1	"	5010	5.0	.6989	
	2HB1	"	6110	6.1	.7853	
	1HA1	"	25600	25.6	1.4082	
	1HB1	"	800	.8	-.0969	
	4HA2	78000	43524	55.8	1.7460	
	4HB2	"	2420	3.1	.4913	
	2HA2	"	7645	9.8	.9912	
	2HB2	"	10493	13.4	1.2171	
	1HA2	"	40248	51.6	1.7136	
	1HB2	"	5460	7.0	.8450	9 ± 14
feces	4HA1	100000	60000	60.0	1.1781	
	4HB1	"	45110	45.1	1.6541	
	2HA1	"	33540	33.5	1.5250	
	2HB1	"	20330	20.3	1.3074	
	1HA1	"	25610	25.6	1.4082	
	1HB1	"	55700	55.7	1.7458	
	4HA2	78000	25662	32.4	1.5105	
	4HB2	"	76985	98.7	1.9943	
	2HA2	"	32602	41.8	1.6211	
	2HB2	"	41340	53.0	1.7242	
	1HA2	"	6705	8.6	.9344	
	1HB2	"	61620	79.0	1.8976	39 ± 27

Table 36. Continued.

Tissue	Bird	dpm. Introduced Per Bird	Average Recovered dpm. per Sample	Percent Recovery	Log. Value of Percent	Average Recovery Per Tissue Type ± S.D.
liver	4HA1	100000	0			
	4HB1	"	0			
	2HA1	"	0			
	2HB1	"	0			
	1HA1	"	0			
	1HB1	"	0			0
kidney	4HA2	78000	0			
	4HB2	"	0			
	2HA2	"	0			
	2HB2	"	0			
	1HA2	"	0			
	1HB2	"	0			0
Blood Samples						
	2HA1	100000	300	0.3	-.5228	
1 hour	2HB1	"	600	0.6	-.2218	
post-	1HA1	"	3610	3.6	.5563	
intro.	1HB1	"	410	0.4	-.3979	
	2HA2	78000	0			
	2HB2	"	0			
	1HA2	"	0			
	1HB2	"	0			0.9±0.7
	4HA1	100000	0			
2 hours	4HB1	"	0			
post-	2HA1	"	170	0.17	-.7695	
intro.	2HB1	"	310	0.30	-.5228	
	4HA2	78000	0			
	4HB2	"	0			
	2HA2	"	0			
	2HB2	"	0			0.7±0.5
	4HA1	100000	0			
4 hours	4HB1	"	0			
post-	4HA2	78000	0			
intro.	4HB2	"	0			

*Data obtained from 12 birds with duplicate analysis of tissues.

Table 37. Recovery of ^{14}C Observed in Various Tissues of Adult White Leghorn Males Following Injection of ^{14}C-8-Uric Acid-Glycerol into the Brachial Vein.*

Tissue	Bird	dpm. Injected Per Bird	Average Recovered dpm. Per Sample	Percent Recovery	Log. Value of Percent	Average Recovery Per Tissue Type ± S.D.
ceca	4HA	108000	0	0		
	4HB	"	0	0		
	2HA	"	0	0		
	2HB	"	0	0		
	1HA	"	0	0		
	1HB	"	0	0		0
kidney	4HA	"	0	0		
	4HB	"	0	0		
	2HA	"	0	0		
	2HB	"	0	0		
	1HA	"	0	0		
	1HB	"	0	0		0
large intes.	4HA	"	8100	7.5	.8750	
	4HB	"	0	0		
	2HA	"	2376	2.2	.3424	
	2HB	"	3564	3.3	.5185	
	1HA	"	11998	11.1	1.0453	
	1HB	"	12204	11.3	1.0530	4.3±5.0
feces	4HA	"	56912	52.7	1.7218	
	4HB	"	95470	88.4	1.9464	
	2HA	"	64908	60.1	1.7788	
	2HB	"	105192	97.4	1.9885	
	1HA	"	54001	50.0	1.6989	
	1HB	"	102058	94.5	1.9754	71±22
Blood Samples						
1 hour post-inject.	2HA	"	4104	3.8	.5797	
	2HB	"	3241	3.0	.4771	
	1HA	"	1940	1.8	.2552	
	1HB	"	2484	2.4	.3617	2.6±0.9

Table 36. Continued.

Tissue	Bird	dpm. Injected Per Bird	Average Recovered dpm. Per Sample	Percent Recovery	Log. Value of Percent	Average Recovery Per Tissue Type ± S.D.
2 hours post-inject.	4HA	"	0	0		
	4HB	"	0	0		
	2HA	"	2482	2.3	.3617	
	2HB	"	1621	1.5	.1760	1.4±0.6
4 hours post-ject.	4HA	"	0	0		
	4HB	"	0	0		0

*Date obtained from 6 birds with duplicate analysis of tissues.

Table 38. Recovery of ^{14}C Observed in Various Tissues of Adult White Leghorn Males Following Injection of ^{14}C-8-Uric Acid-Glycerol into the Ligated Ceca.

Tissue	Bird	dpm. Injected Per Bird	Average Recovered dpm. Per Sample	Percent Recovery	Log. Value of Percent	Average Recovery Per Tissue Type ± S.D.
ceca	4HA	220000	67100	30.5	1.4842	
	4HB	"	62700	28.5	1.4548	
	2HA	"	63580	28.9	1.4608	
	2HB	"	109560	49.8	1.6972	
	1HA	"	154994	72.7	1.8615	
	1HB	"	113080	51.4	1.7109	41±16
large intes.	4HA	"	0			
	4HB	"	0			
	2HA	"	1694	.8	-.1135	
	2HB	"	0			
	1HA	"	1848	.8	-.0752	
	1HB	"	0			1±0.1
kidney	4HA	"	0			
	4HB	"	0			
	2HA	"	0			
	2HB	"	0			
	1HA	"	0			
	1HB	"	0			0
Blood Samples						
1 hour post-inject.	2HA	"	1628	.8	-.1249	
	2HB	"	2497	1.2	.0791	
	1HA	"	264	.1	-.9208	
	1HB	"	220	.1	-1.000	0.3±0.5

Table 38. Continued.

Tissue	Bird	dpm. Injected Per Bird	Average Recovered dpm. Per Sample	Percent Recovery	Log. Value of Percent	Average Recovery Per Tissue Type ± S.D.
2 hours post-inject.	4HA	"	2200	1.0	0	
	4HB	"	880	.4	-.3979	
	2HB	"	1300	.6	-.2218	
	2HB	"	1320	.6	-.2218	0.6±0.2
4 hours post-inject.	4HA	"	0			
	4HB	"	0			0

*Data obtained from 6 birds with duplicate analysis of tissues.

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