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STUDY OF THE NORMAL FECAL BACTERIAL FLORA OF MAN

by Lorraine S. Gall

Prepared under Contract No. NASw-738 *by*
REPUBLIC AVIATION CORPORATION
Farmingdale, Long Island, N. Y.
for

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION • WASHINGTON, D. C. • JUNE 1966

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SECTION I

INTRODUCTION

Scientific reports demonstrate that the balance of normal flora in the intestinal tract influences the nutrition, health, and well-being of humans in several important respects. For example, Spaulding[1] recently stated that the stability of adult fecal bacteria is an important factor in natural resistance and in protecting against overt infection. At a recent symposium on bacteria of the intestine, Dr. H. Haenel presented a paper[2] in which he stated: "It is well known from the results of germ-free research that the metabolic links between macro-organisms and body flora are numerous and important, and may concern many metabolic reactions of the host. The microbial metabolism - mainly that of the intestinal flora - may influence the metabolism of cancers, serum proteins, cholesterol, hormones, and vitamins, and affect the incidence of caries and the functioning of the body defence mechanism, to cite only some important examples." It is also well documented[3] that intestinal bacteria synthesize B vitamins which are necessary for good nutrition and this flora may well make other contributions to the digestive process. Experiments reported by various scientists[4] indicate that conditions such as alteration in diet, radiation or atmospheric pressure and composition such as will be experienced in space travel may well upset this balance of intestinal flora and thus influence the nutrition, health, and well-being of the spaceman. For these reasons, a knowledge of the normal intestinal flora of man and the effect of conditions encountered in space flight and exploration on this flora is essential.

To conduct this research, a knowledge of the normal intestinal flora of man is essential as a basis for evaluation of any changes in intestinal flora induced by space conditions. Unfortunately data adequately describing the

intestinal flora of man, especially the predominating anaerobic bacteria of humans, are lacking, as noted authorities in the field have recently pointed out in scientific publications.

A paper[5] by Dr. J. Dehnert of Heidelberg published in 1961 entitled, "The normal intestinal flora. Limits of cultural analysis of the fecal flora" was abstracted in Excerpta Medica[6] as follows:

> "Escherich introduced the routine study of the 'typical conditions.' However, composition and capacity of the normal intestinal flora have not been elucidated as yet. Our knowledge of the obligate intestinal microbes' impedes an accurate qualitative and quantitative analysis. For most species the suitable culture media are even not known. The insufficiency of 'Bifidum culture media' for the isolation of Gram-positive, anaerobic fecal germs can be clearly demonstrated. Normalization of the disturbed intestinal flora by administration of living bacteria will have to remain a hypothesis so long as the normal flora cannot be determined."

In addition, Dr. I. Juhlin et al, of University of Lund (Sweden) in 1961 published a paper[7] expressing the view that while there is an immense literature on media for use in the examination of stools, "none has offered indisputable advantages."

Also, Dr. E. H. Spaulding et al of Temple University in a paper [1] published in 1962 on normal human fecal flora showing that the bacteroides, which are delicate anaerobic rods, constitute the most numerous group of bacteria in the normal stool. He points out the confusion that exists in this field, and cites statements in standard texts and in several scientific publications that are in direct contradiction on important aspects of this work. Spaulding states, "Misconceptions about the adult fecal flora appear to be based on results of studies in which inadequate identification methods such as Gram stains and broth cultures or too few selective culture media were employed." He continues by emphasizing that this has led to "wide variation in the findings

of different investigators", and "lack of conformity of published opinions on this point." Spaulding finally states, "Comprehensive culturing methods are needed to reveal the total fecal flora," and then concludes that it is "likely that all the viable organisms in the feces have not been accounted for."

Dr. Haenel[2] states the case strongly by saying, "although there is no doubt about the importance of the body flora to the host, our knowledge, even of simple facts, is surprisingly small. The data concerning the composition of any natural microflora, and the criteria upon which the terms 'normal' or 'abnormal' microflora are based, are insufficient and poor." He cites examples that may illustrate that "common opinions are dubious or wrong."

These above mentioned publications clearly demonstrate the need for a systematic study of the normal intestinal flora of man, and this fact was further emphasized during the 8th International Microbiological Congress held in Montreal in August 1962, in a paper presented by Dr. H. Beerens of Pasteur Institute[8]. He suggested reclassification of the important group of fecal anaerobic bacteria, the Bacteroides, in an attempt to reduce the confusion that exists in this field. The discussion following this paper by several noted international scientists led to agreement that: 1) information existing at present on normal human fecal flora was inadequate, 2) studies must be made in this field, 3) the important fecal flora were mainly anaerobic bacteria, and 4) adequate techniques for culturing these anaerobic organisms were the primary requirement for doing this vital work.

For these reasons research was undertaken at Republic Aviation Corporation for NASA under contract NASw-738[9] to study the predominating normal fecal flora of the adult human male. The results collected from these studies offer baseline data for evaluating the effect of various conditions of space flight on the fecal flora of subjects in experiments simulating space travel or of the astronauts themselves, with particular emphasis on any changes in the flora which might influence the health, well-being and performance of these men.

The study of the normal fecal flora of man has several logical phases, which include a determination of the broad class of bacteria which are pre-

dominating, the study of their physiology to characterize them and to assess their probable role in the body. To achieve these objectives, aerobic and anaerobic microbiological studies were made at approximately monthly intervals on a series of four to six fecal samples taken from 25 adult men, and the predominating bacteria were isolated for morphological and physiological studies.

SECTION II

METHODS

The methods used in this contract for collection, isolation, characterization and study of the aerobic and anaerobic bacterial flora of the normal young human adult male will be described briefly in this section, while the details of the procedures which deviate from the standard published methods will be contained in Appendix A.

A. COLLECTION OF SAMPLES

Twenty-five young men between the ages of 18 and 35 years, employed in the research and development laboratories at Republic Aviation Corporation were selected as subjects. They were in good health, were not taking oral medication of any kind, and did not regularly eat food peculiar to one particular ethnic or racial group or indulge in food fads. These men were asked to contribute a series of 4 to 6 fecal samples at approximately monthly intervals and were supplied with sterile pint-sized ice cream cartons placed in sterile paper bags. The men were instructed to eliminate (during working hours) directly into the ice cream carton from which the cover had been removed, replace the cover, replace the sample in the paper bag and call our laboratory immediately. The sampling was carried out so that the time lapse between elimination and culturing was about fifteen minutes and never more than one-half hour. The samples were considered to be acceptable and were cultured only if they retained the body heat which ensured that there had been no delay in notifying the laboratory. The fecal sample for culturing was obtained by inserting a standard loop into the fecal sample in five different areas in rapid succession, giving a composite sample of these five areas. The loop with the fecal sample was inserted immediately into 10 ml of Gall's broth to which had been added enough cysteine to reduce the potential of the medium to approximately -200 mv and manipulated until the loop was free of fecal matter, giving approximately 10^{-3} dilution of the feces. The samples were then labled according to subject number and sample period and were serially cultured immediately into Gall's broth with cysteine.

B. PRIMARY BACTERIAL CULTURING TECHNIQUE

Both the aerobic and anaerobic primary culturing on each sample was done from the 10^{-3} tube of Gall's broth. The aerobic cultures were made by streaking various differential media designed to culture selectively certain types of aerobic bacteria. The media used included MacConkey's agar, Mitis salivarius agar, Rogosa's agar as a pour plate, phytone yeast media and two blood agar plates, one of which was incubated in evacuated jars to which had been added 10% CO_2. An aerobic counting plate was also made from each sample and the plates were observed after the appropriate incubation period for each type of media. The details of the media used will be found in Appendix A. A gram stain was made from the first tube of the dilution series and observed for morphological types of bacteria present in the original fecal sample. The anaerobic culturing of the fecal sample was performed immediately by serial dilution of the 10^{-3} sample prepared in Gall's broth. The procedure followed is shown in Figure 1 and the serially diluted material was incubated anaerobically in an anaerobic jar which had been evacuated and then gassed with CO_2 to a level of 10%. Observation for growth were made at 16, 20, 24 hours and 2, 3 and 5 days. When growth was observed, gram stains were made and agar shakes of the cultures were made to serve as a carrying medium and to determine the degree of anaerobiosis of the cultures. In addition the primary culturing included two anaerobic pour plates made from appropriate dilutions of the fecal samples from which isolated colonies were picked into Gall's broth plus cysteine. The exact details of the primary anaerobic isolation procedure are contained in Appendix A.

The anaerobic cultures to be studied were selected from the agar shakes and from the colonies picked from the anaerobic pour plates which represented the billionth dilution of the fecal material or above. Transfers were made into Gall's broth with cysteine from the agar shakes and from selected individual colonies on the anaerobic pour plates and were incubated in the same manner as the primary broth dilution series. When growth occurred gram stains were made from these cultures and when necessary the cultures were purified by anaerobic plating. The pure cultures were then studied by a series of screen tests in order to group those organisms which appeared to be similar. The screen tests consisted of the patterns of growth shown by each organism in the following media: Gall's broth less carbohydrate plus 2 drops 10% cysteine bicarbonate and 1 drop 10% $NaHCO_3$; to which was added the following: 1) glucose (0.1%), 2) sucrose (0.1%, 3) lactose

(0.1%), 4) dextrin (0.1%), 5) blank (no added sugar); litmus milk plus 0.05% bovine albumin, 0.1% cysteine bicarbonate, 1 drop 10% $NaHCO_3$; gelatin; final pH in 0.1% glucose broth; Gall's agar shake for anaerobiosis. The results of these tests coupled with the morphological characteristics of these organisms and the character of the growth in broth and agar shake formed the basis for the key used to group similar organisms. On the basis of these screen tests, 18 different groups of obligate anaerobes and 5 groups of facultative anaerobes were found to occur repeatedly in the feces of the subjects studied and were designated FA-1 to FA-18 and FN-1 to FN-5 respectively. These 23 groups of organisms were used as a key to group similar bacteria and thus to establish the frequency of their occurrence as the predominating fecal flora of these 25 subjects.

The most frequently occurring groups of fecal anaerobes were studied physiologically in an attempt to further characterize these organisms. Warburg techniques were used to study the carbohydrate metabolism of these organisms with respect to glucose and their ability to decarboxylate certain amino acids and to deaminate constitutent amino acids in a pancreatic digest of casein. The exact procedure used for these studies are contained in Appendix A.

The vitamin use or production of the type cultures were tested with respect to five B vitamins - B2, B12, folic acid, pantothenic acid and niacin. This was done by growing under anaerobic conditions the type cultures in Gall's broth modified by decreasing the amounts of yeast extract and beef extract so that the broth contained minimal amounts of each of these B vitamins. After maximum growth of each culture had been obtained, the supernatant fluid was collected from these cultures and was tested for the increase or decrease in the amount of each of these B vitamins present. Control broths with and without cysteine were also tested. The samples were assayed by the Wisconsin Alumni Research Foundation Laboratories in Madison, Wisconsin using the recognized methods of vitamin analysis (see Appendix A).

The sensitivity of the type cultures to several commonly used oral antibiotics was tested by the standard disc test procedure. In addition the supernatant broth from well grown cultures of each of the type cultures was tested for growth inhibitory action against each of the other type cultures and a control using the usual cup method of analysis. Certain modifications in

technique were required because the cultures are strict anaerobes. Plates were inoculated by streaking all over with 0.1 ml of cultures in Gall's broth with added cysteine and bicarbonate. The antibiotics discs or cups were placed on the inoculated plates using sterile technique. The plates were placed in anaerobic jars, flushed with CO_2, and incubated under hydrogen at 37-38°C. After 16-24 hours incubation, the plates were examined for growth and zones of inhibition. If no growth was apparent, the plates were reincubated under anaerobic conditions. The tests were run on both blood agar plates (BBL) and on Gall's agar.

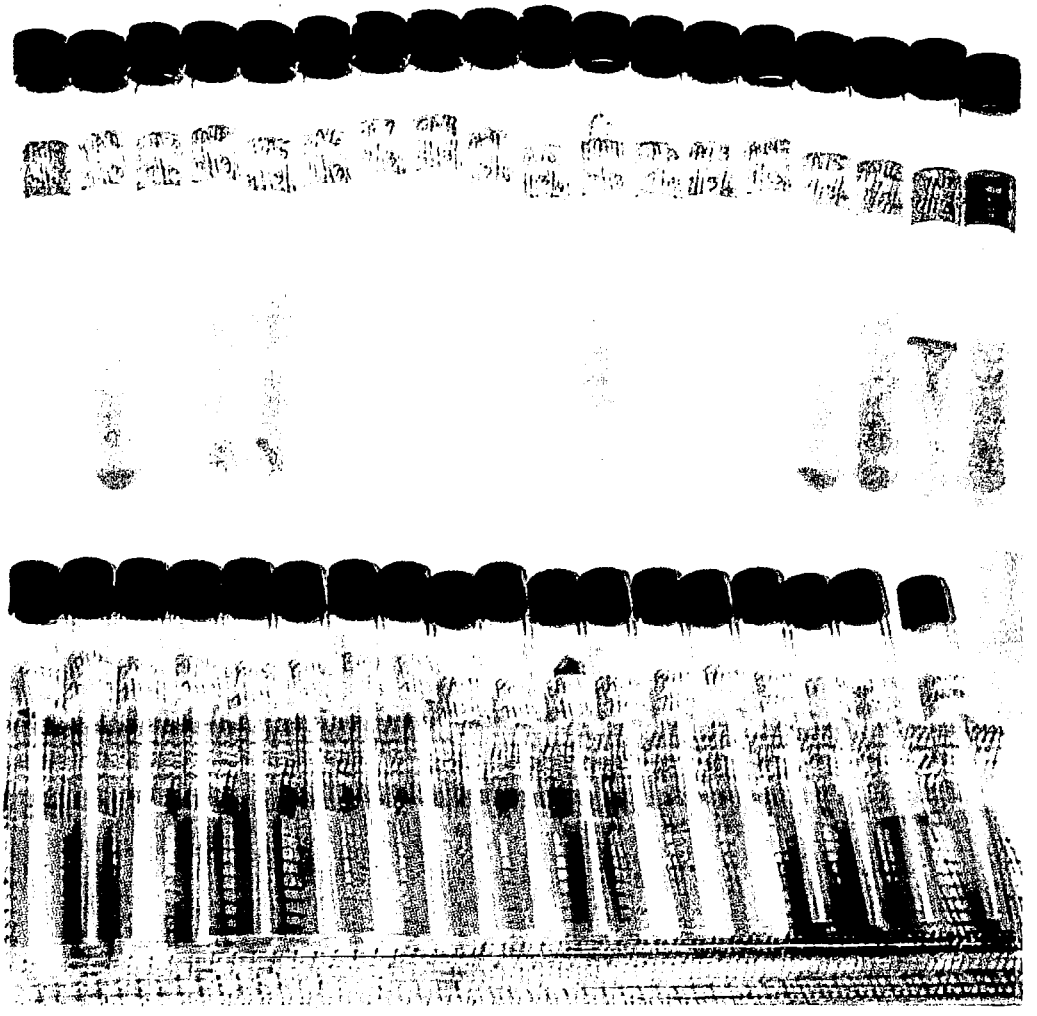
The possible pathogenicity of selected type cultures was tested by using germfree rats. This work was done in conjunction with Drs. H. A. Dymsza, G. S. Stoewsand, and J. J. Enright of the Nutrition Branch, Food Division, U. S. Army Natick, Massachusetts and Dr. P. C. Trexler, Gnotobiotic Research Foundation, 251 Ballardvale Street, North Wilmington, Massachusetts. Groups of five or six germfree animals were inoculated with the type culture as a monocontaminant and were sacrificed after three weeks. The intestinal contents were examined to determine that the organisms had in fact become established in the gut of the germfree animal and the liver, kidney and other organs were examined for size or pathology, and for the cholesterol content of the blood serum and liver by techniques described in Appendix A.

The cultures were also examined for motility, spore formation, temperature optima, pH optima, proteolysis of gelatin and casein and indol formation according to standard accepted microbiological procedures in an attempt to characterize these cultures.[10]

Bacto Spirit Blue Agar was prepared and the requisite amount of Bacto Lipase Reagent was added as directed.[11] This medium is designed for use in plates and slants on which the inoculum is streaked. Positive lipase production is indicated by formation of a dark blue precipitate under the colonies. Efforts to grow the anaerobic type cultures on plates, both surface streaks and in deep plates, with and without cysteine, under strict anaerobic conditions were unsuccessful because cysteine interfered with the

color change of the spirit blue. Control plates seeded with lipase enzyme were positive. When deep butts of the lipase medium were inoculated with 0.1 ml of culture by pipette from heavily grown Gall's broths of the type cultures, a color change to a dull blue occurred in some cultures and this change was regarded as indicative of positive reaction for lipase production. In other cultures, the medium became yellowed as compared with the uninoculated medium, as is demonstrated in color photograph on the following page.

LIPASE TEST CONDUCTED IN DEEP AGAR SHAKES OF BACTO-SPIRIT BLUE AGAR



From Left to Right: FA-1 through FA-16, plus
Lipase Enzyme Control, and Uninoculated Medium Control

Note blue color in control (lipase enzyme) and positive reactions of FA-2, FA-4, FA-5, FA-11, FA-15 and FA-16. Uninoculated control medium remains unchanged. Negative reactions produce a muddy yellow-grey color in the medium.

Prior to photography, the cultures were allowed to come to room temperature to avoid clouding. At refrigerator temperature the blue color is much more pronouned. Lipase production seemed somewhat greater at the bottom of the positive anaerobe cultures, perhaps because of settling-out. The lighter lipase enzyme was held in suspension at the surface of the agar in the positive control, resulting in formation of a blue ring.

SECTION III

THE PREDOMINANT FECAL FLORA

There has long been a controversy over which types of microorganisms are predominant in the fecal flora of the human. In an attempt to settle this question, both aerobic and anaerobic cultural techniques were used to study a series of 4 to 6 fecal samples taken at approximately monthly intervals from 25 adult males in the employ of Republic Aviation Corporation. The initials and ages of the men comprising the three series of subjects are listed in Table 1. The fecal samples were cultured within 15 minutes following elimination.

The comparative numbers of aerobic versus anaerobic microorganisms contained in each sample were determined. The enumeration of aerobic organisms was made from the aerobic plate counts prepared in the conventional way using Gall's agar with inoculum from the same dilution series that was used to determine the height of anaerobic bacterial growth in the sample. The number of colonies found on the aerobic plates was counted and the data are summarized in Table 2. The aerobic counts were in the millions with extremes falling between one million and about 750 million. The counts seemed to be higher in the first period which may have been due to less rapid completion of the culturing than occurred after the routine became more familiar. The counts also seemed to be influenced to some extent by the individual, since certain subjects such as 11 and 18 carried considerably higher counts than other subjects cultured during the same sampling periods.

In contrast to the aerobic organisms which occur in millions per gram, the anaerobic organisms were present in billions per gram or above. The height of anaerobic growth in the broth series for each subject in each sampling period is recorded in Table 3, and the data show that the numbers of anaerobic organisms present in the feces seldom fell below the one hundred billionth

dilution of the sample and often exceeded it. Although it is not possible to compare directly the values obtained from the aerobic plates and the anaerobic dilution series because the former represents actual counts whereas the latter is based on ten times dilutions, it is possible to assess the order of magnitude of the occurrence of the aerobic versus the anaerobic bacteria.

Considering all aerobic counts ranging from 1 to 9 million as representing one million, from 10 to 99 million as 10 million, and from 100 million to 999 million as 100 million, comparisons were made between the number of 10 times differences between the aerobic and anaerobic growth. These data are summarized in Table 4 . Although the number of 10 times differences fluctuates between samples, with the subjects carrying the highest aerobic count, showing the lowest value, the overall average indicates that the anaerobes outnumbered the aerobes by four logs or by 10,000 times on an average.

These comparative data firmly establish the fact that the predominating fecal flora are the anaerobic bacteria. Since this study was designed to learn more about the predominating fecal flora of the normal adult human male, the emphasis in this study centers around the anaerobic microorganisms.

Since the anaerobic bacteria have been established to be the predominating fecal flora, it is necessary to assess the degree of anaerobisis of these fecal organisms because both facultative and strict anaerobes can grow in the anaerobic broth series. Accordingly, a tabulation was made of the number of times that strict anaerobes appeared in the three top serial dilutions of the sample compared to the number of facultative anaerobes appearing in these three dilutions. This comparison is summarized in Table 5 . It is evident that the strict anaerobes occurred in the top three dilutions of the broth series more frequently than did the facultative anaerobes by an overwhelming majority and the number of times in which the strict anaerobe occurred in the last dilution of the sample showing growth exceeded 95% of the determinations. One subject, 18, showed an unusually large number of facultative anaerobes, and was the only subject showing a facultative anaerobe in the top dilution of the broth series which may be due to the fact that this subject was of foreign extraction and to some extent ate the

highly seasoned foods characteristic of his country of origin. In three instances in the samples taken from the last group of subjects, aerobic organisms occurred in one of the top three dilutions which in the case of Subject 23, in period 3 was probably due to contamination of the broth series. With these minor exceptions, the predominance of strict anaerobes over facultative anaerobes was clear for every subject and establishes that the strict anaerobes are the most predominating fecal bacteria.

SECTION IV

TYPES OF FECAL ANAEROBES AND DISTRIBUTION

Since it has become established that the strict anaerobe bacteria are the predominating fecal flora, the next problem is in the characterization of the most frequently isolated anaerobic cultures. No adequate recognized classification is available for identifying these fecal anaerobes, which made it necessary to establish a "key" in order to group similar organisms, which allows an evaluation of the frequency of occurrence of similar organisms and permits a selection of cultures to be studied physiologically based on their frequency of occurrence. Accordingly, a "screen test" was set up which was based on several fundamental physiological and morphological characteristics of the fecal anaerobes isolated, for the purpose of grouping similar organisms. These tests include a gram stain to observe morphology, growth in glucose, sucrose, lactose and dextrin broth as well as in broth with no added carbohydrate, final pH in 0.1% glucose broth, liquification of gelatin, reaction in litmus milk and the degree of anaerobiosis as shown by growth in an agar shake. The cultures isolated from the top three dilutions of the anaerobic broth showing growth supplemented by selected colonies isolated from the Brewer anaerobic plates were screened according to the schema outlined above.

Based on the results obtained from these screen tests, like cultures of strict anaerobes were grouped and each group was assigned a number such as FA-1 (Fecal Anaerobe 1), FA-2, etc. and the facultative anaerobes were designated as FN. The screen tests with the FA and FN designations are included in Table

Those groups of organisms which occurred repeatedly were considered as "type cultures" of fecal anaerobes. During the first months of this study, sixteen types of strict anaerobes and four types of facultative anaerobes occurred frequently enough to be placed in the type culture key. The original sixteen FA type

cultures were subjected to further intensive physiological studies which will be included in this report in the section on physiology. During the latter part of the investigation, two more strict anaerobes and one more facultative anaerobe occurred sufficiently often for inclusion into the key as FA-17 and FA-18 and FN-5. Four of the FN type cultures (facultative anaerobes) were closely related to enterococci and were not further investigated. The other FN type, FN-3, may be a peptostreptococcus, but was isolated so seldom that no physiological studies were done on it.

In another study conducted under contract AF33(615)-1748,[12], Determination of Aerobic and Anaerobic Microflora of Human Feces, for AMRL at Wright-Patterson Air Force Base, young men were fed a space-type diet for 42 days and the fecal flora was studied by methods similar to those developed in this study. A large proportion of the strict fecal anaerobes isolated from the young men on the AMRL Study after they had eaten the test diet for several days did not fit in the key devised during this NASA study, and an addition to the key designated the GD series was necessary to group these organisms. Upon re-examining the "unidentified" cultures from the subjects on this NASA study, a small number, less than 4%, of the strict anaerobes fell into one of the seven GD types and the screen test data for the GD types are recorded in the same table as the FA and FN types (see Table 6).

Using the anaerobic key described above, the occurrence of the various type cultures was determined for each subject in each period and the results are recorded in Table 7 and summarized for each of the three groups of subjects in Table 8 . There was considerable individual variation in the distribution of the fecal anaerobes and certain subjects did carry a disproportionate number of a certain type cultures. This was probably more an apparent than a real difference, as only the predominating organisms in any one sample were cultured and screened. Therefore the organism that was most predominant in any one sample might well be present but not be the most predominating in later samples. This postulation was supported when the gram stains from the lower broth dilutions were observed and the bacteria which had predominated on previous samples were present in smaller numbers. However, in no case did one subject

carry any type of culture exclusively, nor was any type culture isolated exclusively from any one individual. No one person accounted for a majority of the isolates of any one type of culture and all types of cultures were isolated from each of the three groups of subjects. Because no records were kept of the exact composition of the individual's diet prior to the elimination of the fecal sample, no assessment can be made of the influence of individual nutrients on the types of fecal organisms occurring in any one sample. This information was not obtained since the primary purpose of this study was to get a representative picture of the organisms occurring in the feces of adult males eating the usual American diet and no attempt was made to exclude subjects unless they were food faddists or on oral medication. The occurrence of the type cultures isolated in all sampling periods for each subject is summarized in Table 9. In general, the sampling period, which did not represent the same day for each subject, seemed to have little consistent effect on the distribution of the various type cultures. The variation, while rather marked for individual cultures in certain sampling periods, did not appear to be correlated to any traceable factor and probably was due more to individual choice of diet than to any factor connected with sample period.

The frequency of occurrence of the sixteen type cultures in each of the three separate groups of subjects is summarized in Table 10, and the total occurrence of each type of organism is included in this table. It is evident that certain types of strict anaerobes occurred much more frequently than others and since such small numbers of subjects were included in each group, it is important to note the comparative frequency of occurrence of each of these types among the three groups of men studied. The total numbers of each of the types isolated in each of the three groups of subjects and their total occurrence is summarized in Table 11, listed in order of total occurrence. In addition, the frequency of occurrence of each type culture was ranked so that the most frequently occurring organism was given the number one and the rank of each type culture in the overall total and for each of the three groups of subjects tested is contained in Table 12. From these data it is evident that the most frequently occurring organisms in the entire series were FA-1, FA-15 and FA-3, and these bacteria ranked among those isolated most often in all three series

of subjects. In fact, with the notable exception of the decrease in occurrence of FA-5 in the third period, the first six organisms in the total series were isolated consistently among the top six bacteria throughout the entire experiment. In general, the least frequently occurring organisms in the overall rank from the total series were the least frequently occurring organisms throughout the entire study in all groups of subjects and considering those types of organisms ranked 12 and below in the overall total, there was remarkable agreement among the three separate groups of subjects. As might have been expected, FA-18, which was not a recognized group during the first experimental period, ranked low in occurrence in that period and for an unexplained reason FA-14 was found less frequently in the second group of subjects than in the others. Except for these relatively minor variations, occurrence of these 16 types of fecal anaerobes throughout the experiment is remarkably consistent, particularly considering the state-of-the-art in this field of scientific study.

To summarize briefly, the 18 types of strict fecal anaerobes which were found to comprise 91.5% of the strict anaerobes isolated during this study were quite consistent among the three groups of subjects studied and their frequency of occurrence, when considered for each of the three groups, was remarkably consistent. Certain types of organisms occurred much more frequently than others with a range of from 126 isolations for FA-1 to 7 isolations for FA-4, but most of the organisms were isolated at least 26 times and the 4 most frequently occurring organisms accounted for almost exactly half of the strict anaerobes identified. The remaining 12 types, therefore, accounted for half of the organisms identified. No one person or group of subjects or sampling period accounted for an undue proportion of any of the types of organisms considered. Therefore, it can be concluded that using the techniques employed in this study the 18 type of fecal anaerobes considered represent a fair cross-section of the strict fecal anaerobes which might be expected to be found in the normal adult male in this section of the country.

SECTION V

PHYSIOLOGY OF TYPE CULTURES

The complex ecological role of the many different types of microorganisms which exist in the human intestine is extremely difficult to simulate and study. However, there are some fundamental physiological activities of the predominant microorganisms isolated during this study which can be chemically resolved. Probably the most significant of the biochemical reactions which take place in the human intestine are those which subsequently affect human nutrition and performance. For example, microorganisms may compete with the body for nutrients, produce essential nutrients or useful precursers of human nutrients, or produce metabolic end products or autolysis products which are toxic or have other physiological effects on man.

Microorganisms may consume amino acids which exist in the intestine as a result of protein and amino acid metabolism. One mechanism of microbial amino acid utilization is the direct consumption of the amino acid and incorporation of it into the bacterial cell. Another mechanism is the decarboxylation of amino acids with the production of carbon dioxide and amines, many of which are toxic to man or have pronounced physiological effects. Still other mechanisms are deamination with the formation of carboxylic acids, transamination to keto acids, and dehydrogenation reactions. Studies on the physiology of the type cultures have been arbitrarily divided into four topics in this report; deamination, decarboxylation, carbohydrate fermentation, and miscellaneous studies.

A. DEAMINATION STUDIES

Major sources of nutritional energy available for bacterial metabolism in the human colon are the amino acids and peptides. The "anaerobic" microenvironment of the colon is well suited for microbial fermentation of these substrates. A study of some physiological pathways of amino acids and peptide

metabolism by the FA type cultures was undertaken to 1) further group and separate the FA type cultures on the basis of their activities, 2) learn more about potential by-products of bacteria-metabolism which could accumulate in the colon, and 3) evaluate the possibility that bacteria could compete with man for dietary amino acids in the digestive tract.

There are many different pathways for the deamination of amino acids reported in the literature. Other workers, for example, found that a major mechanism for fermentative dissimilation of amino acids is deamination to ammonia and corresponding keto acids. However, a study of the fermentation of single amino acids is complicated by the fact that some anaerobic bacteria only deaminate pairs of amino acids, not individual amino acids. Other deaminating enzymes only release ammonia when coupled to reactions of additional enzymes. Therefore, rather than attempt to study deamination of amino acids individually, it was decided to simulate a mixture of some amino acids that microorganisms might encounter in the digestive tract. FA type cultures were tested for their ability to deaminate a pancreatic digest of casein by means of the Conway microdiffusion procedure for ammonia.

The results given in Table 13 and 14 were obtained using the sixteen FA type cultures. Dry weight determinations of cell preparations are shown in Table 15. Standard curves are also shown. A summary of the results is given in Table 13. It is apparent that six of the type cultures did not produce detectable levels of ammonia; FA-2, 4, 6, 11, 14 and 16. FA-12 and FA-8 were very active and the rest of the cultures produced intermediate amounts. A discussion of the results is in the following section.

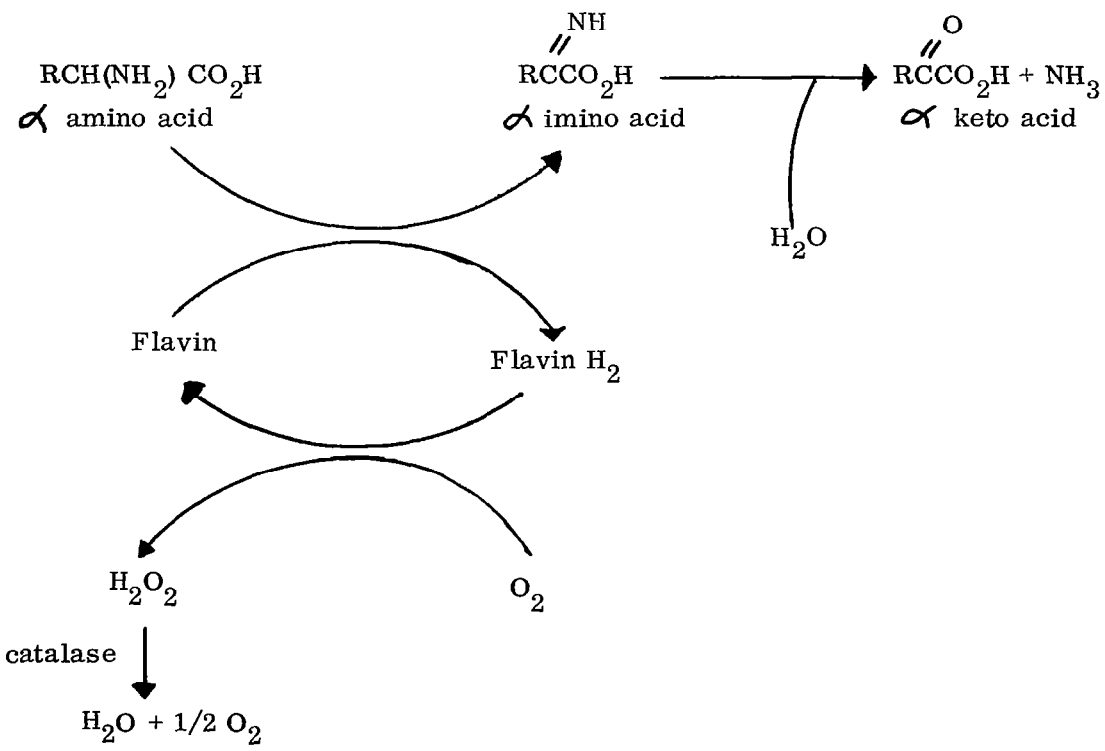
Bacterial and human growth involves protein synthesis. In turn a supply of usable nitrogen, carbon, sulfur, and energy are essential raw materials for this process. Growth includes the reorganization of environmental nitrogen to cellular nitrogen. However, if protein molecules are the source of nitrogen, they cannot be metabolized by man or used by bacteria unless they are first degraded into smaller assimilable molecules. The excretion of bacterial proteolytic enzymes for this purpose is restricted to comparatively few types

of bacteria, but even the highly proteolytic bacteria will not grow when native proteins are the sole source of nitrogen. Proteins used may be the hydrolyzed proteoses, peptones, peptides, and amino acids produced by many bacterial and human hydrolases. Constituent amino acids may be further fermented by bacteria to various end products, often including ammonia.

The human host accommodates its intestinal bacteria with a variety of assimilable nitrogen sources which are residues from the digestive process. A brief review of this process is pertinent to this discussion since it provides insight into the physiological basis of the microfloral ecology which exists in the digestive tract. The first stage of protein (casein, for example) digestion by man involves the formation of proteoses and peptones by the action of pepsin and renin: these enzymes are secreted by the chief and parietal cells of the stomach. Very little absorption of food occurs at this stage of digestion. The pancreas then continues the degradation of proteins by means of the proteolytic enzymes trypsin, chymotrypsin and carboxypeptidase: proteins, proteoses, peptones, and peptides are further hydrolyzed to polypeptides, dipeptides and ultimately to free amino acids. Further along in the digestive process, Brünner's glands and glands of Leiberkühn secret a variety of enzymes including amino-peptidase and dipeptidase which convert residual peptides and dipeptides to amino acids. At this stage of digestion the dietary proteins will be almost completely hydrolyzed to their constituent amino acids and are rapidly absorbed into the portal blood. Most ingested food (about 90%) is absorbed from the small intestine. The residue then passes into the large intestine where considerable absorption of water takes place and the semi-liquid intestinal contents gradually become more solid.

Bell[13] summarized some of the literature on the occurrence of bacteria in the human digestive tract. As one would expect, the stomach and small intestine contain only a sparse transient bacterial flora due to gastric hydrochloric acid and the so-called "antibacterial mechanism" of the small intestine. The small intestine even contains very few viable microorganisms when the stomach contains large numbers. However, enormous numbers of normal bacterial inhabitants are found in the colon. According to Bell these bacteria deaminate and decarboxylate amino acids to amines and phenols.

The role of bacteria in the human digestive tract, therefore, is primarily limited to their metabolic activities in the ecology of the colon. There are obvious reasons why the biochemical ecology of the colon can significantly affect man's performance. For example, Silen[14] showed that the large intestine is the source of a considerable quantity of ammonia which is absorbed into the portal circulation. A normal liver will rapidly remove the ammonia from the portal blood. Otherwise, however, ammonia may reach toxic levels in the peripheral blood and result in serious performance impairment. Minute quantities of ammonia are very toxic to the central nervous system. Symptoms of ammonia intoxication include tremor, slurring of speech, blurring of vision, and coma and possibly death. Hemorrhage into the gastrointestinal tract, for example, introduces large quantities of blood protein and may produce ammonia intoxication. Silen found that the amount of ammonia normally delivered from the intestine to the blood may be reduced by the oral administration of neomycin: inhibition of intestinal bacteria stops the normal deamination of dietary amino acids. There are many metabolic pathways used by bacteria for the deamination of amino acids. One type of deamination, oxidative deamination, is not pertinent to this discussion because it involves molecular oxygen. However, the general mechanism is:

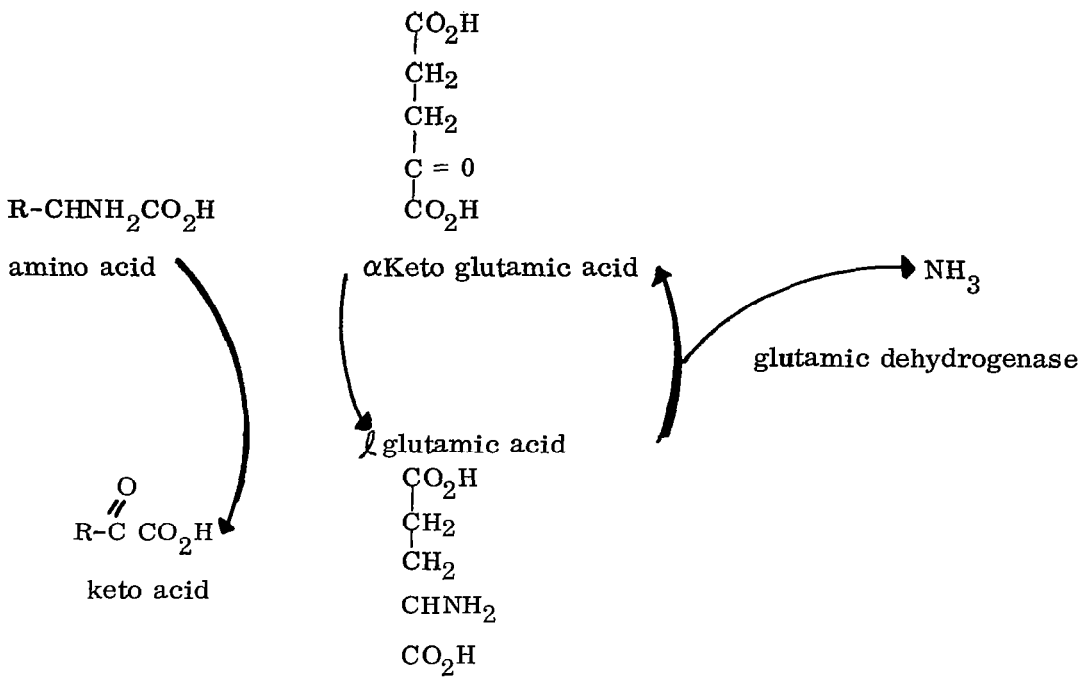


Early studies on Clostridia demonstrated the surprising result that incubation of single alpha-amino acids with cell preparations did not result in the formation of ammonia. However, when various redox dyes were added, some amino acids acted as hydrogen donors and others as hydrogen acceptors. Their oxidation or reduction was concomitant with liberation of ammonia. When an amino acid acceptor in the presence of cell preparations, ammonia was formed according to the general equation:

$$\acute{R}CH(NH_2)CO_2H + R\ CH(NH_2)CO_2H + H_2O$$
$$\downarrow$$
$$\acute{R}CH_2CO_2H + R\ COCO_2H + 2NH_3$$

An important ecological consideration is that the end products of deamination may be further metabolized by several mechanisms in the human host (if they can be absorbed), the deaminating bacteria, or other microorganisms in the gut. For example, the keto acids may be reconverted to amino acids by reamination or transamination. Other mechanisms would include direct catabolization or conversion to glycogen or fat. Similarly, the fate of ammonia in the intestinal ecology could involve many routes. For example, ammonia could be used in the amination of keto acids or used in amidation of amino acids such as the formation of glutamine from glutamic acid by the enzyme glutamine synthetase. The human liver normally converts most of the intestinal ammonia to urea through the Krebs-Henseleit cycle. This general phenomenon of coupled deamination called the "Stickland reaction". The above equation describes only one of many possible variations of the Stickland reaction.

Another mechanism, transdeamination, is reversable in humans and provides an important mechanism for the synthesis of the so-called dispensable amino acids from keto acids and ammonia. Transdeamination also commonly occurs in the anaerobes:



The microbiological deamination of amino acids may be studied by several methods. Measurement of disappearance of several amino acids from casein hydrolyzate or the assay of keto acids (which may be transient) is obviously a complex analysis to perform. The Conway microdiffusion procedure was chosen for these studies because it has several unique advantages over other available techniques for the quantative determination of deamination activity. Although ammonia formation may be measured in various ways, it would be difficult to assay accurately ammonia in a mixture of casein hydrolyzate, cell preparations and ammonia because of substances which interfere with the analysis. Since ammonia is volatile, a process of distillation is an obvious way to separate the ammonia. Standard Conway diffusion dishes were used for this distillation (see Materials and Methods section for details). Cell preparations, buffer, and casein hydrolyzates were placed in the outer well and standard sulfuric acid in the center well. After an adequate incubation period alkali was added to the outer well to drive any ammonia out of solution so that it could be trapped in the H_2SO_4 and subsequently analyzed by Nesslerization.

There are many environmental factors described in the literature which significantly influence the formation and activity of bacterial deaminases. This information is useful because it facilitates extrapolation of the results of our laboratory experiments to the dynamic physiological environment bacteria encounter in the human colon. For example, it is known that carbohydrate in the growth medium supresses deaminase formation. Technologically, one may argue that carbohydrate should spare the breakdown of amino acids, since energy is available in the presence of carbohydrate which would otherwise have to be obtained from amino acids. Since bacterial enzymes are elaborated from a limited protein pool, it may be that an increased production of enzymes involved in glucose metabolism results in a decreased production of enzymes involved in other forms of metabolism. Because of this consideration, cells in this study were prepared for deamination studies by growth in a high amino acid, low carbohydrate modification of Gall's broth. In the colon relatively little carbohydrate is available for bacterial fermentation. This may explain the high rate of bacterial NH_3 production, since conditions are eminently suited for deamination.

Gale[15] determined that the main factor which determined whether deamination or decarboxylation of an amino acid will take place is the pH of the medium during growth. Both mechanisms will not occur at the same time and both are adaptive enzymes. Gale[15] hypothesized that the amino and carboxyl groups must be in the appropriate ionization state for deamination or decarboxylation. Amino acids are amphoteric: in an acid medium a $-CO_2H$ group is produced, but if the medium is alkaline a $-NH_2$ group is produced, but if the medium is alkaline a $-NH_2$ group is produced and attacked (if at all) by deamination. The optimum activity for the enzymes occurs in the range of pH 4.0 - 5.5 for decarboxylase and pH 8.0-9.0 for deaminases. Therefore, the pH chosen for cultivation of microorganisms in this test was 7.5, for a more optimum enzyme synthesis; the deaminase testing was done at pH 8.0 for optimum enzyme activity. At a pH near 6 neither is active in a culture. However, in a more alkaline environment, like the colon, one would predict that deamination would be more prevalent than decarboxylation on the basis of favorable pH. Deamination takes place in an alkaline environment but shifts the pH to a more acid range. Decarboxylation takes place in an acid condition and shifts the pH to a more alkaline

range. Therefore, microorganisms may be instrumental in pH stabilization of the colon by this mechanism.

The values reported in Table 13 represent the total free ammonia formed from the action of the various FA type cultures. It includes ammonia which could arise from (1) the Stickland reaction, (2) other coupled deamination reactions, and (3) various other types of deamination of single amino acids. The values may obviously be a function of (1) peptidases and their characteristics (such as pH optima and oxygen lability), (2) the number and degree of amino acids deaminated in the casein hydrolyzate and (3) the rate of ammonia re-utilization. It is obvious from the data that distinct differences exist between the FA type cultures when they are tested for their ability to deaminate a pancreatic digest of casein. It is also apparent that the microbiological deamination of amino acids by the bacteria in the colon may potentially have profound effects on a man's performance and well-being.

B. DECARBOXYLATION OF AMINO ACIDS

The microbiologically mediated enzymatic decarboxylation of amino acids may have important effects on the human colon ecology. Some of the reasons that elucidation of this ecology is significant are: potent vasoconstricting compounds may be formed as by-products of bacterial growth, dietary amino acids may be competitively scavenged, and amine-dependent as well as carbon-dioxide-dependent bacteria may inhabit the colon. Therefore, a screening program was undertaken to establish which of the type cultures are capable of decarboxylating some selected amino acids. Amino acids were chosen which have significant roles in human nutrition and whose decarboxylation products are potentially toxic amines.

The results of the decarboxylation tests are reported in Table 16. Control broths inoculated with known cultures of Salmonella sp. and Pseudomonas sp. gave the expected pattern of decarboxylation reactions. Growth of all cultures, especially FA-8 and FA-13, was relatively poor compared to control cultures grown in Gall's broth. Although the preliminary data shown in Table 16 should be confirmed manometrically, these preliminary tests indicate that substantial apparent decarboxylase activity of the type cultures took place under the conditions of this experiment. The significance of the results is discussed below.

The enzymatic decarboxylation of intestinal alpha amino acids by microorganisms may result in the formation of primary amines by the following general relationship:

$$RCH(NH_2)CO_2H \xrightarrow[B6\text{-}PO_4]{\text{decarboxylase}} RCH_2NH_2 + CO_2$$

A series of specific enzymes have been described in the literature each of which will catalyze the decarboxylation of a specific 1-alpha amino acid. For example, the following amino acids used in these studies may be decarboxylated to the corresponding amines - all of which are vasoconstrictors:

Amino Acid	Corresponding Amine
lysine	cadaverine
histidine	histamine
tyrosine	tyramine
arginine	agmatine

Lysine is an essential dietary amino acid for man, so its fate in the digestive tract is particularly important. Enzymes have also been discovered which will decarboxylate ornithine, phenylalanine, aspartic acid and glutamic acid. Decarboxylases have been found among various species of Clostridium, Lactobacillus, Proteus, Bacillus and Streptococcus. Other bacteria, such as some Veillonella sp. recently have been reported by Rogosa[16] to have nutritional requirements for cadaverine and putrescine. The concentration of the amines could therefore be limiting factors in the growth of amine-dependent bacteria. Bacteria which have the enzymes to metabolize intestinal amines could play a significant role in man's intestinal ecology, since amines could be symbiotically scavenged from the metabolic by-products of associated bacteria. In addition to microbiological synthesis of amines in the colon, amines may also enter the digestive tract in the form of cheese, beer, wine and certain drugs such as certain cold tablets and reducing pills.

The removal of toxic amines from the human intestine has recently been shown to have a profound effect on man's performance. There are certain drugs

which inhibit the action of intestinal enzymes which render otherwise toxic amines incapable of causing damage to man. Administration of these drugs in the presence of excess concentrations of vasopressor amines may have devastating consequences. Because the drugs inhibit the detoxification of the intestinal amines, there may be a resultant rise in blood pressure, creating the symptoms of acute high blood pressure. Under these conditions some persons experience severe distress, pounding in the chest, dizziness, and throbbing headaches. However, others who suffer from arterial or cardiovascular ailments may even experience cerebral hemorrhage or a heart attack.

Some of the enzyme-inhibiting drugs include nialamide (Niamid), isocarboxazid (Marplan), pargyline hydrochloride (Entonyl), tranylcypromine (Parnate), and phenelzine sulfate (Nardil). Alone, these drugs may be beneficial; however, in combination with cheese, other foods high in certain amines or any of the following drugs, the effects may be disasterous: Amphetamine, Dextroamphetamine, Methamphetamine, Ephedrine, Metaraminol, Phenylephrine, Phenylpropanolamine, Mephentermine Imiparamine, or Amitriptyline.

It is apparent, then, that amine-producing and amine-utilizing bacteria in the human digestive tract may also have a similarly significant role in the delicate balance of the concentration naturally occuring physiologically active substances in the digestive tract. Furthermore, this balance may have profound effects on man's performance if the amines are absorbed from the colon.

A substantial amount of research on decarboxylases is reported in the literature: Gale[17] extensively reviewed factors which affect the formation and expression of bacterial decarboxylases. For example, it is known that decarboxylases are adaptive and that their formation is influenced by such environmental factors as pH, temperature, substrate concentration, and pyridoxal or pyridoxamine concentration. In addition, decarboxylase activity is related to the stage of bacterial population growth. These environmental conditions were considered in the design of these experiments. Since decarboxylases are pyridoxal phosphate dependent (Bellamy[18]), a concentration of 40 mg per liter of pyridoxine HC1 was used in the test medium to assist those microorganisms which can use it for pyridoxal phosphate synthesis. This

relatively excessive concentration was used because the amount required for optimum decarboxylase activity has been shown to be many times the amount required to give optimum growth. Another critical environmental factor for decarboxylase activity is temperature. Room temperature (25 ± 2°C) was used because it is closer to the optimum for enzyme synthesis and activity. Another requirement for decarboxylase formation is that the specific substrate must be present, since the enzymes are adaptive. Possible exceptions to this requirement may exist where an amino acid is synthesized during normal growth and which may consequently be present in sufficient amounts to induce enzyme synthesis. This consideration was satisfied, since the medium used contained high concentrations of substrate. Decarboxylases express themselves most dramatically in a population in which active cell division has ceased. Therefore, results were read after four and five days.

In addition to the factors listed above, an important consideration in the expression of bacterial decarboxylase activity is the effect of pH. The optimum pH for activity for most decarboxylases is 4.0-5.5, although this is not the optimum for growth of most microorganisms. Gale[15] reported that the pH of the medium is the primary factor which determines whether the nature of the enzymatic attack on an amino acid shall be by deamination or by decarboxylation. The basis for this may be in the amphoteric nature of the amino acids. As was mentioned previously under deamination studies, the state of ionization of an alpha amino acid changes so that under acid conditions a $-CO_2H$ group is formed and attacked. Conversely, an alkaline medium will possess more $-NH_2$ groups which may be attacked by deamination. In our studies glucose was added to the medium according to the procedure of Falkow[19]. Fermentation of the carbohydrate results in the lowering of the pH in a poorly buffered medium. Consequently more optimum pH conditions for decarboxylation are achieved. However, the results of this procedure are obviously not valid with bacteria such as FA-13 which do not ferment glucose. It is apparent then that these preliminary data indicate that amines are being formed from bacterial decomposition of amino acids. In particular, lysine - an essential dietary amino acid for man is decomposed and, powerful vasodepressors; cadaverine, histamine, tyramine

and agmatine are being formed by the enzymatic decarboxylation of lysine, histidine, tyrosine, and arginine. Several of the FA type cultures also decarboxylated lysine - supposedly with the formation of cadaverine, an essential nutrient for some Veillonella sp.

C. CARBOHYDRATE METABOLISM

Another important area in the study of intestinal ecology is the utilization of carbohydrate by microorganisms. Since the intestinal environment is "anaerobic" it is likely that the major end products can be predicted by the use of established pathways. The first step was a study of glucose fermentation. It is significant because it is a beginning in an understanding of competitive removal of food from the intestine, ecological relationships between fecal bacteria, and taxonomy of the predominant fecal anaerobes.

The approach to the study of carbohydrate fermentation was to start with glycolysis, since the anaerobic utilization of glucose is important taxonomically and in the intestinal ecology. Two techniques were used in the glycolysis studies:

1. Assay of lactic acid formation in a glucose-containing growth medium

2. Manometric determination of gas and acid by produced from glucose by resting cells.

The data in Table 17 illustrate several obvious points. FA-13 not only failed to produce lactic acid; it consumed lactic acid from the culture medium. The possibility of symbiotic lactic acid removal by FA-13 from a mixed culture containing a lactic acid producer could theoretically have ecological significance: autointoxication of the lactic acid producer could be prevented and the non-glucose fermenting symbiont could benefit by the lactic acid substrate.

Table 18 also illustrates that under the conditions of this experiment FA-4, FA-5 and FA-11 produced relatively large quantities of lactic acid, but FA-1, FA-3, FA-9 and FA-14 all produced relatively little. Another group FA-2, FA-6, FA-7, FA-8, FA-12 and FA-15 produced an intermediate amount. FA-1 and FA-13 do not ferment glucose.

1. Manometric Studies of Carbohydrate Fermentation

The second part of the carbohydrate fermentation studies was concerned with manometric estimation of metabolic and products. By combining the results of manometric studies with the lactic acid production data, it was possible to group some microorganisms on the basis of their common characteristics and separate others on the basis of unique biochemical capabilities.

The results of the manometric studies are reported in Table 18 and are discussed below.

Many end products from bacterial glucose fermentation have been reported in the literature. These include carbon dioxide and hydrogen gas, formic, acetic, propionic, butyric, lactic and succinic acids; glycerol, acetoin, butylene glycol, ethyl, isopropyl, and butyl alcohols; acetone, and many other compounds. Since a complete fermentation balance is both complex and tedious, it was decided to limit the preliminary screening of type cultures to manometric estimation of CO_2, H_2, lactic acid, and total carboxylic acid groups formed. The data is expressed as production of hydrogen, carbon dioxide and total organic acids produced from glycolysis. The Q values are used to correlate enzymatic activity with cell dry weight. However, these values should be interpreted with caution and comparison of FA types by this criteria may be unsound. The obvious reasons for this is that the activity of cell preparations is a function of stage of population growth as well as variations in handling, and washing, and errors in dry weight determinations. Perhaps the most serious variable is the length of time a particular cell preparation is exposed to oxygen. However, the values given for quantity of carboxylic acid groups formed per unit of glucose is probably a more valid basis for comparison of type cultures.

Some of the values differ slightly from the preliminary data given in earlier reports because more refined analysis was used for these current tests. It is obvious that there are several FA types listed in Table 18 which have unique characteristics. For example, FA-14 is the only culture producing measurable hydrogen. FA-8 could not be studied because of its scanty growth in Gall's broth. FA-9 and FA-12 characteristically produced relatively little

lactic acid compared to the high concentration of carboxylic acids formed by fermentation. FA-12 was unique because it failed to ferment glucose with either acid or gas formation. It also appears that there is a correlation between the relative lactic acid values in Table 18 and those shown in Table 17. The slight variations may be due to the fact that resting cells were used in the manometric studies, but actively growing cells which use glucose for cell synthesis were used in the screening studies listed in Table 17.

D. MISCELLANEOUS STUDIES

1. Nitrogen Fixation

During the studies on glucose fermentation, it was inadvertently observed that trace amounts of nitrogen were apparently consumed by the endogenous control cells of FA-14. Since this phenomena has not been reported in the literature, the study was further pursued, primarily because nitrogen fixation could significantly make amino acids available for human nutrition in the colon. The approach to the problem was first to establish unequivocally that nitrogen gas was indeed consumed. In order to do this, 99.996% nitrogen was used in the gas phase of a Warburg flask. Control flasks were used with both potassium pyrogallate and potassium hydroxide to oxygen and carbon dioxide respectively. The results confirm that neither carbon dioxide nor oxygen was initially available for gas exchange, and that the nitrogen used in the flask was pure and free of carbon dioxide and oxygen. In order to link nitrogen fixation specifically to the presence of cells, various concentrations of bacterial cells were examined for their ability to fix nitrogen. It was observed that the quantity of nitrogen gas fixed was proportional to cell concentrations. Parallel experiments employed argon as the inert gas. In these experiments no adsorption or absorption of argon was observed. However, follow on studies have resulted in negative results. This phenomenon should be pursued further before definite conclusions about the role of this type culture can be made.

2. Indol Production

The FA type cultures were tested for their ability to produce indol as a by-product of growth. Type cultures were inoculated into Gall's broth and

tryptone broth (Difco) with added cysteine and then incubated anaerobically. The distillation procedure of Gore[20] and para-dimethyl-amino benzaldehyde reagent was used to test for indol. The results of these tests indicate that only one of the sixteen type cultures, FA-3, produced detectable quantities of indol under the conditions of this experiment.

A major metabolic pathway to indol is through the amino acid tryptophane. Both Gall's broth and tryptone broth contain enzymatic hydrolyzates of caein which are relatively high in tryptophane content. Since only one indol-producing culture was detected, additional tests were run using Gall's broth and tryptone broth supplemented with tryptophane. However, FA-3 remained the only indol-rpoducing culture of the sixteen FA and seven GD types. As expected, control tests with stock cultures of Escherichia coli were positive and Aerobacter aerogenes were negative. These studies indicate that FA-3 has the unique capability of indol accumulation and may partially explain the characteristic odor of broth cultures.

3. Carbohydrate and Protein Metabolism

Other major metabolic activities of these sixteen microorganisms tested with respect to substrates commonly found in the intestinal tract include the breakdown of carbohydrates other than glucose and the proteolysis of gelatin and casein as representative proteins. In the screen tests each culture was inoculated into media containing four sugars, glucose, sucrose, lactose and dextrin and two media with the proteins casein and gelatin to determine their differential reactions to these major nutrients. Certain type cultures grew and produced acid in media containing all four sugars, thus indicating the ability to utilize these sugars. None of the cultures attacked gelatin in the test media used; but many of the cultures caused proteolysis of the casein in litmus milk, ranging from slight to marked. Slight proteolysis was judged to be present when the curd was pock marked with clear spots or showed marked channels not attributable to gas formation, and strong proteolysis was judged by the advanced digestion or peptonization of the casein. These observations indicate that many of the type cultures may break down casein, but not gelatin.

4. Vitamins

Among the important characteristics of the fecal anaerobes is their ability to produce or their ability to use certain B vitamins. In order to determine these characteristics, the important type cultures were grown in media that was marginal in the B vitamins and the cultures were then tested for the increase or decrease in vitamin B2, B12, pantothenic acid, niacin and folic acid. The results of these studies are presented in Table 19. It is evident that almost all of the type cultures either produced small amounts of vitamin B2 or at least did not remove it from the medium. Cultures FA-5, FA-3 and FA-14 appeared to synthesize this B vitamin. Vitamin B12 was produced in significant amounts by almost all of the cultures with the exception of FA-16. FA-9, FA-10, FA-12 and FA-13 produced particularly large amounts of B12 and none of the type cultures used this vitamin. Pantothenic acid showed a more varied pattern. The vitamin was apparently produced by about seven of the type cultures with the largest amount being produced by FA-8, while four of the cultures apparently used this vitamin with the greatest loss from the medium being shown by FA-13 and FA-14. The other type cultures appeared neither to use nor produce this vitamin to any extent. Niacin apparently was not produced by any of the cultures but was used by five cultures, the greatest amount being used by FA-9 and FA-14. The folic acid data presented an interesting picture. None of the cultures appeared to use this vitamin, whereas ten appeared to produce it; the largest amounts being produced by FA-1 and FA-13 followed by FA-7 and FA-10.

From these data it appears that the fecal anaerobes are active with respect to vitamin production and use and this may very well explain some of this symbiotic relationships seen in vitro. For example, FA-1 and FA-3 frequently occur in symbiotic relationship and upon attempted purification the FA-3 organism is frequently lost. It is interesting to note that whereas FA-1 produces pantothenic acid, FA-3 used a considerable amount of this vitamin in the test medium. Thus symbiotic relationships probably exist between the various fecal organisms. The possible relationship between the fecal anaerobes and the human host is not clearly defined, but it is evident that certain of these

fecal anaerobes that produce B vitamins may, potentially at least, supply these vitamins to the host. Conversely the fecal anaerobes that use certain B vitamins may compete with the host for these B vitamins.

5. Antibiotic Sensitivity

Frequently, oral antibiotics are used in the treatment of human disease. For this reason, tests were made with standard sensitivity discs on blood agar and Gall's agar without cysteine to determine the possible effect of commonly used antibiotics upon the sixteen fecal anaerobes which predominated during this study. The results of this test are recorded in Table 20 and show that certain of the fecal bacteria are sensitive to antibiotics. The cultures reacted differently on the two different media. Two of the type cultures, FA-1 and FA-7, did not grow under the test conditions on either media; and seven other cultures did not grow on Gall's agar while only one other culture failed to grow on the blood plate.

Of the fourteen type cultures that could be tested for sensitivity, eleven were sensitive to at least one antibiotic on one test medium, while three, FA-9, FA-12 and FA-13, were not sensitive to any of the antibiotics tested. When the culture grew on both media, sensitivity often was shown on one medium, usually Gall's agar, but not on the other.

The cultures sometimes were sensitive to all the antibiotics tested, but most cultures were selective in their sensitivities. The two antibiotics to which the fewest cultures were sensitive were Unipen and neomycin. It is interesting to speculate whether therapeutic use of these antibiotics would eliminate or diminish the sensitive type cultures.

There apparently is some mechanism which maintains the balance of flora in the intestinal tract. One possible means of control which might be involved is the production of an anti-bacterial substance by one organism to which other organisms in the gut might be sensitive. Accordingly, sensitivity tests were carried out using the autoclaved supernatant broth from each of the sixteen type cultures to test for the inhibition of growth by the usual sensitivity

procedures against each of the other type cultures. In no instance did any of the supernatant broths show any inhibitory activity toward any of the other type cultures. This is not an unexpected finding since these organisms apparently live together in the normal human gut with no antagonism, but at least it does indicate that any combination of these organisms should be compatible.

6. Germfree Rats

It is possible that even the bacterial flora normally present in the gut may be pathogenic and in order to explore this possibility, collaborative work was undertaken with members of the Nutrition Branch Food Division of the U.S. Army Laboratories at Natick, Massachusetts, and Dr. Trexler of the Gnotobiotic Research Foundation. Selected type cultures were sent to this group for mono-contamination of germfree rats. It was reasoned that since these bacteria are normally present in the body within the intestinal lumen, any pathogenic activity which they might exert would best be demonstrated by placing them in the intestinal tract of a test animal. After the organism had been growing in the intestinal tract of the germfree rat for a period of three weeks, the animal was weighed to determine weight gain and was then sacrificed and autopsied. Examination of the organs of these mono-contaminated rats revealed possible pathology only in that group of animals which were inoculated with FA-1. In three of these rats slight liver or kidney pathology was noted. This organism is probably closely related to Catenabacter which has been associated with intestinal pathology in humans. The other fecal anaerobes tested showed no tendency to produce disease, or pathology of the organs examined.

However, one interesting development occurred. Tests were made for the cholesterol content of the blood plasma and the liver. The data from these determinations are recorded in Table 21 and show that there was a statistically significant rise in plasma cholesterol in the rats mono-contaminated with FA-15 and FA-9 and that the liver content of cholesterol was elevated in the animals inoculated with FA-9. This is of possible significance in the

problem related to high cholesterol content of the human blood, since apparently these organisms growing in the gut of the germfree rats produced cholesterol or a precursor of cholesterol which was absorbed through the intestinal wall and entered the circulating blood.

7. Lipase

Among the important functions carried out in the digestive tract is the digestion of fat. Although the enzyme lipase is excreted into the gut at a higher level, it seemed pertinent to determine whether any of the fecal organisms are capable of breaking down fat. Accordingly, the lipolytic activity of the sixteen type cultures were studied and the data are recorded in Table 22. It will be noted that several of the fecal anaerobes are capable of lipolytic activity under the test conditions used. It is particularly interesting to note that FA-4, FA-5 and FA-11 which are preseumed to be identical or very closely related types of strictly anaerobic lactobacilli, gave positive reactions. FA-16, another positive culture, is probably a microaerophilic lactobacillus.

8. pH and Temperature Optima

In order to attempt to characterize these fecal anaerobes and to understand more about their physiology, tests were made to determine the optimum pH and temperature for growth. Accordingly, Gall's broth plus cysteine adjusted to final pH of 5.0, 6.6 and 7.5 was inoculated with each of the sixteen type cultures and none of the cultures grew at a pH other than 6.6, as is shown in Table 23.

To test the optimum growth temperature for these cultures, incubation was carried out in Gall's broth at room temperature, 37.5°C and 45°C. The results are presented in Table 23. Only one culture, FA-14, was able to grow at any temperature other than 37.5°C. The optimum growth temperature for FA-14 was 37.5°C but this culture could grow at both room temperature and 45°C. However, on raising the temperature to 50°C this culture also was unable to grow.

No culture was observed to be motile, or to form spores, and no other media studied proved to be better for primary isolation of the fecal anaerobes than the media used.

The physiological data will be summarized briefly in the next section of this report and will be slanted toward assessing the possible role of these bacteria in the body.

SECTION VI

CHARACTERIZATION AND POSSIBLE ROLE IN THE BODY

The major emphasis in the physiological studies has been slanted toward the determination of the probable role of these organisms in the body rather than a definite identification of these bacteria. This was done for several reasons. First of all the "key" which was set up to group similar organisms was designed to lump rather than to split groups of organisms that had similar physiological traits. Thus the organisms included in any one FA group are probably closely related but not necessarily identical. This fact coupled with the unsatisfactory status of the recognized published classification of non-sporulating strictly anaerobic bacteria discouraged attempts to definitely classify these bacteria.

Table 24 contains, in brief summary form, many of the main physiological characteristics of the sixteen type cultures and Table 25 groups these organisms according to several important physiological reactions. These data together with additional information obtained from the screen tests, lipase activity and antibiotic sensitivities may indicate their possible role in the body.

The discussion of the physiology of these organisms will be centered around their possible function in the body and an opinion will be given concerning the broad class of organisms to which they may belong using Bergey's[21] manual as the authority. The first group of organisms considered in Table 25 are the lactic acid forming bacteria. All five type cultures listed convert glucose into lactic acid in substantial quantities, but convert little or no amino acids into ammonia and with rare exceptions are not active in decarboxylation of lysine, histidine, tyrosine and arginine. These organisms appear to be mainly of the lactobacillus-type and probably FA-4, FA-5 and FA-11 are very similar if not identical cultures. They resemble Bergey's Lactobacillus bifidus, with certain consistent variations including the absence of bifurication, maintenance of strict anaerobic

character after many transfers and the strong production of the acid reduced curd in litmus milk. In addition, these organisms were isolated from the feces of adult males rather than infants. FA-16 is probably also a lactobacillus, but it is different from the other three FA types because it is microaerophilic, makes tadpole-like formations and grows less well in anaerobic media. This organism also decarboxylates arginine and produces no B vitamins. All four of these organisms show lypolytic activity and FA-5 produces vitamins B2, B12 and folic acid. (B vitamin analyses were not done on FA-4 and FA-11 because at the time these analyses were carried out, it was felt that FA-4, FA-5 and FA-11 were probably the same organism.)

From these data it would appear that these four organisms may have a similar role in the body, consisting of the metabolism of carbohydrates with the production of lactic acid, the production of lipase and, in the case of FA-5, at least, the production of certain B vitamins. The other type culture placed in the lactic acid-forming group of organism is FA-2. This organism is not considered to be a lactobacillus because of morphology and it may belong to the Eubacterium or Catenabacterium group. This organism converts glucose into lactic acid actively, but does not produce ammonia from amino acids, and its decarboxylating activities are confined to arginine. This organism also produces lipase and forms B12, folic acid and panthothenic acid. The possible role of this organism in the body may be carbohydrate metabolism, the production of lipase and the production of certain B vitamins.

The next group of organisms are considered under the heading of deaminating and decarboxylating fecal anaerobes. This group of organisms, although having quite similar physiology in certain aspects, are a diverse group morphologically. The organism FA-1 produces little acid from glucose, but is quite active in converting amino acids into ammonia and in decarboxylating three out of the four amino acids tested. FA-1 has a very distinct morphology, which probably classes it in the Catenabacterium group, and this is further indicated by the fact that this was the only organism tested which showed a tendency to produce pathology in the germfree mice since many of the Catanabacteria are pathogenic.

This organism is capable of active decarboxylation, deamination and the production of vitamin B12, folic acid and pantothenic acid.

The organisms FA-9, FA-10 and FA-12 are all capable of actively converting glucose to lactic acid amino acids to ammonia, especially FA-12, and of decarboxylating all four amino acids tested. These organisms are probably members of the Eubacterium or Catenbacterium group and seem to be closely related in function, although differing rather noticably in morphology. Their role in the body may include deamination, decarboxylation, the formation of lactic acid from carbohydrates, and the production of vitamin B12, pantothenic acid and folic acid. In addition, FA-9 does have one unusual characteristic. When this organism was used as a monocontaminant in germfree rats, an elevation of cholesterol was found both in the blood plasma and liver of that group of rats which were significantly higher than those found in the control animals.

FA-7 is also capable of converting glucose to lactic acid in large quantities and of deaminating and decarboxylating three of the four amino acids tested. It produces B12, pantothenic acid and folic acid and it is probable that its role in the body is similar to the other members of this group. Because of morphology it would seem to fit into the group of Bacteroides. FA-8 is a very tiny organism which may belong to the Dialister group. Despite its size it is physiologically very active producing lactic acid from glucose and converting amino acids to ammonia. This organism can also decarboxylate histidine and tyrosine, and produce B12 and pantothenic acid and folic acid, so its possible role in the body is similar to the other fecal anaerobes in this group.

The other five remaining organisms are less homogeneous in their physiological characteristics and have been termed miscellaneous predominating fecal anaerobes. One of these organisms, FA-13 appears to be a veillonella and shows the reaction characteristic of that group of bacteria by using the lactic acid in the media. This organism has little deaminating activity and it is difficult to determine whether or not it can decarboxylate amino acids since the heavy gas formation by this culture interferes with the test for this characteristic. FA-13 can produce B12, riboflavin and folic acid.

FA-3 is an interesting organism which is tentatively classed as a Fusobacterium by virtue of the morphology. This organism produces small amounts of lactic acid from glucose and carries out some deamination, but is active in decarboxylation of the four amino acids tested. FA-3 also produces vitamin B12 and is the only organism in the sixteen type cultures that produces indol. It is also characterized by the alkalinization of the culture media. The other organism which is tentatively classes in the Fusobacteria, largely on the basis of morphology, is FA-15. This organism differs from FA-3 in the production of large amounts of lactic acid from glucose, its greater deaminating activities and the lack of decarboxylation of the amino acids tested with the exception of arginine. This organism also produces lipase, but does not produce indol. When FA-15 is used to mono-contaminate germfree rats, it causes a statistically significant elevation of plasma cholesterol over the control animals. FA-15 is probably capable of fermenting carbohydrates, performing some deamination, producing lipase, B12 and pantothenic acid as well as cholesterol in the body.

One of the most physiologically interesting cultures is FA-14. This organism produces small amounts of lactic acid from glucose and has virtually no deaminating activity, but is capable of decarboxylation of all four of the amino acids tested and produces not only carbon dioxide, but hydrogen from glucose. Surprisingly, this organism is capable of fixing nitrogen. Due to this peculiar combination of physiological characteristics, it is difficult to place FA-14 into any category, but because of the hydrogen production, nitrogen fixation, wider temperature growth range and certain aspects of morphology, it might appear to be related to the clostridia. However, at no time was there any indication that this culture formed spores and the organism appeared to be more gram negative than gram positive. This organism produces B12 and riboflavin, and this vitamin production coupled with its decarboxylating capacity may suggest its possible role in the body.

The last organism in the miscellaneous group is a curiously inert but frequently isolated bacterium, FA-6. It produces small amounts of lactic

acid from glucose, but has no decarboxylating or deaminating activity. However, FA-6 produces some B12 and folic acid. This organism may belong in the Eubacterium or Catenabacterium groups.

Many of the type cultures are capable of metabolizing the four carbohydrates tested, glucose, sucrose, lactose and dextrin, which represent carbohydrates of increasing complexity which commonly occur in foodstuffs in the intestine and of breaking down casein, a representative food protein, which may further indicate the role of these bacteria in the body.

From these data it can be seen that certain of the strict anaerobes present in such large numbers in the feces of the normal adult human male can carry out many functions associated with the digestive process in the human body including the breakdown of glucose with the formation of lactic acid, the metabolism of simple and somewhat more complex carbohydrates, proteolysis of casein, the deamination and decarboxylation of amino acids and the production of lipase. In addition many of these organisms can produce several of the B vitamins including riboflavin, B12, pantothenic acid and folic acid. None of these organisms has been demonstrated to be pathogenic when contained in the intestinal lumen of germfree rats with the possible exception of some pathology of the liver or kidney associated with FA-1. Cholesterol levels in the plasma of germfree rats increased in the presence of FA-9 and FA-15. The exact identification of these fecal anaerobes must await more intensive physiological studies on these organisms, as well as a more definite authoritative classification.

SECTION VII

AEROBIC BACTERIA

Microbiological cultures using both aerobic and anaerobic techniques were made on 125 freshly eliminated fecal samples obtained from 25 adult male employees of Republic Aviation Corporation who were grouped into three series. The research was conducted to isolate and study the predominating fecal microflora and certain conclusions can be drawn from the data.

The results of the aerobic studies were unremarkable, showing E. coli and similar common gram negative rods to be the predominant aerobic flora. Potentially pathogenic gram negative rods were rare, but shigella was isolated from one subject (who incidentally worked with the chimpanzees when they were carrying the same type of shigella).

Other gram negative rods isolated included occasional Alkalescens dispar, klebsiella, pseudomonas, hafnia, aerobacter, and organisms in the Providence group.

Non-hemolytic streptococci isolated included Streptococcus salivarius, S. faecalis, S. mitis, S. viridans, and S. bovis. A moderate number of enterococci were found; as well as relatively few beta hemolytic streptococci.

Both non-hemolytic and MSA negative strains of staphylococci (presumably non-pathogenic) predominated over the hemolytic and MSA positive, coagulase positive strains (possible pathogens).

Miscellaneous microorganisms present in small numbers were Micrococcus sp., Corynebacterium sp. Bacillus sp., lactobacilli, and Hemophilus sp. In addition, a few yeasts Candida sp., C. stellatoiden, C. tropicalis, Pityrosporum sp., and actinomyces were recovered.

In no sample were the aerobic bacteria more numerous than the anaerobic microflora, and there was no instance in which pathogens or potential pathogens assumed important proportions.

SECTION VIII

DISCUSSION OF EXPERIMENTAL RESULTS

The purpose of this study was to establish the nature of the predominating fecal microflora to serve as baseline data for the interpretation of similar studies conducted on men subjected to space conditions. It was popularly supposed that aerobes or facultative organisms such as E. coli were the predominating fecal organisms. However, a study of the fecal flora employing both aerobic and anaerobic techniques showed conclusively that the anaerobic bacteria outnumbered the aerobic by at least 1,000 and probably 10,000 times. Once it had been established that the anaerobic organisms outnumbered the aerobic organisms by such a large margin and were therefore the predominating fecal flora, it became necessary to establish the degree of anaerobiosis of the predominating organisms. Over 95% of the fecal organisms isolated from the highest dilution of sample were strict anaerobes and therefore it can be concluded that the obligate anaerobes are the predominating fecal flora.

It was then necessary to establish which types of fecal anaerobes occurred the most frequently, so that the cultures selected for study would indeed be representative of the major part of the predominating flora. Ordinarily this is accomplished by referring to a recognized authoritative microbiological classification, but since there is so much controversity by authoritative workers in the field over the acceptance of any of the existing classifications, to avoid becoming involved in this controversy a screen test based on ten fundamental characteristics was used to design a key particularly for this study. This key was established for the sole purpose of grouping like organisms to recognize the types which occur most frequently.

The strict anaerobes were grouped according to the key and their distribution was recorded according to subject and period. In all about 125

fecal specimens from 25 men in three groups were examined and the results were tabulated as described above. Although the individual variation from sampling period to sampling period was fairly large, the overall results were quite consistent. When the results were considered on the basis of frequency of occurrence of the predominating organisms from each of the three series of men, the occurrence of the sixteen type cultures was quite uniform, both with respect to the other groups and to the overall average. The reasons for the individual variation have been thoroughly discussed in the section on distribution of fecal anaerobes, but in general it is probably true that this individual variation stems from an artifact of the technique in which only the most predominating organisms are cultured. Also the variety of food eaten in the period directly preceding the elimination of the fecal sample probably contributes to the individual variation.

Not only were the most predominating organisms consistent in this basic NASA study but the initial samples obtained from young men on two other studies, NAS-9-4172[22] and AF33(615)-1814[23] showed a distribution of FA types comparable to the results obtained in this study. Since these men came from widely scattered geographical areas in the case of NAS-9-4172 study and from Ohio in the case of the AF33(615)-1814 contract, these findings are rather reassuring. The data from the NASA study coupled with that found in the other two studies suggest that the sixteen type organisms studied intensively under this contract probably represent a good overall picture of the predominating fecal anaerobes present in adult males in this section of the country eating an average diet, within the limitations of the techniques employed.

After the most predominating fecal flora had been established, an intensive study of their basic physiology was necessary to understand their relationship to their host and to each other. The physiological studies conducted under this contract were slanted toward determining these possible relationships, rather than in trying to identify the organisms according to some recognized classification. Therefore the main physiological functions tested centered around determining the type of nutrient that could be metabolized

by these organisms and the resulting end products, as well as the synthetic activities of these organisms. It is true that whether or not these metabolic activities do indeed influence the host is still a point of debate, because the organisms which were studied had been eliminated from the body. The extent of their influence, if any, on the host is dependent upon the extent to which the fecal organisms reflect the composition of the flora higher up the digestive tract and the extent to which food materials and adequate absorption into the blood stream are present in the part of the intestinal tract where these organisms are active. For this reason the best statement that can be made at present regarding the relationship between the host and these strict anaerobic organisms is that their metabolic activities represent their possible role in the body. Studies of the bacteria present in the various levels of the digestive tract are strongly indicated to resolve this debate.

However, the question of the relationship of these organisms to each other is on much firmer ground, as there are ample opportunities for symbiotic relationships between these organisms. The results of these and other studies indicate that there are factors at work which tend to maintain the balance of the normal intestinal flora under normal circumstances. What these factors are is unknown but represents a fruitful field for investigation. However, symbioses between organisms probably enter into the maintenance of this balance. For example, one member of the fecal flora may produce a B vitamin which is required by another, such as may be the case with FA-1 and FA-3. Also FA-13 may well benefit by the production of lactic acid by any of the other fecal anaerobes. Thus, the symbiotic relationship between the organisms, which represents the action of these organisms as a group, may well play an important role in maintaining the normal balance of flora in the body.

The influence of antibacterial substances upon the intestinal flora is of importance. These antibiotic substances may come from two main sources - the ingestion of therapeutic doses of antibiotic or an antibacterial substance may be produced by one of the fecal anaerobes present in the intestinal tract. In fact, the latter instance might represent one of the factors which play an important role in maintaining the balance of intestinal flora. Accordingly, the

tests of the influence of each of the individual type cultures upon the growth of the others were designed to attempt to determine whether any of the type cultures produced an antibacterial substance to which the other bacteria might be sensitive. No antibacterial substances appeared to be produced by any of the type cultures that would influence the growth of any of the other type cultures, as might have been expected as these bacteria ordinarily live together in the gut. On the other hand the tests of the sixteen type cultures against several commonly employed antibiotics showed that many of them were sensitive to several of the antibiotics tested, including such commonly used broad spectrum antibiotics as tetracycline, chloromycetin and erythromycin. The effect of these antibiotics upon the strict anaerobes in the intestine in vivo should prove to be an interesting study.

The potential pathogenicity of selected cultures of the sixteen types of fecal anaerobes was tested by feeding the cultures to germfree animals, since it was felt that this was the most authentic way of determining the pathogenicity of intestinal organisms rather than using the more usual techniques of injecting the culture into the peritoneal cavity of a conventional mouse. Only one culture tested, FA-1, showed any tendency to produce pathology in the germfree rat. This is not surprising since most of the known intestinal pathogens are members of the aerobic flora.

Some organisms which may not be frankly pathogenic in the sense that they produce disease or pathology in certain organs may, however, form certain substances which are deleterious to the host. Among these are certain vasoconstricting amines such as histamine, tyramine, agmatine and cadaverine, which are commonly formed by the decarboxylation of certain amino acids by microorganisms that can metabolize these amino acids. The production of such deleterious substances in any area of the gut from which absorption takes place could prove harmful to the host. In another light the buildup of cholesterol in the blood vessels of humans has been considered a serious cause of illness often leading to death in humans and attempts have been made by dietary regimentation and other means to lower the amount of plasma cholesterol. The ability of two of the type organisms, FA-9 and FA-15, to

increase the plasma cholesterol in germfree rats may indicate that these organisms can contribute to an undesirable pathology.

The data obtained from this basic study on the predominating fecal flora of the adult male may well serve as a baseline to evaluate changes which might take place in this flora in studies of certain simulated conditions of space flight. For example, a space type diet tested under contract AF33(615)-1748[12], was shown to influence markedly the types of predominating fecal anaerobes present in the subjects fed this diet for relatively short periods of time. A more intensive study of the physiology of these fecal anaerobes would allow an evaluation of whether or not these changes were desirable. Further studies designed to link specific type cultures with the presence of specific major components of the diet would be valuable in two ways, to evaluate whether a certain dietary component encouraged a desirable or undesirable type of intestinal flora, and to permit the identification of possible "indicator" organisms, which might appear early in the test period and allow an evaluation of the desirability of the diet, long before significant physiological reactions occur in the host. In this way frankly undesirable feeding regimens could be screened out of the test with a minimum waste of time and risk to the subject. These baseline data may be used in a similar way to evaluate changes brought about by other conditions of space flight, such as atmospheric pressure and composition, stress, confinement and even changes in geographical location prior to space flight.

SECTION IX

CONCLUSIONS AND RECOMMENDATIONS

As the result of studies made on 125 freshly eliminated fecal samples from 25 adult human male employees of Republic Aviation Corporation who were divided into three series and cultured microbiologically using both aerobic and anaerobic techniques for the purpose of isolating and studying the predominating microflora, certain conclusions can be drawn:

1. Anaerobes were found to outnumber aerobes by 1,000 to 10,000 times, clearly establishing the anaerobic microflora as the predominating microorganisms in the feces. Further investigation showed that strictly anaerobic bacteria rather than facultative anaerobes compose over 90% of the most predominating microorganisms.

2. All of the obligate anaerobes isolated were studied by screen tests based on ten fundamental morphological and physiological characteristics for the purpose of grouping similar bacteria, and over 90% of the strict anaerobes fitted into the key established by the screen tests.

3. The occurrence of the various "type cultures" as designated in the key was determined for each subject in each sampling period, and the distribution of the type cultures was also compared with respect to occurrence in each of the three series of subjects as well as in all 25 men. Although the frequency of occurrence of the type cultures showed considerable variation between individuals and between different samples from the same individual, the comparison of the results from the three groups with each other and with the overall total were remarkable consistent with minor exceptions. Five out of six of the type cultures that occurred most frequently in the total series were also isolated the greatest number of times from each of the three series of subjects tested and this was also true of the type cultures

found the least often. The consistent nature of these results indicates that these strict anaerobes do represent the predominating microflora of these subjects on a normal diet when cultured by the techniques employed in this study. These findings have been substantiated by results of fecal studies conducted on young men under two contracts NAS-9-4172[22] and AF33(615)-1814[23] prior to these men being subjected to experimental conditions.

4. Sixteen type cultures representing the most frequently occurring strict anaerobes isolated when the physiological studies were initiated were studied in an attempt to assess their possible role in the body. The basic approach included tests indicating the ability of these type cultures to metabolize simple and more complex carbohydrates, certain protein and fat and to decarboxylate or deaminate selected amino acids, typical of those which are present in the gut. Several type cultures among those studied were capable of metabolizing all classes of substrates tested.

5. Production or use of selected B vitamins by these sixteen type cultures was tested in a rich medium containing minimal amounts of the B vitamins, and many cultures produced vitamin B2, B12, pantothenic acid and folic acid, while a few cultures used one or more of these vitamins.

6. The antibiotic studies included two considerations. The sensitivity of the sixteen type cultures to commonly used antibiotics was tested, and most of the cultures were sensitive to the broad spectrum antibiotics such as tetracycline and chloromycetin, as well as to other antibiotics. The other consideration was the possible production of an antibacterial substance by one type culture that might inhibit the growth of the other fecal anaerobes. No such sensitivity was found.

7. Monocontamination of germfree rats by feeding selected type cultures was carried out in collaborative efforts with Dr. Henry Dymsza and colleagues at the U. S. Army Natick Laboratories, Nutrition Branch, Food Division, Natick, Massachusetts and Dr. P. C. Trexler of the Gnotobiotic Research Foundation, 251 Ballardvale Street, North Wilmington, Massachusetts

to determine the effect of the type culture on the rat in the absence of other bacteria. Of the cultures tested, only FA-1 produced pathology in the liver or kidneys of the rats. However, two cultures, FA-9 and FA-15, produced a rise in the cholesterol content of the blood plasma.

8. These physiological studies indicate that certain type cultures among the sixteen studied can carry out many of the major processes associated with digestion, including the metabolism of certain carbohydrates, fats and proteins and the production of certain B vitamins. The fecal anaerobes tested, with the possible exception of one culture, do not produce disease or pathology when fed to germfree rats, and most of the cultures are sensitive to commonly used oral antibiotics. Certain cultures raise the level of cholesterol in the blood plasma of the germfree rat. These physiological reactions indicate the possible role of the bacteria in the body. Whether or not these bacteria are present at higher levels of the intestinal tract where their influence may be exerted is not proven by these studies.

9. These physiological reactions also suggest that there may well be symbiotic relationships between the various microorganisms, which may be important in maintaining the normal balance of microflora in the gut.

10. These microorganisms may also exert a deleterious effect upon the host by the production of cholesterol or the formation of toxic amines by the decarboxylation of certain amino acids.

11. The data obtained from these studies on the predominating fecal anaerobic bacteria can serve as a baseline for the evaluation and interpretation of changes in this microflora brought about by simulated or actual conditions of space flight, such as diet, atmospheric pressure and composition or stress and confinement.

These studies suggest certain recommendations for further research on related problems.

1. Diet is known to influence the intestinal flora and recent studies at Wright-Patterson Air Force Base (AF33(615)-1748[12] and AF33(615)-1814[23]) and Philadelphia Navy Yard (NAS-9-4172[22]) have shown the influence of space type diets on the predominating fecal anaerobes. Further studies on the effect of space type diets on the strictly anaerobic fecal flora are indicated to attempt to link the occurrence of a specific type culture with a specific nutrient and to find "indicator" organisms that will allow the establishment of a screening technique for the evaluation of the adequacy or desirability of a certain diet. A physiological study similar to that conducted on the sixteen type cultures in this project should be conducted on additional groups of fecal anaerobes which may become the predominating flora on any dietary regimen.

2. Conditions of space flight other than diet, such as varied atmospheric composition and pressure, stress, or confinement may influence the balance of the intestinal microflora and fecal microbiological studies should be included whenever experimental space flights, either simulated or actual involve these variables.

3. Since the astronauts travel extensively to various parts of the country and the world, often rather close to flight time, the influence of such shifting of environment on the fecal flora may be important and should be studied.

4. Studies should be conducted on the microflora of the gut at several levels higher than that represented by the feces.

5. Further studies with germfree rats should be conducted on other type cultures with particular emphasis on the cholesterol content of the blood plasma.

APPENDIX A

TECHNIQUES

A. ANAEROBIC CULTURING TECHNIQUES

The anaerobic culturing techniques to be described include the primary culturing and the screen tests.

1. Primary Culture

The anaerobic broth series for the primary culture of the fecal sample was essentially the same as that used previously by Gall, et al [24] for culturing rumen anaerobes, and which has been recently successfully adapted in the Republic laboratories to the culture of human feces.[25] This is a technique that can be adapted easily to work under field conditions. Figure 1 gives a schematic representation of the primary culturing technique, which was modified to culture from a standard loop full (100 of a gram) of freshly eliminated fecal material. Samples were cultured within fifteen minutes of elimination.

The fecal material from the standard loop was placed directly into a tube containing 9 ml of Gall's broth prepared with two drops of cysteine and one drop of sodium bicarbonate. This tube was considered to represent roughly a 10^{-3} dilution to the fecal contents. Serial dilutions were made into 11 additional tubes with 9 ml of Gall's broth prepared as above by transferring 1 ml from the inoculated tube into the next tube, etc., the top 10 of which were labeled 1 to 10 and were incubated in evacuated Torbal jars with 10% CO_2. Observations were made at 16 and 24 hours and daily thereafter. These ten tubes were considered to approximate a dilution of the sample from 10^{-4} to 10^{-13}. No dilution blanks were used, as each tube containing broth acts as a dilution blank for the next tube in the series. From tubes 5 and 6 pour plates were made into anaerobic Petri dishes using Gall's medium with cysteine and bicarbonate added. The top three tubes showing growth were subcultured into agar shakes using Gall's medium to observe the anaerobic or aerobic character of the growth and to preserve the cultures for purification and study. Each culture was stained by Hucker's modification of the Gram stain and the slide was observed microscopically. Cultures from the top three dilutions of feces showing two or more distinct morphological types of bacteria were purified by plating and picking colonies using Gall's agar in an anaerobic Petri dish. Selected colonies on the anaerobic Petri dishes originating from tubes 5 and 6 were picked and treated like the subcultures from the agar shakes as described above. Usually 4-6 different colony types appear on each anaerobic Petri plate adding 6-8 pure cultures to be run through the screen tests.

In addition blood plates were streaked from the anaerobic swabs from the rectum by the same technique as the aerobic plates, and were incubated in the same anaerobic jar as the anaerobic broth series. Growth was recorded after 24 hours and the plates were treated in the same manner as the aerobic blood plates.

The compositions of the media and solutions used in this technique are listed below:

a. Gall's Media

1% Peptone C (Albimi)
1% Peptone S (Albimi)
1% Beef Extract (Difco)
1% Yeast Extract (Difco)
0.1% K_2HPO_4
0.1% KH_2PO_4
0.1% Glucose

Make up to 100 ml with distilled water and tube in 9 ml amounts (pipetted for exactness of dilution) and sterilize exactly 10 minutes by autoclaving. Immediately before use, add aseptically 1 drop of sterile 10% $NaHCO_3$ and two drops of 10% cysteine-bicarbonate solution. This gives a pH of approximately 6.8 and an Eh of approximately -200 mv. Add 1.5% agar to the above when agar is needed for shakes and plates. This is done when originally making the media. In agar omit cysteine except where noted otherwise. To all broth and agar media 0.05% of bovine serum is added.

b. 10% Cysteine-Bicarbonate Solution

20 gm Cysteine Hydrochloride
100 ml 1N NaOH
7% $NaHCO_3$

Add the cysteine hydrochloride to the NaOH, giving an approximate pH of 7.0.

More or less NaOH will be needed depending on the particular batch of cysteine hydrochloride.

To 4 ml of this solution (15% as cysteine) in a test tube, add 2 ml of 7% $NaHCO_3$. Seal with melted vaspar. Autoclave at 15 lb for 10 minutes.

2. Screen Tests

The physiological studies of the pure cultures of predominating flora included the following screen tests:

a. Gram stain to observe morphology
b. Final pH in 0.1% glucose broth
c. Fermentation of the following sugars in Gall's media with glucose omitted:
   (1) Glucose
   (2) Sucrose
   (3) Lactose
   (4) Dextrin
   (sugars added at 0.1% level aseptically after autoclaving)
d. Growth in Gall's broth with no carbohydrate added
e. Liquefaction of gelatin in Gall's media minus carbohydrate
f. Growth and reaction in litmus milk (to which 0.05% bovine albumin and 0.1% of peptone have been added)
g. Growth in agar shake containing Gall's media

All media contained bicarbonate and all media except the agar shake contained cysteine to produce an Eh of about -200 mv. The results of the screen test on each anaerobic culture were compared with a "key" (see Table 6).

3. Test to Characterize Groups

a. Gram stain to observe morphology and Gram reaction [10 pg. 15]
b. Motility[10 pg. 150]
c. Spore formation[10 pg. 151]
d. Oxygen relationship (agar shake)
e. Temperature tolerances and optima
f. pH tolerances and optima
g. Fermentation of various carbohydrates
h. Proteolytic activity[10 pg. 158]
i. Lipolytic activity[11 pg. 141]
j. End-products formed from chief metabolic activity
k. Indol formation[10 pg. 155-7]

Where appropriate the media will contain bicarbonate and cysteine to give an Eh of -200 mv.

B. AEROBIC CULTURING TECHNIQUE

The commonly accepted aerobic culturing techniques were used to study the aerobic fecal flora. The exact procedures and media are outlined below.

1. Primary Culturing Media

BLOOD AGAR PLATE

Purpose: Cultivate fastidious microorganisms

Formula:

Base	Gms/Liter
Infusion from beef heart	10.0
Peptone "M"	10.0
Sodium chloride	5.0
Agar	15.0

pH 6.9

Then add:

5% defibrinated sheep blood

Technique: Streak the plate with the original specimen or a subculture from broth.

Procedure: Incubate 37°C for 18-24 hours

Reaction: Colonies of bacteria usually grow luxuriantly, and the hemolytic types exhibit clear distinct degrees of hemolysis.

Reference: Difco Manual,[26] p. 88.

MITIS SALIVARIUS AGAR

Purpose: The detection of fecal streptococci. Incubate exactly 24 hours at 37°C

Formula:

Peptone "M"	20.0 gms/liter
Dextrose	1.0 gms/liter
Sucrose	50.0 gms/liter
Di Potassium Phosphate	4.0 gms/liter
Agar	15.0 gms/liter
Trypan Blue	0.075 gm/liter
Crystal Violet	0.0008 gm/liter

pH 7.0

Technique: Streak the plate with the inoculum.

Reaction:

Streptococcus mitis: small or minute colonies
Streptococcus salivarius: blue (smooth or rough), gum drop colonies 1-5 mm
Enterococcus: dark blue or black raised colonies
Coliform: brown colonies
Pleuro-pneumonia: colorless mucoid colonies

Reference: Albimi Laboratories[27]

ROGOSA'S SL AGAR

Purpose: SL Agar is a selective medium for the cultivation of oral and fecal lactobacilli

Formula:

	Gms/Liter
Peptone "C"	10.0
Yeast extract	5.0
Monopotassium phosphate	6.0
Ammonium citrate	2.0
*Salt solution	5.0 ml
Dextrose	20.0
Sorhitan Mono-oleate	1.0
Sodium Acetate hydrate	25.0
Agar	15.0
Acetic acid	1.32

pH 5.4

*Salt Solution:

Magnesium sulfate $7H_2O$	11.5 gms
Magnesium sulfate $2H_2O$	2.4 gms
Magnesium sulfate $4H_2O$	2.8 gms
Ferrous sulfate $7H_2O$	0.68 gms
Distilled water	1000.0 ml

Technique: Melt agar then cool in water bath to 45°C. Add a drop of broth culture to agar; then make a pour plate.

Procedure: Incubate under partial anaerobic conditions

Reaction: Selective for cultivation of lactobacilli

Reference: Difco Supplementary Literature[11] p. 59

PHYTONE YEAST (BBL)

Purpose: For the isolation of dermatophytes especially T. nerrucosa from human and animal specimens.

Formula:

Phytone	10 gms
Yeast Extract	5 gms
Dextrose	40 gms
Streptomycin	.03 gms
*Chloramphenicol	.05 gms
Agar (dried)	17 gms

*Chloromycetin TM Parke Davis & Co.

Technique: Streak slant directly with heavy inoculum of fecal suspension or other suspicious material

Reaction: Typical colonies of the dermatophytes grow rapidly on phytone yeast agar.

Reference: Baltimore Biological Laboratories[28]

MAC CONKEY'S AGAR

Purpose: Primary differential plating media for coliforms

Formula:

Peptone "M"	10.0 gms/liter
Lactose	10.0 gms/liter
Bile salts	1.5 gms/liter
NaCl	5.0 gms/liter
Agar	15.0 gms/liter
Neutral Red	0.025 gms/liter

pH 7.1

Technique: With an inoculating loop, streak the plate with the original specimen or subculture from a broth culture.

Procedure: Incubate plate at 35-37°C for 16-18 hours. Prolonged incubation may lead to confusion of results.

Reaction: Isolated colonies of coliform bacteria are brick red in color and may be surrounded by a zone of precipitated bile. This reaction is due to the action of the acids, produced by fermentation of lactose, upon the bile salts and the subsequent absorption of neutral red. Typhoid, paratyphoid and dysentery bacilli do not ferment lactose and do not greatly alter the appearance of the medium. These colonies are uncolored and transparent.

Reference: Difco Manual[26] p. 131-2.

* * *

Where appropriate the standard procedures for identifying the organisms were carried out. [10 pg. 140-168]

C. MICROBIOLOGICAL-PHYSIOLOGICAL TECHNIQUES

1. Manometric Studies of Carbohydrate Fermentation

a. Materials and Methods

A rather complex indirect manometric technique for the measurement of CO_2 formation in the absence of oxygen was used. The principle of the technique is illustrated in Figure 2. The procedure consists of equilibration of a bicarbonate buffer solution in a manometer flask and an atmosphere containing 5% CO_2 gas according to the following sequence:

$$\underset{\left[\begin{array}{c}\text{gas in}\\ \text{gas phase}\end{array}\right]}{CO_2} \rightleftharpoons \underset{\left[\begin{array}{c}\text{gas in}\\ \text{solution}\end{array}\right]}{CO_2} + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons H^+ + CO_3^=$$

It is obvious that this procedure is helpful in metabolic studies of anaerobic bacteria, since it will quantitatively account for the following metabolic end products: acetaldehyde, CO_2, lactic acid, total acid. If a sugar such as glucose is used as a substrate, a carbon balance can be estimated and the presence of significant quantities of additional end products will be indicated.

It is obvious that the procedure outlined above is only valid when carbon dioxide is the only gas produced during the fermentation. Therefore, a procedure for the measurement of gases other than carbon dioxide was also used in conjunction with the bicarbonate-buffer technique. A conventional pH 6.7 phosphate buffer was used with a nitrogen atmosphere to measure hydrogen. Umbreit[29] outlined a procedure to overcome the problem of carbon dioxide retention by phosphate buffer:

$$Na_2HPO_4 + CO_2 + H_2O \rightarrow NaH_2PO_4 + NaH\,CO_3$$

To overcome this difficulty acid was added from flask sidearms at the termination of experiments to recover the retained carbon dioxide.

2. Lactic Acid Production

a. Materials and Methods

A modification of the procedure of Hullin and Nobel[30] was developed for lactic acid. It consists of the following steps:

(1) The sample is first freed of bacterial cellular debris and unmetabolized proteins by centrifugation and precipitation with tungstic acid.

(2) Glucose (the substrate) and pyruvic acid (a common end product of bacterial metabolism) are removed by triple extraction with copper sulfate and calcium hydroxide, since they interfere with step 4.

(3) The solution is heated with concentrated sulfuric acid to convert lactic acid to acetaldehyde: an untreated control is run to determine background acetaldehyde.

(4) Acetaldehyde from the previous step is reacted with para-hydroxydiphenyl in the presence of copper catalyst. The resulting solution is read colorimetrically at 560 mμ and compared to a standard lactic acid curve.

Samples were run in duplicate from each of two stationary phase cultures of bacteria. The arithmetic mean of the four determinations for the cultures is shown in Appendix B. . The stock cultures are listed according to the percent lactic acid formed on a weight basis per unit weight of glucose. All tubes initially contained 0.1% glucose by weight: this quantity was chosen so glucose would be a limiting factor in the medium. Of course a complete carbon balance will require more comprehensive quantitative chemical analysis; this study was done only as a preliminary screening procedure to establish the relative quantities of lactic acid formed by the type cultures which were grown to the stationary growth phase on the same quantity of glucose. The purpose was to detect those microorganisms which have the capability to consume or form lactic acid.

3. Deamination Studies

a. Materials and Methods

The following references may be consulted for procedural details: Conway,[31] Pelczar,[32] and Kersey.[33]

(1) Medium

In order to test the cultures for their ability to deaminate constituent amino acids in a pancreatic digest of casein, Gall's broth was modified in the following ways:

Phosphate buffer was used as outlined below for the normal phosphate salts. The pH was adjusted to 7.5. Only 0.1 of the normal concentration of glucose was used and 0.1% cysteine was incorporated into the broth. Twice the normal concentration of tryptone was used. The broth was filtered through Whatman #40 filter paper. Screw top centrifuge bottles were filled to the brim, sealed, and autoclaved at 15 lbs. for 15 minutes. The reasons for these modifications is outlined in the discussion section of this report.

(2) Cell Preparations

Broth was withdrawn from centrifuge bottles at room temperature (26 ± 2°C) to allow seeding with an appropriate test culture previously grown to the middle to late "log phase" of growth. Cultures were again grown to the log phase and harvested at room temperature by centrifugation of the unopened growth flask at 30,000 x gm for ten minutes. Cells were washed twice with the phosphate buffer as described below, only diluted 1:1 with double distilled water. The cells were used in the protocol outlined in Table 26 and below. A standard curve was prepared for each experiment as described in Table 27.

The following materials were used:

1. Distilled Water: tap water was processed through a Barnstead still, then through a four liter Bellco Glass Co. glass still and used fresh.

2. Standard NH_3 Solution: 66.1 mg $(NH_4)_2SO_4$ was dissolved in 500 ml double distilled H_2O, and 1.0 ml concentrated H_2SO_4 was added as a preservative. The volume was adjusted to 1000 ml.

3. Casein Hydrolyzate: 1.0 gm Bacto-Tryptone,[26] a pancreatic digest of casein, was diluted to 100 ml with double distilled water.

4. Gum Arabic: 500 mg gum arabic[34] was dissolved in 250 ml double distilled water, allowed to stand 3 hours, and filtered through #30 filter paper.

5. Nessler's Reagent: The procedure for the preparation of Jackson's Modification was used as is described in Snell and Snell.[25]

6. Phosphate Buffer pH 8.0: 9.07 gm KH_2PO_4 was dissolved in 1 liter double distilled water and 0.465 of Na_2HPO_4 in 1 liter double distilled water: 94.5 ml of Na_2HPO_4 was combined with 5.5 ml KH_2PO_4 solution.

The following procedure is used in performance of Conway microdiffusion tests for bacterial deamination of amino acids:

1. Additions to the microdiffusion dishes were made according to Table with the exception of cell suspensions.
2. The entire frosted surface of the glass covers were covered with a 1:5 paraffin-vaseline mixture about two mm in thickness.
3. The top surface of the inner ring was coated with a thin film of the same mixture.
4. Cell suspensions were added to the outer well with Warburg pipettes and the glass covers were quickly attached to the units.
5. All units were incubated 15 minutes at 37°C to form a good seal, then two hours at room temperature (26 ± 2°C).
6. Lids were pushed back just far enough to allow the addition of 1.0 ml saturated K_2CO_3 with a Warburg pipette. Units were then quickly resealed and incubated for two hours at 37°C, then for one-half hour at room temperature.
7. At the end of the incubation period 0.5 ml of solution was removed from the inner well and placed in a test tube. Then 7.5 ml of distilled water, 1 ml of gum arabic solution, and 1 ml of Nessler's reagent were added.
8. Tubes were stoppered, mixed, and allowed to stand at room temperature for one-half hour.
9. Absorption was read on a Bausch and Lomb Spectronic-20 at 450 mμ. Readings of the test samples were compared to the standard curve prepared as outlined in Table

4. Decarboxylation of Amino Acids

a. Materials and Methods

A modified form of the procedure developed by Falkow [19] was used to screen the FA type cultures for decarboxylase activity.

The decarboxylase broth of Falkow[19] was modified by the addition of 0.1% cysteine, 40 mg per liter of pyridoxine HCl, and 5% of the appropriate l-amino acid (lysine, histidine, tyrosine or arginine). FA type cultures in the late log phase of growth were used to inoculate the test broths in triplicate. Cultures were then incubated anaerobically at room temperature (26 ± 2°C) and observed after four and five day incubation periods.

5. Vitamin Analysis Techniques

Standard procedures for vitamin analysis were used to determine vitamin B_2,[36] B_{12},[37] niacin,[38] pantothenic acid,[39] and folic acid[40] on the filtrates from each type culture.

D. GERMFREE ANIMAL TECHNIQUES

A semisynthetic diet was prepared in agar-gel form by the method of Miller and Allison,[41] sealed in No. 2 cans and sterilized by irradiation at 5 Mrds. in the U.S. Army Natick Laboratories CO^{60} source. Diet composition on a dry basis in percent is as follows: casein-22, sucrose-18, dextrose-18, dextrine-19, lard-7, corn oil-3, Wesson salt mix-4, Vitamin mix-1*, cellulose-4, and agar-4. Agar was dissolved in hot water before being added to a dry mix of the other dietary components. The ratio of dry ingredients to added water was 1:1.

After sterilization, the above diet was fed for 3-week periods to weanling germfree Fischer rats from The Charles River Breeding Laboratories and maintained in Trexler-type[42] flexible film isolators. At the start of the experiments, animals were inoculated with desired microorganisms which were brought into the isolators in sealed ampules and then introduced into the diet which had been withheld from the rats for 12 hours. The study consisted of 3 runs conducted 2 months apart with experimental groups as shown in Table 21 . Germfree rats uninoculated and another group inoculated with normal rat cecum contents served as controls. The establishment of inoculated organisms was determined by

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* A kg. of the vitamin mix contained 9750 I.U. vitamin A, 975 I.U. vitamin D2, 2000 mg. ascorbic acid, 125 I.U. vitamin E, 100 mg. vitamin K, 60 mg. thiamine HCL, 30 mg. ribloflavin, 100 mg. folic acid, 1 mg. biotin, 50 mg. para aminobenzoic acid, 25 mg. vitamin B12 and glucose diluent to make up the balance of the kilograms.

culturing fresh fecal samples obtained in the isolators and the contents of the gastrointestinal tract at the termination of the runs. Samples were cultured aerobically and anaerobically in thioglycollate broth and Gall's medium.[12] In addition, gram stain smears of the cecal contents were examined microscopically. The animals were examined at autopsy for any signs of abnormality of the major organs.

In addition, the blood plasma and liver from each animal was tested for cholesterol.[10]

APPENDIX B

TABLES AND FIGURES

TABLE 1

DESIGNATION AND AGE OF SUBJECTS IN EACH SERIES

Subject Number		Name		Age
1	Series 1	R. S.		32
2		D. S.		33
3		J. B.		24
4		L. W.		30
5		D. R.		33
6		B. K.		34
7		M. O.		26
8		J. M.		40
9		R. C.		36
10		H. W.		34
			Average	32.2
11	Series 2	R. D.		34
12		C. S.		35
13		J. K.		34
15		K. H.		37
16		R. K.		27
17		J. Mc.		35
18		L. O.		32
19		N. R.		30
20		C. H.		38
			Average	33.5
21	Series 3	R. K.		35
22		A. A.		49
23		S. B.		40
24		D. B.		44
26		L. W.		44
27		A. S.		38
			Average	41.6
		Overall Average		35.6

TABLE 2

AEROBIC PLATE COUNT IN MILLIONS

Series 1

Subject Number	Sample Number 1	2	3	4	5
1	< 10	760	100	100	6
2	< 10	550	100	< 10	200
3	350	600	700	3	180
4	< 10	100	100	400	60
5	30	15	500	200	11
6	200	< 10	500	10	440
7	6	440	200	425	325
8	206	390	100	520	250
9	20	30	70	300	175
10	52	40	100	100	300

Series 2

Subject Number	Sample Number 1	2	3	4	5	6
11	130	75	10	37	35	25
12	50	71	90	14		
13	4	8	6	0	1	
15	136	missing	200	210	400	190
16	72	0	1	35	0	55
17	20	24	18	160	150	
18	700	800	300	15	400	560
19	35	1	3	2	600	45
20	1	1	17	0	17	

Series 3

Subject Number	Sample Number 1	2	3	4	5	6
21	TNTC	70	< 1	< 1	< 1	
22	51	150	1200	2	3	
23	< 1	1	240	12	16	
24	1	93	< 1	108	59	10
25	< 1	1	3	5	20	
26	1	81	21	4		

TABLE 3

HEIGHT OF ANAEROBIC GROWTH BY TUBE

Series 1

Subject Number	Sample Number 1	2	3	4	5
1	10	8	8	9	9
2	8	9	9	9	8
3	8	8	8	10	9
4	8	9	9	9	8
5	8	10	9	9	9
6	8	9	8	8	9
7	8	10	10	9	9
8	9	9	10	9	9
9	9	9	10	8	8
10	8	10	8	8	8

Series 2

Subject Number	Sample Number 1	2	3	4	5	6
11	8	9	7	8	9	8
12	9	10	10	10		
13	9	8	9	9	9	
15	10	9	10	10	9	8
16	10	8	9	8	9	8
17	8	9	7	9	8	
18	10	10	9	10	9	8
19	9	8	9	10	9	10
20	10	8	9	8	8	7

Series 3

Subject Number	Sample Number 1	2	3	4	5	6
21	8	8	8	8	9	
22	10	8	8	10	9	
23	8	8	8	8	9	
24	8	10	8	9	9	10
25	8	8	9	8	9	
26	8	8	9	8		

Tube 7 = 10^{-10}; 8 = 10^{-11}; etc.

TABLE 4

DIFFERENCES BETWEEN AEROBIC PLATE COUNT AND ANAEROBIC GROWTH IN BROTH DILUTION SERIES

Series 1

Subject Number	Sample Number 1	2	3	4	5	Average
1	6	3	3	4	6	4.4
2	4	4	4	5	3	4.0
3	3	3	3	7	4	4.0
4	4	4	4	4	4	4.0
5	4	5	5	4	5	4.6
6	3	5	3	4	4	3.8
7	5	5	5	4	4	4.6
8	4	4	5	4	4	4.2
9	5	5	6	3	3	4.4
10	4	6	3	3	3	3.8
						4.2

Series 2

Subject Number	1	2	3	4	5	6	Average
11	3	4	3	4	5	4	3.8
12	5	5	6	5			5.2
13	6	4	6	6	5		4.3
15	5	*	5	5	4	3	4.4
16	6	5	6	4	6	5	5.3
17	4	6	5	4	3		4.4
18	5	5	4	6	4	3	4.5
19	4	5	6	6	5	6	5.1
20	7	5	5	5	4		5.2
							4.8

Series 3

Subject Number	1	2	3	4	5	6	Average
21	**	4	5	5	6		5.0
22	6	3	2	7	6		4.8
23	5	5	3	4	5		4.4
24	5	5	5	3	5	6	4.8
25	5	5	6	5			5.2
26	5	3	5	5			4.5
							4.8

Overall average 4.5

* Missing Aerobic count
** Aerobic plate too numerous to count

TABLE 5

NUMBER OF TIMES STRICT ANAEROBES VS FACULTATIVE ANAEROBES APPEARED IN THE TOP THREE DILUTIONS OF GROWTH

Series 1

Subject Number	Sample Number 1		2		3		4		5	
	A	F	A	F	A	F	A	F	A	F
1	3	0	3	0	3	0	3	0	3	0
2	3	0	3	0	3	0	3	0	3	0
3	3	0	3	0	2	1	3	0	3	0
4	3	0	3	0	3	0	3	0	2	1
5	3	0	3	0	3	0	2	1	3	0
6	3	0	3	0	3	0	3	0	3	0
7	3	0	3	0	3	0	3	0	2	1
8	3	0	3	0	3	0	3	0	3	0
9	3	0	3	0	3	0	3	0	3	0
10	3	0	3	0	3	0	3	0	3	0

Series 2

Subject Number	1		2		3		4		5		6	
	A	F	A	F	A	F	A	F	A	F	A	F
11	2	1	3	0	3	0	2	1	3	0	3	0
12	2	1	2	1	3	0	3	0				
13	3	0	3	0	3	0	3	0	3	0		
15	3	0	3	0	2	1	3	0	2	1	3	0
16	3	0	3	0	3	0	3	0	3	0	3	0
17	3	0	3	0	3	0	2	1	3	0		
18	3	0	0	3	3	0	0	3	3	0	2	1
19	3	0	3	0	3	0	3	0	3	0	3	0
20	3	0	3	0	3	0	3	0	3	0	3	0

Series 3

Subject Number	1		2		3		4		5		6	
	A	F	A	F	A	F	A	F	A	F	A	F
21	2	1	3	0	3	0	3	0	3	0		
22	3	0	3	0	3	0	3	0	3	0		
23	3	0	2	1	1	2*	3	0	3	0		
24	3	0	3	0	3	0	3	0	2	1	3	0
25	2	1*	3	0	3	0	3	0	3	0		
26	3	0	3	0	3	0	3	0				

*Aerobic ; A = Strict Anaerobes; F = Facultative Anaerobes

TABLE 6

SCREEN TESTS FOR PREDOMINATING ANAEROBIC FECAL BACTERIA (OBLIGATE)

Culture Designation	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
FA-1	slender gram + rod singly and in chains; distinct rods uniformly spaced	very fine colonies; very anaerobic	heavy turbidity with slime developing	4+ 4+ slimy sediment	4+ 4+ slimy sediment	4+ 4+ slimy sediment	2+ 2+ slight slime	+ 2+	delayed Arc with proteolysis	no liquefaction	7.0
FA-2	slender gram + rod in chains, with tadpole formation	diffuse colonies very anaerobic	heavy with slime	4+ with silky turbidity 4+ slime	3+ with silky turbidity 3+ slime	3+ with silky turbidity 3+ slime	± +	± ±	delayed Arc with proteolysis	no liquefaction	6.4
FA-3	medium to small gram negative elongate pointed rods in pairs	diffuse growth; heavy gas; very anaerobic	heavy with slimy sediment	4+ slimy sediment 4+ black sediment	4+ slimy sediment 4+ black sediment	4+ slimy sediment 4+ black sediment	4+ slimy sediment 4+ black sediment	4+ slimy sediment 4+ black sediment	delayed Arc with proteolysis and gas	no liquefaction	7.5
FA-4	slender gram positive, sometimes slightly curved rod, singly	small colonies; very anaerobic	moderate turbidity	4+ slime 4+ slime	4+ slime 4+ slime	4+ slime 4+ slime	2+ sediment 2+ sediment	2+ sediment 2+ sediment	Arc strong; delayed proteolysis	no liquefaction	5.6

* Acid reduced curd

TABLE 6 (cont'd)

Culture Designation	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
FA-5	short, medium slightly curved gram positive rod, singly; often developing clusters	medium colonies, very anaerobic	moderate turbidity	4+ slime 4+ slime	4+ slime 4+ sediment	4+ slime 4+ sediment	4+ slime 4+ slime	± + -	delayed Arc* with proteolysis	no liquefaction	5.5-5.8
FA-6	gram positive medium rods, tending to form clusters some slightly curved	medium colonies, very anaerobic	clear slimy sediment	4+ slime 4+ slime	4+ slime 4+ slime	4+ slime 4+ slime	3+ slime 4+ slime	+ slight slime + slight slime	Arc	no liquefaction	6.6
FA-7	small gram negative slender rod tendency towards bipolar staining	fine colonies; very anaerobic	moderate turbidity slime	4+ slime 4+ slime	4+ slime 4+ slime	4+ slime 4+ slime	+ + slime	+ +	Arc delayed proteolysis	no liquefaction	6.6
FA-8	tiny gram negative slender rods, slightly curved	fine colonies; very anaerobic	clear with sediment	+ 3+	+ 3+	+ 3+	+ 3+	+ 3+	partial reduction orange color	no liquefaction	6.9

* Acid reduced curd

TABLE 6 (cont'd)

Culture Designation	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
FA-9	medium to large pleomorphic gram positive rod in pairs and short chains; chain has characteristic hooked or loop shape - older cultures form heavy gram positive aggregation	haze; very anaerobic	moderate turbidity	3+ slight slime 3+ moderate slime	3+ slight slime 3+ moderate slime	+ slime 3+ slime	± slime + slight slime	clear with slight slime +	delayed Arc* with ± proteolysis	no liquefaction	7.0
FA-10	very small gram positive rods in chains with a tendency for bipolar staining, sometimes slightly pointed	fine colonies very anaerobic	heavy with floccular sediment	4+ fluffy sediment 4+ sediment	4+ fluffy sediment 4+ sediment	4+ fluffy sediment 4+ sediment	3+ 4+ sediment	+ sediment 4+ sediment	delayed Arc with proteolysis	no liquefaction	6.7
FA-11	medium short gram positive rods, some slightly curved, older cultures tend toward gram positive aggregation	fine colonies very anaerobic	heavy turbidity	3+ 3+ sediment	3+ 3+ sediment	3+ sediment 3+ sediment	3+ 3+ sediment	± sediment clear with slight sediment	Arc with proteolysis	no liquefaction	6.5

* Acid reduced curd

TABLE 6 (cont'd)

Culture Designation	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
FA-12	gram positive tiny pointed rods in chains with many coccoid forms	medium colonies very anaerobic with slight gas	heavy with slime	3+ slime 3+ slime	3+ slime 3+ slime	+ with slime 3+ slime	± slime + slime	± slime ± slime	delayed Arc* with proteolysis	no liquefaction	7.2
FA-13	small gram negative cocci in masses	fine colonies; heavy gas; very anaerobic	moderate turbidity	3+ gas black slime 3+ black slime	3+ gas black slime 3+ black slime	3+ gas black slime 3+ black slime	3+ gas black slime 3+ black slime	3+ gas black slime 3+ black slime	R**	no liquefaction	6.7
FA-14	gram negative rods long slender with gram positive areas	tiny colonies very anaerobic with heavy gas	heavy turbidity gas	4+ slight slime gas 4+	4+ slight slime 4+	+ 3+ sediment	± 3+ slime	± 3+ slime	R, whey carmelization	no liquefaction	6.75
FA-15	short fat gram negative rod, singly and in pairs; some with pointed ends	delayed haze; heavy gas: very anaerobic	heavy with slight slime	4+ slight slime 4+ slight slime	4+ slight slime 4+ slight slime	+ 4+ black slime	2+ slight slime 4+ slime	± ±	delayed Arc with whey	no liquefaction grey sediment	6.7

* Acid reduced curd
** Reduced

TABLE 6 (cont'd)

Culture Number	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
FA-16	gram positive pleo rods; some curved and some tadpole forms	haze with anaerobic collar	heavy with slime	+ curly slime 3+ slime	+ curly slime 3+ slime	+ curly slime 3+ slime	clear slime + slime	-	ARC**	no lique-faction	6.8
FA-17*	large gram positive rod singly and in pairs forming palisades and V's	fine colonies very anaerobic slight gas, occasionally	slight with finely granular sediment and side growth	clear with finely granular sediment	clear with finely granular sediment	clear with finely granular sediment	clear with finely granular sediment	clear with finely granular sediment	ARC with proteolysis	no lique-faction	6.6
FA-18	gram positive long slender rods, irregular staining	fine colonies, very anaerobic	slight with slime	± moderate slime ± moderate slime	± moderate slime ± moderate slime	± moderate slime ± moderate slime	± moderate slime ± moderate slime	± moderate slime ± moderate slime	ARC delayed	no lique-faction	6.3 to 6.6

*First week readings were the same as the 24 hour readings.
**Acid reduced curd

TABLE 6 (cont'd)

Screen Tests for Predominating Anaerobic Fecal Bacteria (Facultative)

Culture Number	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
FN-1	gram positive pointed rods in pairs and short chains	fine colonies facultative anaerobic	heavy with slime	4+ slime 4+ slime	4+ slime 4+ slime	3+ slime 4+ slime	3+ slime 4+ slime	3+ slime 4+ slime	delayed ARC *	no liquefaction	6.7
FN-2	gram positive coccobacillus pairs and chains	medium colonies facultative anaerobic	clear with growth on sides and white sediment	3+ granular sediment 3+ granular sediment	3+ granular sediment 3+ granular sediment	3+ granular sediment 3+ granular sediment	+ granular sediment 3+ granular sediment	± + with sediment	ARC with proteolysis	no liquefaction	6.5
FN-3	small round cocci in short chains becoming less discrete with age	discrete colonies with heavy gas facultative anaerobic	moderate with white sediment	3+ granular sediment 4+ granular sediment	3+ granular sediment 4+ granular sediment	4+ sediment 4+ granular sediment	3+ 3+ granular sediment	± ±	ARC with proteolysis	no liquefaction	6.4
FN-4	gram positive elongate cocci in short chains	fine colonies facultative anaerobic	moderate	4+ slime 4+ slime	4+ slime 4+ slime	3+ slime 4+ slime	3+ slime 4+ slime	3+ slime 4+ slime	delayed; soft ARC	no liquefaction	6.5

* Acid reduced curd

TABLE 6 (cont'd)

Culture Number	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
FN-5	gram positive diplococci in pairs and short chains; pleomorphic	fine colonies, facultative anaerobic	moderate with floccular sediment	3+ floccular sediment	3+ floccu-lar sedi ment	3+ floccu-lar sedi-ment	3+ floccu-lar sedi-ment	+ sedi-ment	* ARC with slight proteolysis	no lique-faction	7.3 to 7.7
				4+ floccular sediment	4+ floccu-lar sedi-ment	4+ floccu-lar sedi-ment	4+ floccu-lar sedi-ment	+ sedi-ment			

* Acid reduced curd

TABLE 6 (cont'd)

SEVEN NEW TYPES OF OBLIGATE ANAEROBES (SPACE DIET - GD SERIES)

Culture Number	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
G.D. 1	short gram negative rod in pairs and chains, some pointed	fine colonies heavy gas very anaerobic	heavy floccular sediment	4+ with slime 4+ with black slime	4+ with slime 4+ with black slime	4+ with slime 4+ with black slime	2+ with slime 4+ with black slime	1+ with slime 4+ with black slime	delayed Arc* with proteolysis	black bottom no liquefaction	6.7
G.D. 2	gram negative short rod in pairs	small colonies very anaerobic	moderate with floccular slime	4+ with heavy slime 3+ with heavy slime	4+ with heavy slime 3+ with heavy slime	4+ with heavy slime 3+ with heavy slime	4+ with heavy slime 3+ with heavy slime	3+ with floccular + slight floccular slime	Arc with proteolysis	no liquefaction	6.2 6.4
G.D. 3	gram negative pointed rods	tiny colonies very anaerobic	moderate with moderate black sediment sometimes fluffy	2+ with slime 3+ with slime sometimes dark	2+ with slime 3+ with slime sometimes dark	2+ with slime 3+ with slime	2+ with slime 3+ with slime	2+ with slime 3+ with slime	reduced	no liquefaction	6.8

* Acid reduced curd

** Results obtained under contract AF33(615)-1748, "Determination of Aerobic and Anaerobic Microflora of Human Feces

TABLE 6 (cont'd)

Culture Number	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litnus Milk	Gelatin	pH
G.D. 4	gram negative slender rods in pairs some pleomorphic	tiny colonies heavy gas very anaerobic	moderate with granular sediment sometimes dark	4+ with slime and gas 4+ with slime sometimes dark	4+ with slime and gas 4+ with slime sometimes dark	4+ with slime and gas 4+ with slime sometimes dark	4+ with slime and gas 4+ with slime sometimes dark	3+ with slime and gas 3+ with slime sometimes dark	delayed Arc* with slight proteolysis	no liquefaction	6.3 6.4
G.D. 5 and G.D. 5a	gram ± medium rods in short chains	small colonies very anaerobic	clear to moderate with balls of sediment	4+ with granular sediment or slime 4+ with slime or granular sediment sometimes black	4+ with granular sediment or slime 4+ with slime or granular sediment sometimes black	4+ with granular sediment or slime 4+ with slime or granular sediment sometimes black	4+ with granular sediment or slime 4+ with slime or granular sediment sometimes black	2+ with granular sediment 3+ with slime or granular sediment sometimes black	Arc with proteolysis	no liquefaction	6.6**

* Acid reduced curd
** G.D. 5a pH 6.2 to 6.4

TABLE 6 (concluded)

Culture Number	Morphology	Agar Shake	Broth	Glucose	Sucrose	Lactose	Dextrin	Blank	Litmus Milk	Gelatin	pH
G.D. 6	gram negative short pleomorphic rods singly and in pairs	tiny colonies heavy gas very anaerobic	slight to moderate with slimy sediment	3+ with granular sediment 4+ with brown slime	3+ with granular sediment 4+ with brown slime	3+ with granular sediment 4+ with brown slime	3+ with granular sediment 4+ with brown slime	+ with slimy sediment 3+ with brown slime	delayed Arc* with proteolysis	no liquefaction	5.9
G.D. 7	gram ± short pleomorphic rods in pairs some pointed	tiny colonies heavy gas very anaerobic	4+ with dark slime	4+ with slime and heavy gas 4+ with heavy black slime	4+ with slime and heavy gas 4+ with heavy black slime	4+ with slime and heavy gas 4+ with heavy black slime	3+ with heavy slime and gas 4+ with heavy black slime	3+ with heavy slime and gas 4+ with heavy black slime	reduced	no liquefaction black bottom	6.8

* Acid reduced curd

TABLE 7

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 1 - Subject 1

Anaerobes	Sample Number				
	1	2	3*	4	5
FA-1					2
FA-2		1		1	
FA-3	1			1	1
FA-4					
FA-5		2			
FA-6					1
FA-7					
FA-8				1	
FA-9					
FA-10					
FA-11					
FA-12		1			
FA-13					
FA-14	1	1			3
FA-15		1			1
FA-16				2	
FA-17			1		
FA-18					
GD-1					
GD-2					
GD-3			2		
GD-4					
GD-5					
GD-6		1			
GD-7					
Unkeyed	1	3	2		1
TOTAL	3	10	5	5	9
FN-1					
FN-2					
FN-3					
FN-4					
FN-5					
Unkeyed					
Lactobacillus					
Enterococci					
Miscellaneous					
TOTAL	0	0	0	0	0

* 5 cultures of large pointed rod, very anaerobic did not subculture

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 1 - Subject 2

Anaerobes	Sample Number				
	1	2	3	4	5
FA-1					1
FA-2					
FA-3	1	2	2		
FA-4					
FA-5			2		
FA-6		1	1		
FA-7				1	
FA-8				2	
FA-9					
FA-10	1				
FA-11				1	1
FA-12		2			
FA-13	1				
FA-14			1		
FA-15	1	1	1	1	
FA-16					
FA-17			1		1
FA-18					1
GD-1					
GD-2					
GD-3					
GD-4					
GD-5					
GD-6					
GD-7					
Unkeyed	1	1			
TOTAL	5	7	8	5	4
FN-1		2			
FN-2		1			
FN-3					
FN-4					
FN-5					
Unkeyed					
Lactobacillus					
Enterococci					
Miscellaneous					
TOTAL	0	3	0	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 1 - Subject 3

Anaerobes	Sample Number				
	1	2	3	4	5
FA-1		2	2		
FA-2					
FA-3				4	2
FA-4					
FA-5					
FA-6			1		
FA-7					
FA-8			1		
FA-9					
FA-10		1			
FA-11					
FA-12	3	1			
FA-13					
FA-14				1	1
FA-15	1			1	3
FA-16					
FA-17		1	1		
FA-18					
GD-1					
GD-2					
GD-3					
GD-4					
GD-5					
GD-6					
GD-7					
Unkeyed					
TOTAL	4	5	5	6	6
FN-1					
FN-2					
FN-3					
FN-4					
FN-5					
Unkeyed					
Lactobacillus					
Enterococci					
Miscellaneous					
TOTAL	0	0	0	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 1 - Subject 4

Anaerobes	Sample Number				
	1	2	3	4	5
FA-1	1			1	
FA-2					
FA-3			1	1	
FA-4					
FA-5	1				
FA-6			1		
FA-7					
FA-8					4
FA-9					
FA-10		1		3	
FA-11		1	1		
FA-12			4		
FA-13					
FA-14		1			
FA-15		1	2	2	1
FA-16					
FA-17				1	
FA-18					
GD-1					
GD-2					
GD-3					
GD-4					
GD-5					
GD-6					
GD-7					
Unkeyed	1		1	1	
TOTAL	3	4	10	9	5
FN-1					
FN-2					
FN-3	1				
FN-4					
FN-5					
Unkeyed					
Lactobacillus					
Enterococci					
Miscellaneous					
TOTAL	1	0	0	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 1 - Subject 5

Anaerobes	Sample Number				
	1	2	3	4	5
FA-1	1	1		3	
FA-2			1		
FA-3	1		1		1
FA-4					1
FA-5					1
FA-6					
FA-7					
FA-8					
FA-9		1			
FA-10					
FA-11					
FA-12		1	1	1	
FA-13					
FA-14			1	2	
FA-15			2	1	
FA-16					
FA-17			1		
FA-18					
GD-1					
GD-2					
GD-3				1	
GD-4					
GD-5					
GD-6					
GD-7					
Unkeyed	1	1			1
TOTAL	3	4	7	8	4
FN-1					
FN-2					
FN-3					
FN-4					
FN-5					
Unkeyed					
Lactobacillus					
Enterococci					
Miscellaneous					
TOTAL	0	0	0	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 1 - Subject 6

Anaerobes	Sample Number				
	1	2	3	4	5
FA-1			1		
FA-2					
FA-3					
FA-4	1				
FA-5					
FA-6	1	4			1
FA-7					
FA-8				2	1
FA-9					
FA-10					
FA-11					
FA-12				1	
FA-13					
FA-14				1	1
FA-15		1			
FA-16	1				
FA-17		1		1	1
FA-18					1
GD-1					
GD-2					
GD-3					
GD-4					
GD-5					
GD-6					
GD-7					
Unkeyed			1		4*
TOTAL	3	6	2	5	9
FN-1					
FN-2					
FN-3					
FN-4					
FN-5					
Unkeyed					
Lactobacillus					
Enterococci					
Miscellaneous					
TOTAL	0	0	0	0	0

* 4 cultures of large pointed rod, very anaerobic did not subculture

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 1 - Subject 7

Anaerobes	Sample Number				
	1	2*	3	4	5
FA-1	1		2		
FA-2	1		1		5
FA-3	2	1	1		
FA-4				2	
FA-5					
FA-6				1	
FA-7					
FA-8				2	1
FA-9					
FA-10					
FA-11					
FA-12					
FA-13					
FA-14					
FA-15			2		
FA-16					
FA-17				2	
FA-18					
GD-1					
GD-2					
GD-3					
GD-4					
GD-5					
GD-6			1		
GD-7					
Unkeyed			2		1
TOTAL	4	1	9	7	7
FN-1					
FN-2					
FN-3					
FN-4					
FN-5					
Unkeyed					
Lactobacillus					
Enterococci					
Miscellaneous					
TOTAL	0	0	0	0	0

*Temporary equipment shortage, no anaerobic plates poured

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 1 - Subject 8

Anaerobes	Sample Number				
	1	2	3	4	5
FA-1	1		2		
FA-2					
FA-3					
FA-4		1		1	
FA-5	2	1			
FA-6					
FA-7	1				
FA-8					
FA-9					
FA-10		3	3		
FA-11					
FA-12			1		
FA-13					
FA-14					2
FA-15	1				1
FA-16				1	
FA-17				1	
FA-18					
GD-1					
GD-2					
GD-3			1		
GD-4					
GD-5					
GD-6					
GD-7					
Unkeyed	1	1		1	2
TOTAL	6	6	7	4	5
FN-1					
FN-2					
FN-3					
FN-4					
FN-5					
Unkeyed					
Lactobacillus					
Enterococci					
Miscellaneous					
TOTAL	0	0	0	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 1 - Subject 9

Anaerobes	Sample Number				
	1	2	3	4	5
FA-1	2				
FA-2		1			1
FA-3					
FA-4					
FA-5	3	2	1		1
FA-6	2		2		
FA-7					
FA-8					
FA-9					
FA-10					
FA-11			1	3	
FA-12				1	
FA-13	1				
FA-14	1				
FA-15					1
FA-16	4				
FA-17				1	
FA-18			1		
GD-1					
GD-2					
GD-3					
GD-4					
GD-5					
GD-6					
GD-7					
Unkeyed			1		1
TOTAL	13	3	6	5	4
FN-1					
FN-2					
FN-3					
FN-4					
FN-5					
Unkeyed					
Lactobacillus					
Enterococci					
Miscellaneous					
TOTAL	0	0	0	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 1 - Subject 10

Anaerobes	Sample Number				
	1	2	3	4	5
FA-1			2		
FA-2					
FA-3			1	1	1
FA-4					
FA-5	5				1
FA-6		2	1		
FA-7					
FA-8			1	1	2
FA-9					
FA-10					
FA-11					
FA-12			1		
FA-13			1		
FA-14		2			
FA-15			1		2
FA-16					
FA-17				1	
FA-18					
GD-1					
GD-2					
GD-3					
GD-4					
GD-5					
GD-6					
GD-7					
Unkeyed					1
TOTAL	5	4	8	3	7
FN-1		1			
FN-2					
FN-3					
FN-4		1			
FN-5					
Unkeyed					
Lactobacillus					
Enterococci					
Miscellaneous					
TOTAL	0	2	0	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 2 - Subject 11

Anaerobes	Sample Number					
	1	2	3	4	5	6
FA-1	1	3	2	1	2	
FA-2	1					
FA-3	2		3			
FA-4						
FA-5		3			1	1
FA-6			1	1		
FA-7						
FA-8						
FA-9						
FA-10						
FA-11						
FA-12			4			
FA-13						
FA-14						
FA-15	2		1			
FA-16						
FA-17			1	1		
FA-18				2		
GD-1						
GD-2						
GD-3	1					
GD-4						
GD-5						
GD-6						
GD-7						
Unkeyed	1	1		2		
TOTAL	8	7	12	7	3	1
FN-1	1					
FN-2	1					
FN-3						
FN-4						
FN-5						
Unkeyed						
Lactobacillus						
Enterococci						
Miscellaneous						
TOTAL	2	0	0	0	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 2 - Subject 12

Anaerobes	Sample Number					
	1	2	3	4	5	6
FA-1	4	3				
FA-2						
FA-3	4					
FA-4						
FA-5	4	5				
FA-6						
FA-7						
FA-8						
FA-9						
FA-10		1				
FA-11						
FA-12		1				
FA-13						
FA-14	1					
FA-15	2	2	4	2		
FA-16						
FA-17						
FA-18						
GD-1						
GD-2						
GD-3						
GD-4						
GD-5						
GD-6						
GD-7						
Unkeyed	3		2	1		
TOTAL	18	12	6	3	0	0
FN-1						
FN-2						
FN-3						
FN-4		1				
FN-5						
Unkeyed Lactobacillus Enterococci Miscellaneous	1					
TOTAL	1	1	0	0	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 2 - Subject 13

Anaerobes	Sample Number					
	1	2	3	4	5	6
FA-1	1	1	1			
FA-2	1	1				
FA-3						
FA-4						
FA-5		2				
FA-6	1			1		
FA-7						
FA-8						
FA-9						
FA-10						
FA-11	1					
FA-12						
FA-13			1			
FA-14						
FA-15	2	2	5	1	2	
FA-16						
FA-17				1		
FA-18						
GD-1						
GD-2						
GD-3						
GD-4			1			
GD-5						
GD-6						
GD-7						
Unkeyed				1		
TOTAL	6	6	8	4	2	0
FN-1						
FN-2						
FN-3						
FN-4						
FN-5						
Unkeyed		2	1			
Lactobacillus			2		1	
Enterococci						
Miscellaneous						
TOTAL	0	2	3	0	1	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 2 - Subject 15

Anaerobes	Sample Number					
	1	2	3	4	5	6
FA-1		7	4	1	1	
FA-2		1		2		
FA-3				1	1	
FA-4				1		
FA-5		3	5	3	1	
FA-6	1				1	
FA-7						
FA-8						
FA-9						
FA-10						
FA-11						
FA-12						
FA-13						
FA-14						
FA-15	1	1	2	2		1
FA-16	1			1		1
FA-17						
FA-18					1	1
GD-1						
GD-2						
GD-3						
GD-4						
GD-5						
GD-6						
GD-7						
Unkeyed	1		2	1		
TOTAL	4	12	13	12	5	3
FN-1						
FN-2						
FN-3						
FN-4						
FN-5						
Unkeyed						
Lactobacillus		1				
Enterococci						
Miscellaneous						
TOTAL	0	1	0	0	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 2 - Subject 16

Anaerobes	Sample Number					
	1	2	3	4	5	6
FA-1		2	3	2	1	1
FA-2						
FA-3		1				
FA-4						
FA-5			4			1
FA-6						
FA-7						
FA-8	3			2		
FA-9						
FA-10		3				
FA-11						
FA-12	3					
FA-13						
FA-14						2
FA-15		2			2	
FA-16					2	
FA-17						
FA-18		4				
GD-1						
GD-2						
GD-3		1	1			
GD-4						
GD-5						
GD-6		1				
GD-7						
Unkeyed	1	1		1	2	
TOTAL	7	15	8	5	7	4
FN-1				1		
FN-2						
FN-3						
FN-4						
FN-5						
Unkeyed						
Lactobacillus						
Enterococci						
Miscellaneous						
TOTAL	0	0	0	1	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 2 - Subject 17

Anaerobes	Sample Number					
	1	2	3	4	5	6
FA-1					2	
FA-2	1					
FA-3	2					
FA-4						
FA-5		1		1		
FA-6	2			1	1	
FA-7						
FA-8						
FA-9						
FA-10	1					
FA-11						
FA-12			3	1		
FA-13						
FA-14	1			3		
FA-15	3				1	
FA-16						
FA-17						
FA-18	1					
GD-1						
GD-2			2			
GD-3						
GD-4						
GD-5						
GD-6						
GD-7						
Unkeyed	1					
TOTAL	12	1	5	6	4	0
FN-1						
FN-2						
FN-3						
FN-4						
FN-5						
Unkeyed						
Lactobacillus						
Enterococci						
Miscellaneous						
TOTAL	0	0	0	0	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 2 - Subject 18

Anaerobes	Sample Number					
	1	2	3	4	5	6
FA-1		6	2			
FA-2		1				1
FA-3			2		1	
FA-4						
FA-5		2				
FA-6					1	
FA-7						1
FA-8	4					
FA-9						
FA-10			1			
FA-11						
FA-12	1				1	4
FA-13				1		
FA-14			1			
FA-15	2	1	1	1	2	
FA-16						
FA-17	1			1		
FA-18						
GD-1						
GD-2						
GD-3					1	
GD-4						
GD-5						
GD-6						
GD-7						
Unkeyed	1	3		1	1	1
TOTAL	9	13	7	4	7	7
FN-1						
FN-2						
FN-3						
FN-4						
FN-5						
Unkeyed		3		3		
Lactobacillus						
Enterococci						
Miscellaneous						
TOTAL	0	3	0	3	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 2 - Subject 19

Anaerobes	Sample Number					
	1	2	3	4	5	6
FA-1		3	2	3	1	
FA-2						
FA-3	1		4			
FA-4						
FA-5				3		
FA-6		1				1
FA-7	1					
FA-8	1					1
FA-9			1	1		1
FA-10	1		1			
FA-11	1					
FA-12						
FA-13		1				
FA-14			1			
FA-15	1	2	2	2	1	
FA-16						
FA-17		1			1	
FA-18					1	
GD-1						
GD-2		1				
GD-3			4			1
GD-4						
GD-5						
GD-6					1	
GD-7						
Unkeyed	1	3	1	1		
TOTAL	7	12	16	10	5	4
FN-1						
FN-2						
FN-3						
FN-4						
FN-5						
Unkeyed						
Lactobacillus			1			
Enterococci			1			
Miscellaneous						
TOTAL	0	0	2	0	0	0

TABLE 7 (Cont'd)

DISTIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 2 - Subject 20

Anaerobes	Sample Number					
	1	2	3	4	5	6
FA-1	3				1	1
FA-2						
FA-3						
FA-4						
FA-5		2	2	4		
FA-6			1	3		1
FA-7						
FA-8					1	
FA-9						
FA-10			1			
FA-11						
FA-12						
FA-13						
FA-14						
FA-15						1
FA-16						
FA-17					1	
FA-18	2	1				
GD-1						
GD-2						
GD-3						1
GD-4						
GD-5						
GD-6						
GD-7						
Unkeyed	1		2	1		
TOTAL	6	3	6	8	3	4
FN-1						
FN-2						
FN-3						
FN-4						
FN-5						
Unkeyed						
Lactobacillus		1				
Enterococci						
Miscellaneous						
TOTAL	0	1	0	0	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 3 - Subject 21

Anaerobes	Sample Number				
	1	2	3	4	5
FA-1	2	1		2	
FA-2					2
FA-3					
FA-4					
FA-5			1		
FA-6	3	1	1	1	
FA-7					2
FA-8	2	1	1		
FA-9					
FA-10	1			1	
FA-11		1			
FA-12	1		1		1
FA-13					
FA-14					1
FA-15		1	1		1
FA-16					
FA-17		1	1		1
FA-18			2	1	
GD-1					
GD-2					
GD-3		1		1	
GD-4					
GD-5					
GD-6					
GD-7					
Unkeyed					
TOTAL	9	7	8	6	8
FN-1					
FN-2					
FN-3					
FN-4					
FN-5					
Unkeyed					
Lactobacillus		1			
Enterococci				1	
Miscellaneous					
TOTAL	0	1	0	1	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 3 - Subject 22

Anaerobes	Sampling Period				
	1	2	3	4	5
FA-1	1	2	1		
FA-2	1	1		1	
FA-3			1	2	1
FA-4					
FA-5					
FA-6	1		1		
FA-7					
FA-8					
FA-9	2	1		1	
FA-10					
FA-11					
FA-12	1	2	1	2	1
FA-13					
FA-14		1	1		2
FA-15			1	3	
FA-16					
FA-17	1	2		1	1
FA-18		1			
GD-1					
GD-2					
GD-3					
GD-4					
GD-5					
GD-6					
GD-7					
Unkeyed					1
TOTAL	7	10	6	10	6
FN-1					
FN-2					
FN-3					
FN-4					
FN-5					
Unkeyed					
Lactobacillus					1
Enterococci		1			
Miscellaneous					
TOTAL	0	1	0	0	1

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 3 - Subject 23

Anaerobes	Sampling Period				
	1	2	3	4	5
FA-1	1	1	1	2	2
FA-2					
FA-3		1	1	1	2
FA-4					
FA-5	2	1			
FA-6					
FA-7			1	1	1
FA-8					
FA-9					
FA-10					
FA-11					
FA-12					1
FA-13				1	1
FA-14	1				1
FA-15	1	1	1	1	1
FA-16					
FA-17					
FA-18	1	2	1	2	1
GD-1					
GD-2					
GD-3					
GD-4					
GD-5					
GD-6					
GD-7					
Unkeyed	2	1		1	
TOTAL	8	7	5	9	10
FN-1					
FN-2					
FN-3					
FN-4					
FN-5					
Unkeyed					
Lactobacillus					
Enterococci		1			
Miscellaneous					
TOTAL	0	1	0	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 3 - Subject 24

Anaerobes	Sampling Period					
	1	2	3	4	5	6
FA-1	2	1	2	1		1
FA-2						
FA-3			2	2		
FA-4						
FA-5						
FA-6		2	1	1	3	3
FA-7				1		
FA-8	1		1	1	2	
FA-9						
FA-10			2			1
FA-11						
FA-12	1	1	1			
FA-13						
FA-14	1	2	1		5	2
FA-15		1			5	3
FA-16			2			
FA-17						1
FA-18	2		1			
GD-1						
GD-2						1
GD-3						
GD-4	2					
GD-5						
GD-6						
GD-7						
Unkeyed			1			
TOTAL	9	7	14	6	15	12
FN-1						
FN-2						
FN-3						
FN-4						
FN-5						
Unkeyed						
Lactobacillus				1		
Enterococci						
Miscellaneous						
TOTAL	0	0	0	1	0	0

TABLE 7 (Cont'd)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 3 - Subject 25

Anaerobes	Sampling Period				
	1	2	3	4	5
FA-1	1		1	2	2
FA-2					
FA-3	6	5	4	5	2
FA-4					
FA-5					
FA-6	1				
FA-7					
FA-8	1		1	1	1
FA-9					
FA-10		1	1		
FA-11					
FA-12					2
FA-13					
FA-14		1			2
FA-15			2	1	2
FA-16		1			
FA-17					
FA-18					
GD-1					
GD-2					
GD-3					
GD-4					
GD-5					
GD-6					
GD-7					
Unkeyed					
TOTAL	9	8	9	9	11
FN-1					
FN-2					
FN-3					
FN-4					
FN-5					
Unkeyed					
Lactobacillus				1	
Enterococci				1	
Miscellaneous					
TOTAL	0	0	0	2	0

TABLE 7 (Concluded)

DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES

Series 3 - Subject 26

Anaerobes	Sampling Period			
	1	2	3	4
FA-1	2	2	2	1
FA-2				
FA-3	2	1	2	4
FA-4				
FA-5				1
FA-6				1
FA-7				
FA-8				
FA-9	1			
FA-10				
FA-11				
FA-12			3	3
FA-13				
FA-14	1	2	1	1
FA-15	1	1	2	3
FA-16				
FA-17				1
FA-18	1	1		2
GD-1				
GD-2				
GD-3				
GD-4				
GD-5				
GD-6				
GD-7				
Unkeyed	1	1	2	4
TOTAL	9	8	12	17
FN-1				
FN-2				
FN-3				
FN-4				
FN-5				
Unkeyed				
Lactobacillus				
Enterococci				1
Miscellaneous				
TOTAL	0	0	0	1

TABLE 8

TOTAL DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES BY SUBJECT

Series 1

Anaerobes	Subject Number										
	1	2	3	4	5	6	7	8	9	10	Total
FA-1	2	1	4	2	5	1	3	3	2	2	25
FA-2	2	0	0	0	1	0	7	0	2	0	12
FA-3	3	5	6	2	3	0	4	0	0	3	26
FA-4	0	0	0	0	1	1	2	2	0	0	6
FA-5	2	2	0	1	1	0	0	3	7	6	22
FA-6	1	2	1	1	0	6	1	0	4	3	19
FA-7	0	1	0	0	0	0	0	1	0	0	2
FA-8	1	2	1	4	0	3	3	0	0	4	18
FA-9	1	0	0	0	1	0	0	0	0	0	2
FA-10	0	1	1	4	0	0	0	6	0	0	12
FA-11	0	2	0	2	0	0	0	0	4	0	8
FA-12	1	2	4	4	3	1	0	1	1	1	18
FA-13	0	1	0	0	0	0	0	0	1	1	3
FA-14	5	1	2	1	3	2	0	2	1	2	19
FA-15	2	4	5	6	3	1	2	2	1	3	29
FA-16	2	0	0	0	0	1	0	1	4	0	8
FA-17	1	2	2	1	1	3	2	1	1	1	15
FA-18	0	1	0	0	0	1	0	0	1	0	3
GD-1	0	0	0	0	0	0	0	0	0	0	0
GD-2	0	0	0	0	0	0	0	0	0	0	0
GD-3	2	0	0	0	1	0	0	1	0	0	4
GD-4	0	0	0	0	0	0	0	0	0	0	0
GD-5	0	0	0	0	0	0	0	0	0	0	0
GD-6	1	0	0	0	0	0	1	0	0	0	2
GD-7	0	0	0	0	0	0	0	0	0	0	0
Unkeyed	6	2	0	3	3	5	3	5	2	1	30
TOTAL	32	29	26	31	26	25	28	28	31	27	283
FN-1	0	2	0	0	0	0	0	0	0	1	3
FN-2	0	1	0	0	0	0	0	0	0	0	1
FN-3	0	0	0	1	0	0	0	0	0	0	1
FN-4	0	0	0	0	0	0	0	0	0	1	1
FN-5	0	0	0	0	0	0	0	0	0	0	0
Unkeyed	0	0	0	0	0	0	0	0	0	0	0
Lactobacillus	0	0	0	0	0	0	0	0	0	0	0
Enterococci	0	0	0	0	0	0	0	0	0	0	0
Miscellaneous	0	0	0	0	0	0	0	0	0	0	0
TOTAL	0	3	0	1	0	0	0	0	0	2	6

TABLE 8 (Cont'd)

TOTAL DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES BY SUBJECT

Series 2

Anaerobes	Subject Number									
	11	12	13	15	16	17	18	19	20	Total
FA-1	9	7	3	13	9	2	8	9	5	65
FA-2	1	0	2	3	0	1	2	0	0	9
FA-3	5	4	0	2	1	2	3	5		22
FA-4	0	0	0	1	0	0	0	0	0	1
FA-5	5	9	2	12	5	2	2	3	8	48
FA-6	2	0	2	2	0	4	1	2	5	18
FA-7	0	0	0	0	0	0	1	1	0	2
FA-8	0	0	0	0	5	0	4	2	1	12
FA-9	0	0	0	0	0	0	0	3	0	3
FA-10	0	1	0	0	3	1	1	2	1	9
FA-11	0	0	1	0	0	0	0	1	0	2
FA-12	4	1	0	0	3	4	6	0	0	18
FA-13	0	0	1	0	0	0	1	1	0	3
FA-14	0	1	0	0	2	4	1	1	0	9
FA-15	3	10	12	7	4	4	7	8	1	56
FA-16	0	0	0	3	2	0	0	0	0	5
FA-17	2	0	1	0	0	0	2	2	1	8
FA-18	2	0	0	2	4	1	0	1	3	13
GD-1	0	0	0	0	0	0	0	0	0	0
GD-2	0	0	0	0	0	2	0	1	0	3
GD-3	1	0	0	0	2	0	1	5	1	10
GD-4	0	0	1	0	0	0	0	0	0	1
GD-5	0	0	0	0	0	0	0	0	0	0
GD-6	0	0	0	0	1	0	0	1	0	2
GD-7	0	0	0	0	0	0	0	0	0	0
Unkeyed	4	6	1	4	5	1	7	6	4	38
TOTAL	38	39	26	49	46	28	47	54	30	357
FN-1	1	0	0	0	1	0	0	0	0	2
FN-2	1	0	0	0	0	0	0	0	0	1
FN-3	0	0	0	0	0	0	0	0	0	0
FN-4	0	1	0	0	0	0	0	0	0	1
FN-5	0	0	0	0	0	0	0	0	0	0
Unkeyed	0	0	3	0	0	0	6	0	0	9
Lactobacillus	0	0	3	1	0	0	0	1	1	6
Enterococci	0	1	0	0	0	0	0	1	0	2
Miscellaneous	0	0	0	0	0	0	0	0	0	0
TOTAL	2	2	6	1	1	0	6	2	1	21

TABLE 8 (Concluded)

TOTAL DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES BY SUBJECT

Series 3

Anaerobes	Subject Number						
	21	22	23	24	25	26	Total
FA-1	5	4	7	7	6	7	36
FA-2	2	3	0	0	0	0	5
FA-3	0	4	5	4	22	9	44
FA-4	0	0	0	0	0	0	0
FA-5	1	0	3	0	0	1	5
FA-6	6	2	0	10	1	1	20
FA-7	2	0	3	1	0	0	6
FA-8	4	0	0	5	4	0	13
FA-9	0	4	0	0	0	1	5
FA-10	2	0	0	3	2	0	7
FA-11	1	0	0	0	0	0	1
FA-12	3	7	1	3	2	6	22
FA-13	0	0	2	0	0	0	2
FA-14	1	4	2	11	3	5	26
FA-15	3	4	5	9	5	7	33
FA-16	0	0	0	2	1	0	3
FA-17	3	5	0	1	0	1	10
FA-18	3	1	7	3	0	4	18
GD-1	0	0	0	0	0	0	0
GD-2	0	0	0	1	0	0	1
GD-3	2	0	0	0	0	0	2
GD-4	0	0	0	2	0	0	2
GD-5	0	0	0	0	0	0	0
GD-6	0	0	0	0	0	0	0
GD-7	0	0	0	0	0	0	0
Unkeyed	0	1	4	1	0	4	10
TOTAL	38	39	39	63	46	46	271
FN-1	0	0	0	0	0	0	0
FN-2	0	0	0	0	0	0	0
FN-3	0	0	0	0	0	0	0
FN-4	0	0	0	0	0	0	0
FN-5	0	0	0	0	0	0	0
Unkeyed	0	0	0	0	0	0	0
Lactobacillus	1	1	0	1	1	0	4
Enterococci	1	1	1	0	1	1	5
Miscellaneous	0	0	0	0	0	0	0
TOTAL	2	2	1	1	2	1	9

TABLE 9

TOTAL DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES BY PERIOD

Series 1

Anaerobes	Sample No.					
	1	2	3	4	5	Total
FA-1	6	3	9	4	3	25
FA-2	1	2	2	1	6	12
FA-3	5	3	6	7	5	26
FA-4	1	1	0	3	1	6
FA-5	11	5	3	0	3	22
FA-6	3	7	6	1	2	19
FA-7	1	0	0	1	0	2
FA-8	0	0	2	8	8	18
FA-9	0	1	0	1	0	2
FA-10	1	5	3	3	0	12
FA-11	0	1	2	4	1	8
FA-12	3	5	7	3	0	18
FA-13	2	0	1	0	0	3
FA-14	2	4	2	4	7	19
FA-15	3	4	8	5	9	29
FA-16	5	0	0	3	0	8
FA-17	0	2	4	7	2	15
FA-18	0	0	1	0	2	3
GD-1	0	0	0	0	0	0
GD-2	0	0	0	0	0	0
GD-3	0	0	3	1	0	4
GD-4	0	0	0	0	0	0
GD-5	0	0	0	0	0	0
GD-6	0	1	1	0	0	2
GD-7	0	0	0	0	0	0
Unkeyed	5	6	7	2	10	30
TOTAL	49	50	67	58	59	283
FN-1	0	3	0	0	0	3
FN-2	0	1	0	0	0	1
FN-3	1	0	0	0	0	1
FN-4	0	1	0	0	0	1
FN-5	0	0	0	0	0	0
Unkeyed	0	0	0	0	0	0
Lactobacillus	0	0	0	0	0	0
Enterococci	0	0	0	0	0	0
Miscellaneous	0	0	0	0	0	0
TOTAL	1	5	0	0	0	6

TABLE 9 (Cont'd)

TOTAL DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES BY PERIOD

Series 2

Anaerobes	Sample Number						
	1	2	3	4	5	6	Total
FA-1	9	25	14	7	8	2	65
FA-2	3	3	0	2	0	1	9
FA-3	9	1	9	1	2	0	22
FA-4	0	0	0	1	0	0	1
FA-5	4	18	11	11	2	2	48
FA-6	4	1	2	6	3	2	18
FA-7	1	0	0	0	0	1	2
FA-8	8	0	0	2	1	1	12
FA-9	0	0	1	1	0	1	3
FA-10	2	4	3	0	0	0	9
FA-11	2	0	0	0	0	0	2
FA-12	4	1	7	1	1	4	18
FA-13	0	1	1	1	0	0	3
FA-14	2	0	2	3	0	2	9
FA-15	13	10	15	8	8	2	56
FA-16	1	0	0	1	2	1	5
FA-17	1	1	1	3	2	0	8
FA-18	3	5	0	2	2	1	13
GD-1	0	0	0	0	0	0	0
GD-2	0	1	2	0	0	0	3
GD-3	1	1	5	0	1	2	10
GD-4	0	0	1	0	0	0	1
GD-5	0	0	0	0	0	0	0
GD-6	0	1	0	0	1	0	2
GD-7	0	0	0	0	0	0	0
Unkeyed	10	8	7	9	3	1	38
TOTAL	77	81	81	59	36	23	357
FN-1	1	0	0	1	0	0	2
FN-2	1	0	0	0	0	0	1
FN-3	0	0	0	0	0	0	0
FN-4	0	1	0	0	0	0	1
FN-5	0	0	0	0	0	0	0
Unkeyed	0	5	1	3	0	0	9
Lactobacillus	0	2	3	0	1	0	6
Enterococci	1	0	1	0	0	0	2
Miscellaneous	0	0	0	0	0	0	0
TOTAL	3	8	5	4	1	0	21

FA & GD = Obligate Anaerobes
FN = Faculative Anaerobes

TABLE 9 (Concluded)

TOTAL DISTRIBUTION OF ANAEROBES IN FECAL SAMPLES BY PERIOD

Series 3

Anaerobes	Sampling Period						
	1	2	3	4	5	6	Total
FA-1	9	7	7	8	4	1	36
FA-2	1	1	0	1	2	0	5
FA-3	8	7	10	14	5	0	44
FA-4	0	0	0	0	0	0	0
FA-5	2	1	1	1	0	0	5
FA-6	5	3	3	3	3	3	20
FA-7	0	0	1	2	3	0	6
FA-8	4	1	3	2	3	0	13
FA-9	3	1	0	1	0	0	5
FA-10	1	1	3	1	0	1	7
FA-11	0	1	0	0	0	0	1
FA-12	3	3	6	5	5	0	22
FA-13	0	0	0	1	1	0	2
FA-14	3	6	3	1	1	2	26
FA-15	2	4	7	8	9	3	33
FA-16	0	1	2	0	0	0	3
FA-17	1	3	1	2	2	1	10
FA-18	4	4	4	5	1	0	18
GD-1	0	0	0	0	0	0	0
GD-2	0	0	0	0	0	1	1
GD-3	0	1	0	1	0	0	2
GD-4	2	0	0	0	0	0	2
GD-5	0	0	0	0	0	0	0
GD-6	0	0	0	0	0	0	0
GD-7	0	0	0	0	0	0	0
Unkeyed	3	2	3	1	1	0	10
TOTAL	51	47	54	57	50	12	271
FN-1	0	0	0	0	0	0	0
FN-2	0	0	0	0	0	0	0
FN-3	0	0	0	0	0	0	0
FN-4	0	0	0	0	0	0	0
FN-5	0	0	0	0	0	0	0
Unkeyed	0	0	0	0	0	0	0
Lactobacillus	0	1	0	2	1	0	4
Enterococci	0	2	0	3	0	0	5
Miscellaneous	0	0	0	0	0	0	0
TOTAL	0	3	0	5	1	0	9

TABLE 10

DISTRIBUTION OF SIXTEEN TYPE CULTURES IN THREE SEPARATE GROUPS AND TOTAL

Anaerobes	Series 1	Series 2	Series 3	Total
FA-1	25	65	36	126
FA-2	12	9	5	26
FA-3	26	22	44	92
FA-4	6	1	0	7
FA-5	22	48	5	75
FA-6	19	18	20	57
FA-7	2	2	6	10
FA-8	18	12	13	43
FA-9	2	3	5	10
FA-10	15	13	7	35
FA-11	8	2	1	11
FA-12	18	18	22	58
FA-13	3	3	2	8
FA-14	19	9	26	54
FA-15	29	54	33	116
FA-16	8	5	3	16
FA-17	15	8	10	33
FA-18	3	13	18	34
TOTAL	250	305	256	811
Number of Subjects	10	9	6	25

TABLE 11

DISTRIBUTION OF SIXTEEN TYPE CULTURES IN THREE SEPARATE GROUPS AND TOTAL RANK ACCORDING TO TOTAL OCCURRENCE

Anaerobes	Series			
	1	2	3	Total
FA-1	25	65	36	126
FA-15	29	54	33	116
FA-3	26	22	44	92
FA-5	22	48	5	75
FA-12	18	18	22	58
FA-6	19	18	20	57
FA-14	19	9	26	54
FA-8	18	12	13	43
FA-10	15	13	7	35
FA-18	3	13	18	34
FA-17	15	8	10	33
FA-2	12	9	5	26
FA-16	8	5	3	16
FA-11	8	2	1	11
FA-7	2	2	6	10
FA-9	2	3	5	10
FA-13	3	3	2	8
FA-4	6	1	0	7
TOTAL	250	305	256	811

TABLE 12

SIXTEEN TYPE CULTURES ARRANGED ACCORDING TO RANK OF OCCURRENCE IN TOTAL AND THREE SERIES

Anaerobes	Overall Rank	Series		
		1	2	3
FA-1	1	2	1	2
FA-15	2	1	2	3
FA-3	3	3	4	1
FA-5	4	4	3	12-13-14
FA-12	5	7-8	5-6	5
FA-6	6	5-6	5-6	6
FA-14	7	5-6	9-10-11	4
FA-8	8	7-8	8	8
FA-10	9	9-10	9-10-11	10
FA-18	10	15-16	7	7
FA-17	11	9-10	12	9
FA-2	12	11	9-10-11	12-13-14
FA-16	13	12-13	13	15
FA-11	14	12-13	16-17	17
FA-7	15-16	17-18	16-17	11
FA-9	15-16	17-18	14-15	12-13-14
FA-13	17	15-16	14-15	16
FA-4	18	14	18	18
TOTAL	878	250	357	271

TABLE 13

SUMMARY OF CONWAY MICRODIFFUSION STUDIES
USING TRYPTIC DIGESTS OF CASEIN

(16 gm N/100 gm of Substrate)

FA Type Culture	$\mu M\ NH_3$/mg Substrate	% Substrate N Converted to NH_3
12	0.32	28
8	0.26	23
9	0.18	16
1	0.15	13
10	0.14	12
7	0.14	12
15	0.10	9
3	0.07	6
13	0.03	2
5	0.02	2
11	<0.02	< 2
4	<0.02	< 2
2	<0.02	< 2
6	<0.02	< 2
14	<0.02	< 2
16	<0.02	< 2

TABLE 14. CALCULATION OF AMMONIA RELEASED BY DEAMINATION OF CASEIN HYDROLYSATE

Type Culture	Amount of Substrate	Optical Density	Standard Curve Interpretation x 2	Corrected For Background	μM NH_3 Per mg. Casein
FA 10	0.5 ml	0.18	1.8 μM/5 mg	0.70	0.14
	0.5 ml	0.175	1.8 μM/5 mg	0.70	0.14
	0.25 ml	0.10	1.0 μM/2.5 mg	-	-
	0.25 ml	0.10	1.0 μM/2.5 mg	-	-
	0.5 ml alone	0.07	0.70 μM		
	Endogenous	0.04	0.40 μM		
FA 9	0.5 ml	off-scale	-	-	-
	0.5 ml	off-scale	-	-	-
	0.25 ml	0.13	1.3	0.40	0.16
	0.25 ml	0.14	1.4	0.50	0.20
	0.5 ml alone	0.04			
	Endogenous	0.045	0.5		
FA 7	0.5 ml	0.18	1.8	0.70	0.14
	0.5 ml	0.18	1.8	0.70	0.14
	0.25 ml	0.10	1.0	-	-
	0.25 ml	0.10	1.0	-	-
	0.5 ml alone	0.07	0.7		
	Endogenous	0.04	0.4		

TABLE 14 (Cont'd)

CALCULATION OF AMMONIA RELEASED
BY DEAMINATION OF CASEIN HYDROLYSATE

Type Culture	Amount of Substrate	Optical Density	Standard Curve Interpretation x2	Corrected For Background	μM NH_3 Per mg. Casein
FA4	0.5 ml	0.07	0.70	-	0
	0.5 ml	0.064	0.68	-	0
	0.25 ml	0.065	0.68	-	0
	0.25 ml	0.80	0.84	-	0
	0.5 ml	0.03	0.30		
	Endogenous	0.05	0.50		
FA 5	0.5 ml	0.09	0.90	0.12	0.02
	0.5 ml	0.09	0.90	0.12	0.02
	0.25 ml	0.07	0.70	-	-
	0.25 ml	0.06	0.64	-	-
	0.5 ml	0.03	0.30		
	Endogenous	0.04	0.48		
FA 6	0.5 ml	0.050	0.25	-	0
	0.5 ml	0.053	0.27	-	0
	0.25 ml	0.049	0.26	-	0
	0.25 ml	0.058	0.29	-	0
	0.5 ml	0.044	0.22		
	Endogenous	0.040	0.21		

TABLE 14 (Cont'd)

CALCULATION OF AMMONIA RELEASED
BY DEAMINATION OF CASEIN HYDROLYSATE

Type Culture	Amount of Substrate	Optical Density	Standard Curve Interpretation x2	Corrected For Background	μ M NH_3 Per mg. Casein
FA 2	0.5 ml	0.045	0.46	-	0
	0.5 ml	0.045	0.46	-	0
	0.25 ml	0.035	0.38	-	0
	0.25 ml	0.035	0.38	-	0
	No Cells 0.5 ml	0.030	0.32		
	Endogenous	0.020	0.20		
FA 3	0.5 ml	0.123	1.22	0.44	0.088
	0.5 ml	0.100	1.00	0.22	0.044
	0.25 ml	0.059	0.58	-	-
	0.25 ml	0.060	0.60	-	-
	No Cells 0.5 ml	0.049	0.50		
	Endogenous	0.028	0.28		
FA 16	0.5 ml	0.030	0.16	-	0
	0.5 ml	0.38	0.19	-	0
	0.25 ml	0.028	0.14	-	0
	0.25 ml	0.028	0.14	-	0
	No Cells 0.5 ml	0.030	0.16		
	Endogenous	0.006	0.01		

TABLE 14 (Cont'd)

CALCULATION OF AMMONIA RELEASED
BY DEAMINATION OF CASEIN HYDROLYSATE

Type Culture	Optical Density	Interpretation x2	Corrected For Background	μM NH_3 Per mg Casein
FA 10	0.17	1.7	0.9	0.18
	0.14	1.4	0.6	0.12
	0.10	1.0	0.2	-
	0.09	0.9	0.1	-
	0.045	0.5	-	
	0.03	0.3	-	
FA 14	0.06	0.60	-	0
	0.05	0.50	-	0
	0.03	0.30	-	0
	0.025	0.24	-	0
	0.05	0.50	-	
	0.01	0.10	-	
FA 15	0.14	1.4	0.4	0.08
	0.16	1.6	0.6	0.12
	0.09	0.9	-	-
	0.10	1.0	-	-
	0.05	0.5	-	-
	0.05	0.5	-	

TABLE 14 (Cont'd)

CALCULATION OF AMMONIA RELEASED
BY DEAMINATION OF CASEIN HYDROLYSATE

Type Culture	Optical Density	Standard Curve Interpretation x2	Corrected For Background	μ M NH_3 Per mg Casein
FA 1	0.203	1.96	1.08	0.215
	0.125	1.26	0.38	0.076
	0.085	0.84	-	-
	0.110	1.08	0.20	0.08
	0.009	0.04	-	-
	0.083	0.84	-	-
FA 11	0.025	0.24	0.02	-
	0.020	0.24	0.02	-
	0.030	0.30	0.06	0
	0.026	0.26	-	-
	0.009	0.04	-	
	0.0	0.22	-	
FA 12	0.198	1.96	1.64	0.33
	0.188	1.84	1.52	0.31
	0.108	1.06	0.74	0.30
	0.110	0.08	0.34	0.14
	0.010	0.10	-	
	0.022	0.22	-	

TABLE 14 (Concluded)

CALCULATION OF AMMONIA RELEASED
BY DEAMINATION OF CASEIN HYDROLYSATE

Type Culture	Optical Density	Standard Curve Interpretation x2	Corrected For Background	μ M NH_3 Per mg Casein
FA 5	0.028	0.26	0.04	0
	0.19	0.20	-	0
	.022	0.22	-	0
	.029	0.28	0.06	0
	0.	0.2	-	-
	0.005	0.02	-	-
FA 8	0.16	1.50	1.44	0.29
	0.14	1.34	1.28	0.26
	0.07	0.70	0.64	0.26
	0.07	0.70	0.64	0.26
	0.005	0.02	-	-
	0.01	0.04	-	-
FA 13	0.002	0.02	-	-
	0.039	0.38	0.16	0.032
	0.085	0.84	0.62	0.025
	0.071	0.68	0.46	0.018
	0.020	0.20	-	
	0.001	0.002	-	

TABLE 15

DRY WEIGHTS OF CELL SUSPENSIONS USED IN MICRODIFFUSION ANALYSIS EXPERIMENTS

FA Type	Dry Weight Mg. Per Ml.
1	25
2	27
3	31
4	29
5	23 (35 in 2nd test)
6	39
7	27
8	23
9	23
10	39 (30 in 2nd test)
11	21
12	28
13	33
14	30
15	24
16	28

TABLE 16

AMINO ACID DECARBOXYLASE SCREENING TESTS

Culture Designation	Control*	Lysine	Histidine	Tyrosine	Arginine
FA1	0	0	+	+	+
FA2	0	0	0	0	+
FA3	0	+	+	+	+
FA4	0	0	0	0	0
FA5	0	0	0	0	0
FA6	0	0	0	0	0
FA7	0	0	+	+	+
FA8	0	0	+	+	0
FA9	0	+	+	+	+
FA10	0	+	+	+	+
FA11	0		0	0	0
FA12	0	+	+	+	+
FA13	0	(+)	(+)	(+)	(+)
FA14	0	+	+	+	+
FA15	0	0	0	0	+
FA16	0	0	0	0	+
Pseudomonas sp.	0	0	--	--	0
Salmonella sp.	0	+	--	--	+

* Amino acid deficient

KEY: + = decarboxylation
0 = negative
-- = not tested

TABLE 17

PERCENT CONVERSION OF GLUCOSE TO LACTIC ACID BY TYPE CULTURES

Culture	% Lactic Acid/Weight Glucose
FA-13	*
FA-1	5
FA-14	9
FA-6	9
FA-3	9
FA-12	19
FA-15	21
FA-2	26
FA-9	26
FA-7	28
FA-8	28
FA-11	37
FA-4	39
FA-5	40

* These assays were 64% below the concentration of lactic acid in the uninoculated culture medium (18 mg/100 ml.)

TABLE 18

SUMMARY GLUCOSE FERMENTATION BY TYPE CULTURES

FA	Q CO_2*	Q-CO_2H	M RCO_2H/2.5 M glucose	M lactic acid per 2.5 M glucose
1	0	1.5	1.0	0.5
2	0	3	5.0	3.0
3	0	10	5.0	0.5
4	0	1.6	7.0	3.0
5	0	1.5	6.5	2.5
6	0	1.4	7.0	1.8
7	0	1.5	1.5	1.0
8	-	-	-	-
9	0	10	8.0	0.5
10	0	5	6.0	2.1
11	0	1.5	6.0	3.0
12	0	10	8.0	0.6
13	0	0	0	0
14	2.5	0(QH_2=0.4)	0 CO_2=5.0 M H_2=1.0 M	
15	0	3	5	3.2
16	0	3	6	4.0

* M CO_2/hr/mg. dry wt. cells

** M-CO_2H/hr/mg dry wt cells

TABLE 19

B VITAMIN PRODUCTION OR USE BY THE TYPE CULTURES

Type Culture	Vitamin B-12 mμ/cc	Riboflavin μ/cc	Niacin μ/cc	Pantothenic Acid μ/cc	Folic Acid mμ/cc
FA-1	0.288	0.096	3.1	0.37	35.0
FA-2	0.237	0.078	3.6	0.37	14.5
FA-3	0.125	0.099	3.0	0.0463	10.0
FA-5	0.262	0.102	3.2	0.0814	15.5
FA-6	0.262	0.093	3.35	0.243	16.5
FA-7	0.262	0.093	2.65	0.393	25.0
FA-8	0.225	0.087	3.60	0.532	14.5
FA-9	0.362	0.078	2.45	0.208	15.5
FA-10	0.400	0.084	2.74	0.301	25.0
FA-12	0.325	0.090	2.65	0.359	17.0
FA-13	0.300	0.111	3.10	0.0116	35.0
FA-14	0.200	0.114	2.50	0.0231	11.0
FA-15	0.255	0.096	3.40	0.301	10.0
FA-16	0.0953	0.093	3.6	0.254	10.5
Control	0.084	0.084	3.6	0.254	10.0

TABLE 20

SENSITIVITY OF SIXTEEN ANAEROBIC TYPE CULTURES TO VARIOUS ANTIBIOTICS

Anaerobic Type Culture		Antibiotics							
		Penbritin 10 mcg.	Unipen 1 mcg.	Lincocin 2 mcg.	Novobiocin 5 mcg.	Neomycin 5 mcg.	Erythromycin 2 mcg.	Chloromycetin 5 mcg.	Tetracycline 5 mcg.
FA-1	GA	0	0	0	0	0	0	0	0
	BP	0	0	0	0	0	0	0	0
FA-2	GA	+	+	+	+	+	+	+	+
	BP	-	-	-	-	-	-	-	-
FA-3	GA	+	-	+	+	+	-	+	+
	BP	0	0	0	0	0	0	0	0
FA-4	GA	+	+	+	+	+	+	+	+
	BP	-	-	-	±	-	-	-	-
FA-5	GA	+	+	-	+	-	-	-	-
	BP	-	-	-	-	-	-	-	-
FA-6	GA	0	0	0	0	0	0	0	0
	BP	-	-	±	-	-	+	+	+
FA-7	GA	0	0	0	0	0	0	0	0
	BP	0	0	0	0	0	0	0	0
FA-8	GA	0	0	0	0	0	0	0	0
	BP	+	-	+	+	+	+	+	+
FA-9	GA	0	0	0	0	0	0	0	0
	BP	-	-	-	-	-	-	-	-
FA-10	GA	0	0	0	0	0	0	0	0
	BP	+	±	+	+	-	+	+	+

\+ sensitive
\- not sensitive

0 = no growth
GA = Gall's agar without cysteine

BP = blood agar plate

TABLE 20 (Concluded)

SENSITIVITY OF SIXTEEN ANAEROBIC TYPE CULTURES TO VARIOUS ANTIBIOTICS

Anaerobic Type Culture		Antibiotics							
		Penbritin 10 mcg.	Unipen 1 mcg.	Lincocin 2 mcg.	Novobiocin 5 mcg.	Neomycin 5 mcg.	Erythromycin 2 mcg.	Chloromycetin 5 mcg.	Tetracycline 5 mcg.
FA-11	GA	+	+	+	+	+	+	+	+
	BP	-	-	-	-	-	-	-	-
FA-12	GA	0	0	0	0	0	0	0	0
	BP	-	-	-	-	-	-	-	-
FA-13	GA	0	0	0	0	0	0	0	0
	BP	-	-	-	-	-	-	-	-
FA-14	GA	+	-	+	+	-	+	-	+
	BP	+	+	±	+	±	+	±	+
FA-15	GA	+	-	+	+	-	+	+	+
	BP	-	±	+	+	-	+	+	+
FA-16	GA	+	±	+	+	±	+	+	+
	BP	+	±	+	+	±	+	+	+

\+ sensitive
\- not sensitive

0 = no growth
GA = Gall's agar without cysteine

BP = blood agar plate

TABLE 21

EFFECT OF PREDOMINATING FECAL ANAEROBES ON GERMFREE RATS

Type Culture	3-Wk. Body Wt. Gain (gm)	Cecum as % of Body Wt.	Plasma Cholesterol mg/100 ml	Liver Cholesterol mg/100 g
GF	74	7.1	95	2.52
FA-1	95	6.8	97	2.78
FA-9	80	6.2	124	3.26
FA-13	98	5.6	99	2.16
FA-15	91	5.1	158	2.64
GF	83	7.1	88	
Cecum organism	101	2.4	78	
FA-1, 9, 13, 15	83	6.6	95	
FA-13 and L. acidophilus	77	6.1	73	
FA-13 and contaminant	87	7.3	71	
L. acidophilus	90	7.3	74	
GF	91	7.1	100	
FA-3	94	5.3	102	
FA-5	93	5.7	94	
FA-10	83	6.3	96	
L. acidophilus and L. bulgarius	88	6.3	101	
L. ATC 332	86	5.2	104	
GF	-	6.3		
Cecum organism	-	2.8		
FA-3	86	5.0		
GD-2	68	7.2		
GD-7	62	5.7		

GF = Germ Free

TABLE 22

LIPASE PRODUCTION BY ANAEROBIC TYPE CULTURES

Type Culture	Spirit Blue Agar Shake	Type Culture	Spirit Blue Agar Shake
FA-1	-	FA-10	-
FA-2	+	FA-11	+
FA-3	-	FA-12	-
FA-4	+	FA-13	-
FA-5	+	FA-14	-
FA-6	-	FA-15	+
FA-7	-	FA-16	+
FA-8	-	Control (lipase enzyme)	+
FA-9	-	Uninoculated control	-

Blue color = positive

TABLE 23

OPTIMAL TEMPERATURE AND pH RANGE FOR GROWTH OF SIXTEEN ANAEROBIC TYPE CULTURES

Anaerobic Type	5.0	6.6	7.5	R. T.	37.5°C	45°C
FA-1	-	+	-	-	+	-
FA-2	-	+	-	-	+	-
FA-3	-	+	-	-	+	-
FA-4	-	+	-	-	+	-
FA-5	-	+	-	-	+	-
FA-6	-	+	-	-	+	-
FA-7	-	+	-	-	+	-
FA-8	-	+	-	-	+	-
FA-9	-	+	-	-	+	-
FA-10	-	+	-	-	+	-
FA-11	-	+	-	-	+	-
FA-12	-	+	-	-	+	-
FA-13	-	+	-	-	+	-
FA-14	-	+	-	+ sl no gas	+ hy gas	+ hy no gas*
FA-15	-	+	-	-	+	-
FA-16	-	+	-	-	+	-

RT = room temperature

+ = growth

- = no growth

*FA-14 failed to grow at 50-52°C

sl = slight

hy = heavy

TABLE 24

PHYSIOLOGICAL CHARACTERISTICS OF THE PREDOMINATING FECAL ANAEROBES

Type Culture	Morphology	Agar Shake	pH Broth*	Deamination % Substrate Converted to NH_3	Decarboxylation***				% Lactic Acid/ Wt. Glucose	Gas From Glucose	Vitamins***				
					Lysine	Histidine	Tyrosine	Arginine			B_{12}	B_2	Niacin	P. A.	Folic Acid
FA-1	sl gr + rods	v an	7.0 4.6	13	0	+	+	+	5		+	0	0	+	+
FA-2	sl gr + rod, tadpole	v an	6.4 4.5	<2	0	0	0	+	26		+	-	0	+	+
FA-3**	gr neg elongate pt rds in pr	v an heavy gas	7.5 6.1	6	+	+	+	+	9	CO_2	+	0	0	-	0
FA-4	sl gr + rods	v an	5.6 4.65	<2	0	0	0	0	39		X	X	X	X	X
FA-5	sl med gr + rod clusters	v an	5.5 4.55	2	0	0	0	0	40		+	+	0	-	+
FA-6	gr + med rods clusters	v an	6.6 4.45	<2	0	0	0	0	9		+	0	0	0	+
FA-7	sm gr neg sl rod bipolar	v an	6.6 4.85	12	0	+	+	+	28		+	0	-	+	+
FA-8	tiny gr neg sl rods, sl curve	v an	6.9 8.0	23	0	+	+	0	28		+	0	0	+	+
FA-9	pleo gr + rod hooked chains	v an	7.0 4.85	16	+	+	+	+	26		+	-	-	-	+

* Top number pH = 1/10% glucose heavily buffered
Bottom = 5/10% glucose not buffered
** Produces indol

X Test not done
*** + = activity or production
- = utilization
0 = no reaction

TABLE 24 (Concluded)

PHYSIOLOGICAL CHARACTERISTICS OF THE PREDOMINATING FECAL ANAEROBES

Type Culture	Morphology	Agar Shake	pH Broth*	Deamination % Substrate Converted to NH_3	Decarboxylation				% Lactic Acid/ Wt. Glucose	Gas From Glucose	Vitamins				
					Lysine	Histidine	Tyrosine	Arginine			B_{12}	B_2	Niacin	P. A.	Folic Acid
FA-10	v sm gr + rods in chain bipolar sl pt	v an	6.7 4.90	12	+	+	+	+	20	CO_2	+	0	-	+	+
FA-11	sh med gr + rods	v an	6.5 4.5	2	X	0	0	0	37		X	X	X	X	X
FA-12	tiny pt gr + rods chains coccoid	v an occ sl gas	7.2 4.65	28	+	+	+	+	19		+	0	-	+	+
FA-13	sm gr neg cocci in masses	v an hvy gas	6.7 8.1	2	(+)	(+)	(+)	(+)	used		+	+	0	-	+
FA-14**	gr neg rods long sl with gr + areas	v an hvy gas	6.7 5.3	2	+	+	+	+	9	CO_2H_2	+	+	-	-	0
FA-15	sh fat gr neg rods pt ends	v an hvy gas	6.7 4.65	9	0	0	0	+	21		+	0	0	+	0
FA-16	gr + pleo rods tadpole	anaerobic collar	6.8 4.62	2	0	0	0	+	40		0	0	0	0	0

* Top number pH = 1/10% glucose heavily buffered
Bottom = 5/10% glucose not buffered
** Fixes nitrogen

X = Test not done
( ) = Questionable results due to gas formation by culture
v = very
pt = pointed

TABLE 25

LACTIC ACID FORMING PREDOMINATING FECAL ANAEROBES

Type Culture	% Lactic acid/ Wt. Glucose	% Substrate Converted to NH_3	Decarboxylation			
			Lysine	Histidine	Tyrosine	Arginine
FA-2	26	2	0	0	0	+
FA-4	39	2	0	0	0	0
FA-5	40	2	0	0	0	0
FA-11	37	2	X	0	0	0
FA-16	40	2	0	0	0	+
DEAMINATING AND DECARBOXYLATING PREDOMINATING FECAL ANAEROBES						
FA-1	5	13	0	+	+	+
FA-9	26	16	+	+	+	+
FA-10	20	12	+	+	+	+
FA-12	19	28	+	+	+	+
FA-7	28	12	0	+	+	+
FA-8	28	23	0	+	+	0
MISCELLANEOUS PREDOMINATING FECAL ANAEROBES						
FA-3	9	6	+	+	+	+
FA-6	9	2	0	0	0	0
FA-13	used	2	(+)	(+)	(+)	(+)
FA-14	9	2	+	+	+	+
FA-15	21	9	0	0	0	+

( ) = Questionable results due to gas formation by culture
X = Test not done

TABLE 26

PROTOCOL FOR MICRODIFFUSION ANALYSIS

	Inner Well	Outer Well (milliliters of addition)			
Unit Number	H_2SO_4	pH 8.0 Phosphate Buffer	0.1% Casein Hydrolyzate	Cell Preparation	Water
1	1.0	0.5	0.5	1.0	0
2	1.0	0.5	0.5	1.0	0
3	1.0	0.5	0.25	1.0	0.25
4	1.0	0.5	0.25	1.0	0.25
5	1.0	0.5	0.5	1.0	0
6	1.0	0.5	-	1.0	0.5

TABLE 27

PROTOCOL FOR PREPARATION OF STANDARD CURVE

Tube	1 mg/ml. NH_3 Solution	DDH_2O	0.2% Gum Arabic Solution	Nessler's Solution
1	0.5 ml	7.5 ml	1.0 ml	1.0 ml
2	1.5	6.5	1.0	1.0
3	2.5	5.5	1.0	1.0
4	0	8.0	1.0	1.0
5	3	5.0	1.0	1.0
6	3.5	4.5	1.0	1.0
7	4.0	4.0	1.0	1.0
8	4.5	3.5	1.0	1.0

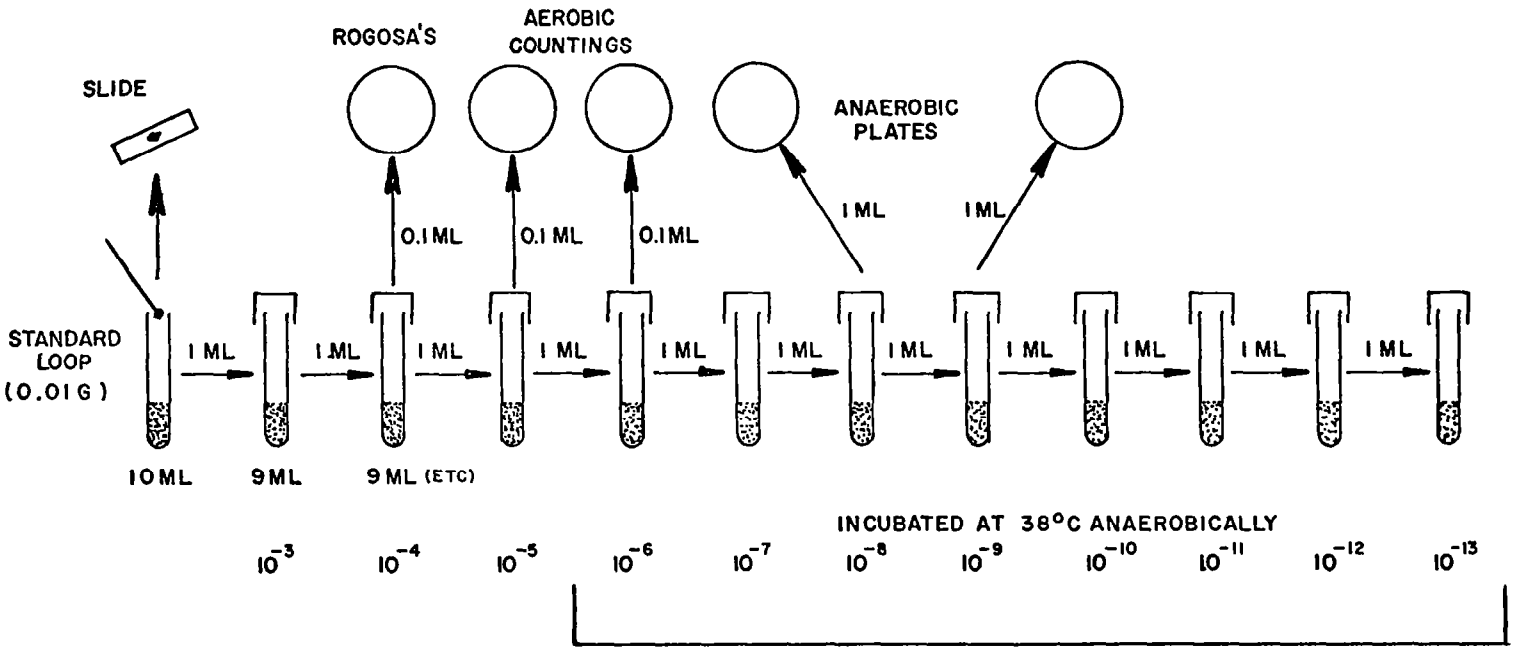
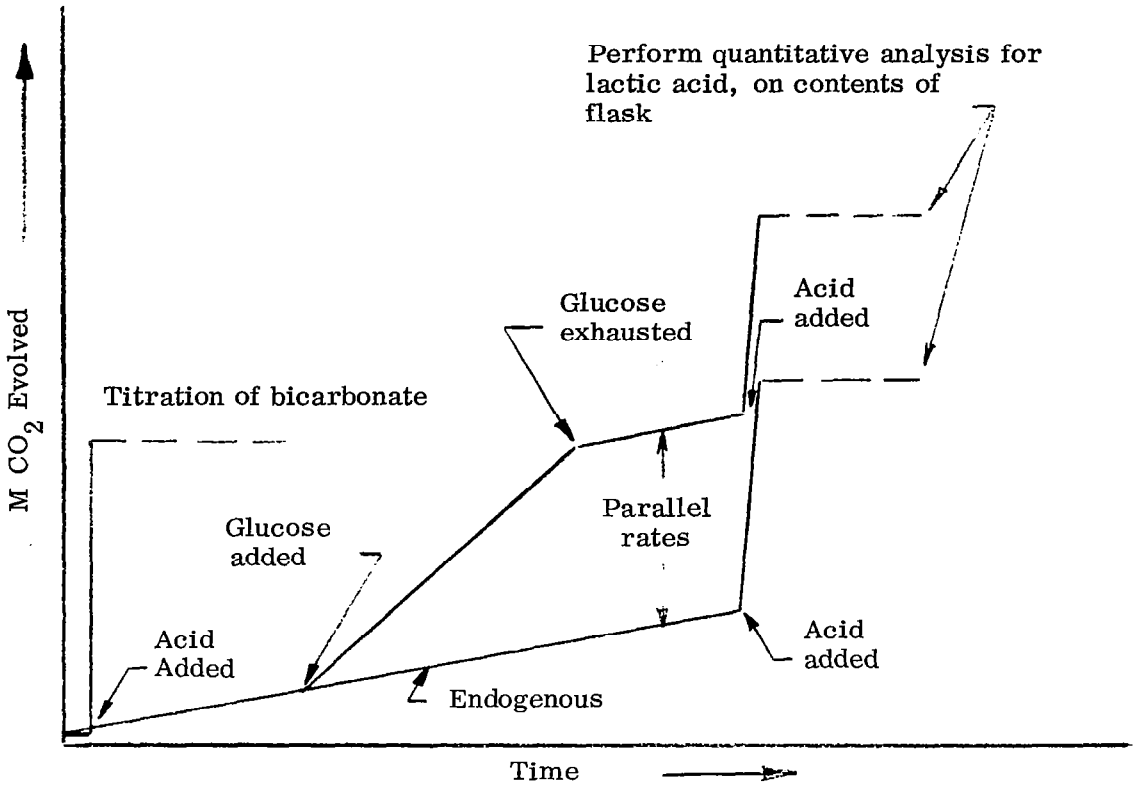


Figure 1. Anaerobic Dilution Series



Procedure for Studies on Fermentation

1. Run an endogenous control.

2. Sacrifice one reaction vessel by adding acid prior to the run in order to titrate bicarbonate.

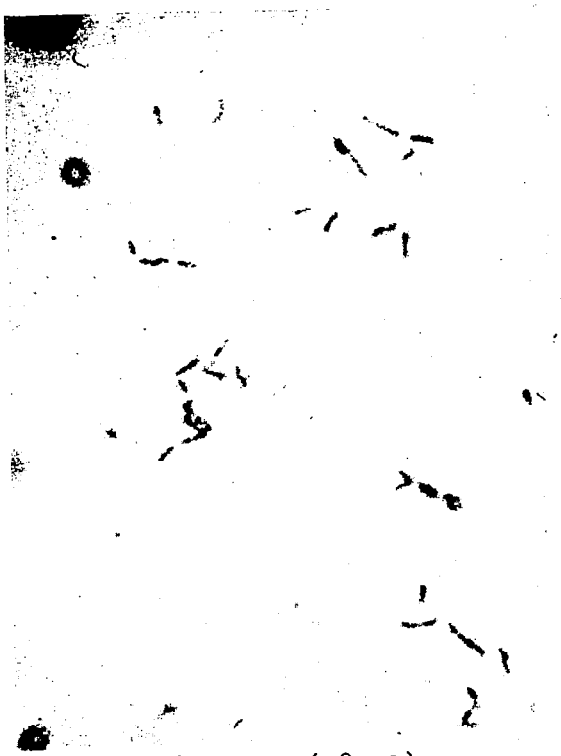
3. Run an experimental flask:

   a. Equilibrate and measure endogenous rate
   b. Add substrate until all is utilized and endogenous rate is parallel to the endogenous flask
   c. Tip in acid to titrate residual bicarbonate and kill the cells
   d. Perform analysis on the flask contents to assay:

      (1) Acetaldehyde
      (2) Lactic acid

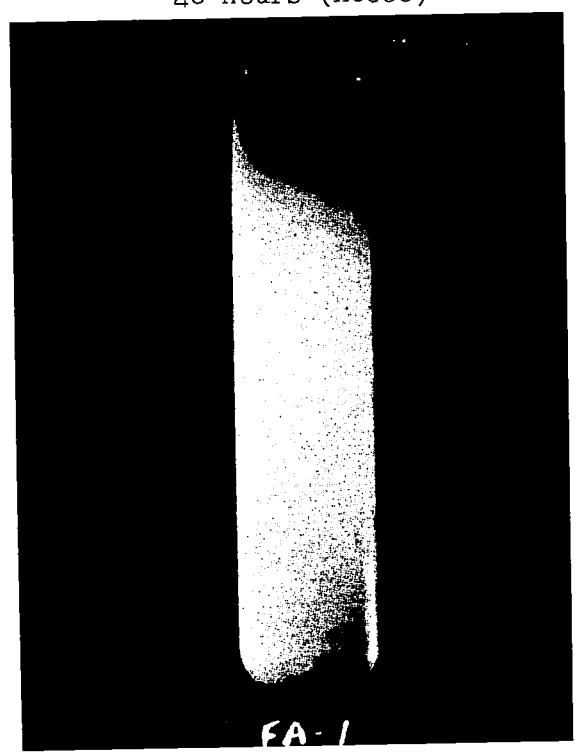
Figure 2. Idealized Theoretical Results From Typical Experiment in Which CO_2 and Organic Acids Are Produced by Fermentation.

FA-1



24 Hours (X8000)

48 Hours (X8000)

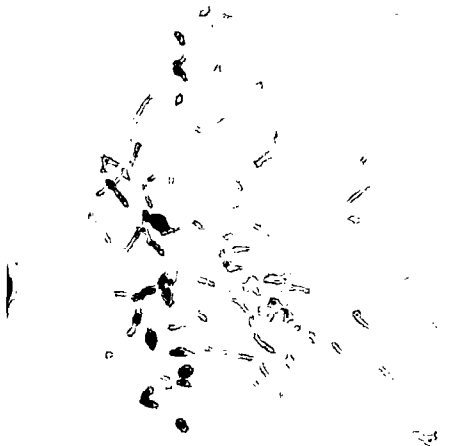


Agar Shake

1 Week (X8000)

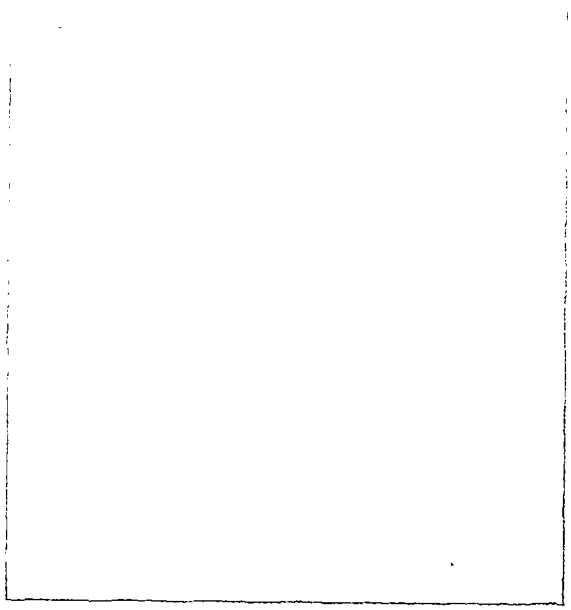
Figure 3. Pictures of Type Cultures

FA-2

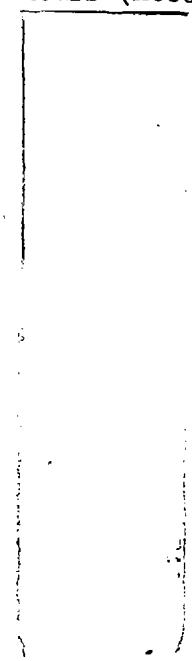


24 Hours (X8000)

48 Hours (X8000)



1 Week (X8000)



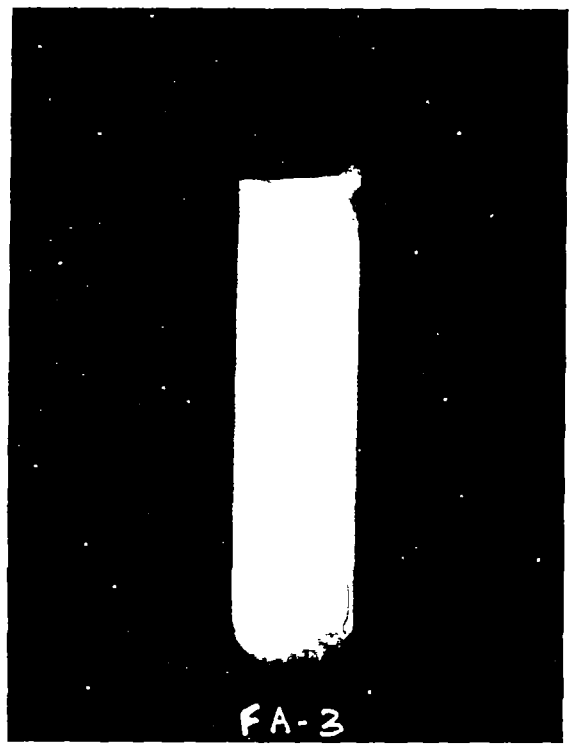
Agar Shake

Figure 3 (cont'd)

FA-3

24 Hours (X8000)

48 Hours (X8000)



1 Week (X8000)

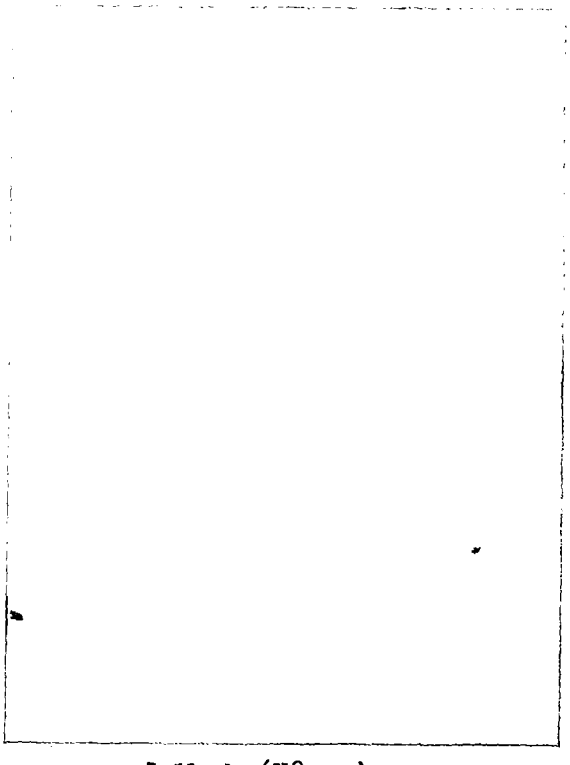
Agar Shake

Figure 3. (cont'd)

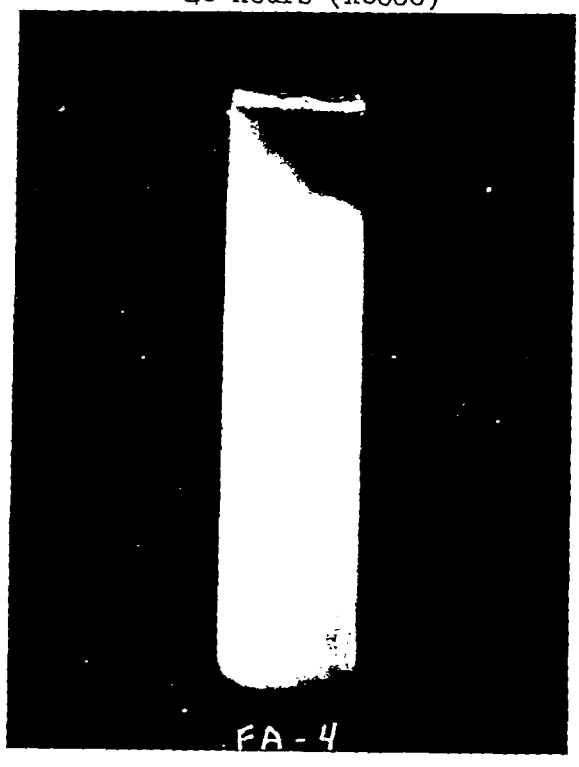
FA-4

24 Hours (X8000)

48 Hours (X8000)



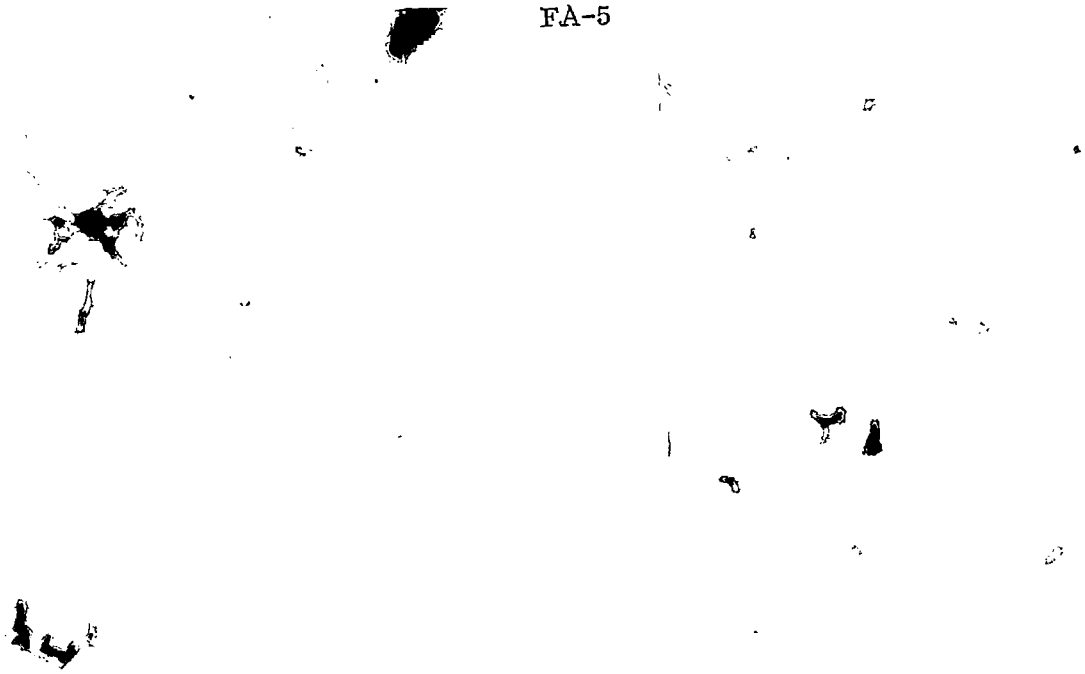
1 Week (X8000)



Agar Shake

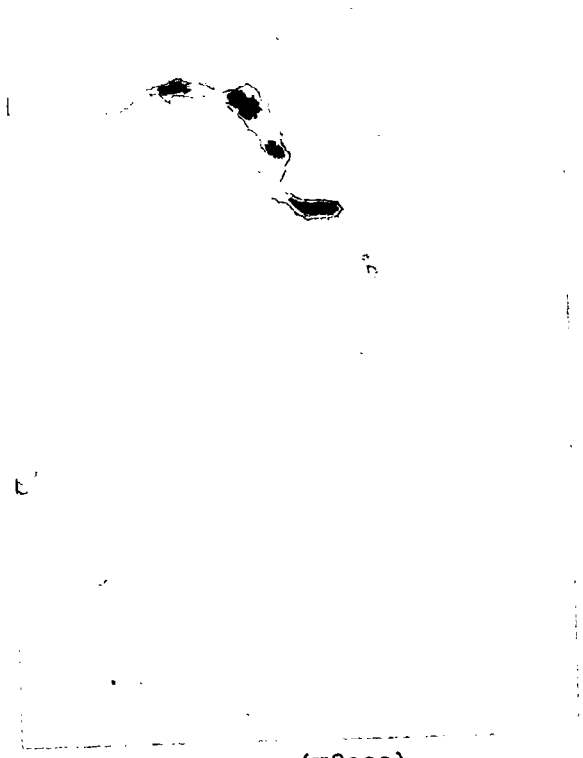
Figure 3 (cont'd)

FA-5

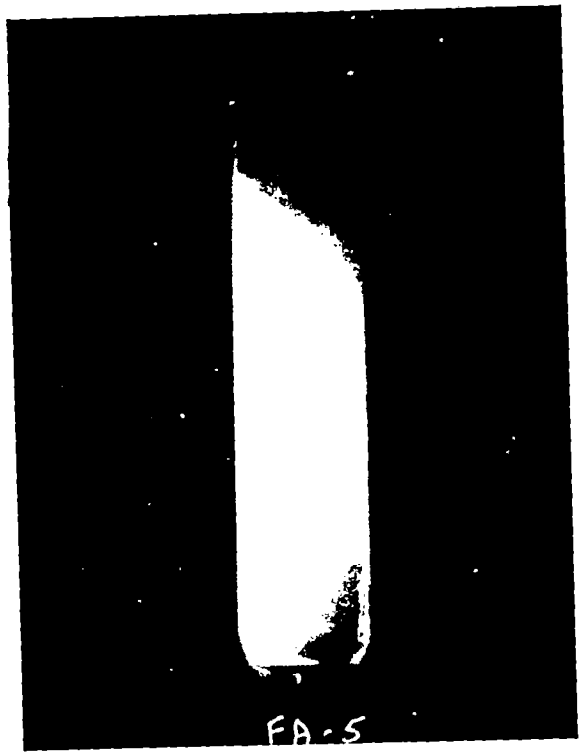


24 Hours (X8000)

48 Hours (X8000)



1 Week (X8000)



Agar Shake

Figure 3 (cont'd)

FA-6

24 Hours (X8000)

48 Hours

1 Week (X8000)

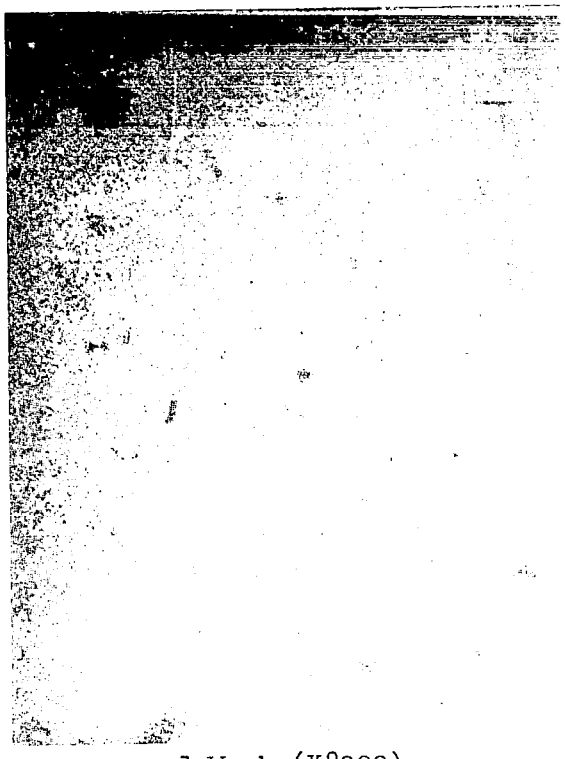
Agar Shal

Figure 3 (cont'd)

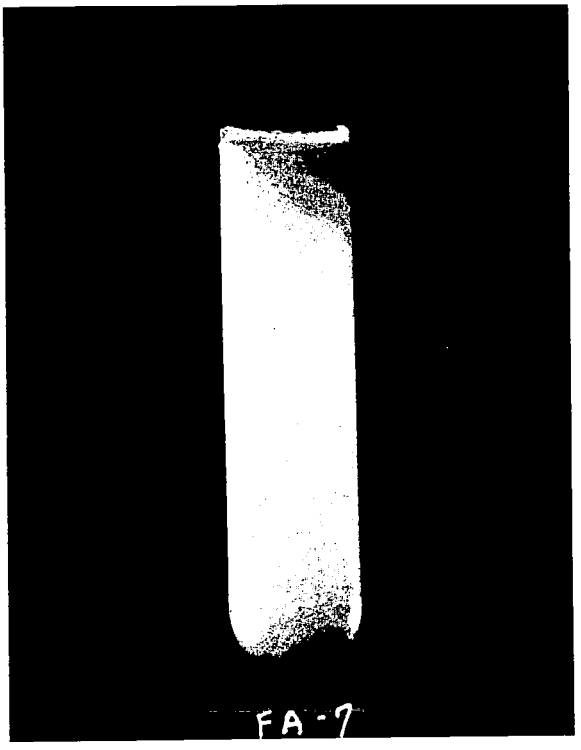
FA-7

24 Hours (X8000)

48 Hours (X8000)



1 Week (X8000)



Agar Shake

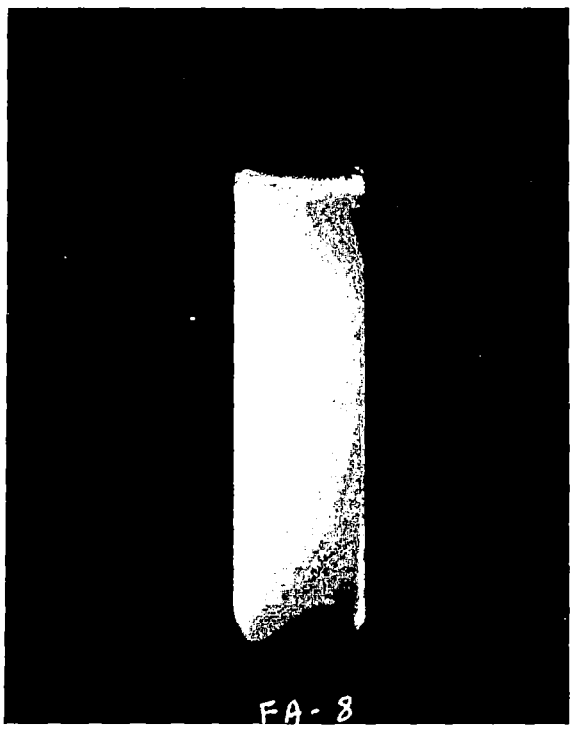
Figure 3 (cont'd)

FA-8

24 Hours (X8000)

1 Week (X8000)

48 Hours (X8000)



Agar Shake

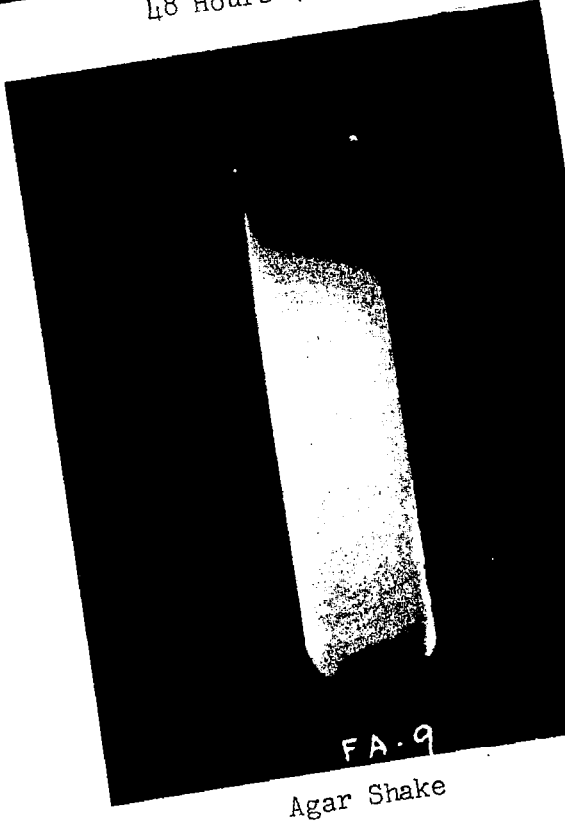
Figure 3 (cont'd)

FA-9

24 Hours (X8000)

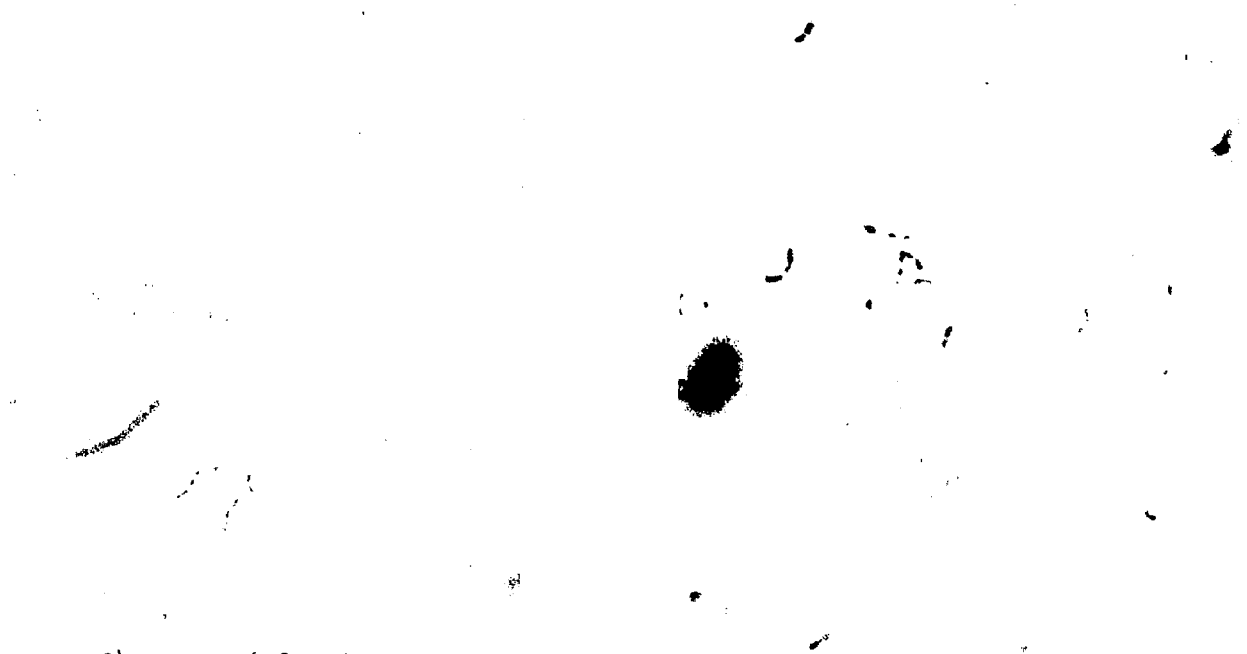
48 Hours (X8000)

1 Week (X8000)



Agar Shake

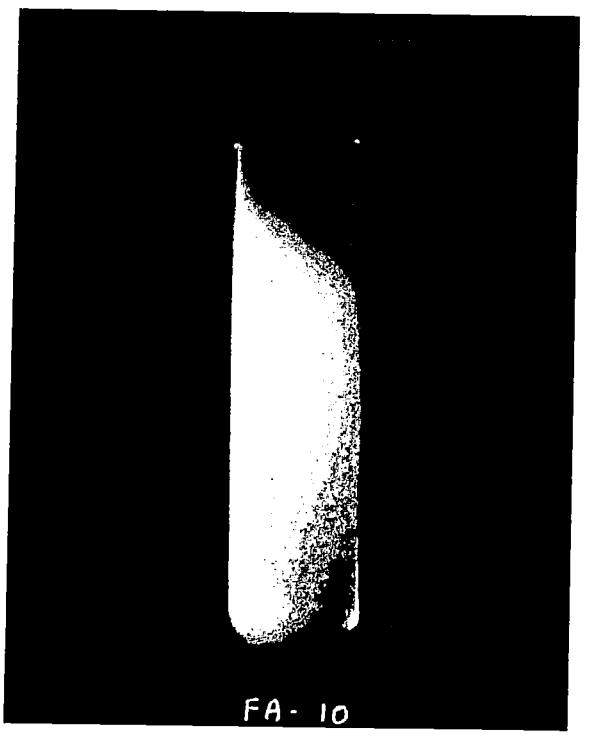
Figure 3 (cont'd)



24 Hours (X8000)

48 Hours (X8000)

1 Week (X8000)

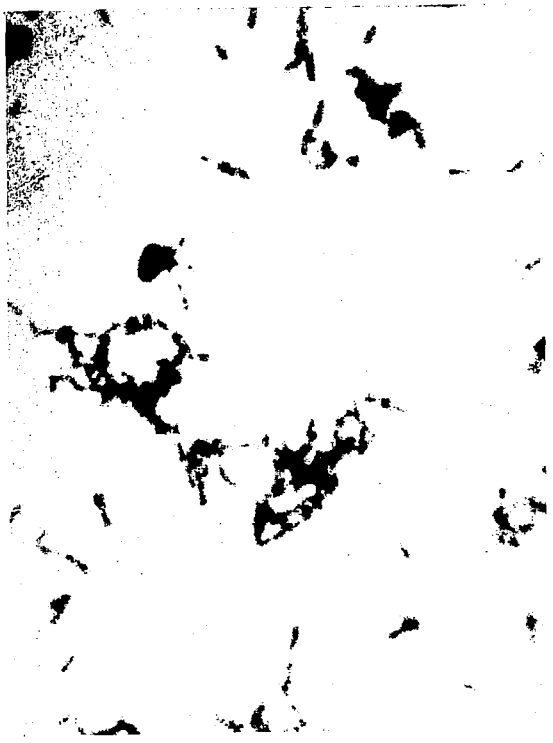


Agar Shake

Figure 3 (cont'd)

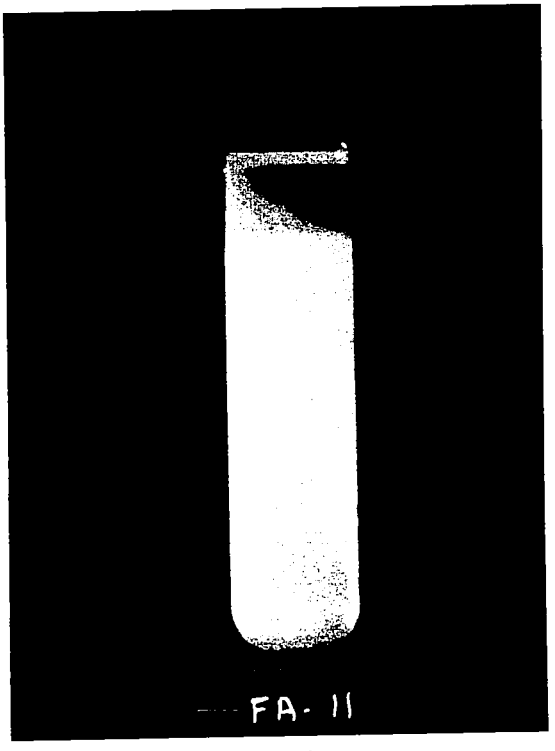
FA-11

24 Hours (X8000)



48 Hours (X8000)

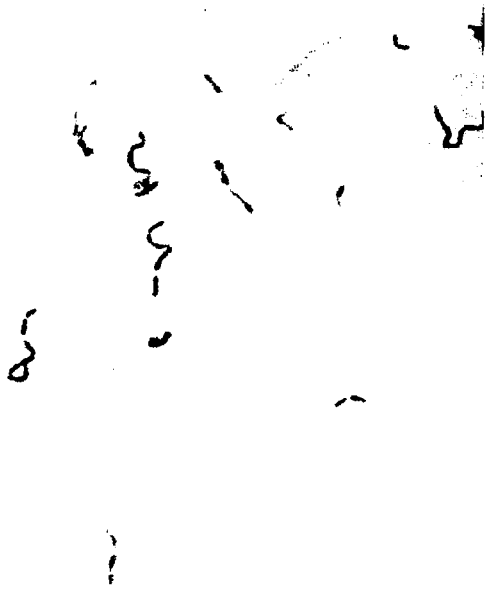
1 Week (X8000)



Agar Shake

Figure 3 (cont'd)

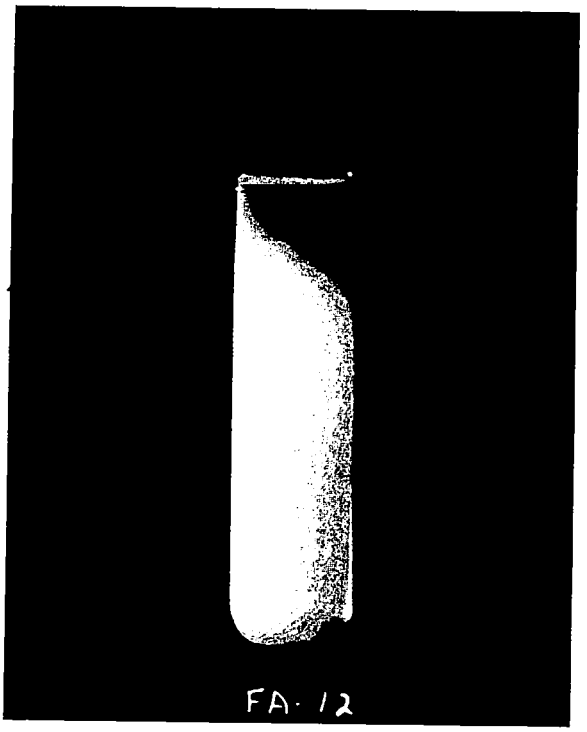
FA-12



24 Hours (X8000)

48 Hours (X8000)

1 Week (X8000)

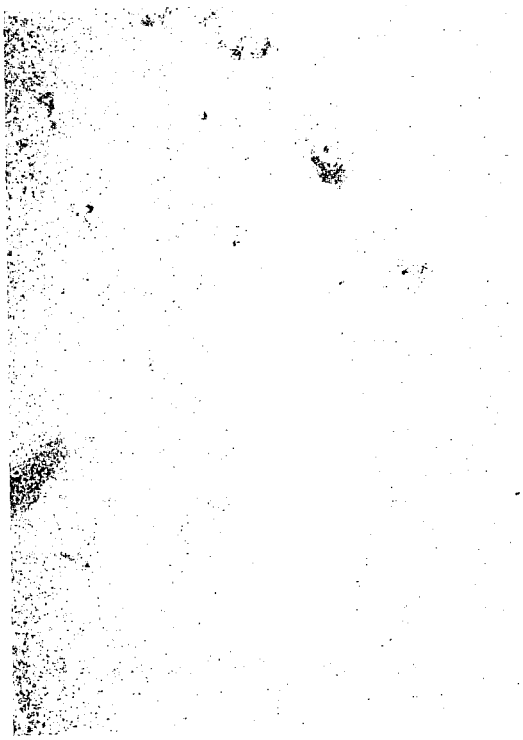


Agar Shake

Figure 3 (cont'd)

24 Hours (X8000)

48 Hours (X8000)



1 Week (X8000)

Agar Shake

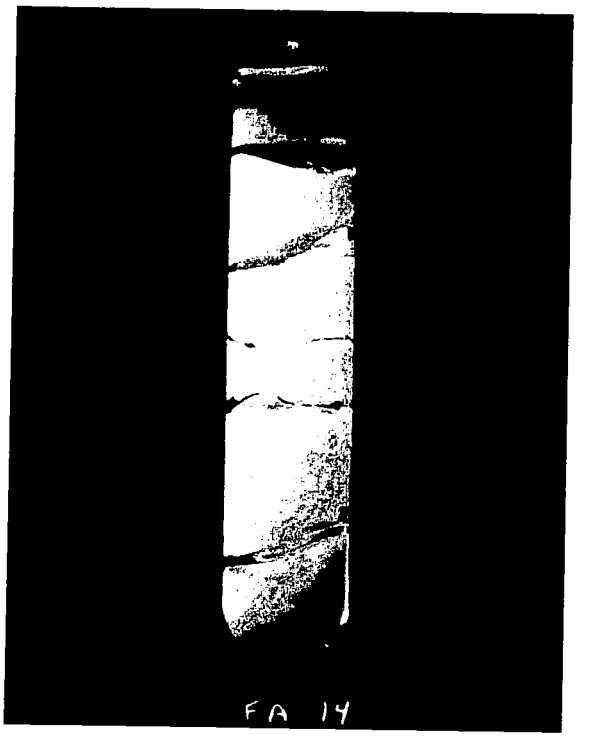
Figure 3 (cont'd)

FA-14

24 Hours (X8000)

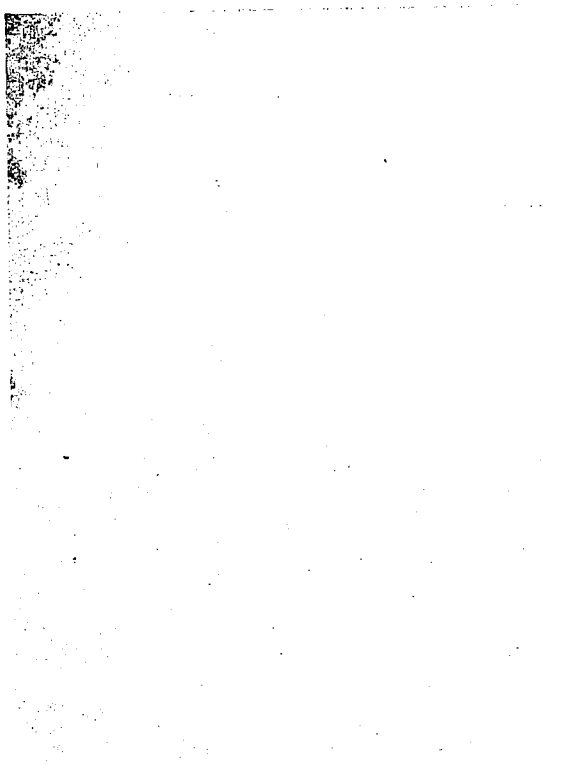
1 Week (X8000)

48 Hours (X8000)

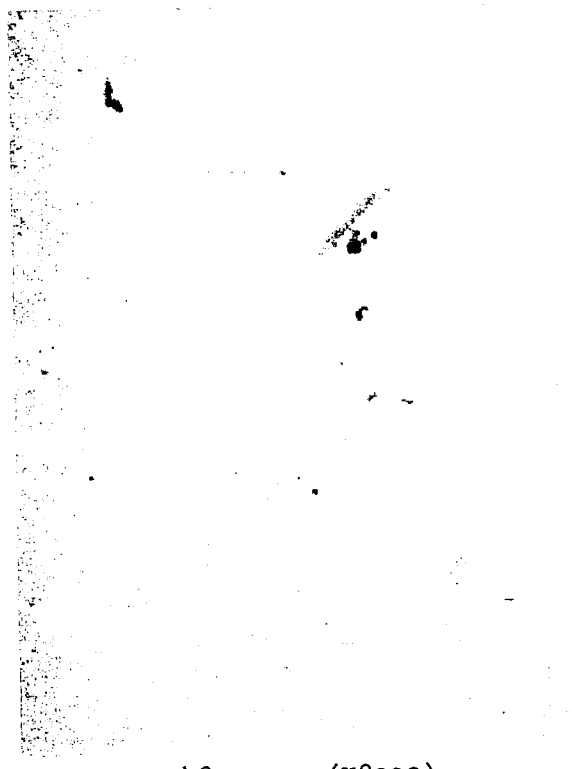


Agar Shake

Figure 3 (cont'd)

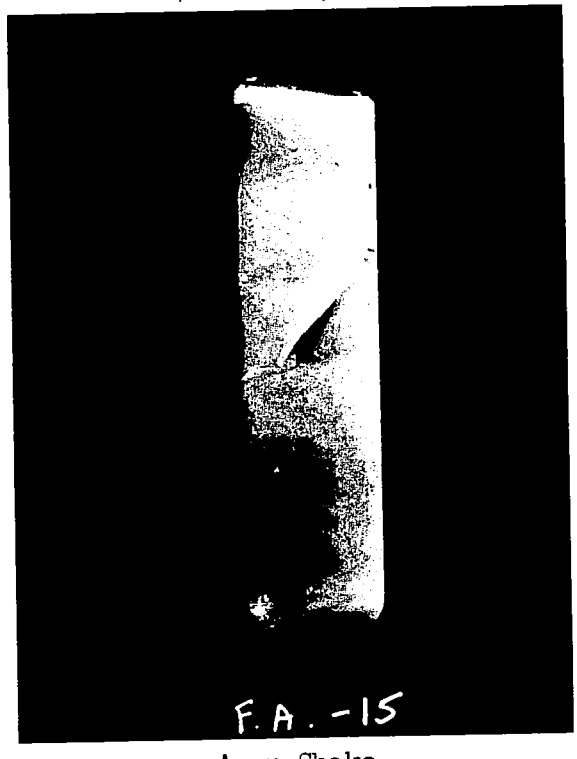


24 Hours (X8000)



48 Hours (X8000)

1 Week (X8000)



Agar Shake

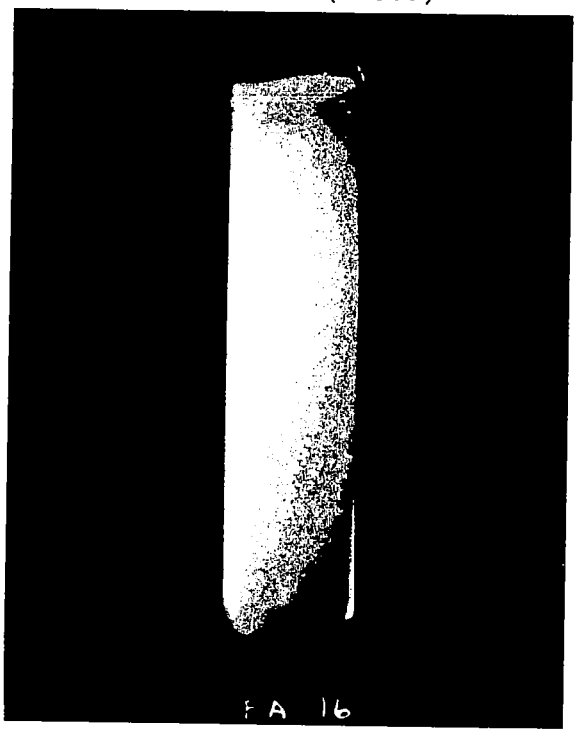
Figure 3 (cont'd)

FA-16

24 Hours (X8000)

48 Hours (X8000)

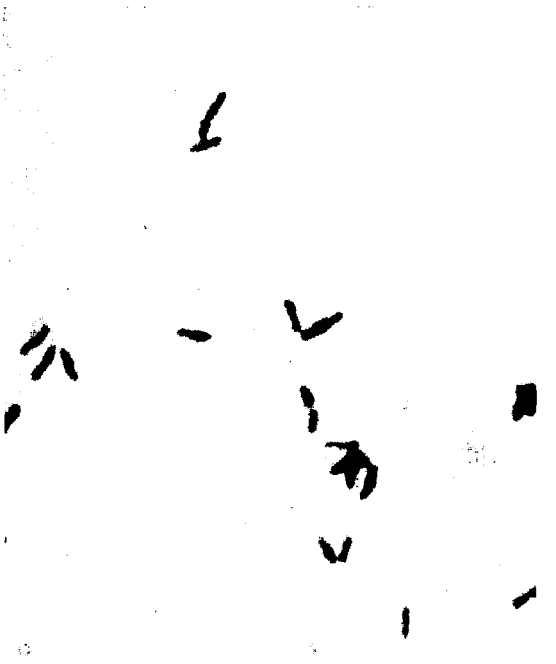
1 Week (X8000)



Agar Shake

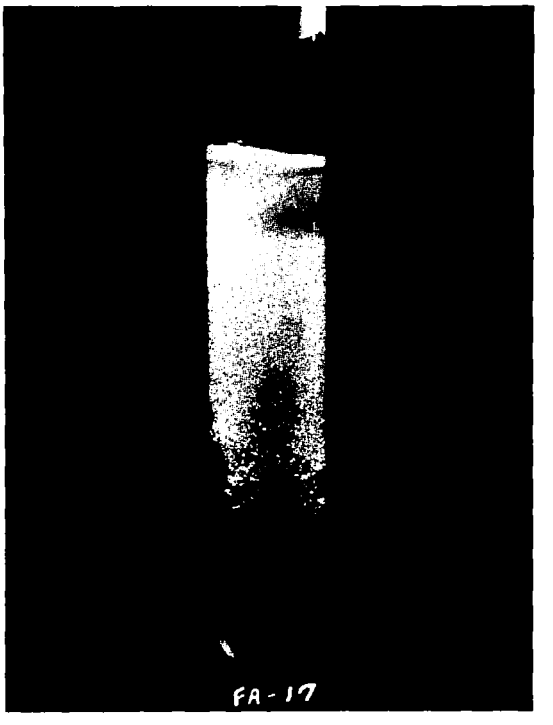
Figure 3 (cont'd)

24 hours (X1250, enlarged 8X)



48 hours (X1250, enlarged 8X)

1 week (X1250, enlarged 8X)

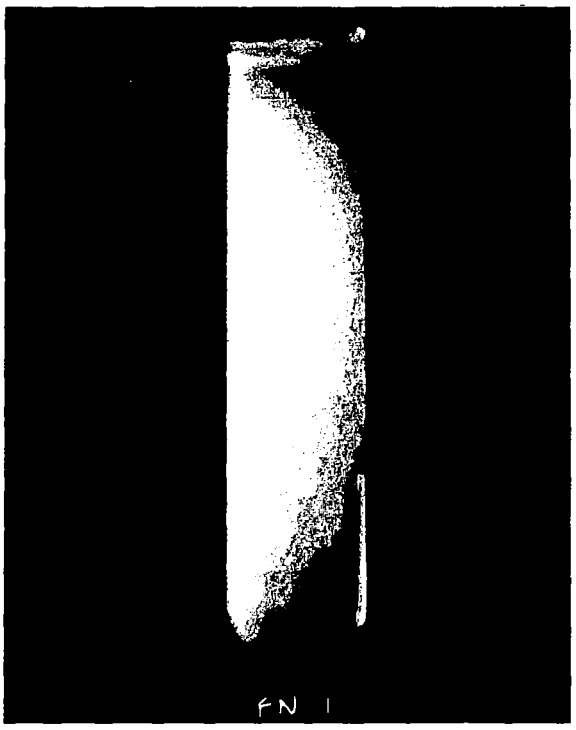


Agar Shake

Figure 3 (cont'd)

FN-1

24 Hours (X8000)



48 Hours (X8000)

1 Week (X8000)

Agar Shake

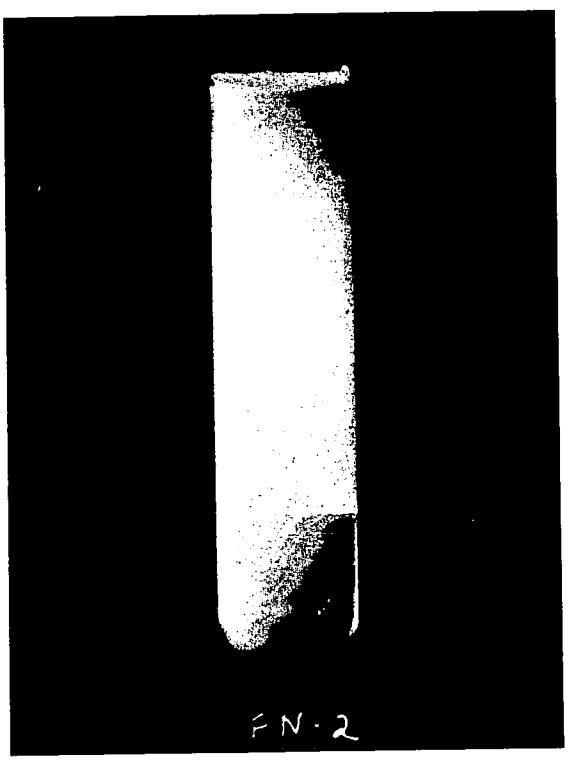
Figure 3 (cont'd)

FN-2

24 Hours (X8000)

48 Hours (X8000)

1 Week (X8000)



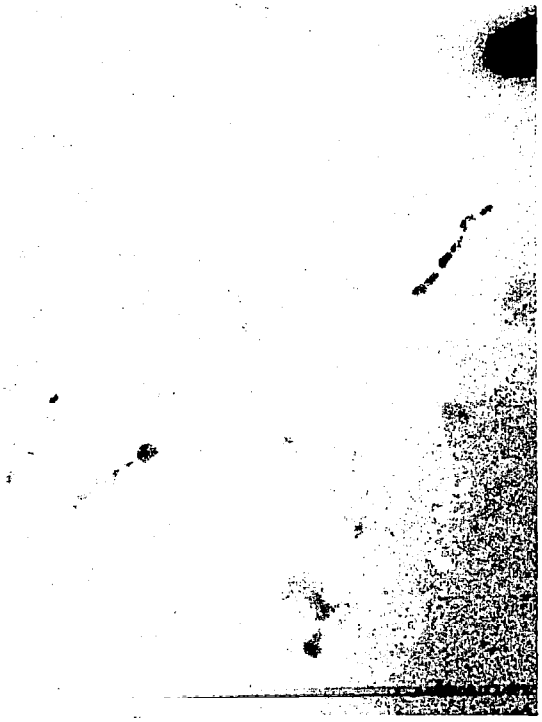
Agar Shake

Figure 3 (cont'd)

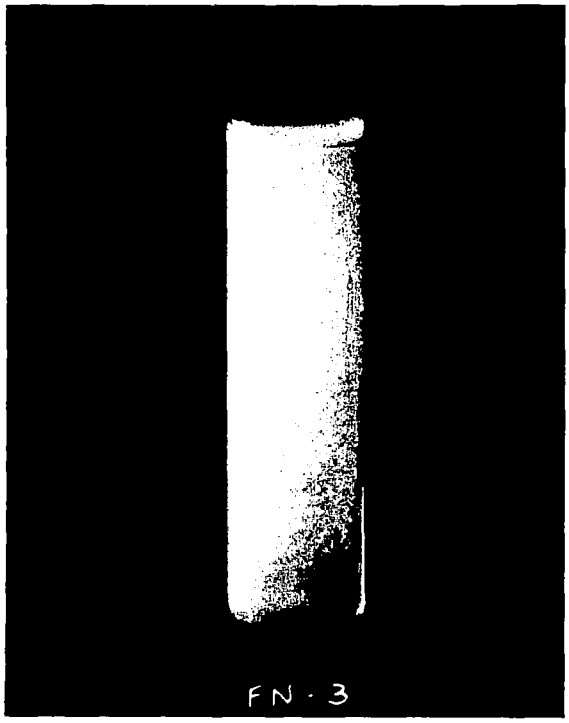
FN-3

24 Hours (X8000)

48 Hours (X8000)



1 Week (X8000)

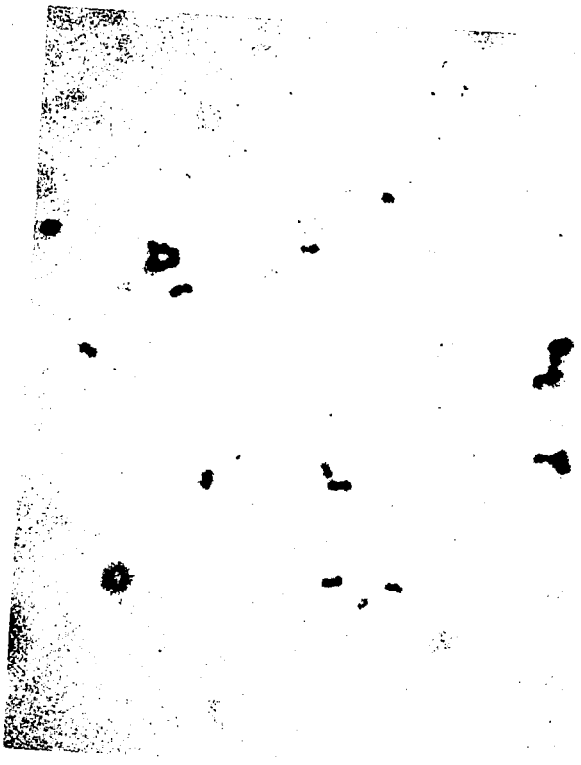


Agar Shake

Figure 3 (cont'd)

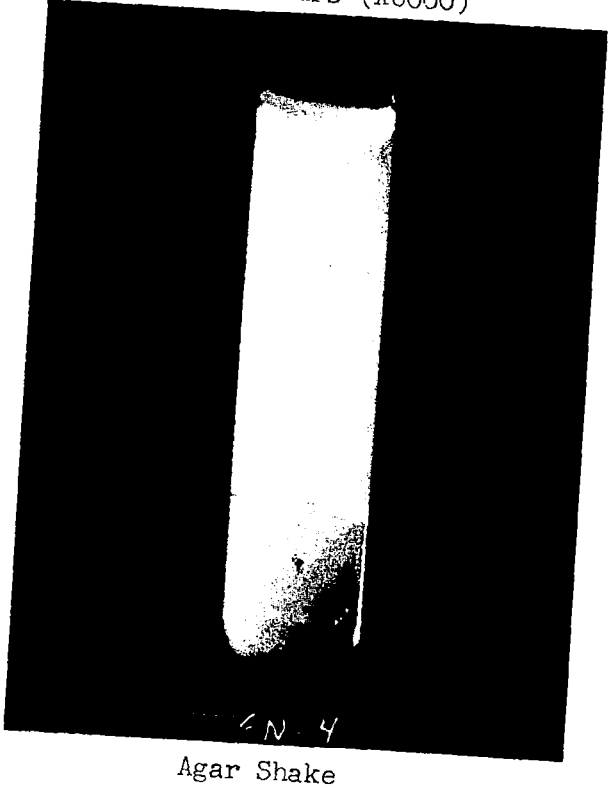
FN-4

24 Hours (X8000)



48 Hours (X8000)

1 Week (X8000)



Agar Shake

Figure 3 (cont'd)

SECTION X

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