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DETERMINATION OF AEROBIC AND ANAEROBIC MICROFLORA OF HUMAN FECES

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REPUBLIC AVIATION CORPORATION

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FOREWORD

This is the final report of a study conducted both at the Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, and the Paul Moore Research and Development Center of Republic Aviation Corporation at Farmingdale, L.I., New York, under Air Force Contract AF 33(615)-1748. The study was initiated in support of Project No. 7164, "Biomedical Criteria for Aerospace Flight," Task No. 716405, "Aerospace Nutrition," by the Air Force technical monitors, Dr. Sheldon London and Dr. Alton Prince of the Biospecialties Branch, Physiology Division of the Biomedical Laboratory. The study was funded by National Aeronautics and Space Administration Manned Spacecraft Center, Houston, Texas, under NASA Defense Purchase Request No. T23387-G, as directed by Dr. Elliot Harris.

The study was under the direction of Dr. Lorraine S. Gall, Group Biology Leader, Life Sciences and Space Environment Laboratory, Paul Moore Research and Development Center, Republic Aviation Corporation. Field investigation was under the direction of Mrs. Phyllis Riely. The authors wish to acknowledge the invaluable assistance of Dr. George A. Albright, Mrs. Shirley Dunwoody, Mr. Charles Huhtanen, Mrs. Fay Ames, and Mr. Norman Richards.

This technical report has been reviewed and is approved.

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ABSTRACT

The purpose of this study was the determination of the aerobic and anaerobic microflora of human feces and any effect, real or relative, of a space-type diet upon this flora. Fecal specimens from four young men confined in the experimental activity facility at the Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, were cultured both aerobically and anaerobically thirteen times during a six-week period. During certain periods the men wore space suits. Two of the men were on an experimental space-type diet which was freeze-dehydrated. During this same time period the other two subjects were on a "control" diet which contained identical foods, fresh and canned, in order to duplicate as nearly as possible the dehydrated diet from a nutritional viewpoint. Midway in the experiment the diets of the subjects were switched. The procedures used emphasized the anaerobic isolation of the predominating microorganisms using Gall's specialized technique[1]. The aerobes were isolated and identified by standard procedures. An attempt was made to roughly quantitate the flora by the use of triplicate aerobic dilution series and by the plating from each dilution into differential media. Although the obligately anaerobic character of the flora remained constant, a shift was found in the types of anaerobic organisms isolated. This change in the biochemically distinct flora occurred after a sufficiently lengthy period on the diet to suggest that the diet was a contributing factor. The aerobic flora differed from that cited in the literature by the frequent presence of *Shigella* and enteropathogenic types of *E. coli*.

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SECTION I

INTRODUCTION

The objective of this study was to culture the feces of four young men at the Aerospace Medical Research Laboratories (AMRL) on two specific space-type diets (freeze-dehydrated and fresh) in order to test the possible effects both real and relative of these diets upon the normal fecal microflora. The "test subjects" were in normal health according to history, physical examination and routine clinical laboratory tests. The four men were confined in the experimental activity facility at the AMRL, Wright-Patterson AFB, Ohio during the experiment.

In order to determine the effects of the diet upon the fecal microflora, the data obtained while the subjects were on test were compared to: 1) the microbiological data obtained from the relatively short control period (0-3 days) immediately after the subjects were confined, 2) the normal predominating fecal flora as delineated in the current National Aeronautics and Space Admin. Study NASw-738[1] being conducted by Republic Aviation Corporation, and 3) to the summary of the normal fecal flora derived from the critical literature survey prepared by T. Rosebury[2].

The purpose of the NASA study NASw-738 is the determination of the predominating fecal flora of healthy human males between 21 and 34 years of age. This baseline study is in the second year and while not complete, sufficient data has been compiled to allow meaningful comparisons to be made. In direct contrast to the AMRL study the NASA subjects are not on controlled diets or under confinement stress, or even in close proximity to each other.

The experimental design is shown in Table I* which also plots the periods when fecal samples were obtained and indicates the presence of a semi-liquid stool. The random distribution of the samples was the result of the subjects' inability to provide samples on specific days.

Two men, Number I and III, wore space suits from Day 5 through Day 20 and Subjects II and IV wore space suits from Day 25 through Day 40. The space suit was unpressurized and the torso and boots were worn continuously during the 16-day period. Gloves and helmet were worn for four hours a day.

The space-type diet was composed of precooked freeze-dehydrated and bite-size compressed foods. The sixty different foods were arranged in a four-menu, four-day cycle. The control diet was matched to the dehydrated diet as closely as possible. The diets contained an average of 2,500 kilocalories, 51% of the calories were derived from carbohydrates, 32% from fat, and 17% from protein[3]. The diets were switched midway in the experiment.

On the fourth sampling day gamma globulin was administered to the subjects who had been exposed to the measles. The possible effect of this upon the microbiological results is unknown.

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*This table and all succeeding tables appear in Section VIII

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SECTION II

METHODS

1. Quantification of the Aerobic Fecal Microflora

The following modification of the technique used by Zubrzycki and Spaulding was followed[3]. A standard loop of fecal sample was placed directly into 10 ml of nutrient broth. This tube was considered to represent roughly a 10^{-3} dilution of the rectal contents. Serial dilutions were made into 11 additional tubes containing 9 ml of nutrient broth, by transferring 1 ml from the inoculated tube into the next tube, the top 10 were labeled 1-10 and incubated, aerobically. These 10 tubes were considered to approximate a dilution of the sample from 10^{-4} to 10^{-13}. From each of the dilution series immediately after dilution, 0.1 ml was placed on the surface of the following "dry plates" and spread evenly over surfaces, incubated 24 hrs - 48 hrs - and counted with the aid of a Quebec Colony Counter.

The media used in this study were carefully chosen to recover the maximum number of the differing components of the fecal microflora. Blood Agar (Albimi) was selected as one of the primary media because of its ability to provide basic essentials for fastidious microorganisms and in order to read the type and degree of hemolysis present. Bacto Mitis Salivarius Agar was selected for the isolation of Streptococcus mitis, Streptococcus salivarius and enterocci. Its high selectivity and the ease of primary identification by colony morphology[4] make it an ideal media for use in the determination of fecal microflora. PEA (Albimi) - Phenylethyl Alcohol[5] Agar is a selective medium for the isolation of staphylococci and streptococci from fecal specimens since gram negative organisms are inhibited.

Bacto MacConkey's Agar was selected primarily for the case with which its differential action makes possible the differentiation of lactose and non-lactose fermentation by the gram negative bacilli. Bacto S-S- Agar (Salmonella-Shigella Agar) was used for the isolation of potential pathogens since its inhibitory action against other coliform organisms should allow a higher recovery of pathogens. Bismuth Sulfite Agar (Albimi) was used to enhance the recovery of enteric pathogens since its inhibitory action against both gram positive and coliform organisms allows the use of a large inoculum. It is usually used in the isolation of Salmonella typhosa. Tetrathionate broth was used as an enrichment medium for the possible recovery of enteric pathogens.

Rogosa's medium obtained from Albimi Laboratories was selected as most suitable for cultivation of lactobacilli[6]. Unpublished experimental work in this laboratory has demonstrated superior recovery of lactobacilli with the use of this medium. Phytone Yeast Medium (B.B.L.) with its excellent suppression of bacteria due to chloramphenicol and streptomycin gives excellent recovery of fungi. Mannitol Salt Agar (Albimi) was selected for the screening of staphylococci in an attempt to demonstrate possible pathogenicity. The high 7.5% sodium chloride content of the medium inhibits most bacteria, and the peculiar ability of the organism to ferment mannitol is easily read from the plate. All colonies appearing on mannitol salt agar were tested for their ability to coagulate rabbit plasma.

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The Gram stain reaction and colonial appearance was used to tentatively identify the bacteria. Further identification of the predominating aerobic groups was made in accordance with the following aerobic procedures:

2. Aerobic Procedure

   A. Blood Plate - Aerobic

      1. Colonies

         a. Described

         b. Representative colonies planted in nutrient broth

      2. Broth

         a. Incubated

         b. Slides made for morphological identification

      3. Morphology

         a. Gram positive cocci in clumps and masses (Staphylococci)

            (1) Mannitol salt agar

            (2) All positives confirmed with coagulase test

         b. Gram positive cocci in chains (Streptococci)

            (1) Alpha hemolysis

            (2) Beta hemolysis

            (3) Gamma hemolysis

         c. Tiny gram negative rod (Haemophilus)

            (1) Identified with typing antisera:
                a, b, c, d, e, f

         d. Gram negative cocci (Neisseria)

            (1) Sugar screen test

               (a) Glucose

               (b) Maltose

               (c) Sucrose

         e. Gram positive rods

            (1) Loefflers (Microscopic identification by morphology)

            (2) All negative on Loefflers carried to:

               (a) Glycerol agar

                  1. All colonies stained by Ziehl-Neelsen method

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(3) All negative on Glycerol agar carried to:

(a) Rogosa's S. L. agar

(4) All negatives observed for spore formation

f. Gram negative rods (Enterobacteriaceae)

(1) Expanded "Imvic" screen test

(a) TSI

(b) Indol

(c) Methyl red

(d) Voges-Proskauer

(e) Simmon's Citrate

(f) Urease

(g) Nitrate

(h) Litmus milk

(i) Motility

(j) Gelatin

(k) Hemolysis

(l) KCN

(m) Phenylalanine

(n) Cytochrome oxidase

1. On all alkaline over alkaline TSI's

(2) Shigella typing antisera

Poly Groups A, B, C, D and
Alkalescens - Dispar Group

(3) Salmonella typing antisera

a, b, c, d, i and Groups A, B, C_1, C_2,
D and E

(4) E. coli typing antisera

(a) 026:B6

(b) 0127:B8

(c) 0111:B4

(d) 055:B5

(e) 086:B7

(f) 0128:B12

(g) 0119:B14

(h) 0125:B15

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(i) 0126:B16

(j) 0124:B17

(5) Klebsiella typing antisera
Types 1, 2, 3, 4, 5, and 6

B. MacConkey's Medium

1. Colonies

a. Described

b. Planted onto TSI

2. TSI

a. Handled as described under gram negative rods

C. Rogosa's S. L. Agar

1. Colonies

a. Described

b. Slides made for morphological identification

D. Phytone Yeast Medium

1. Colonies

a. Described

b. Planted onto cornmeal agar

(1) Growth observed for sporulation

E. Broth

1. Slides made and organisms identified morphologically

2. Organisms not previously isolated from a particular source will be treated as morphology indicates.

F. Aerobic Counting Plate

1. Counted and evaluated

G. All differential media plates from aerobic series counted and evaluated.

3. <u>Anaerobic Series - Culture and Set Up</u>

The anaerobic broth series for the primary culture of the fecal sample was essentially the same as that series used previously by Dr. Gall, et al for culturing rumen anaerobes, and which has been recently successfully adapted in the Republic Aviation Corporation Laboratories to the culture of

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human feces. 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. The technique, as discussed below, can be modified to culture from a rectal swab.

The standard loop of the fecal sample was placed directly in a tube containing 10 ml of Gall's broth prepared with 2 drops of cysteine and 1 drop of sodium bicarbonate. This tube was considered to represent roughly a 10^{-3} dilution to the rectal contents. Serial dilutions were made into 11 additional tubes with 9 ml 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 incubated. 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 acted as a dilution blank for the next tube in the series. From tubes 6 and 7 pour plates were made in anaerobic Petri dishes using Gall's medium with cysteine and bicarbonate added. All 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 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 6 and 7 were picked and treated like the subcultures from the agar shakes as described above. Usually 4-6 different colony types appeared on each anaerobic Petri plate adding 6-8 pure cultures which were run through the following schema.

The pure cultures of predominating bacteria were studied for their physiological characteristics. In general, bacteria isolated in numbers of less than one million per gram of sample were not considered in the physiological studies. Emphasis was placed first on studying those bacteria isolated in the top three dilutions.

A Gram stain was made from the 10^{-3} dilution of the feces to observe the types of bacteria present in the original sample.

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

1. <u>Gall's Medium</u>

   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

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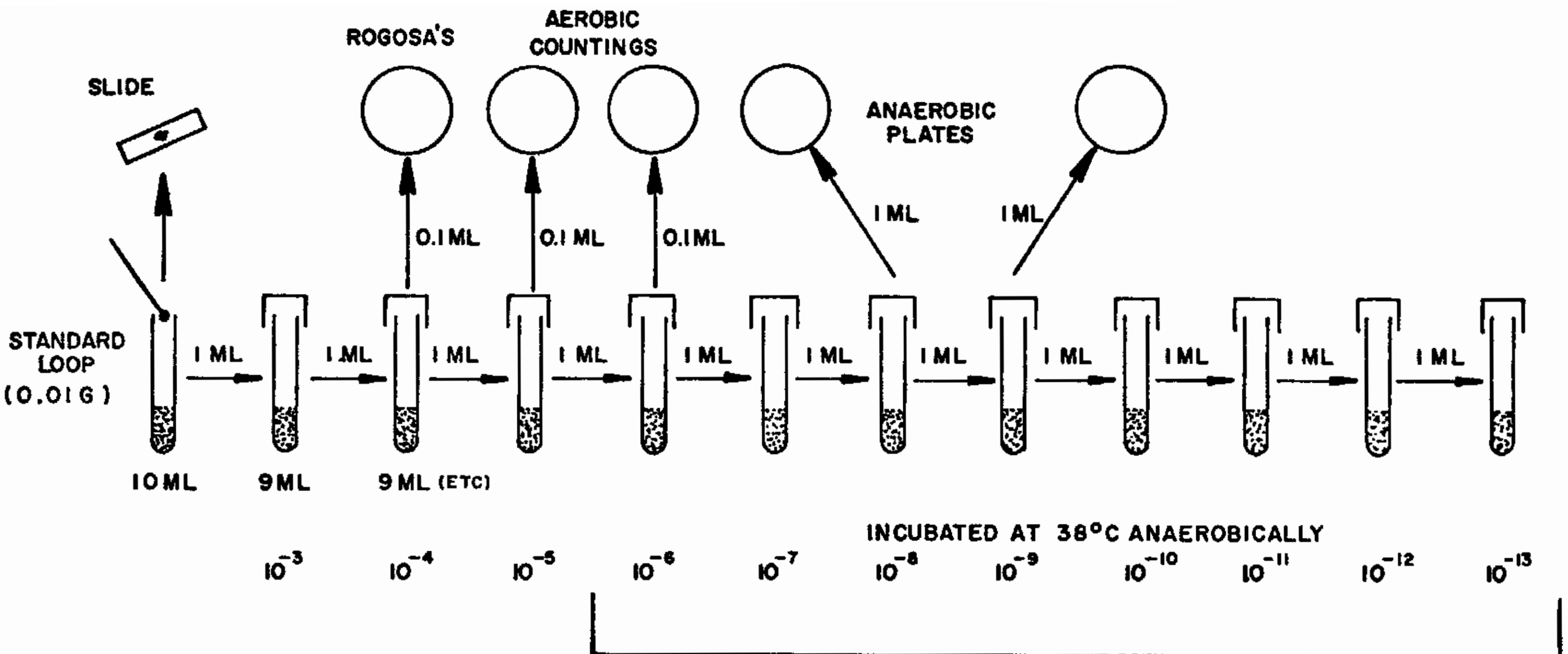

Figure 1. Anaerobic Dilution Series

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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 2 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, omitting cysteine except where noted otherwise. To all broth and agar media 0.05% of bovine serum is added.

2. 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.

3. Physiological Studies

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

1. Gram stain to observe morphology
2. Final pH in 0.1% glucose broth
3. Final Eh in 0.1% glucose broth
4. Fermentation of the following sugars in Gall's medium with glucose omitted:
   a) Glucose
   b) Sucrose
   c) Lactose
   d) Dextrin

   (sugars added at 1% level aseptically after autoclaving)
5. Growth in Gall's broth with no carbohydrate added
6. Liquefaction of gelatin in Gall's medium minus carbohydrate
7. Growth and reaction in litmus milk (to which 0.05% bovine albumin and 0.1% of peptone have been added
8. Growth in agar shake containing Gall's medium

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All media will contain bicarbonate and all media except the agar shake will contain cysteine to produce an Eh of about -200 mv.

4. Tests to Characterize Groups

The predominating anaerobes were assigned to the groups set up under NASA contract NASw-738 in order that a comparison could be made with the normal human volunteers being studied at Republic. Any predominating anaerobes that did not fall into the groups as set up were submitted to further tests in order that new groups could be set up. The additional work (on selected cultures) of the new groups included where indicated:

1. Gram stain to observe morphology and Gram reaction
2. Motility
3. Spore formation
4. Oxygen relationship (agar shake)
5. Temperature tolerances and optima
6. pH tolerances and optima
7. Fermentation of various carbohydrates
8. Proteolytic activity
9. Lipolytic activity

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

Some of the data obtained from these physiological tests duplicated that obtained in the screen tests, but the tests were repeated and extended where indicated during the more intensive physiological study. The results of these physiological tests were used to characterize the types of predominating organisms isolated from the feces.

5. Primary Media

Qualitative Aerobic Analysis

(1) MacConkey's Medium
(2) Blood Plate
(3) Rogosa's Medium
(4) M. S.
(5) P. E. A.
(6) Phytone yeast
(7) Counting plate (Nutrient agar)

Quantitative Aerobic Analysis

(1) EMB
(2) Blood plate
(3) Rogosa's Medium
(4) M. S.
(5) P. E. A.
(6) Nutrient broth series

Anaerobic media as described in Gall's procedure with the addition of an anaerobic blood plate.

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SECTION III

AEROBIC FLORA

The aerobic bacteria were studied by a procedure that gave both qualitative results and allowed relative quantitation of this segment of the flora. A standard loop of fecal material obtained from three different internal areas of the fecal mass was incorporated into 10 ml of broth. After adequate mixing by pipette, serial 10 fold dilutions were made into heart infusion broth for a total of 10 dilutions. This procedure was done in triplicate in order that three comparable dilutions could be run from any one sample, which was designed to determine the homogenous or non-homogenous character of the fecal mass rather than to check a replicate technique. From each tube of each dilution series 0.1 ml was plated on top of the following differential media: MacConkey's, blood agar, PEA and Mitis Salivarius and spread with a glass spreader. Following 24 hours incubation, each plate was counted with the aid of a Quebec colony counter and the total number of differing bacterial types for each dilution was recorded in appropriate tables.

Quantitation

The results obtained from the aerobic triplicate dilution series in broth are summarized in Tables II and III. In Table II the highest tube in each of the triplicate series showing growth was recorded for each subject at each sampling period, while in Table III the results from each sampling period were averaged. The agreement between the three dilution series was quite close and was within 95% confidence limits.

Enterobacteriaceae

The occurrence and distribution of the Enterobacteriaceae isolated during this study were summarized in Table IV according to subject and sampling period. The overall picture presented by the gram negative rods is noteworthy in several respects and will be commented upon as the data for each individual group are presented.

The frequency of occurrence and quantities of the lactose-fermenting gram negative rods is presented in Table V. The numbers are lower than would be expected from a review of the literature, but are quite consistent both between subjects and sampling periods. It is particularly interesting to note in Table VI that many diverse strains of E. coli were isolated and typed. The occurrence of eight different coli types in the culturing period was a deviation from what is considered normal, as usually a resident coli strain is very difficult to replace and other strains are difficult to implant. The encountered strains are considered enteropathogenic serotypes of E. coli: 026:B6 (1x), 055:B5 (5x), 086:B7 (1x), 0124:B7 (2x), 0125:B15 (1x), 0127:B8 (5x), 0126:B16 (1x), and 0111:B4 (2x).

The only difference between ordinary E. coli and those coli involved in disease is in the serological specificity of their somatic and surface antigens.

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Only one to two percent of the adult population according to Sears[7] excrete epidemic serotypes of E. coli at any given time. Since 50 to 75% of the subjects on this study excreted enteropathogenic strains of E. coli at some given sample period, this finding would seem to be highly significant and should be carefully considered. It is possible that a higher percent of people than cited in the literature harbor enteropathogenic strains which can become predominant and are detected only when the balance of the flora is disturbed or that the subjects on the study ingested the serotypes which thrived once the balance of the flora was no longer maintained.

The occurrence of non-lactose fermenting Enterobacteriaceae is noted in Table VII, and it will be noted that these types of bacteria are especially heavy in Subjects I and IV. The prevalence of Shigella group B and A. D. and other shigella-like organisms in all of these subjects is striking and unexpected since it greatly exceeds the normal pattern described in the literature[2]. The occurrences of Shigella group B which also at times agglutinated weakly in Alkalescens Dispar serum, and those shigella which agglutinated only in Alkalescens Dispar were frequent. The etiology of their occurrence suggests ingestion or transference.

Klebsiella was isolated frequently, occurring three times in the first half of the experiment on Subject I, five times in the second half of the experiment on Subject II, four times in the second half of the experiment on Subject III, and six times on Subject IV in the second half of the experiment. It is possible that Subject I brought the Klebsiella into the group and proceeded to "share" or transmit it to Subjects II, III, and IV. This was difficult to demonstrate clearly as the Klebsiella isolated did not give definite types with the standard typing serum.

Other gram negative rods were isolated sporadically. Salmonella group A was isolated only once, on Subject II, in the sixth culturing period. The only occurrence of Citrobacter was in Subject I, from whom it was isolated twice, while Aerobacter B was isolated four times from Subject II in the first half of the experiment.

Pseudomonas aeruginosa occurred twice on Subject III and a pseudomonas of a different group occurred once on Subject IV. Subject IV had Proteus rettgeri in the first two culturing periods and also in one of the last culturing periods.

Thus the Enterobacteriaceae present a notable deviation from the accepted norm with respect to the occurrence of the enteropathogenic strains of the E. coli and the prevalence of Shigella types B and A. D. The presence of many diverse, odd patterns of biochemical reactions was noted suggesting possible "matings". Cultures of E. coli and shigella-like organisms which were positive in the saline control occurred in Subjects I and IV.

In Table VIII the average incidence of the gram negative rods both lactose fermenters and non-lactose fermenters was calculated by the standard methods described in the Standard Methods text[8]. The data present a consistent picture and show that the methods used in this study for these type of organisms are statistically acceptable.

It must be understood that plating of decimal dilutions need not necessarily result in exact agreement of the counts obtained from the dilutions that are plated.

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This can be attributable to the crowding affected; i.e., plating efficiency decreases as more cells are seeded on an agar surface or to the dilution of inhibitory substances such as bacteriocenes which may be present in lower concentrations than the microbes themselves.

Nitrate-Negative, H_2S-Positive Rod

A very unusual organism which was nitrate-negative, but which produced H_2S on bismuth sulfite media was isolated frequently, especially from Subjects I and IV, as is shown in Table IX. The bacterium had a rather peculiar morphology, so that it was easily recognizable and although it was not identified, it seemed to have many characteristics of Chondrococcus columnaris.

Streptococci

The composite picture presented by the streptococci is interesting and unusual as is shown in Table X. The enterococci fell in total numbers until at certain cultural periods they were not recovered, while the Str. salivarius became the predominant member of the streptococci and appeared in relatively large numbers. The fluctuation in the total number of Str. mitis colonies seemed to follow inversely the pattern of Str. salivarius.

The streptococci did not follow the accepted percentage occurrence of the literature. The very high incidence of Str. salivarius and the low number of enterococci isolated seems significant but the interpretation must be considered in the light of the knowledge that a subclinical throat infection could easily load the feces with transient organisms which could distort the total picture.

The interposing of graphs of the numbers of streptococci upon the Enterobacteriaceae graphs (Figure 2) seems to point strongly to an inverse relationship between these principal microbial categories in the feces because as the total level of streptococci increased the total number of gram negatives decreased and this held conversely. The fluctuations within the streptococci group were not as easy to correlate, although a marked positive increase in Str. salivarius seemed to correlate with a decrease in enterococci.

Staphylococci

It seemed difficult to draw any correlations with staphylococci although a minor trend towards increased incidence of staphylococci seemed to follow the decrease in total numbers of streptococci. The staphylococci were isolated from blood agar and PEA media. The incidence of staphylococcus and the possible pathogenic strains as established on mannitol salt media and by coagulase positivity are recorded in Table XI. Possible pathogenic strains occurred to some extent on all subjects but appeared more frequently on Subject I.

Lactobacilli

Microaerophilic forms of lactobacillus remained relatively constant in all four subjects. Subject I came in with a very high total count and held this throughout the experiment. Subject II, III and IV came in with relatively low total counts of lactobacilli which held at about the same levels (Table XII). The averages of lactobacilli in three subjects are substantially lower than the ten subjects on the NASA study.

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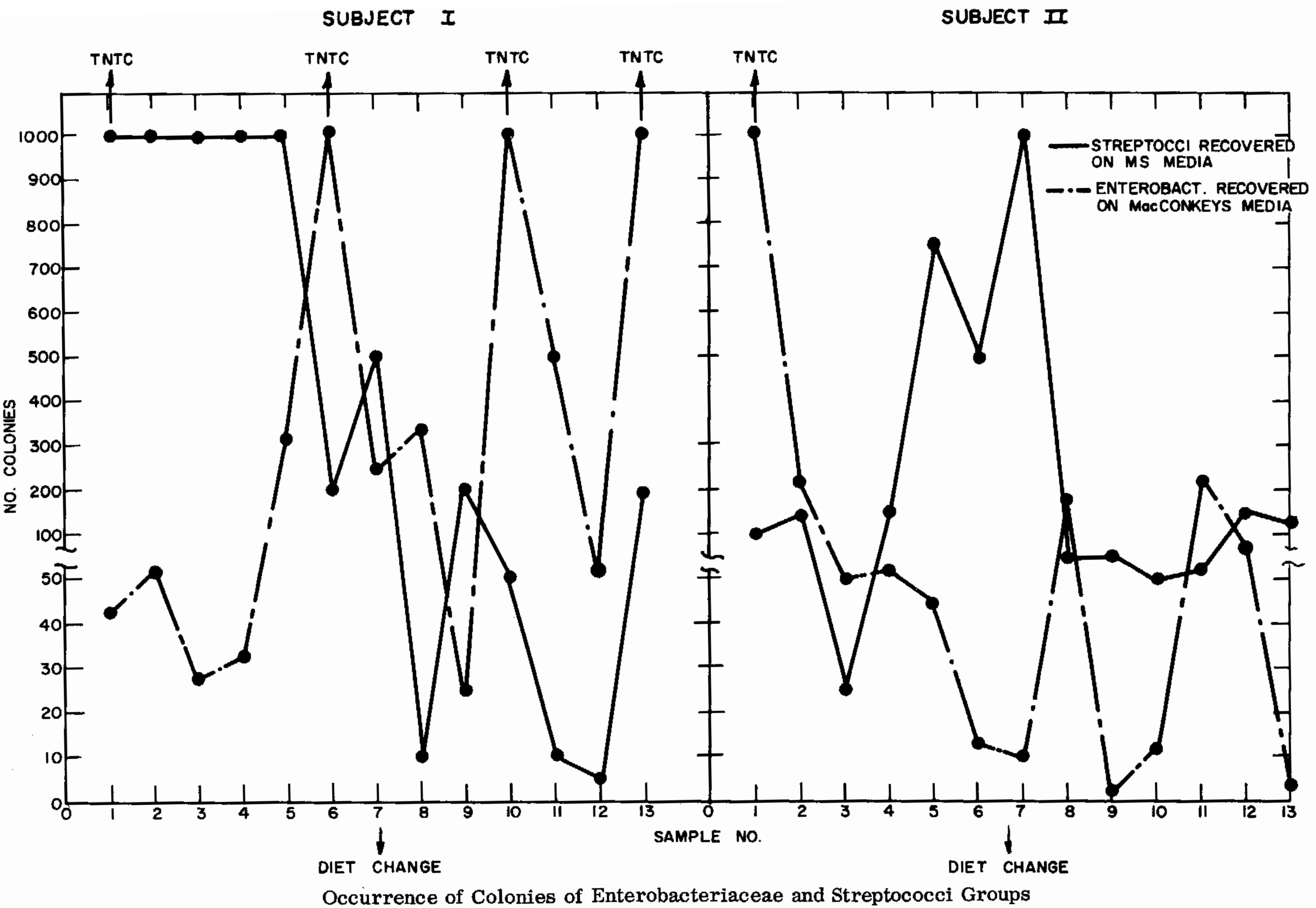

Occurrence of Colonies of Enterobacteriaceae and Streptococci Groups

Figure 2

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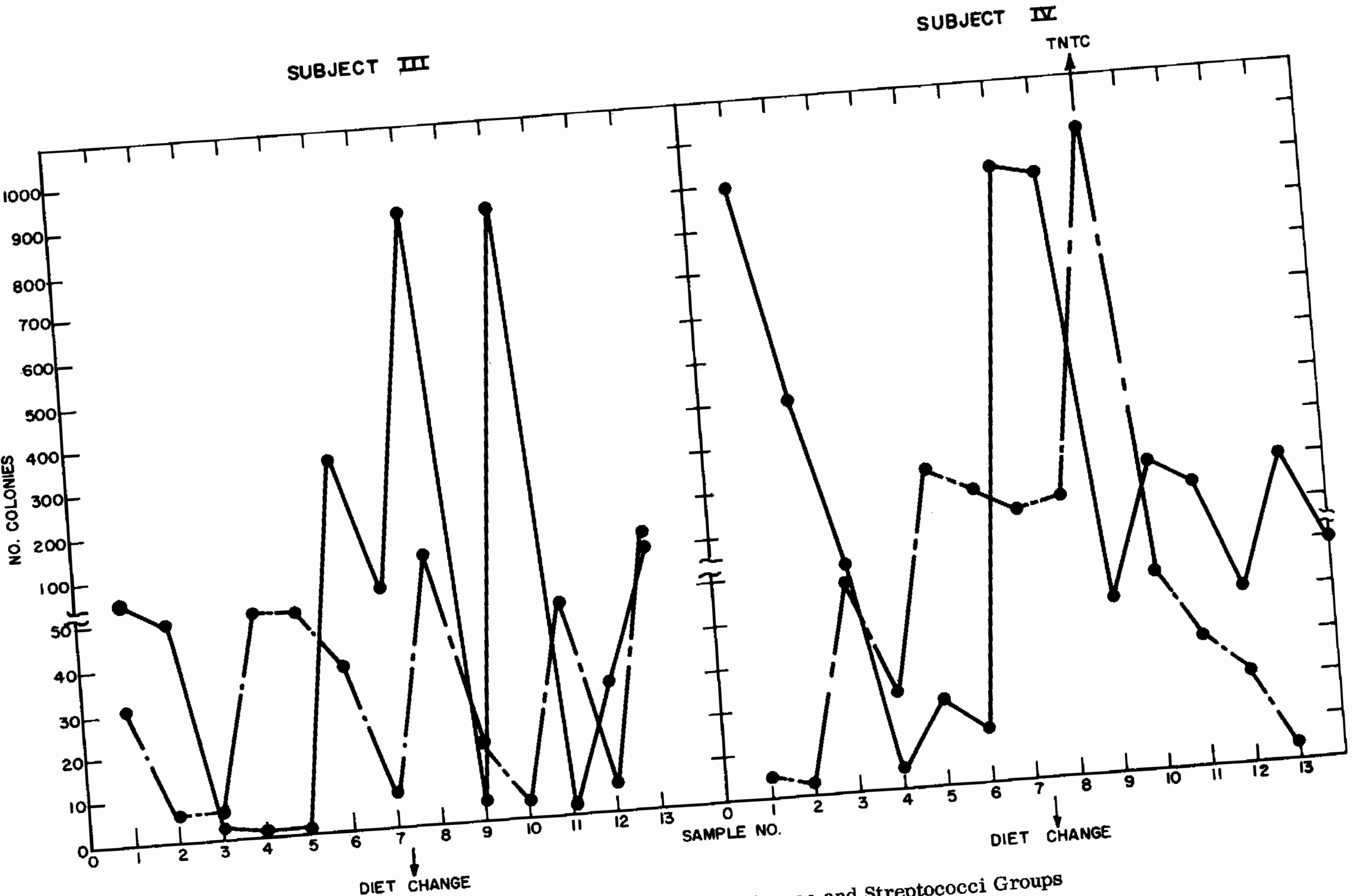

Occurrence of Colonies of Enterobacteriaceae and Streptococci Groups

Figure 2 (cont'd)

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Miscellaneous Bacteria and Fungi

The occurrence of a gram positive spore-forming rod which preferred growth in the dilution series to the differential plate media (although recovery was possible from the blood plate) appeared early on all subjects and persisted until the end of the experiment. This organism belongs in the Bacillus family and is probably a strain of Bacillus polymyxa, or Bacillus subtilis var. aterrimus which could no longer grow in 7% NaCl broth.

Corynebacteria were isolated from all subjects during the last two culture periods.

The pattern of occurrence of Candida albicans and C. parapsilosis was interesting and may be seen in Table XIII. Since none of the subjects was on antibiotic therapy it seemed that the degree of recovery was quite high.

Discussion of Aerobes

The literature on the fecal aerobes is quite voluminous but recently has been summarized admirably by Rosebury(2). Since this author has critically reviewed literature on this topic and has presented an excellent composite picture of this flora in a concise form, his work will be used as a basis for comparison of the aerobic microorganisms generally accepted to occur in the feces. In addition the results of this study will be compared to those obtained in the Republic laboratory using the same techniques in examining the feces of ten normal young male subjects under the NASA contract. The results of these comparisons are tabulated in Table XIV. In general, the organisms isolated on the present study agree well with those listed as probable components of the human feces by Rosebury and compare reasonably well with those found using our techniques in the NASA study. The notable exception was the complete lack of fungi isolated during the NASA study whereas both Rosebury's review and the present AMRL study indicate the frequent presence of fungi.

Thus the qualitative picture of the aerobic fecal flora on the subjects on the AMRL study agreed well with that of numerous other investigators reviewed by Rosebury and with our own experience with the normal young men in the NASA study.

One type of organism was found frequently in the feces of the AMRL subjects which was not reported in the literature or found in the NASA subjects. This was the peculiar nitrate negative H_2S forming rod isolated from the bismuth sulfite plates. The frequent isolation of this organism on all the subjects is not easily explained, but it is possible that it was brought in on the food which was eaten by all subjects or passed to them from one of the handlers and transferred from one subject to the others.

There were interesting quantitative differences observed in the aerobic fecal flora. For example, the number of lactose fermenting gram negative Enterobacteriaceae was lower than that expected from a review of the literature(2). This may be due to the promptness with which the samples on this study were processed as it has been shown by preliminary unpublished work from this laboratory that the longer a sample is held after elimination the higher the coli count is apt to be. This increase in coliforms begins as soon as one-half hour after elimination. Since care was taken to process these samples within fifteen minutes, the

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lower coli counts found in this study may be merely a reflection of the technique employed.

The occurrence and distribution of the Enterobacteriacae as illustrated in Table IV demonstrates the relative consistency of the types within each individual. There is a possibility of the transference of Shigella from I and IV to II and III where it seemed to occupy the position of a "fellow traveler". The fecal microflora seemed to stabilize beginning with Sample No. 12, possibly an effect of diet or confinement[9] as both have been indicated by germfree work in animals.

More important is the frequency of isolation of the enteropathogenic strains of coli and the higher incidence of shigella. Since these organisms are potentially disease producing in the human, the frequency of their occurrence warrants careful consideration.

The inverse relationship between the occurrence of the streptococci and the Enterobacteriaceae are of interest and should be confirmed by further work. Also the relationships between the various types of streptococci present an interesting picture, which may warrant further investigation.

The averages of lactobacilli in three subjects are substantially lower than the ten subjects on the NASA study. This is interesting since it appears from the literature[10] that there is a correlation between dental caries and the presence of lactobacilli both in the mouth and in the feces, those present in the feces being a direct reflection of the mouth flora. It might be possible to correlate the extent of dental caries of the subjects with the varying occurrence of lactobacilli.

The marked difference in the numbers of lactobacilli recovered from No. I in contrast to the other subjects probably is a reflection of that individual's physiological make-up, whether biochemical in origin, or a latent influence of his diet prior to confinement rather than due to endogenous influence.

The high frequency of occurrence of _Candida albicans_ as well as _C. parapsilosis_ is noteworthy particularly in the absence of antibiotic therapy. Both _C. albicans_ and _C. parapsilosis_ are able to utilize keratin as the sole nitrogen source and are able to hydrolyze it to amino acids in a medium containing glucose salts and vitamins. In addition to this rather unique trait, they are able to cross react in agglutination tests with minor O antigens of Salmonella and Arizonia groups and this may point to the presence of an endotoxin.

The pattern of _Candida_ occurrence is worth consideration since Subject IV was the only subject in which _Candida_ was demonstrated in the first two cultural periods. However, by the third sampling period subjects II and III carried candida and by the fourth sampling period it was recovered from the feces of Subject No. I, in whose feces it was then demonstrated through the next seven sampling periods. In Subject III it was recovered seven times while in Subject IV its possible initiator it was recovered twelve times. _Candida_ is a normal constituent of the human fecal flora which is isolated in about one-third of the general population[2] so it could be a normal constituent of the flora of these subjects that was not initially isolated. The pattern of incidence may possibly suggest transference from Subject No. IV. However, it is also necessary to consider the possibility of its introduction through food, a food handler, or other contact.

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Any attempt to qualify and relatively quantitate the aerobic microbial population of the feces is presumptive, and all results must be considered in the light of the magnitude of the job. The bacteria isolated may mask other inhabitants of the region. The use of selective media with its known inhibitory qualities both for those bacteria inhibited and those selected must be considered when trying to derive population figures. It is important to remember that the counting and characterizing of the fecal flora depends upon observing cultures grown on or in bacterial media and the condition cannot approximate the reality of in vivo conditions. Some bacteria, due to injury, character of nutrients, amount of O_2, or unfavorable temperature of incubation, are unable to grow and form colonies within 24 hours. For this reason the figures given represent estimates of bacterial populations under the conditions of the experiment. By the use of platings from successive dilutions and the running of each sample in triplicate it is felt that errors were kept to a minimum. Analysis of variance, performed for each category of microorganisms, indicated a probability of less than 1% that individual trends toward higher or lower counts were due to chance alone.

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SECTION IV

ANAEROBIC FLORA

The composition and role in the body of the anaerobic fecal flora of the young healthy adult male has never been adequately described, even though this flora is of prime importance in maintaining the health and well-being of man. Basic information on the fecal flora is of particular importance in the manned space program, because certain conditions of space travel, such as diet, may influence the balance of the fecal flora with possible unknown effects on the well-being of the space man.

The primary isolation of the fecal anaerobes was carried out on freshly eliminated feces according to the procedure outlined in Section VII. Selected predominating organisms representing those bacterial cultures isolated in the top dilutions (usually representing the billionth dilution of feces or above) were subjected to the screen test outlined in Section II. Aerobic counts were made using 1 ml of the third tube in the anaerobic dilution series by the pour plate technique using 9 ml of agar. The results of these studies will be compared with the results obtained using the same techniques in the NASA study of the normal fecal flora of healthy young men.

It should be emphasized that the study under NASA contract NASw-738 of the normal fecal flora of the young adult male considers only the predominating anaerobes and is still incomplete. Therefore discussion of the data will be limited to the areas studied on the NASA contract completely enough to allow meaningful comparisons such as the proportion of aerobic vs anaerobic bacteria in each sample, the percentage of obligate anaerobes, the types of bacteria isolated based on a "type culture" key which groups similar bacteria, and on broad groupings of certain bacteria based on the formation of specific end-products or on distinctive characteristics.

The baseline information obtained on the NASA study has shown that the aerobes are outnumbered by the anaerobes in proportions 1,000 to 10,000:1 (Table XV) and that over 95% of the predominating anaerobes isolated are obligately anaerobic, as shown in Table XVII. These findings are further substantiated by data obtained from the pre-test samples obtained on twenty-four young men on a previous NASA contract NASr-92, "Effects of Prolonged Exposure to Pure Oxygen on Human Performance," and with data from ten young men representing the second group of subjects on the current NASA contract NASw-738. No attempt was made to regulate the diet of any of these young men prior to obtaining these samples except that no sample was considered valid if oral medication of any kind had been ingested.

The predominating anaerobes isolated on the NASA contract NASw-738 have been studied by means of screen tests based on certain important morphological and physiological characteristics and have been separated into seventeen "type culture" groups (Table XVI). Certain type cultures occurred more frequently than others, as is shown in Table XVII, and about seventy-five percent of the cultures that were screened were keyable.

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In general these "type cultures" divided themselves into several broad "families" with the "families" being distinguished from each other by some fairly well defined differences such as marked gas production; formation of black slime; proteolysis in litmus milk, relative amount of lactic acid produced, etc. One outstanding group is composed of cultures of pointed rods producing marked gas and black slime, usually with proteolysis in milk. Another type of organism which produces gas and black slime closely resembles a Viellonella. Another broad family is the group of organisms which produces lactic acid as the main end product, some of which produce relatively large amounts and may be bona fide lactobacilli, while others produce lactic acid in smaller amounts. Using the nomenclature of the "key" established under the NASA contract the marked gas-formers producing black slime are FA-3 and FA-15 and FA-13. The marked lactic formers are FA-4, FA-5, and FA-11, while the intermediate group are FA-2, FA-7, FA-8, and FA-9. On the NASA study the frequency of occurrence of the marked gas, black slime producers ran about 20% while the incidence of marked lactic acid formers was about 12%, and the intermediate lactic acid formers ran about 10%.

The results from the subjects at AMRL on the space-type diets have proven to be most interesting when compared to the results of the NASA study. The AMRL subjects as a whole showed the same degree of obligate anaerobiosis as experienced with the subjects on the NASA study, both with respect to predominance of anaerobes over aerobes (Tables XVIII and XIX) and with respect to the percentage of obligate anaerobes (Table XX). In general, throughout the entire AMRL study the obligate anaerobes exceeded the aerobes by a proportion of 10,000:1 and over 97% of these cultures were obligate anaerobes. This was true of all individuals on all test diets whether suited or non-suited. Thus the basic anaerobic character of the fecal flora of those subjects did not seem to be influenced by either diet or the wearing of the space suit under the conditions of this test.

The breakdown of the types of obligate anaerobes from the key established in the NASA study, however, presented an extremely interesting picture. For purposes of discussion the initial sample will be used as the "control" sample, as it was taken before the men had been on the test diet for any length of time and comes closest to representing the fecal flora of the men on a "normal" civilian diet comparable to the men on the NASA study. This initial sample will also be used as a reference point for the discussion of the following samples.

During the initial sampling period, the picture with respect to types of cultures isolated was quite similar to that found on the NASA study, that is, about 75% of the cultures screened were keyable using the key from the NASA contract and the variety of cultures isolated compared reasonably well with that found in the subjects on the basic study. Nine out of the seventeen "type cultures" were isolated from the four subjects in the initial sampling period, which compares favorably with the data obtained on a similar number of subjects on the NASA study. Thirteen percent of the cultures isolated on the AMRL study were of the black slime, marked gas-forming type and 6% of the cultures were of the marked lactic acid forming type, while 26% of the cultures were of the type that form moderate amounts of lactic acid. Thus the data from the initial sampling period at AMRL gave results that were very similar to those obtained on the NASA study with the exception that the subjects on the NASA study did not have as many cultures of the moderate lactic acid-forming type as did the subjects on the AMRL study (10% as compared to 26%).

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However, as the experiment proceeded and the men were on the test diets for longer periods of time, it became evident that fewer of the screened cultures fitted into the basic "type culture" key set up from the NASA study. The results are summarized in Tables XXI and XXII. It will be observed that the numbers of the keyable cultures fell rapidly from 77% on the initial sample to considerably less than 40% of the cultures screened during certain sampling periods. It was then observed that many of the cultures which could not be grouped using the basic NASA key did appear to have similar characteristics. As a result seven new groups were set-up based on the observed similar characteristics (Table XXIII) and the number of cultures that fell into the expanded "key" increased considerably, especially in the later samples (Tables XXIV, XXV and XXVI). This would indicate that there had been a rather marked and quite consistent shift in the basic flora of these men as the test progressed. Most noticeably there was a marked increase in the cultures which had the characteristics of gas, black slime formation and proteolysis, ranging from 24 to 40% (Table XXII) as compared with 13% on these same men during the initial sampling and 20% on the young men in the NASA study.

The incidence of strong lactic acid forming bacteria was low in the initial sampling period and remained low until the sixth sample when it increased somewhat and remained at the higher level throughout the remainder of the test (Table XXVII). On the other hand the occurrence of cultures forming lesser amounts of lactic acid which showed a very high incidence in the initial sampling period fell rapidly and had virtually disappeared by the seventh sampling period.

There may have been a slight tendency for the flora to become less diverse, as shown in Table XXVI, especially with respect to the original 17 "type cultures". Except for the initial sampling period when very few of the new groups of bacteria (G.D.) occurred, the variety of new groups of bacteria isolated remained rather constant throughout the remainder of the test period.

In general there did not appear to be strong individual differences among the subjects, and the variations seen between individuals were no greater than those seen between individuals on the NASA study. Some minor differences did occur, however. Subject I carried the strong lactic acid former (FA-5) in eight sampling periods, as opposed to 5, 6 and 2 for Subjects II, III and IV, respectively. FA-17 was found in three different samples from Subject I and was isolated from only one sample from all the rest of the subjects combined. FA-15 was isolated from seven samples from Subject II, but was found in only two or three samples on the other subjects.

The black slime, gas-formers occurred more frequently during the latter half of the test, but the increased occurrence did not result from cultures from any one individual. There were six samples in which three or more cultures of this type were isolated. Three of these episodes occurred during a period of diarrhea, while three did not. It is interesting to note that the new groups of organisms (G.D. series) appeared only seven times (2%) in 250 cultures screened on the NASA study when the unidentified cultures from the NASA study were reexamined and appeared only three times (10%) in the initial sampling period on the AMRL study, but made up a much larger percentage of the "keyable" cultures in the latter part of the test period on the AMRL study. Beginning with the second sample, the percentage increased to 24% and ranged between 30-40% or above after the third sample (Table XXII). No subject caried an undue proportion of any of the new groups of bacteria, although occasionally one of the organisms

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would dominate the cultures found in any one sample, which is not an unusual finding.

A careful examination of the data showed no marked differences in anaerobic fecal flora between subjects on the fresh and dehydrated diets or between the suited vs non-suited individuals.

Discussion of Anaerobic Flora

One of the objects of this experiment was to determine whether a space-type diet, either fresh or dehydrated, affected the fecal flora of young adult males eating this type of diet for a 28-day period. An added variable was the influence of wearing a space suit. Since one of the important components of the fecal flora is the anaerobic bacteria, these bacteria were examined in this experiment by procedures similar to those used on a NASA study on fecal anaerobes of the young adult male so that the results of the two studies can be compared. Although the work on the NASA study is incomplete, a meaningful comparison can be made in several areas where there is sufficient data. These are:

1. the proportion of aerobes to anaerobes
2. the percent of obligate anaerobes
3. the types of bacteria isolated based on the NASA study "key"
4. the broad characteristics of the predominating flora

By careful tabulation of the data on the AMRL experiment and further selective study, a more complete comparison and interpretation of the results can be made at a future time as more is learned about the individual cultures isolated.

The high proportion of anaerobes to aerobes in all phases of this study conforms with the data found on two other studies (NASw-738 and NASr-92[11] and strengthens the conclusion that anaerobes are the predominating fecal flora of the young adult male. The overwhelmingly high percentage of obligate anaerobes among these predominating bacteria in all sampling periods again confirms the results of previous studies that the obligate anaerobes are the predominant flora of the young adult male by a wide margin. These results seem reasonable since none of the conditions in this experiment was radically different from conditions in the previous test, and the test diet was composed of natural foods. No factor in this test would be predicted to cause an upset in the O-R potential in the intestinal tract.

However, there is a different picture when the types of bacteria found on this study are compared with the types found on the NASA study. The types of bacteria isolated from the samples taken before the subjects had been on the test diet for an appreciable length of time were quite similar in most respects to the types found on the NASA study. The one exception was the markedly higher incidence of bacteria producing moderate amounts of lactic acid, which may be the result of the rather high milk diet which the AMRL subjects had favored prior to being placed on the experimental diet. However, by the third sampling period a shift in the types of bacteria being isolated was apparent, and this alteration became more marked as the length of time on the experiment increased. One of the most notable differences was a decrease in the predominance of the types of

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bacteria found most often and "keyed" in the NASA study coupled with a corresponding increase in seven differing types of anaerobic bacteria. The types of bacteria which increased most markedly were those that produced gas, black slime and frequently were quite proteolytic when tested in litmus milk. It might be postulated that these bacteria rose to predominance because of the high protein content of the diet. Another possibility is that one subject or food handler carried these types of bacteria and transferred them to the others, or they may have been ingested with the food. Also marked was the decrease in those types of bacteria which produced moderate amounts of lactic acid while a small increase in the types of bacteria producing large amounts of lactic acid was noted. Both shifts occurred about mid-way in the test period directly preceding the change of the subjects from dehydrated to fresh food and vice versa.

Thus it would appear that the space-type diet did cause a shift in the predominating flora, resulting in the increase in gas-forming, black slime producing, frequently proteolytic bacteria. These types of bacteria had been less frequently isolated on the basic NASA study so that they appear to represent a different type of flora produced in response to the conditions of this experiment - most probably the diet. This finding is not unexpected, since in animal experimentation, diet is a prime factor influencing the bacteria in the digestive tract. Whether or not this shift in bacterial flora toward a gas-forming bacterial population is desirable or undesirable cannot be stated with certainty, but an increase in flatulence presumably would not be desirable under conditions of space flight. Just what type of bacteria these gas-forming, black slime producers may be has not yet been determined. The black color appears to be the result of H_2S production and two of them (G.D. 2 and G.D. 6) produced relatively high concentrations of lactic acid. Several of these types of bacteria are heavily proteolytic in litmus milk although none liquify gelatin as measured in the screen tests. Some consideration was given to the possibility that some of these bacteria were clostridial types, but morphology and lack of evidence of spore-formation do not support this theory. Further tests should be done on these types of bacteria in an attempt to clarify their identity.

There did appear to be a slight simplification of the bacterial flora toward the end of the experimental period, which corresponds to a similar finding when young men were kept on a defined diet of dehydrated foods during the NASr-92[11] study. It may mean that even a rather varied diet, when repeated at frequent intervals and when rigidly controlled so that no random foods are ingested, may induce a less varied fecal flora.

In general no consistent differences appeared in the anaerobic character or types of organisms isolated when the diet was shifted from dehydrated to fresh or vice versa. Since both the fresh and dehydrated diets were matched exactly in composition, this lack of differences in flora between the fresh and dehydrated periods is not unexpected and only serves to point out the excellence of the matching of these two types of diets. It also suggests that diet composition may be more important than physical form.

Also no consistent differences were observed in the fecal flora between the suited and non-suited subjects. It would seem from this finding that the wearing of this type of space suit for a two-week period did not alter the conditions in the intestinal tract to a degree sufficient to alter the intestinal flora.

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As far as the anaerobes are concerned, no clear-cut evidence was found to indicate that one subject carried a culture of bacteria peculiar to him which he subsequently transferred to the other subjects. The appearance of the new types of cultures (the G.D. series) did not give evidence of having been brought in by one subject and passed on to the others, since these new types of cultures occurred rather sporadically at first, and spread among all of the subjects. These findings do not in any way rule out transference, but merely fail to demonstrate such transference in a clear-cut fashion.

The results of this experiment show a considerable degree of variation between different samples on the same subject and samples taken during the same period on different subjects, although certain types of bacteria were sometimes isolated more frequently from one subject than from the others. This finding is consistent with the results from the NASA study and probably reflects the complexity of the problem of isolating and identifying the fecal anaerobes as well as the incompleteness of the picture now available with respect to the predominating fecal anaerobes. The volume of work necessary for a really quantitative study of these anaerobes creates practical problems. Because there are so many fecal anaerobes (about one trillion) per gram of feces and so many varieties of bacteria which may occur even in the top dilutions of the feces (one billion per gram and above), it is not practical to screen every bacterial culture which could be isolated. For this reason only selected cultures of the most predominating bacteria can be studied at this time. The apparent lack of agreement between duplicate dilutions or between different samples from the same individual probably reflect this limitation imposed by the necessity of choosing only certain cultures from the highest dilutions for screening. If every culture from each primary dilution could be studied, no doubt better agreement would be obtained. This supposition is substantiated to a degree by the results obtained from examining the Gram stains from the lower dilutions of feces which were not screened. Those bacteria isolated in high dilutions in previous samples, which can be recognized because of peculiar morphology, are often seen to be present in the lower dilutions of subsequent samples, even though they were not predominating enough to reach the high dilutions on the subsequent samples. This represents a shift in predominance rather than a real change in flora. Only when a significant trend is maintained over several sampling periods using an average figure from several subjects can the finding be considered to be meaningful. Such definite trends were shown by data in this report with respect to the occurrence of the new GD types of organisms and the appearance of the gas-forming, black slime producing proteolytic rods.

For this reason, experiments designed to study the influence of test conditions on the anaerobic fecal flora should continue for long enough periods of time to allow adequate numbers of samples to be studied to minimize these transient fluctuations and should include enough subjects to overcome individual variations and relative lack of precision of the technique at this stage of experimentation.

It was unfortunate on this test that no samples were obtained from the subjects prior to the start of the experimental test diet, as such control samples would have rendered the experimental findings more amenable to true comparison with the basic data obtained on the NASA study. In future experiments such a control period is recommended.

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One finding that was not presented in the results because of the difficulty of offering comparative results is the sporadic isolation of ultra-microscopic forms of bacteria. These organisms are difficult to detect even when they have grown in the primary culture series because of their small almost sub-microscopic size so that their presence is masked by larger bacteria that have not been diluted out. When these tiny organisms are in pure culture, they do not produce turbidity or form colonies in agar and they are difficult to detect on a routine examination of a Gram stain. These tiny bacteria have been found frequently on the NASA study and in this study and they are probably among the predominating fecal bacteria; but until the development of suitable techniques for their study is accomplished, reporting of comparative data on the occurrence of these bacteria would not be meaningful.

Rosebury's[(2)] review of the literature indicates that clostridia are a usual component of the fecal flora. In the study at AMRL and in the young men on the NASA contract, the presence of clostridia has not been reported. This does not imply that clostridia are absent, but simply means that clostridia have not been identified as such for one of several reasons. One or more of the "type" cultures may on closer study turn out to be clostridia and at that time will be identified as a clostridium. Also, the clostridia may be present in too small numbers to reach the billionth dilution of feces which is the lowest dilution of feces which was screened for identification in this study. It is probable that clostridia are present in small numbers which are not detected by the methods used in the present study. One of the objectives of the NASA study on the predominating anaerobic fecal flora of young men is to characterize or identify the organisms which are now designated only by the key number FA-1 or FN-1, etc. When these organisms have been more thoroughly studied it will be possible to place a more precise interpretation upon the data obtained in this and other comparative experiments on fecal anaerobic flora.

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SECTION V

PHYSIOLOGICAL STUDIES OF NEW G.D. TYPES

The seven new "type cultures" isolated during this study are G.D. 1 through G.D. 7. Several aspects of the physiology of this group of anaerobes were studied in detail in order to determine if they have unique characteristics which distinguish them from the "type cultures" isolated during the NASA contract NASw-738. The additional data helped to further separate, or group, the type cultures G.D. 1-7.

One obvious characteristic some of the seven "type cultures" possessed was an ability to form a black pigment in Gall's broth. This characteristic was studied in detail. It was decided to test two hypotheses:

1. A by-product of bacterial metabolism could react with the medium and produce discoloration, and

2. Pigments could be formed by the bacteria.

A series of studies were done to test the first hypothesis. Clark and Cowan[12] demonstrated that most of the cultures they tested produced H_2S from cysteine. Hydrogen sulfide may be detected in bacterial cultures by the black sulfides formed by the salts of lead, nickel, cobalt, bismuth or iron. Zobell and Feltham[13] showed that the lead acetate test strip method has two advantages in the detection of H_2S gas: sensitivity and lack of inhibition of cultures. Difco[14] developed "lead acetate agar", a culture medium which reportedly gives no inhibition due to lead toxicity and gives browning in the presence of hydrogen sulfide; the same concentration of lead acetate was used in Gall's broth.

The possibility that the discoloration was linked to cysteine, a constituent of Gall's medium which was utilized by some of the "type cultures" with the production of hydrogen sulfide which in turn formed iron sulfide from iron in Gall's medium was investigated.

In order to test this possibility, H_2S gas was bubbled through uninoculated culture media. A slight gray precipitate high in iron concentration resulted, indicating the formation of a metallic sulfide. Therefore, Gall's medium is inadvertently a weak differential medium for hydrogen sulfide production.

The second step in the program was designed to determine if darkening of the medium by some of the anaerobes was correlated with cysteine, since this sulfur-containing amino acid has been shown to be substrate for bacterial H_2S production by Clark and Cowan[12]. Cultures were grown in Gall's broth with cysteine deleted and also with various concentrations of cysteine. Two batches of broth were prepared: 1) a lead acetate saturated filter paper strip placed in the lip of each tube of Gall's broth, and 2) modified Gall's medium containing 0.02% lead acetate.

The results indicated that:

(1) Discoloration of the culture was not observed in the absence of added cysteine, although the cultures grew in its absence.

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(2) All of the tested cultures produced hydrogen sulfide in the presence of cysteine (G.D. 1-7), although there was a quantitative difference in H_2S production.

(3) The cultures that produced discoloration of Gall's broth (G.D. 1, 4, 5, and 7) all produced intense blackening in the presence of cysteine.

(4) G.D. 7 was the only culture that produced definite blackening in Gall's medium in 24 hours; it produced the most intense H_2S.

(5) All cultures except GD-2 and GD-6 grew better in the presence of cysteine.

(6) The amount of blackening of the lead acetate medium was directly proportional to the concentration of cysteine.

In summary, then, the blackening of certain cultures grown in Gall's broth is probably due to hydrogen sulfide gas which originates from the cysteine substrate and subsequently reacts with normally present iron in the medium to form black iron sulfide. Cultures which do not spontaneously blacken Gall's broth do not produce as much hydrogen sulfide.

In addition to the hydrogen sulfide studies, assays were done to determine the relative quantities of lactic acid formed by bacteria in Gall's broth in an attempt to further group and separate G.D. 1-7 on the basis of their physiology.

A modification of the procedure of Hullin and Nobel[15] for the quantitative analysis of lactic acid and its salts was developed. 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 parahydroxydiphenyl in the presence of copper catalyst. The resulting solution is read colorimetrically at 560 $m\mu$ 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 is listed in Table XXVIII. The stock cultures are listed according to the percent of 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

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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.

The data in Table XXVIII illustrate several obvious points. Under the conditions of this experiment G.D. 2 and G.D. 6 produced relatively large quantities of lactic acid. These cultures also produce a relatively low pH in culture media. G.D. 7 did not form substantial lactic acid or consume the lactic acid which exists in Gall's medium. This anaerobe was also unique in its high rate of H_2S production. The other anaerobes tested, G.D. 1, G.D. 3, G.D. 4, and G.D. 5, produced an intermediate amount of lactic acid in the culture medium.

The seven new GD type cultures present certain interesting features which warrant further investigation and these cultures should be subjected to the same types of physiological testing as the other cultures.

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SECTION VI

CONCLUSIONS

The fecal population depends upon the effects of interactions between host and biota and between different members of the biota. The important effect of diet has been restressed by the results of this experiment. Man's economy is dependent upon his successful relationship to his flora.

"Mere tolerance is biologically and statistically improbable".[16] Lucas' statement is as appropriate as when it was written. It is improbable that the presence of the bacteria encountered in this study has not affected their host. This effect may be subtle or slow to become apparent, and either beneficial, harmful, or unknown; however, its existence must be recognized.

The results of the study were rather clear-cut. During the "control" period the young men on the AMRL study showed a fecal flora which agreed rather well with that found on the NASA study and the aerobic flora compared well with that cited in the literature. However, as the experiment proceeded and the subjects had been on the space-diet for a longer period of time, differences in both the aerobic and anaerobic flora began to appear. With respect to the anaerobic flora, the types found predominantly on the young men in the NASA study became less predominant and seven new types of organisms which are described in this report became prevalent among the predominating flora. Most of these new types were gas-forming, black slime producing proteolytic organisms. These data represent a significant shift in the predominating anaerobic fecal flora.

The aerobic flora also showed some marked peculiarities when compared with the literature and with the results of the NASA study. This was particularly marked with respect to the frequent recovery of enteropathogenic coli and shigella and shigella-like organisms. There also was prevalent a nitrate-negative, H_2S-producing rod which was isolated on the bismuth sulfite medium, which was not found using similar procedures on the subjects in the NASA study and which was not listed in the literature among the normal fecal bacteria.

Thus it can be concluded that during the course of this experiment in which the subjects ate the space-type diet there was a shift in the predominating anaerobic flora which occurred after the subjects had been on the diet for some days and which contrasted with the "control" period and with the results obtained from the NASA study. The aerobic flora differed from that cited in the literature and found on the subjects in the NASA study. There appeared to be no difference between the subjects on the fresh or freeze-dehydrated food. These data would indicate, therefore, that the space-type diet did exert an influence on the normal fecal flora of the four subjects with respect to anaerobic bacteria, and that the aerobic flora isolated from these subjects was different from that commonly found.

Some individual differences between subjects were noted but these appeared to be well within the limits of variability found on the subjects on the NASA study. The wearing of the space suit did not seem to influence the results.

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SECTION VII

RECOMMENDATIONS

The results of this study of the effect of the space-diet on the fecal flora of the four subjects were clear-cut, but they raise many important questions which warrant further consideration. Some of these involve the experimental design. The lack of a real control period on this study points to the importance of including a pre-experimental diet control period in future studies of this nature. Certain of the results suggested that a longer period of time on a given dietary regimen might allow the balance of flora to become more nearly stabilized so that more meaningful results might be obtained. A relatively small number of subjects (four) were used in this experiment and the entire experimental period was only 28 days. It would be highly desirable to repeat this experiment with more subjects for a longer time in an effort to increase the validity of the results of this experiment.

The occurrence of potentially pathogenic organisms such as the enteropathogenic strains of coli, the shigella and shigella-like organisms, and the coagulase positive staphylococci suggest that these human pathogens may have occurred in these subjects as the result either of their being ingested in the food or transferred from one human to another. These human pathogens may have been introduced into the food at any time during its processing or preparation. Human carriers may have transmitted the organisms to the subjects during any personal contact. It would seem wise in future experiments to strictly control the source of the food, the food handlers and any human contacts with the subjects in an attempt to eliminate these factors as variables in the experiment.

Other recommendations which suggest possible further work are indicated by the results of this experiment. It was demonstrated that there was a shift in the anaerobic flora of these subjects while they were on the experimental diet. However, the limited data available on these strains of organisms which appeared during the dietary test period do not allow conclusions to be drawn regarding the desirability or the undesirability of the presence of these strains of organisms. Further studies on the physiology of these types of organisms might allow an assessment of the importance of these bacterial shifts.

Differences in both the aerobic and anaerobic flora were noted but whether or not one influenced the other was not readily determined. Investigations of the interrelationships between the various bacteria comprising the intestinal flora would help to clarify this point. The interrelationship between the various bacteria would involve a fairly complete study of the physiology followed by a synergistic study of combinations of certain organisms.

The aerobic flora of these subjects was not typical of that recorded in the literature or found on the men on the NASA study, particularly with respect to the presence of certain potential enteric pathogens. The reason for the presence of these enteric pathogens is not clear. The significance and mechanism of these findings may be still further defined at this time by culturing the food to determine whether these organisms were already present in the food (if some of the freeze-dehydrated food from the same lot is available) as well as by an

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examination of the feces of the personnel who were in contact with the subjects. Examination of the food handlers, subjects, and personnel in contact with the subjects for serum antibodies for enteropathogens should elucidate this problem. It is known that colicin production usually inhibits the growth of shigella and the determination of colicin, if possible, from any stored feces from this project might indicate the relative amounts of colicin present in the intestinal tract during the various sampling periods.

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SECTION VIII

TABLES

TABLE I

EXPERIMENTAL PROCEDURE

Days on Test	Date	Diet	Subject I	Subject II	Diet	Subject III	Subject IV
1	4 May	Fresh	X		Dehydrated		
2	5 "		XD	X		X	X
3	6 "		XD				
4	7 "			X		X	X
5	8 "		SUITED	NON-SUITED		SUITED	NON-SUITED
6	9 "						
7	10 "						
8	11 "		X	X		X	X
9	12 "						
10	13 "						
11	14 "		XD				X
12	15 "			X		X	
13	16 "						
14	17 "						XD
15	18 "						
16	19 "		X	X		X	
17	20 "						
18	21 "						
19	22 "		XD	X		XD	XD
20	23 "						
21	24 "	Dehydrated			Fresh		
22	25 "						
23	26 "						X
24	27 "		XD	X		X	

X = fecal sample

D = semi-liquid stool

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TABLE I (cont'd)

EXPERIMENTAL PROCEDURE

Days on Test	Date	Diet	Subject I	Subject II	Diet	Subject III	Subject IV
25	28 May						
26	29 "						
27	30 "		XD			XD	X
28	31 "						
29	1 June			X			
30	2 "						
31	3 "		XD				XD
32	4 "			X		XD	
33	5 "						
34	6 "						
35	7 "		X			X	X
36	8 "			X			
37	9 "						
38	10 "		X			X	X
39	11 "		X	X			X
40	12 "		X	X		X	X
41	13 "	Fresh			Fresh	X	
42	14 "			X			
43	15 "						
44	16 "						
45	17 "						

X = fecal sample

D = semi-liquid stool

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TABLE II

AEROBIC DILUTION SERIES (AGREEMENT)

(10^{-})

Sample No.		Subject Number			
		I	II	III	IV
1	A	7	6	5	6
	B	6	5	6	6
	C	6	6	6	7
2	A	6	6	6	6
	B	7	6	6	6
	C	6	6	6	5
3	A	6	5	4	5
	B	7	5	4	5
	C	7	5	4	6
4	A	7	6	4	4
	B	7	5	4	4
	C	7	6	5	4
5	A	7	7	4	5
	B	7	6	5	5
	C	8	7	5	5
6	A	7	6	7	4
	B	6	5	7	5
	C	6	5	7	5
7	A	7	6	5	6
	B	6	6	5	7
	C	6	6	4	7
8	A	6	4	6	6
	B	7	4	6	7
	C	6	4	6	7
9	A	6	5	4	7
	B	6	5	4	7
	C	6	5	4	7
10	A	8	5	8	6
	B	8	4	8	6
	C	8	5	8	6
11	A	6	5	7	4
	B	6	5	5	5
	C	6	5	5	4
12	A	5	6	4	6
	B	5	6	4	6
	C	5	6	5	6
13	A	6	6	5	5
	B	6	6	5	5
	C	6	6	5	5

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TABLE III

AEROBIC DILUTION SERIES
GROWTH HEIGHT
(Average*)

Sample No.	Subject Number				Average
	I	II	III	IV	
1	6	6	6	6	6
2	6	6	6	6	6
3	7	5	4	5	5+
4	7	6	4	4	5+
5	7	7	5	5	6
6	6	5	7	5	5+
7	6	6	5	6+	6
8	6	4	6	7	5+
9	6	5	4	7	5+
10	8	5	8	6	6+
11	6	5	5	4	5
12	5	6	4	6	5
13	6	6	5	5	5+

* 10^{-}

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TABLE IV

OCCURRENCE AND DISTRIBUTION OF ENTEROBACTERIACEAE

Sample No.	Subject Number			
	I	II	III	IV
1	Klebsiella Citrobacter Shigella B E. coli	E. coli*	E. coli *	E. coli A * Shigella B Proteus rettgeri
2	E. coli Shigella B	E. coli E. coli (Poly A)* Aerobacter B	Pseudomonas	E. coli Shigella B Proteus rettgeri
3	E. coli Poly A * Poly B Shigella B Klebsiella	Aerobacter B E. coli * Alcaligenes faecalis	Pseudomonas	E. coli Shigella B
4	Shigella B E. coli * Alcaligenes faecalis	Aerobacter B E. coli Aerobacter C	E. coli * Shigella	E. coli Shigella B Alcaligenes faecalis
5	Shigella B Klebsiella Aerobacter gr. E. coli * Alcaligenes faecalis	Aerobacter B E. coli	Shigella A. D. E. coli * Klebsiella	Shigella B Klebsiella Alcaligenes faecalis Pseudomonas Aerobacter C
6	E. coli	E. coli * Salmonella group A	Shigella A. D. E. coli	Klebsiella E. coli Shigella B Alcaligenes faecalis
7	Aerobacter C Shigella B Klebsiella Aerobacter B	E. coli *	Shigella	Klebsiella E. coli Serratia
8	E. coli Klebsiella	E. coli* Shigella A. D.	4 unidentified	Shigella B Klebsiella Aerobacter C
9	Klebsiella E. coli * Shigella	E. coli Shigella	E. coli	Shigella B Klebsiella
10	E. coli * Klebsiella	E. coli Aerobacter C	Klebsiella E. coli	Shigella B Klebsiella E. coli

*potentially pathogenic coli

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TABLE IV (Cont'd)

OCCURRENCE AND DISTRIBUTION OF ENTEROBACTERIACEAE

Sample No.	Subject Number			
	I	II	III	IV
11	Klebsiella E. coli Citrobacter	E. coli	E. coli Klebsiella	E. coli Shigella B. Klebsiella
12	E. coli Klebsiella	E. coli	E. coli Klebsiella	E. coli Klebsiella
13	E. coli* Klebsiella	E. coli *	E. coli * Klebsiella	E. coli Klebsiella

*potentially pathogenic coli

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TABLE V

OCCURRENCE OF LACTOSE FERMENTERS ON MacCONKEY'S MEDIUM

Sample No.	Subject Number			
	I	II	III	IV
1	43×10^6	5.7×10^5	0	15×10^5
2	13×10^6	20×10^5	**	13×10^4
3	46×10^4	1.9×10^5	0	6.1×10^5
4	11×10^7	28×10^5	0	**
5	44×10^6	21×10^6	7×10^4	5×10^4
6	13×10^5	50×10^5	31×10^5	1×10^5
7	62×10^5	30×10^5	3.8×10^5	75×10^5
8	1.9×10^5	1.3×10^5	65×10^5	13×10^6
9	7.4×10^5	**	**	3×10^5
10	6×10^5	8×10^4	42×10^6	18×10^5
11	1×10^5	1.9×10^5	**	**
12	7×10^4	17×10^5	5.1×10^5	11×10^5
13	*	6×10^5	33×10^5	3.3×10^5

* Spreaders
** Less than 10 colonies

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TABLE VI

DISTRIBUTION OF COLI TYPES

Sample No.	Subject Number			
	I	II	III	IV
1		Poly A 055:B5	Poly B 0124:B17	Poly A 0127:B8
2		Poly A 0127:B8 (3x)		
3	Poly A 0127:B8	Poly A 055:B5		
4	Poly A 0111:B4		Poly A 0127:B8	
5	Poly B 0125:B15		Poly B 086:B7	
6		Poly A 026:B6		
7		Poly B 0124:B17		
8		Poly A 0111:B4 (2x)		
9	Poly A 055:B5			
10	Poly A 055:B5			
11				
12				
13	Poly A 055:B5	Poly B 0126:B16	Poly A 0127:B8	

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TABLE VII

OCCURRENCE OF NON-LACTOSE FERMENTERS

Sample No.	Subject Number			
	I	II	III	IV
1	3×10^6	0	0	4.8×10^5
2	10×10^6	few	0	10×10^6
3	4×10^6	0	0	few
4	2.9×10^5	0	few	0
5	1×10^6	few	few	1.3×10^5
6	14×10^5	few	6×10^4	3.3×10^4
7	17×10^5	0	0	0
8	0	1.6×10^5	0	few
9	13×10^5	few	0	8×10^4
10	0	few	0	5.9×10^5
11	0	0	0	0
12	0	0	0	1.9×10^5
13	0	0	0	2.3×10^5

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TABLE VIII

COLIFORMS
STANDARD METHODS CALCULATIONS

Subject Number I

Sample		Lactose Fermenters					Non-Lactose Fermenters				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1	A	>	>	22*	14	2	>	>	–	2	–
	B	>	125	27*	4	–	>	>	10	2	–
	C	>	>	31	3	–	>	>	–	–	–
SMC		$43x10^6$					$3x10^6$				
2	A	>	29	*	–	–	>	100	*	2	–
	B	>	32	*	1	–	>	100	*	3	2
	C	>	71	*	–	–	>	*	*	4	–
SMC		$13x10^6$					$10x10^6$				
3	A	46	53	22	–	–	*	18*	5	–	–
	B	*	56	46	2	–	*	23*	2	2	–
	C	46	*	10	2	–	*	*	6	2	–
SMC		$46x10^4$					$4x10^6$				
4	A	>	306	106	23	–	28	32	4	1	–
	B	>	400	107	29	–	35	28	4	1	–
	C	>	310	130	25	–	25	15	8	–	–
SMC		$11x10^7$					$2.9x10^5$				
5	A	14	86	36	4	–	*	4	1	2	–
	B	18	93	26	1	1	*	2	1	–	–
	C	*	*	72	6	2	*	5	1	–	–
SMC		$44x10^6$					$1x10^6$				
6	A	117	18	5	–	–	190	50	8	–	–
	B	154	25	2	–	–	128	20	3	–	–
	C	139	25	3	–	–	101	13	10	–	–
SMC		$13x10^5$					$14x10^5$				
7	A	500	50	5	–	–	140	9	1	–	–
	B	>300	60	12	–	–	210	85	–	–	–
	C	>500	76	11	–	–	144	26	1	–	–
SMC		$62x10^5$					$17x10^5$				

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TABLE VIII (cont'd)

COLIFORMS

STANDARD METHODS CALCULATIONS

Subject Number I (cont'd)

Sample		Lactose Fermenters					Non-Lactose Fermenters				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
8	A	16	1	-	-	-	-	-	-	-	-
	B	18	4	-	-	-	-	-	-	-	-
	C	15	23	-	-	-	-	-	-	-	-
SMC		1.9×10^5									
9	A	72	4	1	-	-	146	22	2	-	-
	B	86	11	4	-	-	135	15	6	-	-
	C	65	6	3	-	-	120	16	2	-	-
SMC		7.4×10^5					13×10^5				
10	A	63	5	-	-	-	-	-	-	-	-
	B	58	4	-	-	-	-	-	-	-	-
	C	60	9	1	-	-	-	-	-	-	-
SMC		6×10^5									
11	A	10	1	-	-	-	-	-	-	-	-
	B	20	2	-	-	-	-	-	-	-	-
	C	9	4	-	-	-	-	-	-	-	-
SMC		1.0×10^5									
12	A	-5	-	-	-	-	-	-	-	-	-
	B	7	-	-	-	-	-	-	-	-	-
	C	10	-	-	-	-	-	-	-	-	-
SMC		7×10^4									
13	A	*	*	4*	-	-	-	-	-	-	-
	B	*	*	3	-	-	-	-	-	-	-
	C	*	40	5	1*	-	-	-	-	-	-
SMC											

Key:

> = Too numerous to count
* = Spreaders
SMC = Standard Methods Calculations

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TABLE VIII (cont'd)

COLIFORMS
STANDARD METHODS CALCULATIONS

Subject Number II

Sample		Lactose Fermenters					Non-Lactose Fermenters				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1	A	53	3	-	-	-	-	-	-	-	-
	B	53	3	2	-	-	-	-	-	-	-
	C	64	6	-	-	-	-	-	-	-	-
SMC		$5.7x10^{5}$									
2	A	124	29	5	-	-	5	3	-	-	-
	B	252	43	5	-	-	-	2	-	-	-
	C	120	12	-	-	-	-	-	2	-	-
SMC		$20x10^{5}$									
3	A	15	3	-	-	-	-	-	-	-	-
	B	29	4	-	-	-	-	-	-	-	-
	C	12	5	-	-	-	-	-	-	-	-
SMC		$1.9x10^{5}$									
4	A	288	40	11	1	-	-	-	-	-	-
	B	220	9	0	-	-	-	-	-	-	-
	C	328	48	15	4	-	-	-	-	-	-
SMC		$28x10^{5}$									
5	A	>800	240	32	5	-	-	-	-	-	-
	B	> 700	200	30	1	-	-	-	-	-	-
	C	>700	181	26	4	-	4	2	-	-	-
SMC		$21x10^{6}$									
6	A	>	66	8	2	-	-	-	-	-	-
	B	>	36	4	-	-	2	-	-	-	-
	C	380	48	4	-	-	2	-	-	-	-
SMC		$50x10^{5}$									
7	A	>	400	40	9	-	-	-	-	-	-
	B	>	308	21	4	-	-	-	-	-	-
	C	>	300	20	3	-	-	-	-	-	-
SMC		$30x10^{5}$									

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TABLE VIII (cont'd)

COLIFORMS

STANDARD METHODS CALCULATIONS

Subject Number II (cont'd)

Sample		Lactose Fermenters					Non-Lactose Fermenters				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
8	A	8	-	-	-	-	16	2	-	-	-
	B	3	-	-	-	-	9	3	-	-	-
	C	27	4	-	-	-	21	2	-	-	-
SMC		1.3×10^5									
9	A	20	2	1	-	-	17	3	4	-	-
	B	2	-	-	-	-	1	-	-	-	-
	C	6	1	-	-	-	-	-	-	-	-
SMC											
10	A	5	1	-	-	-	1	-	-	-	-
	B	20	3	-	-	-	2	1	-	-	-
	C	19	1	-	-	-	2	-	-	-	-
SMC		8×10^4									
11	A	15	2	-	-	-	-	-	-	-	-
	B	15	3	-	-	-	-	-	-	-	-
	C	19	2	-	-	-	-	-	-	-	-
SMC		1.9×10^5									
12	A	122	22	1	-	-	-	-	-	-	-
	B	210	28	4	-	-	-	-	-	-	-
	C	*	20	3	-	-	-	-	-	-	-
SMC		17×10^5									
13	A	40	7	-	-	-	-	-	-	-	-
	B	102	14	1	-	-	-	-	-	-	-
	C	28	3	-	-	-	-	-	-	-	-
SMC		6×10^5									

Key:

> = Too numerous to count
* = Spreaders
SMC = Standard Methods Calculations

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TABLE VIII (cont'd)

COLIFORMS
STANDARD METHODS CALCULATIONS

Subject Number III

Sample		Lactose Fermenters					Non-Lactose Fermenters				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1	A	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-
SMC											
2	A	26	3	1	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-
SMC											
3	A	-	-	-	-	-	-	1	-	-	-
	B	-	-	-	-	-	-	-	-	-	-
	C	2	-	-	-	-	-	-	-	-	-
SMC											
4	A	2	-	-	-	-	2	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-
SMC											
5	A	6	-	-	-	-	-	-	-	-	-
	B	4	1	-	-	-	-	-	-	-	-
	C	10	-	-	-	-	1	-	-	-	-
SMC		$7x10^4$									
6	A	350	38	9	2	2	7	1	-	-	-
	B	300	25	8	2	-	6	-	2	-	-
	C		41	8	5	2	6	4	-	-	-
SMC		$> 31x10^5$									
7	A	51	6	1	-	-	-	-	-	-	-
	B	16	4	-	-	-	-	-	-	-	-
	C	64	3	-	-	-	-	-	-	-	-
SMC		$3.8x10^5$									

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TABLE VIII (cont'd)

COLIFORMS

STANDARD METHODS CALCULATIONS

Subject Number III (cont'd)

Sample		Lactose Fermenters					Non-Lactose Fermenters				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
8	A	>	48	2	-	-	-	-	-	-	-
	B	>	74	13	-	-	-	-	-	-	-
	C	>	72	4	-	-	-	-	-	-	-
SMC		$65x10^5$									
9	A	2	-	-	-	-	-	-	-	-	-
	B	9	-	-	-	-	-	-	-	-	-
	C	2	-	-	-	-	-	-	-	-	-
SMC											
10	A	>	373	5	1	-	-	-	-	-	-
	B	>	410	12	-	-	-	-	-	-	-
	C	>	412	8	-	-	-	-	-	-	-
SMC		$42x10^6$									
11	A	30	3	1	-	-	-	-	-	-	-
	B	1	-	-	-	-	-	-	-	-	-
	C	15	-	-	-	-	-	-	-	-	-
SMC											
12	A	56	3	-	-	-	-	-	-	-	-
	B	53	3	-	-	-	-	-	-	-	-
	C	43	7	-	-	-	-	-	-	-	-
SMC		$5.1x10^5$									
13	A	*	40	2	-	-	-	-	-	-	-
	B	*	32	6	-	-	-	-	-	-	-
	C	*	27	4	1	-	-	-	-	-	-
SMC		$33x10^5$									

Key:

> = Too numerous to count
* = Spreaders
SMC = Standard Methods Calculations

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TABLE VIII (cont'd)

COLIFORMS

STANDARD METHODS CALCULATIONS

Subject Number IV

Sample		Lactose Fermenters					Non-Lactose Fermenters				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1	A	>	160	25	4	-	50	7	1	-	-
	B	>	148	17	7	-	47	6	-	-	-
	C	>	132	40	9	-	48	17	1	-	-
SMC		$15x10^5$					$4.8x10^5$				
2	A	4	-	7	1	-	380	110	-	-	-
	B	2	-	14	-	-	400	96	15	-	-
	C	7	2	-	-	-	375	100	-	-	-
SMC		$13x10^4$					$10x10^6$				
3	A	34	7	1	-	-	-	-	-	-	-
	B	90	5	3	-	-	25	-	-	-	-
	C	60	10	2	-	-	-	-	-	-	-
SMC		$6.1x10^5$									
4	A	3	-	-	-	-	-	-	-	-	-
	B	43	-	-	-	-	-	-	-	-	-
	C	5	-	-	-	-	-	-	-	-	-
SMC											
5	A	6	1	-	-	-	17	8	-	-	-
	B	8	-	-	-	-	9	1	-	-	-
	C	7	-	-	-	-	12	-	-	-	-
SMC		$5x10^4$					$1.3x10^5$				
6	A	12	4	-	-	-	5	-	-	-	-
	B	7	1	-	-	-	1	-	-	-	-
	C	12	1	-	-	-	4	-	-	-	-
SMC		$1x10^5$					$3.3x10^4$				
7	A	>	36	10	2	-	-	-	-	-	-
	B	>	132	32	2	-	-	-	-	-	-
	C	>	58	17	-	-	-	-	-	-	-
SMC		$75x10^5$									

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TABLE VIII (cont'd)

COLIFORMS

STANDARD METHODS CALCULATIONS

Subject Number IV (cont'd)

Sample		Lactose Fermenters					Non-Lactose Fermenters				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
8	A	>	127	17	1	-	*	1	-	-	-
	B	>	132	13	-	-	-	-	-	-	-
	C	>	120	15	1	-	-	1	-	-	-
SMC		$13x10^{6}$									
9	A	31	4	-	-	-	11	-	-	-	-
	B	8	2	-	-	-	7	2	-	-	-
	C	6	2	-	-	-	6	-	-	-	-
SMC		$3x10^{5}$					$8x10^{4}$				
10	A	222	19	-	-	-	46	4	-	-	-
	B	105	17	-	-	-	52	3	-	-	-
	C	224	46	-	-	-	80	31	-	-	-
SMC		$18x10^{5}$					$5.9x10^{5}$				
11	A	9	-	-	-	-	-	-	-	-	-
	B	62	5	-	-	-	-	-	-	-	-
	C	65	3	-	-	-	-	-	-	-	-
SMC											
12	A	100	60	7	-	-	150	5	-	-	-
	B	128	100	16	2	-	160	-	-	-	-
	C	106	74	14	2	-	270	-	-	-	-
SMC		$11x10^{5}$					$1.9x10^{5}$				
13	A	23	3	1	-	-	20	5	-	-	-
	B	29	5	-	-	-	23	-	-	-	-
	C	46	9	3	-	-	26	2	1	-	-
SMC		$3.3x10^{5}$					$2.3x10^{5}$				

Key:

> = Too numerous to count
* = Spreaders
SMC = Standard Methods Calculations

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TABLE IX

DISTRIBUTION OF PECULIAR CULTURE WHICH PRODUCES H_2S ON BISMUTH SULFITE MEDIUM

Sample No.	Subject Number			
	I	II	III	IV
1				+
2			+	
3		+	+	+
4	+	+	+	
5				+
6	+	+	+	+
7	+			+
8	+			+
9	+			
10	+			+
11	+			+
12	+			+
13	+			+

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TABLE X

DISTRIBUTION OF STREPTOCOCCI ON MITIS SALIVARIUS MEDIUM

Sample No.	Subject Number			
	I	II	III	IV
1	Mitis Enterococci	Mitis	Mitis	Mitis Salivarius
2	Mitis Enterococci	Enterococci Mitis	Enterococci	Enterococci Mitis
3	Mitis Enterococci	Mitis Enterococci	Salivarius	Salivarius Mitis
4	Mitis Salivarius Enterococci	Salivarius Enterococci	Enterococci	Enterococci Salivarius
5	Salivarius Enterococci	Mitis Salivarius	Enterococci Salivarius	Salivarius Mitis
6	Salivarius Mitis Enterococci	Salivarius	Salivarius Enterococci Mitis	Salivarius Mitis
7	Salivarius Mitis	Salivarius Mitis Enterococci	Salivarius Enterococci	Salivarius Mitis
8	Salivarius Mitis Enterococci	Salivarius Enterococci	Salivarius Enterococci	Salivarius Enterococci
9	Enterococci Mitis Salivarius	Enterococci	Salivarius	Salivarius Enterococci Mitis
10	Salivarius Enterococci Mitis	Enterococci Salivarius	Enterococci Salivarius	Salivarius Mitis
11	Salivarius Mitis	Salivarius Mitis Enterococci	Salivarius Mitis	Salivarius
12	Salivarius Mitis Enterococci	Salivarius Enterococci	Salivarius Mitis	Salivarius Enterococci
13	Salivarius Enterococci	Salivarius Enterococci	Enterococci Salivarius Mitis	Salivarius Enterococci

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TABLE XI

OCCURRENCE OF STAPHYLOCOCCI

Sample No.	Subject Number			
	I	II	III	IV
1	* 50	40	60	* 10
2	* 20	* 53	32	20
3	* 2	2	4	10
4	* 4	12	80	* 3
5	2	4	48	* 5
6	* 125	6	* 3	5
7	100	5	* 30	50
8	* 10	22	18	* 75
9	* 10	12	32	33
10	* 10	4	5	25
11	5	100	125	10
12	100	50	* 28	5
13	* 100	10	* 19	* 3

* Indicates possible pathogenicity as indicated by growth on mannitol salt agar and positive coagulase test.

Note: Numbers under Subjects I, II, III, IV refer to number of colonies of staphylococci.

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TABLE XII

MICROAEROPHILIC LACTOBACILLI RECOVERED FROM ROGOSA'S MEDIUM

(Colony Count)

Sample No.	Subject Number			
	I*	II**	III**	IV**
1	250	2	3	0
2	30	1	4	4
3	42	3	6	2
4	110	5	2	1
5	37	80	2	2
6	100	5	2	4
7	60	4	0	3
8	338	11	2	3
9	400	4	2	6
10	260	6	4	4
11	140	9	0	2
12	225	3	2	6
13	200	3	110	6

* Count from third tube
** Count from tube one

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TABLE XIII

FUNGI

Sample No.	Subject Number			
	I	II	III	IV
1	Penicillium sp. Candida parapsilosis	0	0	+
2	0	0	0	+
3	0	+ Candida parapsilosis	Aspergillus +	+
4	+	+ Candida parapsilosis	+	+
5	+	+	+	
6	+	+	+ Candida parapsilosis	+
7	+		+	+
8			+	+
9			+	+
10	+ Candida parapsilosis		Rhodotorula Candida parapsilosis	+
11	+			+
12				+
13				+

+ = Indicates Candida albicans

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TABLE XIV

AEROBIC MICROORGANISMS FOUND IN FECES

		Literature*	NASA Study	W. P. Study
Gram + Cocci	1. Coag. neg. staph	+	+	+
	2. Coag. pos. staph	+	+	+
	3. Str. mitis	+	+	+
	4. Str. salivarius	+	+	+
	5. Enterococci	+	+	+
	6. Str. pyogenes (BCFG)	+	**	+
Gram + bacilli	1. Lactobacilli	+	+	+
	2. Corynbacteria	+	+	+
	3. Mycobacteria	+	**	
	4. Act. bifidus	+		+
Gram - bacilli	1. Undiff. coliformes	+	+	+
	2. E. coli	+	+	+
	3. E. coli "intermed"	+	+	+
	4. Klebsiella	+	+	+
	5. Proteus sp.	+	+	+
	6. Pseudomonas aeruginosa	+	+	+
	7. Alcaligenes faecalis	+		+
	8. Vibrio alcaligines	+	+	+
	9. Serratia			+
	10. Mima polymorph	+		
	11. Aerobacter C and B			+
	12. Citrobacter			+
PPLO		+	+	+
Fungi	1. Candida albicans	+		+
	2. Other candidas	+		+
	3. Torulopsis glabrata	+		
	4. Pityrosporum ovale	+		
	5. Rhodotorula			+

* Microorganisms Indigenous to Man, Theodor Rosebury, 1962, McGraw Hill Book Co., New York. (2)

** Not tested for

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TABLE XV *

DIFFERENCES IN APPROXIMATE NUMBERS OF AEROBIC AND ANAEROBIC BACTERIA

Expressed as Serial Dilutions (10^{-})

Subject No.		Sample Number 1	2	3	4	Average Difference
1	A AN	7 13	8 11	8 11	8 12	4
2	A AN	7 12	8 12	8 13	7 12	5
3	A AN	8 12	8 11	8 11	6 13	4
4	A AN	7 12	7 12	7 12	8 12	5
5	A AN	7 12	7 11	8 12	8 12	4
6	A AN	8 12	7 12	8 11	7 11	4
7	A AN	6 12	8 13	8 13	- -	5
8	A AN	8 13	8 13	8 13	8 12	5
9	A AN	7 13	7 13	7 13	8 11	5
10	A AN	7 12	7 12	8 11	8 11	4
Overall Average						4.5

A = Aerobic
AN = Anaerobic
* Results obtained under NASA Contract NASw-738.

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TABLE XVI *

SCREEN TESTS FOR PREDOMINATING ANAEROBIC FECAL BACTERIA

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

* Results obtained under NASA Contract NASw-738

** Acid reduced curd

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TABLE XVI* (cont'd)

SCREEN TESTS FOR PREDOMINATING ANAEROBIC FECAL BACTERIA

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

* Results obtained under NASA Contract NASw-738

** Acid reduced curd

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TABLE XVI* (cont'd)

SCREEN TESTS FOR PREDOMINATING ANAEROBIC FECAL BACTERIA

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

* Results obtained under NASA Contract NASw-738
** Acid reduced curd

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TABLE XVI* (cont'd)

SCREEN TESTS FOR PREDOMINATING ANAEROBIC FECAL BACTERIA

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 lique-faction	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 lique-faction	6.7
FA-14	gram negative rods long slender with gram positive areas	tiny colo= nies 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 lique-faction	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 lique-faction grey sediment	6.7

* Results obtained under NASA Contract NASw-738
** Acid reduced curd
*** Reduced

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TABLE XVI* (cont'd)

SCREEN TESTS FOR PREDOMINATING ANAEROBIC FECAL BACTERIA

Culture Designation	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 li-que-faction	6.8
FA-17	large gram posi-tive rod singly and in pairs form-ing palisades and V's	fine colonies very anaerobic slight gas, occasionally	slight with finely gran-ular sedi-ment and side growth	clear with finely granular sedi-ment	clear with finely granular sedi-ment	clear with finely granular sedi-ment	clear with finely granular sedi-ment	clear with finely granu-lar sedi-ment	Arc with proteolysis	no li-que-faction side growth	6.6

* Results obtained under NASA Contract NASw-738
** Acid reduced curd

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TABLE XVI* (cont'd)

SCREEN TESTS FOR PREDOMINATING FACULTATIVE ANAEROBIC FECAL BACTERIA

Culture Designation	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

* Results obtained under NASA Contract NASw-738

** Acid reduced curd

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TABLE XVII

SUMMARY OF RESULTS FROM ALL SUBJECTS

BY SAMPLE PERIOD**

Type Cult. No.	Sample No.					
	1	2	3	4	5*	Total
FA1	1	2	1			4
FA2	1	2	2	1	6	12
FA3	5	3	6	7	3	24
FA4	1	1		1	1	4
FA5	11	3	3		3	20
FA6	2	4	2		1	9
FA7	1			1		2
FA8			2	7	3	12
FA9		1		1		2
FA10	1	5	3	3		12
FA11		1	2	4		7
FA12	3	5	6	2		16
FA13	2					2
FA14	2	4	1	3	5	15
FA15	3	4	7	5	5	24
FA16	4	1		3		8
FA17	1	1	2	6	1	11
Unknown	9	11	23	7	8	58
FN1		3				3
FN2		1				1
FN3	1					1
FN4		1				1
FN5						

* Results of screening not complete
** Results obtained under NASA Contract NASw-738
FA= fecal obligate anaerobes
FN= fecal facultative anaerobes
Obligate Anaerobes 97.5%

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TABLE XVIII

AEROBIC PLATE COUNT

(Aerobic Counts from the 10^{-5} Dilution of Anaerobic Series)

Sample No.	Subject Number				
	I	II	III	IV	Average
1	47	12	4	68	33
2	38d	9	1	50	32
3	13d	7	4	53	19
4	200	12	3	11	55
5	46d	45	22	10d	30
6	10	8	11	5d	8-1/2
7	25d	12	10	20	17
8	46d	3	11d	46	26
9	97d	6	4	38d	36
10	150d	6	126	11	73
11	91	8	3	10	28
12	4	5	4	10	5-1/2
13	20	6	16	9	12

d = Indicates diarrhea

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TABLE XIX

ANAEROBIC DILUTION SERIES
GROWTH LEVEL
(10^-)

Sample No.	Subject Number			
	I	II	III	IV
1	9	9	8	9 1/2
2	9	9	10	10
3	9	9	9	9
4	9 1/2	10	9	8
5	8	8	10	8
6	8	9 1/2	10	10
7	8 1/2	9	10	9
8	9	10	10	10
9	10	8 1/2	9 1/2	10
10	10	10	10	10
11	10	10	10	9
12	10	10	10	10
13	10	10	10	10

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TABLE XX

OCCURRENCE OF STRICT ANAEROBES IN SUBJECTS

Sample No.		Subject Number I Anaer.	I Facul.	II Anaer.	II Facul.	III Anaer.	III Facul.	IV Anaer.	IV Facul.
1	A	2		3		3		3	
	B	3		3		3		3	
	P	5		4		4		2	
2	A	3		3		3		2	1
	B	3		3		3		3	
	P	6		7		4		1	
3	A	3		3		3		3	
	B	3		3		2	1	3	
	P	5		3		7		7	
4	A	2		1	2	2	1		
	B	3		1	2	1	2	3	
	P	1		5		5	1	6	
5	A	2		3		1		3	
	B	1		3		2		3	
	P	3		9				1	
6	A	3		3		3		3	
	B	3		2		3		3	
	P	5	1	13		12		6	
7	A	3		3		3		3	
	B	3		3		3		3	
	P	2		5		4		2	
8	A	3		3		3		3	
	B	3		3		3		3	
	P	4		4		1		7	
9	A	3				3		2	
	B	3				1		2	
	P	8		2		4		7	
10	A	3		3		2		1	1
	B	3		2		3		1	
	P	9	3					9	
11	A	1		3		3		3	
	B	2		3		3		3	
	P	8		4				2	
12	A	3		3		3		3	
	B	3		3		3		3	
	P	4		3		1		4	
13	A	3		3		3		3	
	B	3		3		3		3	
	P	4		6		4		3	

A, B = dilution series; P - anaerobic plate
97% of cultures strict anaerobes; 3% facultative anaerobes
Blank spaces due to aerobic contaminants (spore-bearing)
Numbers refer to different types of anaerobes isolated

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TABLE XXI

KEYABLE CULTURES WITH NASA KEY

Dilution Series		Subject I	Subject II
1	A	FA-8	FA-12, -,
	B	-	FA-3, -, FA-17, FA-17
	P	FA-8, FA-8, FA-5	FA17, FA17, FA5, FA15, CT2, FA1
2	A	FA-6	-, FA-15, FA-15, FA-5
	B	FA-15, FA-17	-,
	P	-, -, -, FA-5	FA6, -, -, -, FA15
3	A	FA-16	-,
	B	-, -	FA-15, -, -, -, -
	P		
4	A		FA-7, -, -,
	B	-, -	FA-8
	P		FA-1, -, -,
5	A	FA-3	-
	B	FA-17, FA-6	-
	P	-, FA-17, -, -	-, FA-15, -, -, FA-15
6	A	-	-, -, -,
	B	-, -,	
	P	FA1-, -,	FA-3, FA-3, FA-8, FA-8
7	A	-, FA-11	FA-19, FA-15
	B	-, -,	FA-1
	P	FA-11, -, -,	
8	A	-, -	-
	B	FA-5	FA-5, FA-5
	P	FA-17	FA-2
9	A	-,	-, -, -, -,
	B	FA-5	
	P	-,	-
10	A	FA-13	
	B	-, FA-13	-
	P	-, FA-5	-, -, FA5, -
11	A		-, -
	B	-, FA-13	-,
	P	FA-5	-
12	A	FA-15	FA-15
	B	FA-6, FA-2	-, FA3
	P		-, FA-3
13	A		FA-5
	B	-	
	P	FA-1. FA-5, -, -,	FA-15, FA-15, -, -,

Dash indicates a culture was screen tested but unidentified

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TABLE XXI (cont'd)

KEYABLE CULTURES WITH NASA KEY

Dilution Series		Subject III	Subject IV
1	A	FA-9, FA-9	
	B	FA-12	FA-2
	P	FA-15, -, FA-2, FA-8, -	FA-2
2	A		-
	B	FA-9, -, -, -,	FA-3, -
	P		
3	A	FA-12, FA-14	
	B	FA-10, FA-15	FN-, FA8, FA1 (3x), -, FA3
	P	Lacto, -, -, -, FA-9	
4	A	-, FA-1 -	FA-2
	B	-, -, FA-5	FA-1, FA-15
	P	-, -, -, -	-, -,
5	A		
	B	-, FN-1	-, -,
	P		-, -,
6	A	FA-5	FA-15, -, FA-5
	B	FA-3	-, -, -,
	P	-, -, FA5, FA2, FA5	FA-15, -, -,
7	A	FA-13	-,
	B	-, FA-5	-, FA-15
	P		-,
8	A	FA-3, -,	-, -,
	B	-	
	P		FA-1, FA-1, -, -,
9	A	-	-, -, -, -, -,
	B	-, FA-5, FA-5	
	P		FA-6
10	A	-, -, -, -, -,	
	B		
	P		FA-5, -
11	A	-, -, -, -,	
	B		
	P		-, -, -, -,
12	A		
	B	-	
	P	-, FA-5	-, FA-6, -, -,
13	A		
	B		
	P	-, FA-14, FA-5	-, -,

Dash indicates a culture was screen tested but unidentified

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TABLE XXII

COMPARISON OF CULTURES ISOLATED IN EACH PERIOD

Sample No.	NASA Key	NASA Key + G D Types	Difference	Black Gas Producers
1	77%	86%	11%	13%
2	50%	65%	15%	20%
3	56%	76%	20%	20%
4	33%	91%	58%*	24%
5	33%	62%	29%	24%
6	46%	78%	32%	39%
7	44%	89%	45%	33%
8	44%	76%	43%	18%
9	20%	70%	50%	38%
10	28%	72%	44%	22%
11	13%	75%	62%	31%
12	47%	88%	41%	47%
13	41%	82%	41%	18%

* Possible sampling period during which GD series became strongly entrenched, followed by a period of adjustment of flora

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TABLE XXIII

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

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TABLE XXIII (cont'd)

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

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 pleo-morphic	tiny colo-nies heavy gas very an-aerobic	moderate with granu-lar sedi-ment some-times dark	4+ with slime and gas 4+ with slime some-times dark	4+ with slime and gas 4+ with slime some-times dark	4+ with slime and gas 4+ with slime some-times dark	4+ with slime and gas 4+ with slime some-times dark	3+ with slime and gas 3+ with slime some-times dark	delayed Arc* with slight pro-teolysis	no li-que-faction	6.3 6.4
G.D. 5 and G.D. 5a	gram ± medium rods in short chains	small colo-nies very anaerobic	clear to moderate with balls of sedi-ment	4+ with granular sediment or slime 4+ with slime or granular sediment some-times black	4+ with granular sediment or slime 4+ with slime or granular sediment some-times black	4+ with granular sediment or slime 4+ with slime or granular sediment some-times black	4+ with granular sediment or slime 4+ with slime or granular sediment some-times black	2+ with granu-lar sedi-ment 3+ with slime or granu-lar sedi-ment some-times black	Arc with proteolysis	no li-que-faction	6.6**

* Acid reduced curd

** G.D. 5a pH 6.2 to 6.4

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TABLE XXIII (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. 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

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TABLE XXIV

KEYABLE CULTURES WITH G. D.* TYPES

Sample No.		Subject Number I	II	III	IV
1	A				
	B		GD3, GD1		GD3
	P				GD3
2	A				
	B	GD3			
	P	GD3 (2x), GD5	GD2, GD7		
3	A		GD4		
	B				
	P		GDl, 4	GD5	GD3
4	A		GD3	GD2, GD5(2x) GD5a	
	B			GD2	
	P	GD7, GD5	GD7	GD 5 (3x)	GD1 (2x)
5	A				
	B			GD6	
	P	GD7	GD2 (2x), GD3		
6	A		GD6, GD2		GD4
	B	GD5, GD4			GD4, GD6
	P				GDl, GD6
7	A				GD6
	B	GD1		GD2	GD2
	P	GD4, GD7		GD3	GD5
8	A		GD5a		GD4
	B			GD3	
	P	GD1			GD1
9	A	GD2?, GD2	GD1, GD5a		GD1
	B	GD5			
	P	GD4	GD1	GD7	GD1 (4x)
10	A			GD4	
	B		GD2		
	P	GD3	GD3?, GD3 (2x)		GD5, GD1
11	A			GD1 (2x)	
	B				
	P	GD3 (2x)		GD1 (2x)	GD5 (3x), GD5a
12	A				
	B			GD1	
	P	GD1	GD3?, GDl, GD2		GD4 (2x), GD5a
13	A				GD3, GD2, GD5
	B	GD2			
	P	GD3	GD4		GD5

*G. D. = Space Diet
(x) = Number of times isolated
? = Questionable

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TABLE XXV

FREQUENCY OF OCCURRENCE OF GD TYPES

Types	Subject Number				Total
	I	II	III	IV	
GD-1	3	4	1	6	14
GD-2	3	6	4	2	15
GD-3	6	7	1	2	16
GD-4	3	3	2	5	13
GD-5	3	0	5	3	11
GD-5a	0	1	1	1	3
GD-6	0	1	1	3	5
GD-7	3	2	1	0	6

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TABLE XXVI

NUMBER OF DIFFERENT TYPES OF KEYABLE CULTURES
AT EACH SAMPLING PERIOD

Sample No.	NASA Only	GD Only	NASA & GD
1	9	2	11
2	7	4	11
3	10	4	14
4	6	4	10
5	5	4	9
6	7	5	12
7	5	6	11
8	5	4	9
9	3	6	9
10	2	4	6
11	2	3	5
12	5	5	10
13	4	4	8

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TABLE XXVII

LACTIC ACID PRODUCERS

Number of Cultures Providing High and Low Amounts of Lactic Acid by Subject and Period

Sample Number	Subject Number							
	I		II		III		IV	
	High	Low	High	Low	High	Low	High	Low
1	1	3	1	0	0	3	0	2
2	1	0	1	0	0	1	0	0
3	0	0	0	0	0	1	0	1
4	0	0	0	2	1	0	0	1
5	0	0	0	0	0	0	0	0
6	0	0	0	2	3	1	1	0
7	2	0	0	0	1	0	0	0
8	1	0	2	1	0	0	0	0
9	1	0	0	0	2	0	0	0
10	1	0	1	0	0	0	1	0
11	1	0	0	0	0	0	0	0
12	0	1	0	0	1	0	0	0
13	1	0	1	0	1	0	0	0

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TABLE XXVIII

PERCENT FORMATION OF LACTIC ACID
ON THE BASIS OF AVAILABLE GLUCOSE

Culture	% Lactic Acid/Unit Glucose
GD 2	100
GD 6	72
GD 5	47
GD 3	42
GD 4	37
GD 1	28
GD 7	4

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SECTION IX

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13. ABSTRACT

The purpose of this study was the determination of the aerobic and anaerobic microflora of human feces and any effect, real or relative, of a space-type diet upon this flora. Fecal specimens from four young men confined in the experimental activity facility at the Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, were cultured both aerobically and anaerobically thirteen times during a 6-week period. During certain periods the men wore space suits. Two of the men were on an experimental space-type diet which was freeze-dehydrated. During this same time period the other two subjects were on a "control" diet which contained identical foods, fresh and canned, to duplicate as nearly as possible the dehydrated diet from a nutritional viewpoint. Midway in the experiment the diets of the subjects were switched. The procedures used emphasized the anaerobic isolation of the predominating microorganisms using Gall's specialized technique. The aerobes were isolated and identified by standard procedures. An attempt was made to roughly quantitate the flora by the use of triplicate aerobic dilution series and by the plating from each dilution into differential media. Although the obligately anaerobic character of the flora remained constant, a shift was found in the types of anaerobic organisms isolated. This change in the biochemically distinct flora occurred after a sufficiently lengthy period on the diet to suggest that the diet was a contributing factor. The aerobic flora differed from that cited in the literature by the frequent presence of Shigella and enteropathogenic types of E. coli.

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