VOLUME I

RESEARCH ON LONG TERM ISOLATION OF PRIMATES AND MICE

FINAL REPORT

PREPARED FOR

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
MANNED SPACECRAFT CENTER
Houston, Texas 77058

NASA CONTRACT NAS 9-9000

GENERAL ELECTRIC COMPANY
SPACE SYSTEMS ORGANIZATION
BIOSCIENCES OPERATION
VALLEY FORGE SPACE CENTER

VOLUME I OF IV

RESEARCH ON LONG TERM BIOLOGICAL ISOLATION OF PRIMATES AND MICE

MYRON H. BENGSON

T. D. LUCKEY

FOREWORD

This is the Final Report of a study conducted at the Gnotobiology Laboratory of the Biosciences Operation, Life Systems Section of the General Electric Space Systems Organization, Valley Forge Space Center, King of Prussia, Pennsylvania. The work was done for the National Aeronautics and Space Administration, Manned Spacecraft Center at Clearbrook, Texas, under Contract NAS 9-9000 during the period 7 January 1969 to 7 January 1970. The program was technically monitored by Dr. J. Ferguson of the NASA Lunar Receiving Laboratories.

The authors wish to acknowledge the valuable contributions of Mr. John Geating, Mr. Herbert Kaplan, Mr. Norbert Behringer, Mr. Richard Ruby, Miss Susan Tice, Miss Elaine Silver and Mr. R. Green, all of the General Electric Company, Dr. Terry Hand of Temple University and Dr.'s J. Smith, P. Rambaut and M. Smith of the Manned Spacecraft Center.

M. H. Bengson
Program Manager, Gnotobiology

T. D. Luckey
Professor of Biochemistry
University of Missouri

ABSTRACT

Two simultaneous programs were carried out to investigate some of the effects of long term bioconfinement. Part A used classic Rhesus monkeys (Macaca mulatta) and a commercial monkey diet. Part B used gnotobiotic mice and a comminuted Apollo diet.

In Part A, after six months of bio-isolation in a gnotobiotic isolator (sterile air, sterile food and sterile water), a shifting of the indigenous intestinal microflora of Macaca mulatta was observed. Escherichia coli, originally present at 10^7 microorganisms (per gram of feces) dropped in numbers (less than 10^3). Control animals, individually caged and housed in a "clean" environment, receiving the same sterile diet and sterile water, reflected the shift but at a much slower rate. Animals in the clean environment and receiving the same but non-sterilized diet (but sterile water) retained the microorganism in original numbers. Other changes in the majority of the animals kept in the isolators were a two log drop in aerobic microflora and an increase in the anaerobic count. Some microorganisms, originally present in numbers of less than 10^3/gram of sample, increased in the bioconfined animals to 10^7/gram of sample count. After five months, the indigenous Lactobacilli began to disappear in this same manner as the coliforms. In addition to monitoring intestinal tract microflora, weekly samplings were taken of the gingiva, groin, eye, and throat. Shifting of the indigenous population was followed by using a marker organisms. Associated physiological studies included hematology and serum proteins. Implications of these results, in the context of long term space flight are possible deleterious changes in the immunity mechanisms, potential pathogen runaway and loss of the benefits of a protective mixture of microflora.

In Part B, radiation sterilized, communited Apollo diet was fed to classic, germfree and gnotophoric weanling mice. The growth, food efficiency, general appearance, reproduction and autopsy data from classic mice reared in the open laboratory with either γ -radiation sterilized or non-treated diet was satisfactory. Classic mice reared in isolation with sterile diet, water and air showed denudation, poor reproduction and about 50% mortality in 50 days. The performance of germfree mice fed sterile Apollo diet was acceptable. When germfree mice were orally inoculated with Escherichia coli or contaminated with Staphylococcus epidermidis, the mortality was about 70%. The diflora of E. coli with either Candida albicans or Lactobacillus leichmannii did not prevent this while C. albicans in a diflora with S. epidermidis did prevent this high mortality. Monoflora mice with C. albicans had 45% mortality while few of the mice with L. leichmannii monoflora died: when both C. albicans and L. leichmannii were orally inoculated into weanling mice, 80% died in 50 days. Whether present in a mono or a diflora, C. albicans reduced the food efficiency of mice. C. albicans or Bacteroides monoflora provided protection from the alopecia observed in other groups. It is concluded that the nurture and well being of mice is profoundly affected by their intestinal microflora.

TABLE OF CONTENTS

TABLE OF CONTENTS

APPENDICES

LIST OF ILLUSTRATIONS

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF TABLES

I. INTRODUCTION - PART A

Bengt Gustafsson* (1968) has written, ".....the two most hazardous things an astronaut takes into his capsule on an extended flight.....are his brain and his intestinal flora... The big question here is, of course, could..... germfree characteristics and deficiency symptoms....occur in man in space, if the intestinal flora, for one reason or another, is changing or drifting in the astronaut."

There are an infinite variety of microorganism combinations, and it is easy to see that an infinite number of normal and imbalance situations would and do occur. Fortunately, experience has shown that imbalances are usually quickly corrected by the very nature of the environment and the microorganisms themselves.

Somehow, a microbial balance of a number of species is usually achieved in the normal individual under normal terrestrial conditions that enables the subject to remain healthy. When imbalance occurs, the potential for disease increases.

Luckey (1963, 1968), Bengson and Thomae (1965) and others have discussed the possibilities of microfloral simplification and potential hazards thereof to astronauts. However, much of the early work (pre 1960) that gave rise to the assumption that biological isolation per se is harmful has either been disproved or is strongly suspect. The attack on the bio-isolation discussions has centered upon the techniques, the knowledge, and the conclusions drawn, which are evidently based upon insufficient data. Personal examination of the available facts plus interrogation of personnel that were present during the course of some of the early work, though not necessarily directly involved, lead

*Bengt Gustafsson, Discussion Leader at the ONR-NASA Joint Interdisciplinary Conference "Human Ecology in Space Flight", held at Princeton, New Jersey (1965).

to the belief that the critics of the work are correct insofar as their critical claim is concerned: that valid and final conclusions simply could not be drawn from the data that were presented. Notwithstanding these valid criticisms, knowledge that all would not be biologically static and inferences that biological change could bring deleterious effects to the astronaut certainly makes a reasonable statement and presents a case for experiment. To this end, a General Electric program to assess the effects of bio-isolation was begun in 1964. Other parallel programs, both company- and government-funded, have been undertaken to determine the effects of deliberately distorting the intestinal microecology and balance (Bengson and Thomae, 1966; Thomae, Kaplan and Bengson, 1968; Bengson and Thomae, 1968). One program, using mice, was primarily concerned with bio-isolation per se and to a smaller extent with the effect of a modified diet on the isolated animals. Its purpose and nature were purposefully very limited. It was recognized early that only an extremely large experiment extending over several years could fully define the hazard, if any, and the necessary corrective nature of treatment if such a hazard exists. Future plans included the extension of each of the factors or problems developed during the exploratory work. The programs discussed herein were designed to learn some of the effects of bio-isolation with particular reference to its influence on the astronaut requirements for nutrition and microfloral stability.

II. BACKGROUND AND DISCUSSION - PART A

Among the major variables that determine the composition of the body microflora are diet, drugs, exposure to other living creatures, stress, micro-organisms in the atmosphere, food and surroundings, body cleanliness, and perhaps the physical state of the individual concerned. In normal life,

manipulation of these variables is possible only in the grossest context - yet enormous forward progress in the well being of the human race has been made, even with the crude controls now practiced. For example, food sterilization, sewage treatment, potable water treatments, drugs and general sanitation have been shown to be effective means for general controls.

The possibility of changing the indigenous bacterial flora for an extended period, the effect on the host of the altered ecological relationships, the techniques that would be involved in such a study and the possibility of showing compatibility requirements (in the sense of a similar body microflora) for normal humans or animals has had but few studies, although individual parts of the above have been reported in gnotobiotic investigations.

A general picture is, however, taking shape. Investigations on mice, primates, and miscellaneous other animals plus some space data on humans indicates that the changes in indigenous microflora are not host species specific. Animals are furnishing models that have credibility. They (animal studies) do not completely take the place of studies using the eventual subject, the human, but certainly furnish us guidelines to plan required testing on man.

Simplification, Bacterial Antagonism and Control of Microflora

Gordon and Pesti (1969) have performed a series of experiments using isolated and non-isolated, conventional mice, and ex-germfree but conventionalized mice. They followed the microfloral changes in their isolated mice and compared them with open laboratory controls.

All of their mice, at an early age, had the potential pathogens Clostridia and Staphylococci in very low numbers (less than 10^5). As the open air

conventionals aged, "the Clostridia and Staphylococci appeared in substantial numbers ($+10^7$). The isolated mice maintained the pathogens at levels comparable to the original numbers."

Their theory is that the open air mice were continually "reseeded" with pathogens from the open air and the isolated mice were spared this burden. All mice were fed sterile diet.

Several interpretations may be made of this experiment: Some directly from the paper's evidence and others from combining the knowledge gained by the work of Dubos (1967) and our previous General Electric experiments.

1. Pesti and Gordon are correct in that undoubtably reseeding encouraged the proliferation of Clostridia and Staphylococci in the open air colony.
2. Since all the mice were receiving a sterile diet, the multiplication of some bacteria was inhibited (most probably the Escherichia coli and Lactobacillus).
3. In the Pesti-Gordon experiment, the pathogens of the isolated mice were inhibited by the original starting flora successfully exerting the protective mix effect. A pathogen orally introduced earlier in life was successfully combatted.

Pesti and Grodon state, "the open conventional mice (controls) displayed characteristics of their own", but, "the isolator conventional and the ex-germfree conventional mice presented essentially similar results and could be combined into a single group.

Simplification of the isolated mice to the extent of disappearance of E. coli was never observed in the Pesti-Gordon work. This is in direct contradiction to the results of Dubos (1967). We suggest a possible explanation:

A balance was established in the Pesti-Gordon work where, with the particular microorganisms of the experiments and the diet fed, E. coli was never forced out. This could be the result of more than just the original microorganism mix, for example; available nutrients, number of animals in the isolator (Gordon, 1966) and perhaps the subtle but real differences between autochthonous and indigenous microflora and the particular strains present affected the course of the experiment.

Craven and Miniats (1969) have investigated the relationships of different strains of E. coli. Bacterial antagonism between strains has been documented with regard to wound healing and has been discussed by Bengson (1968) with respect to burns. Craven and his co-workers, using gnotobiotic pigs, have demonstrated that one strain of E. coli can quickly become dominant (over another strain) regardless of when the potentially dominant strain is introduced. The complex relationship possible in the Gordon-Pesti experiment may be the result of strain dominance whereby the dominant strain is kept in check by yet another microorganism. The single strain or combined strains of E. coli keep still other microorganisms, Clostridia and Staphylococci, in low numbers. We may have thus a case of E. coli of one strain disappearing or going to low numbers (simplifying) but the result hidden due to the presence of a dominant and aggressive "cousin" strain. The isolation of the animals in this instance, prevented any change in the balance occurring from outside influence.

While simplification in the context of complete disappearance was never observed by Pesti, there yet may have been losses of microflora originally present (but undetected) in very small numbers in the conventional animals. The difficulties of establishing a complete microbiological profile is one

of the important reasons for the use of the germfree animal. In that the total numbers of microorganisms is not normally subject to much change, then the rise in the pathogens had to be accompanied by other losses. The bio-confined animals' failure to increase the numbers of the pathogens may also possibly be explained by the work of Tanami (1967). This experiment is reviewed later. Van der Waaij, et al. (1969) has discussed the difficulties of transference of "strange" microorganisms into animals with a well established "normal" microflora. It is of interest that following "decontamination" of normal mice by orally administered antibiotics, in the treated (decontaminated) mice, transference was simple. "A take was seen in all mice even after very low doses with a few bacteria." Their work points out very strongly how dangerous antibiotic therapy would be in space, for should some pathogens escape antibiotic kill, replication to fill the vacuum created could be rapid. Without the protective mix and with exposure (from crew mates) to other microflora, an extremely hazardous situation is possible. It is fairly well established that innocuous or beneficial organisms are among the most susceptible to broad spectrum antibiotics.

Ducluzeau and Raibaud (1969) investigated some of the mechanisms involved in the establishment of various bacterial strains in the gastrointestinal tract. "In one case, interferences occurred because one of the strains synthesized a metabolite that was toxic for the other; for example, the inhibition of Staphylococcus pyogenes by E. coli. In another case, one of the strains established physico-chemical conditions in the digestive tract which were incompatible with the survival of another; for example, the inhibition of Micrococcus sp. by Staphylococcus pyrogenes."

Even different strains of the same species of microorganism were inhibited by each other! Ducluzeau and Raibaud also noted that several simple mechanisms may act simultaneously on the same strain and that the final level at which a strain is stabilized is the result of an integration mechanism.

All the above leads to the conclusion that the presence of some certain microorganisms at some level is critical to a healthy balance and that when this balance is upset, a new and different balance may be struck. If this new balance is not rapidly achieved or if this balance is not a healthy (for the host) balance, trouble in the form of disease seems to be the certain ending. The most common balance in man, monkeys, and mice seems to be dependent upon E. coli being present in relatively large numbers. To iterate, a potentially dangerous Micrococcus was inhibited by S. pyrogenes which in turn was inhibited by E. coli. The E. coli may allow just enough S. pyrogenes to exist to keep the Micrococcus at an "acceptable" level. Although all three of the cited organisms have been shown to be pathogenic, certainly the level at which these organisms exist in the body determines if recognizable damage is being done. The levels at which an overt pathogenic condition is usually considered to exist is sometimes quoted as 10^7 and above. This is, of course, subject to argument and certainly very low levels can be the basis for a toxic condition, recognized or not.

All of this tends to imply that the microbial balance may be very, very sensitive. The individual immunological defenses, therefore, must assume more and more importance. How the other body defenses, such as the phagocytes, acid-base balance, and even the nutritional habits of the individual are balanced may thus play a major part in prevention, development or limitation of a

disease. This is not new! It does lead one to question whether there is enough "overlap" among the body defenses? Can particular defenses be strengthened or reinforced? Where does the primary line of defense lay? Certainly among the controllable factors should be the microorganism balance. We simply need to know much more in this area. Simply stated one major problem appears to be; given that changes in microflora can cause disease and changes in microflora are inevitable under any circumstances. Can we lead or direct the flora to acceptable and desirable balances from given states of imbalance (or undesirable balance)? Basically, this is what the primate-mouse experiment is all about. We are now learning what, how fast, and why changes occur in our questioned environment -- long term space flight.

Another interesting question may be raised concerning the Pesti experiment. What is simplification? Is the failure of the two pathogens to proliferate to large numbers (in the isolated animals) simplification? Always to date we have considered simplification as loss. Here we have an instance wherein an identical, except for bio-isolation, situation, two organisms multiply in the unprotected situation and fail to multiply in the "protected" regime. Pesti and Gordon theorize "reseeding", this seems strange in that 10^4 are already known present. Intuitively one feels that the unprotected mice did not need "reseeding" to grow to 10^7+ contamination. Even if reseeding were true, how many microorganisms would it take to reseed (10^4, 10^5, 10^6)? How many organisms of this potentially pathogenic type are floating about in a normal laboratory. Certainly enough to contaminate, but then the animals in the isolator also were contaminated and they must have released organisms into their atmosphere and contaminated their surroundings. Why then, unless some very large number differential is involved, did not the isolated mice reseed themselves

and their cage mates. Abrams (1969) suggests the mucosal resistance to pathogens is directly affected by the (existing) flora. His experience indicates that the simple rapidity of gastro-intestinal emptying (most rapid in "normal" animals) is one defense line, but this apparently did not operate equally in the Pesti work. Returning to the original question, what is simplification? The work of Pesti and Gordon forces the conclusion that the definition of simplification in the bio-isolation context must also include the suppression of potential or expected growth of some microorganisms, in this case, the Clostridia and Staphylococci. Ducluzeau (1969) then with his theory of "integrated mechanisms" has given us a major movement forward in our search for the mechanisms and reasons for simplification.

Dietary Influences

Disappearance of some strains of intestinal microflora, while nominally due to extended confinement may also be due to dietary changes, either alone or speeded up by the confinement. Winitz, et al. (1966) and others have shown the composition of the indigenous microflora to be highly diet dependent. Winitz reported almost total disappearance of microorganisms from subjects utilizing a synthetic diet, but a general return (with some species missing) following a return to normal diet. Schwartz Bio-Research (1967) has written in their newsletter that use of their synthetic diet has an effect on the composition (genera and species) of the microflora. The question is raised: Is this dietary change effect equally effective in reducing or changing the autochthonous as well as the indigenous microflora? Dealing only with the general gut population and assuming that a given dietary regime has influenced the composition, then if a species disappears for this (or any other reason) then under conditions of bio-isolation, it cannot return. This would be true

even if a return to normal eating habits and diet occurred. In fact, a return to normal habits would constitute a second change and any new (for crew mates in the aerospace context) microorganisms and some of those that may have become numerically predominant, due to the first change, may have their existence jeopardized. The second "simplification" may be the dangerous one. Initial reports from Schwartz indicated that no deleterious effects were caused by the microorganism changes. What was not reported is whether any pathogens or potential pathogens were ever present (or available).

Assuming that microorganisms within the body have useful functions, including nutrient synthesis, immunity build-up, and protective mixtures as discussed previously concerning pathogen build-up, then the other questions arise: (a) have we lost a microorganism that performs or allows to be performed a critical function; and (b) have we allowed a pathogen to multiply into the vacuum. We assume there is now little question that a pathogen could multiply in a biological vacuum.

In healthy breast-fed children, bifido bacteria are the main bacterial component (Mata, Carrillo and Villatoro, 1969). It has been suggested that a high incidence of bifido bacteria, 10^{11} to 10^{12}/gm of wet feces, is responsible for the low incidence of pathogenic microflora such as Shigella (Mata, L. J., J. J. Urrutia, B. Garcia, R. Fernandez, and M. Behar, 1969). These pathogens have been shown to infect the gut even though the bifido bacteria count was high, but then significantly, rarely cause disease. In contrast, as the bifido count drops, due to change of diet from milk to "normal" adult food, the incidence of diarrheal disease rises with the change in microflora. These conclusions have been suggested by several investigators (Beck, et al., 1957).

As children age, heavy exposure to pathogens and the dietary changes combine to remove the early life protective mix, and the data of Mata tends to confirm Pesti's work. Where Mata's diet changed, the total influence was enough to cause illness.

Wagner and Starr (1968) attempting to manipulate the microorganism population of mono-gnotophoric mice (Lactobacillus bifidus) by means of diet fed them successively: commercial diets, high carbohydrates, high protein and high fat diets.

They established that the organisms infecting mono-gnotophores established in characteristic numbers, i.e., for Lactobacillus, 10^7 to 10^8 per gram of dry feces. Their dietary results were inconclusive for mono-gnotophores. When the same experiments were done using two species of bacteria, (Salmonella typhimurium and L. bifidus) the Lactobacillus was inhibited within a few days regardless of the original relative numbers.

Another strain of Lactobacillus (Lactobacillus brevis) was successfully established in high numbers in a S. typhimurium infected gnotobiote. In this case, a high carbohydrate diet appeared to have some effect on suppressing the S. typhimurium. The experiment was not run long enough to be conclusive.

The work of these two teams of investigators indicate suppression of pathogens by Lactobacillus control (by diet) should be extended.

Gall (1964) describing her findings concerning the microflora of human feces of young men undergoing semi-bioconfinement for thirty days reported a slight simplification of the bacteria flora. The diet composition was changed midway in the test period. The diet was not a rigidly-sterilized diet but was presented to represent as nearly a normal meal as possible. She reported that using their particular diet and conditions, the predominating flora shifted. The subjects had preferred a rather high milk diet prior to

confinement. Expectedly, the incidence of lactic acid producing bacteria was high. By the 12th day under a new dietary regime, the flora shifted to predominantly a gas and black slime producing bacteria. This happened both when the diet was shifted from fresh to dehydrated foods and vice versa. The new dominant forms were certainly not desirable in space flight for an increase in flatulence is to be avoided whereever possible.

Conventionalization

One problem that has worried investigators considering the possible loss of protective microorganisms during extended bioconfinement has been what happens when the animal (astronaut) is returned to normal conditions. Luckey (1966), Wilkins (1967), and others have publicly speculated on this topic. Thomae, Kaplan, and Bengson (1967) have conducted an experiment using a commercial Lactobacillus preparation on microorganism depleted primates in an attempt to develop an indigenous population of Lactobacillus in the subject animal. As an inducement to the Lactobacillus to establish, a high lactose dairy preparation was added to the diet. This failed to induce other than a transient population, most probably because the strains of Lactobacillus used were not monkey derived. A proven non-pathogenic strain of Lactobacillus, primate subject species derived, must be used for a proper repetition of this experiment.

Malyoth and Sickel (1969) have successfully introduced L. bifidus into gnotobiotic pigs. The strain successfully negated the pathogenic effects of B-hemolyzing E. coli serotype 0:26. During later conventionalization, the protective L. bifidus seemed to be eliminated (simplification during conventionalization?). The authors report, however, that "supplementary feedings of Bifido-bacteria in milk culture had a positive influence on the health and

and general condition of the piglets". It may be that during conventionalization, a strain of bacteria was introduced or developed that prevented the Bifidus from ever becoming a normal resident of the pig and that the constant reseeding of Bifidus (and the lactose diet) is necessary to maintain even a transient (but useful) population. Moyer and Lewis (1964) have shown that some microorganisms thought to be indigenous, were truly transients and maintained a population only under certain circumstances such as constant reseeding because the subject had a companion in which the transient was truly indigenous.

When considering the use of bacteria in the diet for astronauts, then it would appear a wise course to determine a protective strain of Lactobacillus that could be induced into the astronaut, perhaps even specific strains for each astronaut, and to have a lypholyzed supply of this microorganism stored ready for use when the astronaut(s) need it. This would be a long project, perhaps continuing throughout astronaut training. This concept must be carefully correlated with the diet to assure that the diet fed does not nullify the reseeding. When simplification occurs on a long flight, conventionalization should be accomplished before return unless an extended period of isolation on earth is planned. Lactobacillus may be only one of a number of microorganisms needed to assure the astronauts' microbial balance is a truly protective mix when he returns. The previously mentioned work of Mata and his colleagues is strong support for the concept.

Van der Waaij (1969) demonstrated that reinfection (not conventionalization) of decontaminated (by antibiotics) animals is very rapid. The organisms previously eliminated (presumably pathogens) rise rapidly to high numbers. This happens in contrast to difficulties experienced when trying to infect

normal or germfree animals with specific microorganisms. If re-infection should be by only one or two species, compatible with each other and pathogenic to the host, the consequences are obvious.

Hopefully, this discussion has brought a number of problems and questions to light. These questions should be answered before long-term space journeys are attempted. The primate experiment was conducted to whether or not microorganisms of the protective mix are lost during bioconfinement. The effects of the change may not be felt for a considerable period after the change in gut flora has occurred. The why the change, and measures to correct or control it are natural outgrowths of the present program. Requirements for frequent analyses of the flora of long term space travelers is indicated. When considering space stations, a bacteriological laboratory is a must.

Other Implications - Return From Flight

Studies on conventionalization are not numerous. Successful methods have included direct insertion of "normal" gut contents into the subject, gradual exposure to selected microorganisms and several others. Generally, agreement is found that sudden exposure of a naive mammal is frequently lethal. Previously mentioned was the work of Van der Waaij (1969) where the "take" on decontaminated animals was extremely rapid. Assuming this to be true for humans, then one requirement would be to proceed with conventionalization or in the space flight context "normalization" very carefully and slowly using carefully selected microorganisms, probably isolated from the individual before launch. This could be done toward latter stages of the flight; if it were done following return, then the complete isolation procedure must be followed, but in this case, all supplies including the atmosphere introduced to the returnee must be sterile. The burden would be enormous. Time to release the crewmen to normal living is at this stage only an educated guess.

The Soviets in their year long manned test are cognizant of this problem. Adamovich (1969) said, "Appreciable changes were found in the intestinal microflora of test subjects. The nature of the changes indicating significant simplification of the microflora made it necessary to develop specific procedures for removing the test subjects from the cabin and take certain preventive measures after the accomplishment of the experiment."

III. THE EXPERIMENT - PART A

Health and Treatment of Monkeys

Ten post-puberty, male Rhesus monkeys (Macaca mulatta) were received at the General Electric Valley Forge Gnotobiology Laboratories on 25 March 1969. All animals weighed between 6 and 8 pounds at the time of delivery. Cage mates were randomly selected and then similarly distributed into five standard primate cages. The experimental plan was to select eight at random from the ten animals purchased.

During the five week quarantine period at the vendor's facilities*, Thiabenazole at 100 mg/Kg was administered twice as an anti-parasite control; all monkeys were also TB tested and found negative. However, bacteriological testing, particularily with respect to Salmonella and Shigella organisms, was not attempted. An earlier delivery date was requested by us of the vendor's laboratory in order to speed up the initiation of the isolation phase. This halved the intended eight week vendor quarantine period precluding bacterial investigation by the vendor.

All monkeys were immediately placed on standard Rockland Primate Diet, supplemented with slices of fresh oranges and bananas to help overcome the normal trauma resulting from the delivery and change of quarters. Fresh tap

*Primate Imports Corporation, 34 Munson Street, Port Washington, Long Island, New York 11050

water was given ad libitum, via mechanical sipping straws. The quantity of food consumed per monkey per day averaged 115 grams. This represents an approximation since it was not possible to completely control food distribution to each of the cage mates.

Two days after delivery, Monkey Number 516 was found to be in obvious distress, with a portion of the lower colon protruding from the anal sphincter. Food consumption, even of fruit, ceased. Palliative measures were undertaken on the advice of Dr. Ramsey Buchanan, veterinary consultant of the Gnotobiology Laboratories.

The animal continued to show signs of extreme distress through the next few days; the appetite remained poor and the stools were thin and watery. It was decided that the monkey represented a serious health hazard to the remainder of the colony and to the laboratory personnel. Since no safe and convenient means of isolation was possible within the animal facilities, and with the concurrence of Dr. Buchanan, the monkey was tranquilized and death induced with a large intravenous injection of sodium pentathol.

To secure maximum commonality of the microflora, redistribution of cage mates was instituted with the use of a table of random numbers. New cage mates would not be tolerated among the monkey colony and further attempts at randomization were abandoned. Wounds from bites and scratches were treated with a hydrogen peroxide solution.

Eight days after arrival, Monkey Number 512 refused food and water, exhibited thin, watery stools, and became increasingly listless. A feces culture taken for bacteriological examinations showed the presence of Shigella organisms. The ailing animal was isolated. Fresh orange slices were offered to the monkey to stimulate his appetite, but to no avail. To overcome the

obvious dehydration and lack of nutriment, 10 to 15 ml doses of a 10% dextrose solution in normal saline were orally administered via a sterile, plastic catheter inserted beyond the epiglostis of the tranquilized monkey. Ten days after onset of the symptoms described above, the monkey expired, apparently from the combined effects of dehydration and malnutrition induced by the Shigella infection.

The remaining monkeys appeared to be in good health, eating and drinking satisfactorily. No evidence of additional infections with Shigella were noted in the bacteriological sampling of the remaining eight animals. Antibiotic therapy was not instituted.

Monkey Isolators*

New isolator covers were designed. Their construction reflects improvements suggested by our previous experience with monkey isolation (Bengson and Thomae, 1968). These improvements are outlined below:

- Four sets of glove ports, instead of two, placed at staggered heights to permit every portion of the isolator to be reached from the outside for more efficient cleaning and to allow more freedom of operation during sampling.
- A parallel double zipper system, the inner one of PVC which acted as a protective barrier during sterilization with peracetic acid and an outer one of metal-nylon to provide strength, both set at waist height for convenience.
- A series of plastic straps to provide support during opening and closing of the double zipper system and to prevent premature weakening of the zippers, particularily the inner one.

*The basic isolators used have been described in publication AMRL TR-67-177, Aerospace Medical Research Laboratories, AMD-AFSC, Wright-Patterson AFB, Ohio, dated May, 1968.

- o A set of three drain plugs at the bottom of each isolator to allow removal of waste and debris during closed-bag cleaning operations and to permit outside maintenance of the urine drain bottle without opening the cover.

Figure 1 shows the isolator system used. Figure 2 shows the cover design.

Bacteriological Testing

Bacteriological sampling of the monkeys was begun the week following their arrival. These initial tests were designed to concentrate not only on the quantitative aspect of the microflora from five selected sites (feces, gingiva, throat, eye, groin) but also on the identification of each species isolated. Each bacterial identification, along with its relative number and distribution among the primate colony, was considered in the selection of "marker" organisms with which to monitor the effect of physical isolation from random bacterial challenge. The specific details of how and where to sample each monkey and the diagnostic bacteriological procedures used are detailed in the Standing Operating Procedures (SOP) Appendix J. Table I shows the initial total quantitative organism count per site in number/gram dry weight of feces or number/swab sample. Anaerobic counts include facultative as well as obligatory anaerobes.

The figures shown in Table II represent initial hematology data on the animals; the figures are compared with the normal as reported by Milville, Whitcomb, and Martinez (1967) on post-puberty, immature male Macaca mulatta monkeys. Our data obtained is complexed by the fact that the animals were still in the process of settling down from the trip from the vendor and adapting to their new environment. Appendix B gives the data on each of the animals

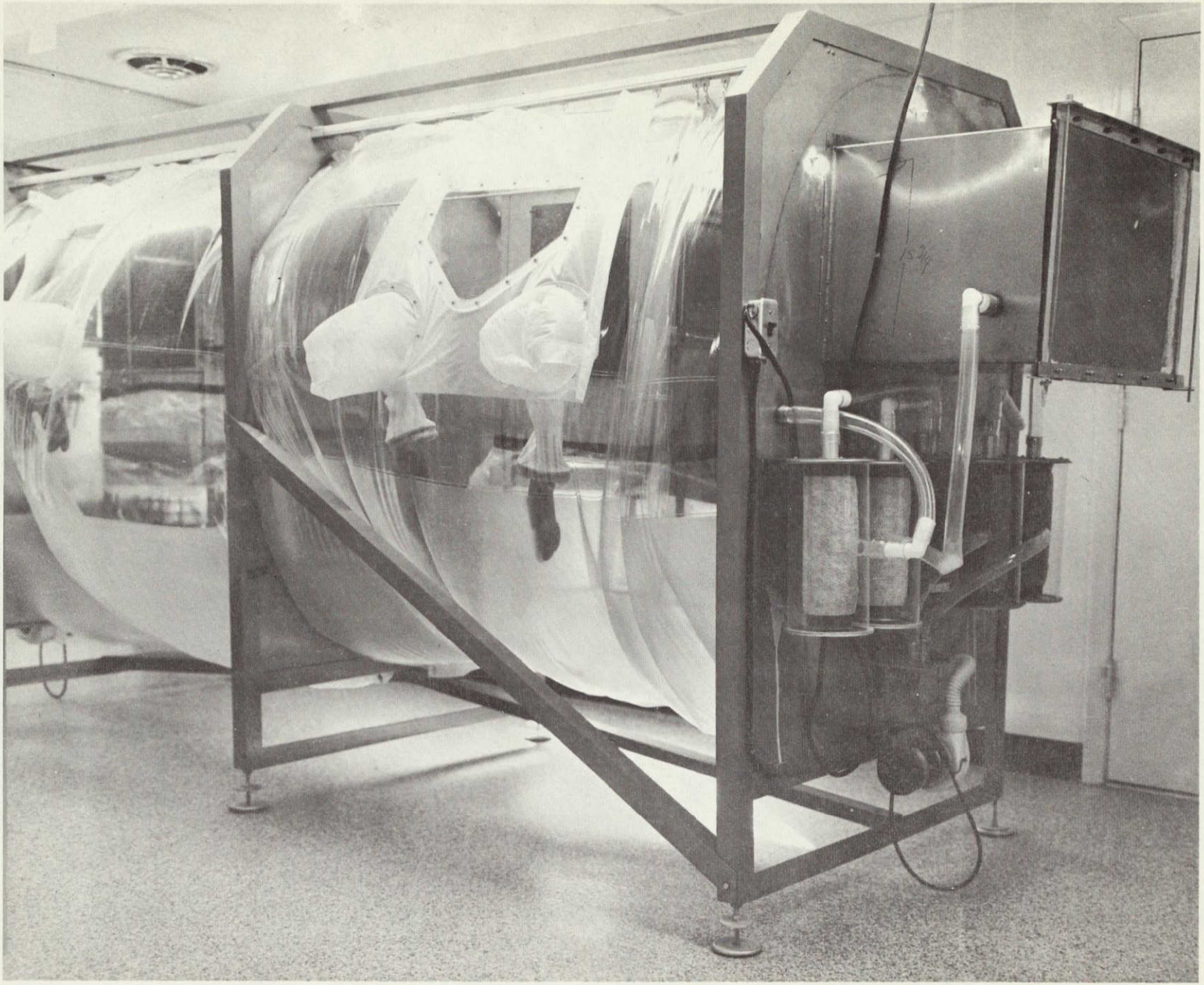


FIGURE 1. ISOLATOR SYSTEM USED ON PRIMATE EXPERIMENT

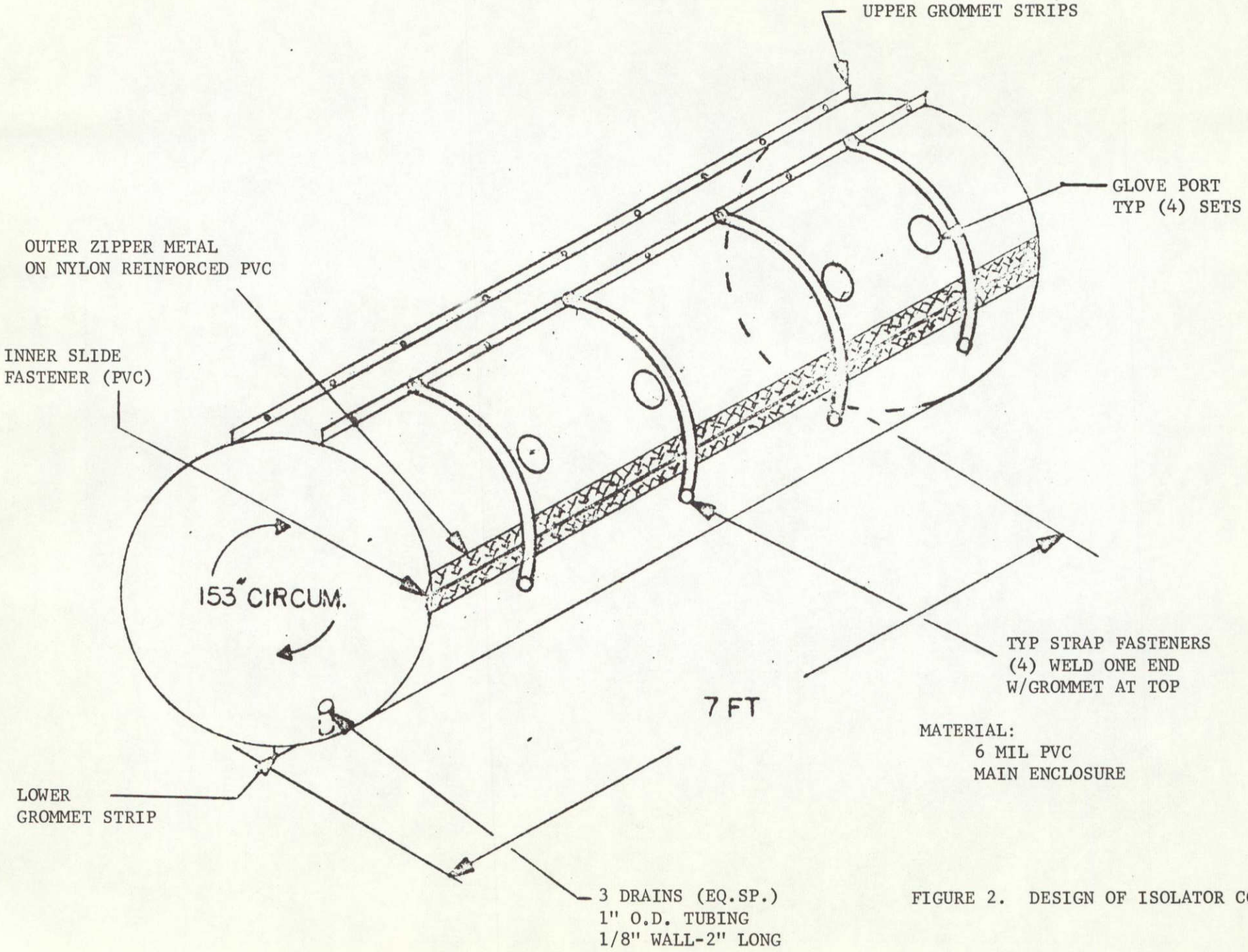


FIGURE 2. DESIGN OF ISOLATOR COVERS

TABLE I. SUMMARY OF INITIAL BACTERIOLOGICAL ASSAY OF MONKEYS - AEROBIC AND ANAEROBIC QUANTITATION

CODE	MONKEY NUMBER	FISCAL WEEK	TOTAL AEROBIC COUNT[1] (48 HOUR INCUBATION @ 35°C) SITES					TOTAL ANAEROBIC COUNT[1] (48 HOUR INCUB. @ 35°C) SITES				
			FECES	GINGIVA	THROAT	EYE	GROIN	FECES	GINGIVA	THROAT	EYE	GROIN
1	512[2]	14	2.2×10^{10}	1.4×10^{6}	8.3×10^{5}	7×10^{2}	9.4×10^{3}	1.6×10^{10}	8.3×10^{6}	8.7×10^{5}	1×10^{3}	9×10^{3}
		16	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2	513	14	1.1×10^{10}	1.3×10^{6}	7.2×10^{5}	5×10^{2}	7.6×10^{3}	3.0×10^{10}	1.6×10^{6}	8.4×10^{5}	2×10^{3}	6.2×10^{3}
		16	2.5×10^{10}	N.D.	N.D.	N.D.	N.D.	6×10^{10}	N.D.	N.D.	N.D.	N.D.
3	514	14	4×10^{10}	6.3×10^{6}	3.8×10^{5}	3.2×10^{3}	3.4×10^{4}	1.2×10^{11}	2.1×10^{7}	1.1×10^{6}	4×10^{3}	4.4×10^{4}
		16	4.9×10^{10}	N.D.	N.D.	N.D.	N.D.	1.4×10^{11}	N.D.	N.D.	N.D.	N.D.
4	518	14	3.1×10^{10}	4.8×10^{6}	2.6×10^{5}	1.5×10^{4}	4.5×10^{4}	5.0×10^{10}	7.1×10^{6}	3.7×10^{5}	4.2×10^{4}	6.7×10^{4}
		16	4.2×10^{10}	N.D.	N.D.	N.D.	N.D.	7.9×10^{10}	N.D.	N.D.	N.D.	N.D.
5	511	14	5.6×10^{10}	3.9×10^{5}	1.3×10^{6}	1.5×10^{4}	1.1×10^{5}	6.6×10^{10}	6.7×10^{5}	1.4×10^{6}	1.3×10^{4}	7.4×10^{4}
		16	1.4×10^{10}	N.D.	N.D.	N.D.	N.D.	2.2×10^{10}	N.D.	N.D.	N.D.	N.D.
6	515	16	2.3×10^{11}	6.1×10^{6}	4.1×10^{6}	2.7×10^{3}	1.1×10^{5}	3.5×10^{11}	9.1×10^{6}	4.1×10^{6}	4.2×10^{3}	1.3×10^{5}
7	517	16	1.0×10^{11}	1.0×10^{6}	8.6×10^{5}	4.7×10^{3}	3.3×10^{5}	3.1×10^{11}	7.6×10^{6}	3.6×10^{6}	8.5×10^{3}	9.6×10^{5}
8	519	16	3.0×10^{11}	3.1×10^{6}	2.5×10^{6}	3×10^{2}	1.5×10^{3}	3.4×10^{11}	1.2×10^{7}	8.0×10^{6}	1×10^{3}	1.7×10^{3}
9	520	16	1.3×10^{11}	1.5×10^{7}	5.2×10^{6}	3×10^{2}	4.6×10^{3}	3.1×10^{11}	9×10^{7}	7×10^{6}	1×10^{3}	5.3×10^{3}

[1] Counts
Feces - Organisms/Gram Dry Weight
Other Sites - Organisms/Swab Sample

[2] Monkey No. 512 Died F.W. 16

N.D. = Not Done

MONKEY NO.	CLASS	MEASUREMENTS				DIFFERENTIAL COUNT					RBC INDICES		
		HGB gm/100 ml	PVC-%	RBC $x10^6/mm^3$	WBC $x10^3/mm^3$	% TOTAL NEUTROS.	% LYMPHS.	% MONOS.	% EOS.	% BASOS.	μ^3 MCV	µµgm MCH	gm/100 MCHC
2	C	10.3	31.7	6.2	15.4	32	55	7	6	0	51	17	32
		10.6	31.0	6.9	12.8	33	55	4	8	0	45	15	34
3	I	11.3	33.5	6.1	6.1	20	64	6	10	0	55	19	34
		12.3	34.9	6.45	8.0	39	54	2	4	1	54	19	35
		12.3	33.4	5.3	7.4	39	46	6	7	2	63	23	36
4	I	12.8	38.2	6.05	6.2	41	40	2	15	2	63	21	34
		13.6	39.9	6.23	8.6	49	42	2	6	1	64	22	34
		13.1	39.2	6.75	3.5	31	49	5	12	3	58	19	33
5	I	12.8	34.4	5.8	6.1	38	45	3	12	2	57	22	37
		11.8	34.0	5.4	6.6	25	62	6	6	1	62	22	34
		12.9	36.8	6.5	11.0	21	64	8	6	1	57	20	35
		12.6	35.0	5.95	7.6	2	76	3	18	1	58	21	35
6	C	11.4	35.1	5.3	9.6	20	50	3	27	0	66	22	32
		11.5	35.9	6.3	6.7	21	50	5	23	1	57	18	32
		12.2	36.3	5.9	7.2	31	45	2	22	0	62	21	33
		12.1	35.1	5.9	8.0	17	57	5	19	2	59	21	34
7	I	11.6	35.7	6.75	8.3	32	57	5	5	1	53	17	32
		10.7	31.8	5.57	8.1	38	53	8	0	1	57	19	33
		10.6	32.0	5.4	7.9	29	64	5	2	0	59	19	33
		10.9	32.2	5.75	8.7	20	67	6	4	3	56	19	33
		10.6	30.0	5.35	10.7	14	81	1	2	2	56	19	35
8	C	9.8	29.9	5.1	12.0	27	60	9	4	0	58	19	33
		11.9	35.6	5.1	7.4	22	67	4	5	2	69	23	33
9	C	11.3	31.2	5.25	4.6	18	74	6	2	0	59	22	36
		12.6	35.3	6.25	6.5	13	73	5	9	0	56	20	36
NORMAL VALUES		11-12.5	39-43	5-6	7-13	20-56	40-76	0.5-2.0	1-3	0-2	65-78	18-23	27-31

I = ISOLATOR MONKEY
C = CONTROL MONKEY

TABLE II. HEMATOLOGY DATA - INITIAL MEASUREMENTS

through the experiment and can be compared to Table II. Individual differences are apparent but do not seem significant.

Experimental Conditions

During the last weeks of gentling, the animals were switched to an autoclaved diet. This was done to accustom them to the taste change which occurs upon sterilization and to verify that the diet planned for use during the isolation period was nutritionally adequate. Since the vitamin loss during autoclave sterilization is relatively severe, supplementary vitamins were fed via the water supply. The water was also autoclave sterilized prior to supplement action. Table VIII gives the nutritional content of the diet; Table IX the contents of the vitamin supplement; and Table X the amount of vitamins given contrasted to the minimum daily requirement of each vitamin required by humans. The vitamins were filter sterilized by millipore filter techniques. The filtering was done within a sterile flexible Trexler type isolator. The glass outer (vitamin) container was sterilized when the isolator was sterilized with peracetic acid. During this pre-test period, the animals were kept in open grill cages, exposed to normal atmosphere, with the usual temperature (75^{o}F and relative humidity of 50%) of the primate holding center. The food after autoclaving was not kept sterile other than enclosure in sealed kraft paper bags. After isolation started, food was kept in glass containers and autoclaved in daily packets within the containers. Immediately following sterilization, the containers were sealed.

Consistent with our past experience, when the animals (primary subjects) were placed in the isolators, timing of the feeding would quickly induce the animals to travel from isolator to isolator as desired. Thus the separate sections of the isolators could be opened, cleaned, stool and urine specimens

removed, food and water placed into the isolators and the whole resterilized with 2% peracetic acid solution at times of our choice. The sterilized food and water, in glass containers, was not, of course, reached by the peracetic acid.

The temperature inside the isolators was at all times essentially the same as those experienced by the controls. (Normal laboratory temperature was kept at 75°F.) When for any reason the air temperature dropped below 75°F, quartz lamp heating units were automatically activated directly onto the animal's cage areas until the air temperature was re-established to the desired point. The relative humidity within the laboratory, control center and interior of the isolators was automatically controlled, additional moisture sometimes being required during the colder months of the year. Our desired RH was 50%. The isolators were placed near windows so that the animals at their option would get both sun and shade during the day. To insure ease in handling during body and skin sampling, the animals had been thoroughly gentled, accustomed to the sounds of voices and personnel and a radio played 24 hours a day. The animals were not cleaned before placement within the isolator systems in order to remove one possible shock variable from the transfer.

Before taking blood samples, the animals were tranquilized by intramuscular injections of Sernylan*. Excellent success was found using this product. No ill effects were ever noticed following usage. This greatly facilitated taking samples inside the isolator. This was an important item for a loose (and angry) monkey inside a plastic isolator is not a joke.

The primates adapted well to their bio-isolation. The isolated animals all had a "view" and seemed alert and interested in their surroundings. We encouraged our laboratory personnel to attract and divert the animals by

*Sernylan, Product of Parke, Davis and Company, Detroit, Michigan.

various means throughout the working day. At night, radio music was played in the isolator laboratory. At present, it seems as if the psychological problems normally attendant upon infra-human primate isolation were alleviated. The animals showed individual behavioral characteristics as might be expected. One animal is a "rocker".

Four monkeys were placed in isolation during Fiscal Week 18 (2 May 1969). One of the isolated animals shortly began to suffer from diarrhea. We did not administer drugs and the condition cleared up by itself. One animal reacted negatively (sulking and refusal to eat or drink); after two days he was returned to the open (control) group and another, randomly picked, substituted. The substitute monkey adapted immediately to his new environment and the sulking monkey immediately upon return to his former cage, began to eat and drink normally.

We found much to our surprise that there were <u>not six</u> microorganism species (in high numbers) common to the eight animals undergoing test. This was contrary to our previous experience. The animals, in spite of mixing, had their individual flora. This required us to make a great many more quantitative and qualitative analyses than planned in order to follow the individual animal's predominant flora. The animals' total number of microflora began to drop after several weeks of isolation. Table III indicates the approximate log change in microorganisms by Week 14 of the experiment. This is due almost entirely to losses in the feces counts.

This change was slight but seemed definite. Later statistical analyses verified this (Appendix D). As shown in the summary charts (Appendix B) for each animal, the composition of the flora had changed. There are many possible reasons for the change: (a) the diet could contain a weak antibiotic - if

TABLE III

APPROXIMATE CHANGE IN TOTAL NUMBERS OF MICROORGANISMS (LOGS)
THREE MONTHS AFTER START

ANIMAL NUMBER	TOTAL AEROBES	TOTAL ANAEROBES	STATUS
2	-1	0	Control
6	-1	-1	Control
8	-2	-1	Control
9	-2	-1	Control
3	-2	0	Isolated
4	-3	0	Isolated
5	0	+1	Isolated
7	-2	-1	Isolated

this were so, the flora would sooner or later overcome this; (b) the diet could be slanted toward encouraging certain microorganisms; (c) there could be a predator upon the most successful species (in the competition for certain nutrients; and (d) the conditions of the experiment (bio-isolation) could be a pseudopredator. These last two possibilities (c and d) have been discussed by Young and Weston (1969). They conclude, "The presence of two species in the same environment with a common limiting resource is paradoxical if competition for the limiting resource is the only consideration. One or the other of the species must be eliminated. This analysis shows that a normally unsuccessful competitor for the limiting resource may persist however when there is a predator upon the otherwise successful species." Their model is of bacterial growth in a chemostat. Our experiment comes quite close to simulating in life their experimental conditions.

In the second quarter, it was noted that the E. coli count for the isolated animals was beginning to drop, indicating that simplification was beginning. This trend continued. Table IV illustrates the progressive reduction in the counts of this microorganism. Table V shows the same phenomena also began for the Lactobacillus.

The animals undergoing bioconfinement showed simplification first and then the control animals! Only one control animal continued to show E. coli in normal numbers. This animal (Number 6) was the one that received normal (non-sterilized) Rockland Monkey Chow. The other control animals received the same sterile diet as the isolated primates. (All animals had sterile water.) The quarters of the control animals were separately air conditioned, and the air within the room was constantly being swept through a HEPA) bacterial filter. This is closely equivalent to a "clean" room. Very low numbers of microorganisms were thus present in the air.

TABLE IV

SUMMARY OF CHANGES IN LOG NUMBERS OF E. COLI IN FECES OF MONKEYS DURING BIO-ISOLATION

ANIMAL NUMBER	CONDITION	DIET	LEVEL OF E. coli IN WEEK OF ISOLATION					
			1	10	20	23	27	37
2	Control	Sterile	7	4	<3	<3	<3	<3
6	Control	Non-Sterile	6	7	7	7	7	7
8	Control	Sterile	6	3	5	4	<3	<3
9	Control	Sterile	6	<3	<3	<3	<3	<3
3	Bio-Isolated	Sterile	7	4	<3	<3	<3	<3
4	Bio-Isolated	Sterile	8	<3	<3	<3	<3	<3
5	Bio-Isolated	Sterile	5	<3	<3	<3	<3	<3
7	Bio-Isolated	Sterile	7	6	6	<3	<3	<3

TABLE V

CHANGES IN LOG NUMBERS OF LACTOBACILLUS* IN FECES OF MONKEYS DURING BIO-ISOLATION

ANIMAL NUMBER	CONDITION	DIET	LEVEL OF LACTOBACILLUS IN WEEK OF ISOLATION								
			1	10	13	16	19	22	25	28	36
2	Control	Sterile	10	9	8	8	9	9	8	8	9
6	Control	Non-Sterile	10	9	8	8	10	10	9	9	10
8	Control	Sterile	10	9	8	8	10	10	8	8	10
9	Control	Sterile	10	9	9	9	9	9	9	9	9
3	Bio-isolated	Sterile	10	9	8	8	10	10	8	7	8
4	Bio-isolated	Sterile	10	8	7	7	8	8	7	6	7
5	Bio-isolated	Sterile	10	10	10	10	10	10	9	6	9
7	Bio-isolated	Sterile	10	9	8	8	8	8	6	3	8

*This includes all strains of Lactobacillus

These results are in agreement with those of Dubos' (1967). Dubos placed conventional mice in bio-isolators and noted the animals lost their E. coli by the third generation. Of importance here is the verification of the phenomena in a second animal species. If this can be related to data on man, then a firmer groundwork is laid for relation of the whole experiment to man in spacecraft isolation.

Thus, in 4½ months, a shift away from the normal flora and "normal" levels has been shown to occur in all the isolated animals. The change was slower in the clean air animals than in those that were bio-isolated, but it still occurred. In one isolated animal (Number 5), Proteus, not previous detected probably because it was present in less than 10^3 per sample, climbed in numbers. The aerobic organisms decreased and the anaerobes increased. Figures 3 through 10 and Table VI presents our findings on total organisms. Proteus numbers increased presumably to fill the void left by the disappearance of E. coli. Of interest is the fact Proteus was present only in very low numbers at the start of the experiment.

The decrease (disappearance?) of E. coli in two species of animals (mice and monkeys) is interesting from another viewpoint. The requirements for water analyses usually use E. coli as the marker species. If checks of the astronaut drinking supply (or any other water) do not disclose E. coli, it obviously does not mean the water is safe from a bacteriological standpoint. It would appear that the standards for water analyses should be checked to make certain not too much reliance would be placed on normal presumptive testing using the presence or absence of E. coli as a standard.

E. coli has been shown by Tanami (1967) in Japan (using germfree animals), to promote the antibacterial power of sera (against Salmonella typhosa). The

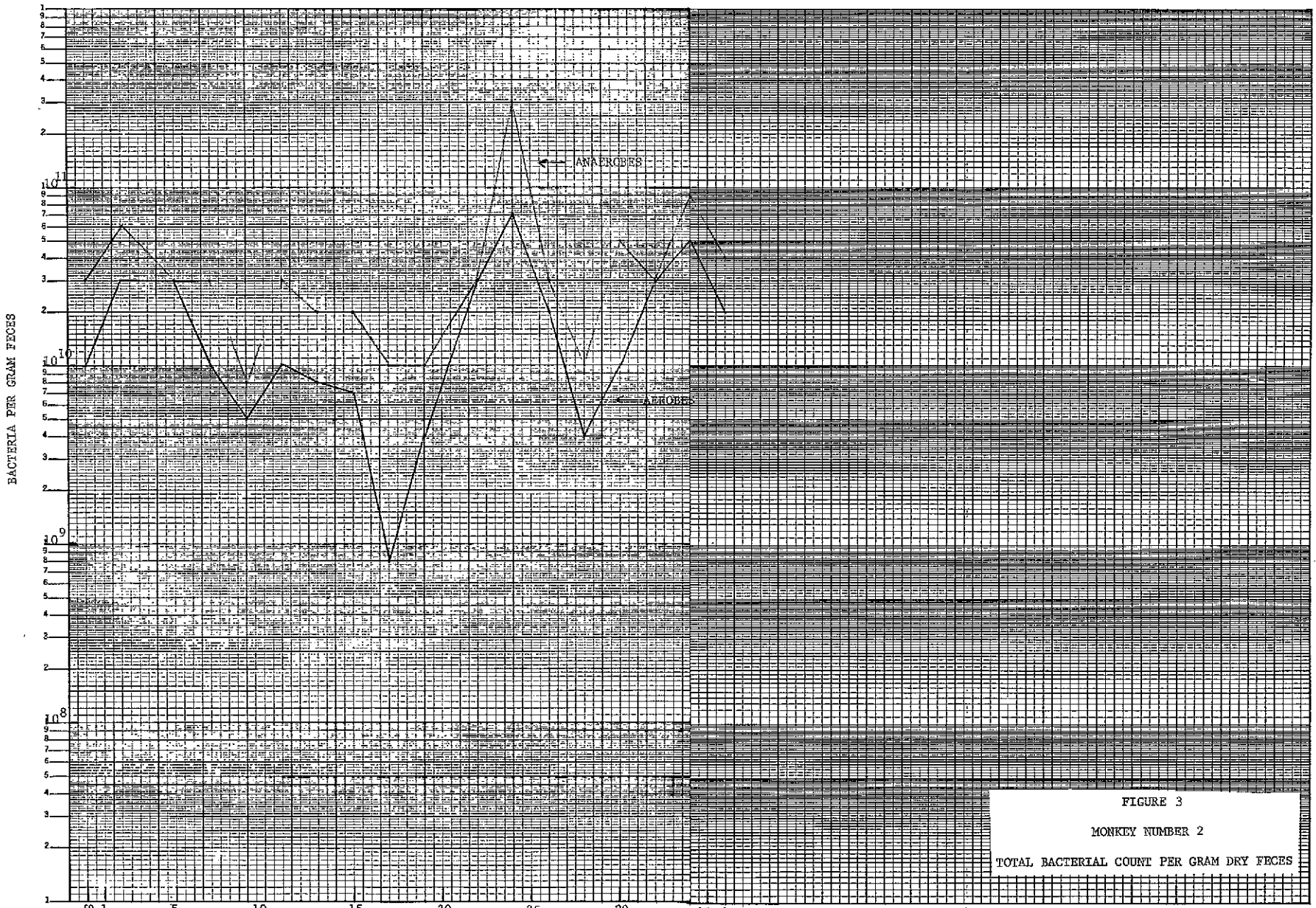


FIGURE 3

MONKEY NUMBER 2

TOTAL BACTERIAL COUNT PER GRAM DRY FECES

FOLDOUT FRAME

FOLDOUT FRAME

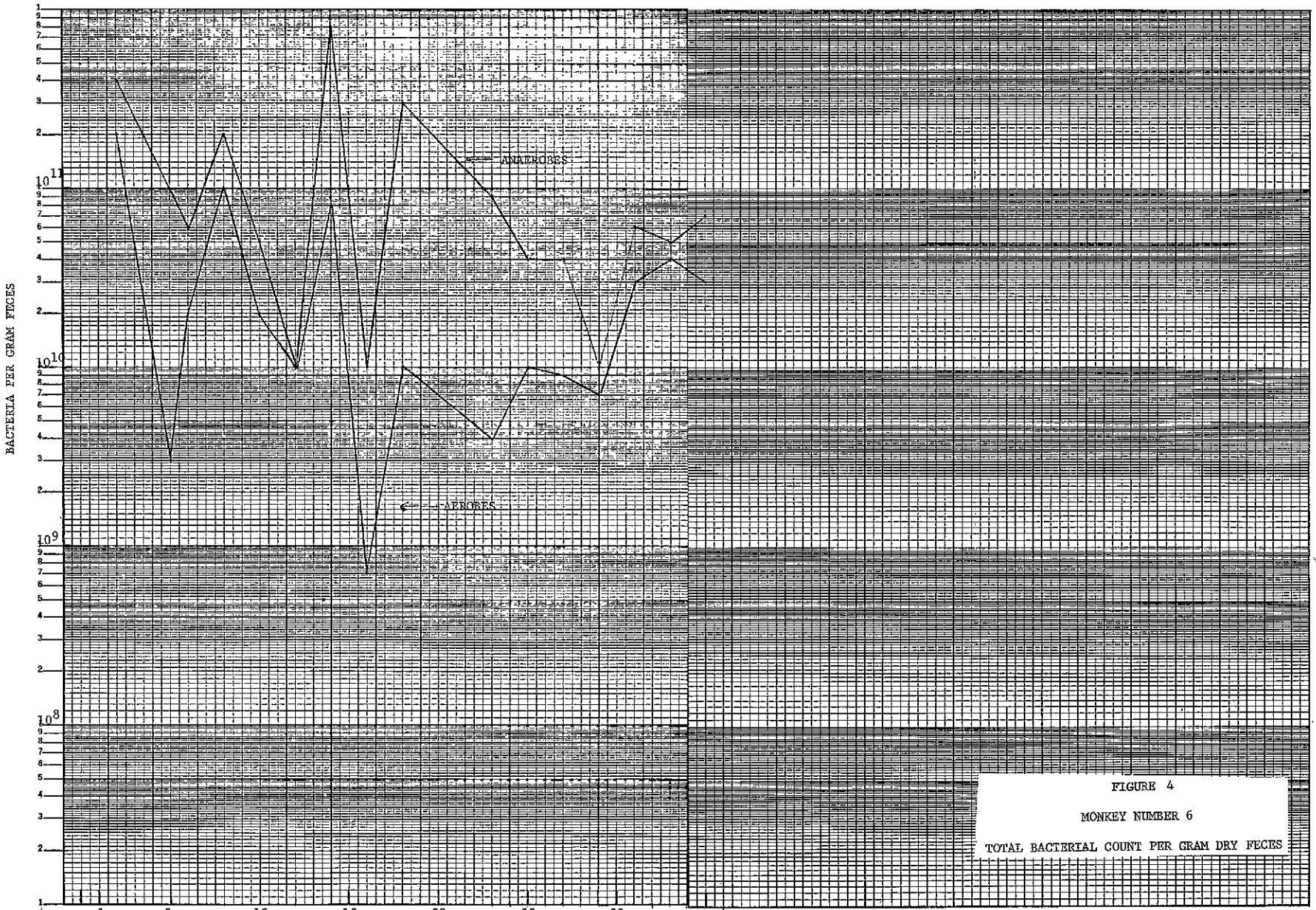


FIGURE 4

MONKEY NUMBER 6

TOTAL BACTERIAL COUNT PER GRAM DRY FECES

FOLDOUT FRAME 2

FOLDOUT FRAME

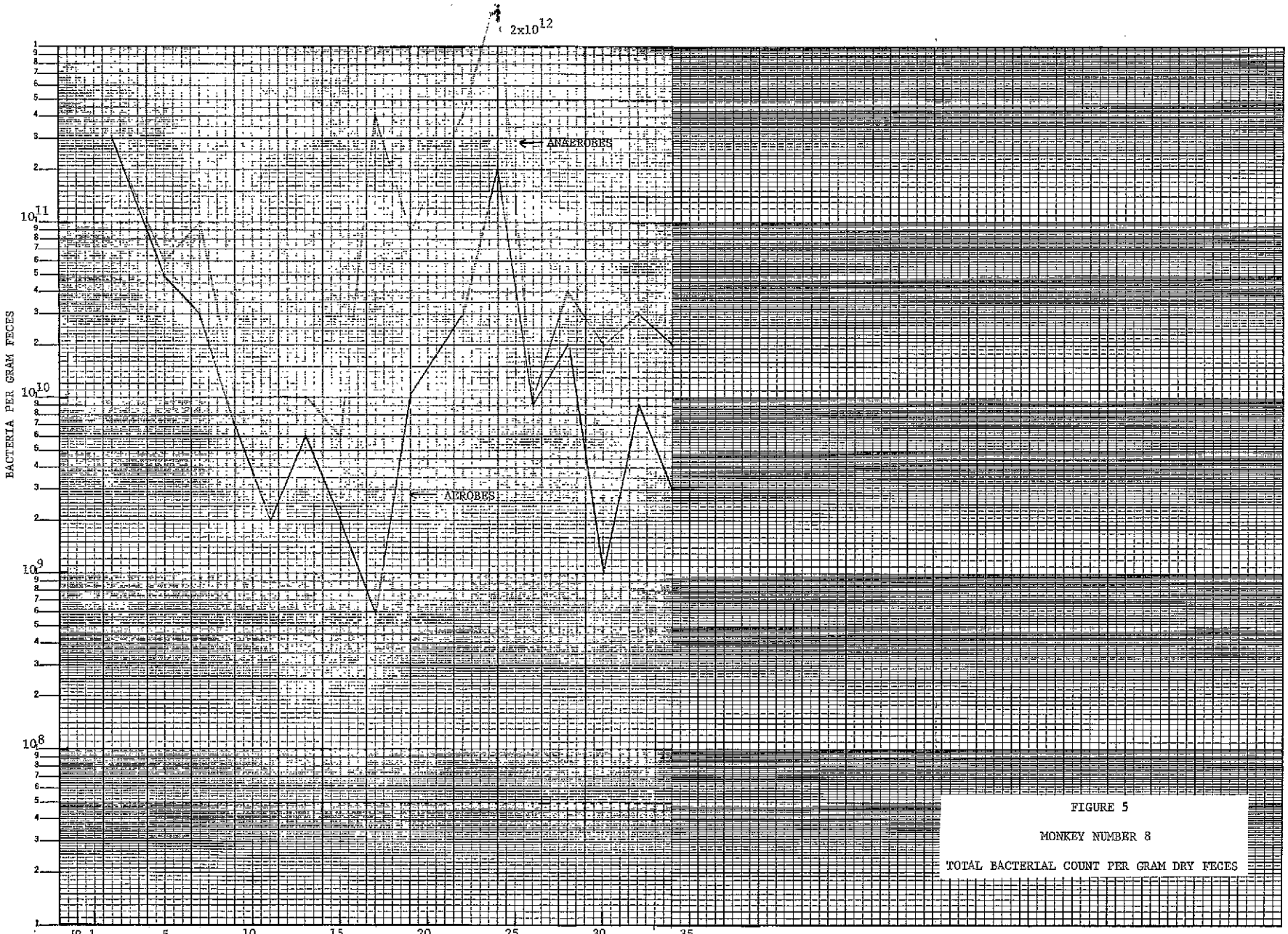


FIGURE 5

MONKEY NUMBER 8

TOTAL BACTERIAL COUNT PER GRAM DRY FECES

FOLDOUT FRAME

FOLDOUT FRAME 2

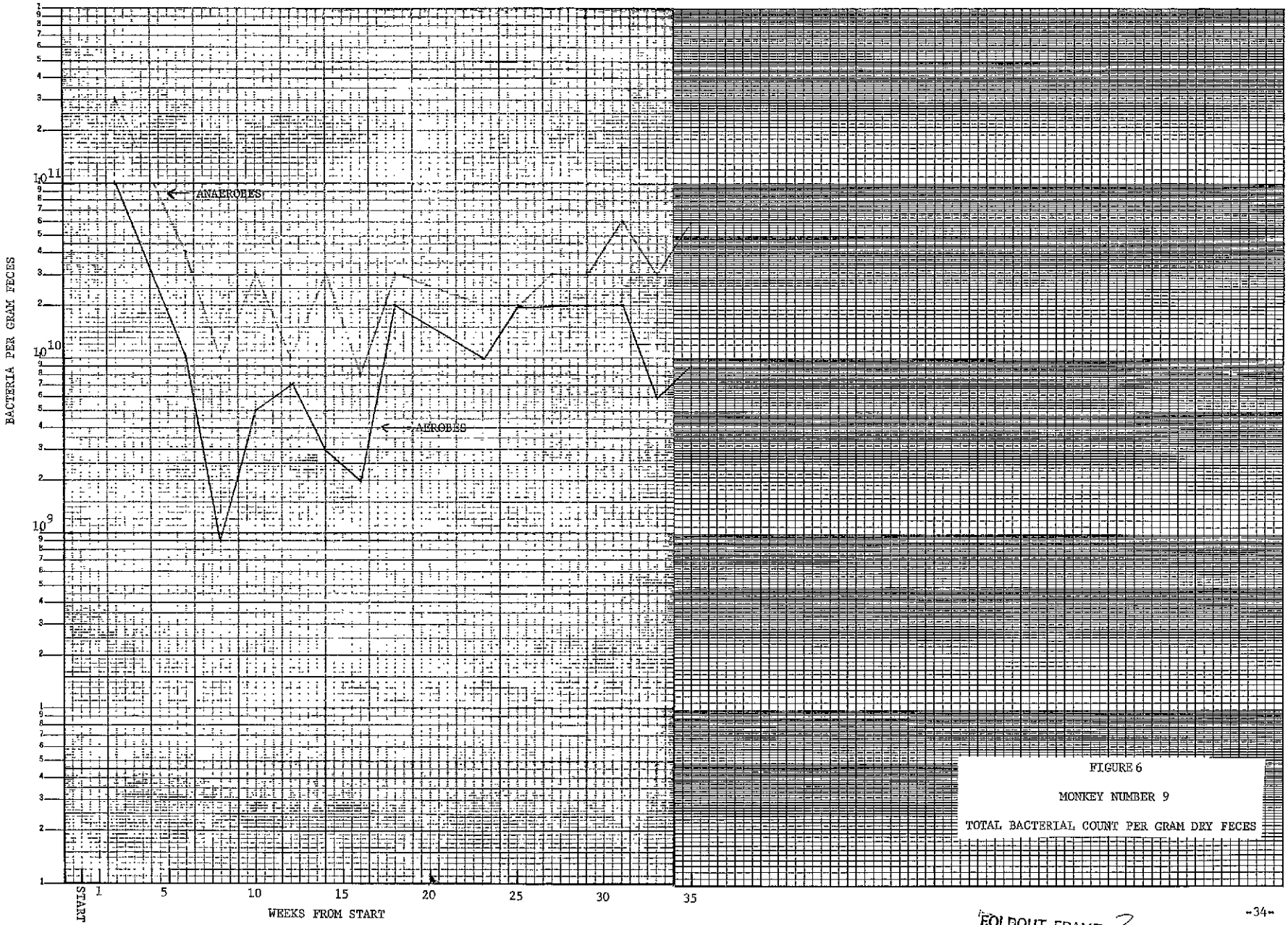


FIGURE 6

MONKEY NUMBER 9

TOTAL BACTERIAL COUNT PER GRAM DRY FECES

FOLDOUT FRAME 1

FOLDOUT FRAME 2

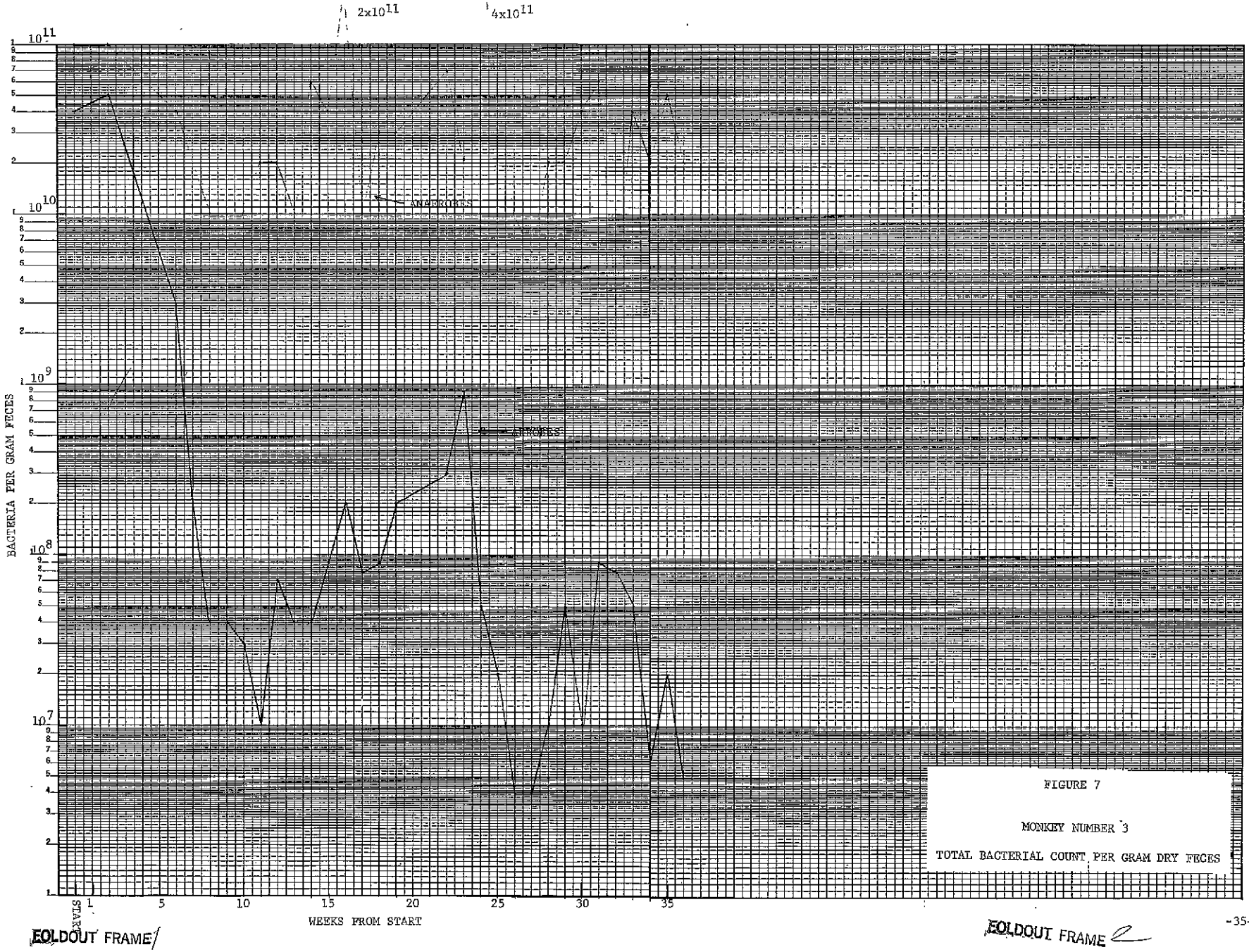


FIGURE 7

MONKEY NUMBER 3

TOTAL BACTERIAL COUNT PER GRAM DRY FECES

FOLDOUT FRAME

FOLDOUT FRAME

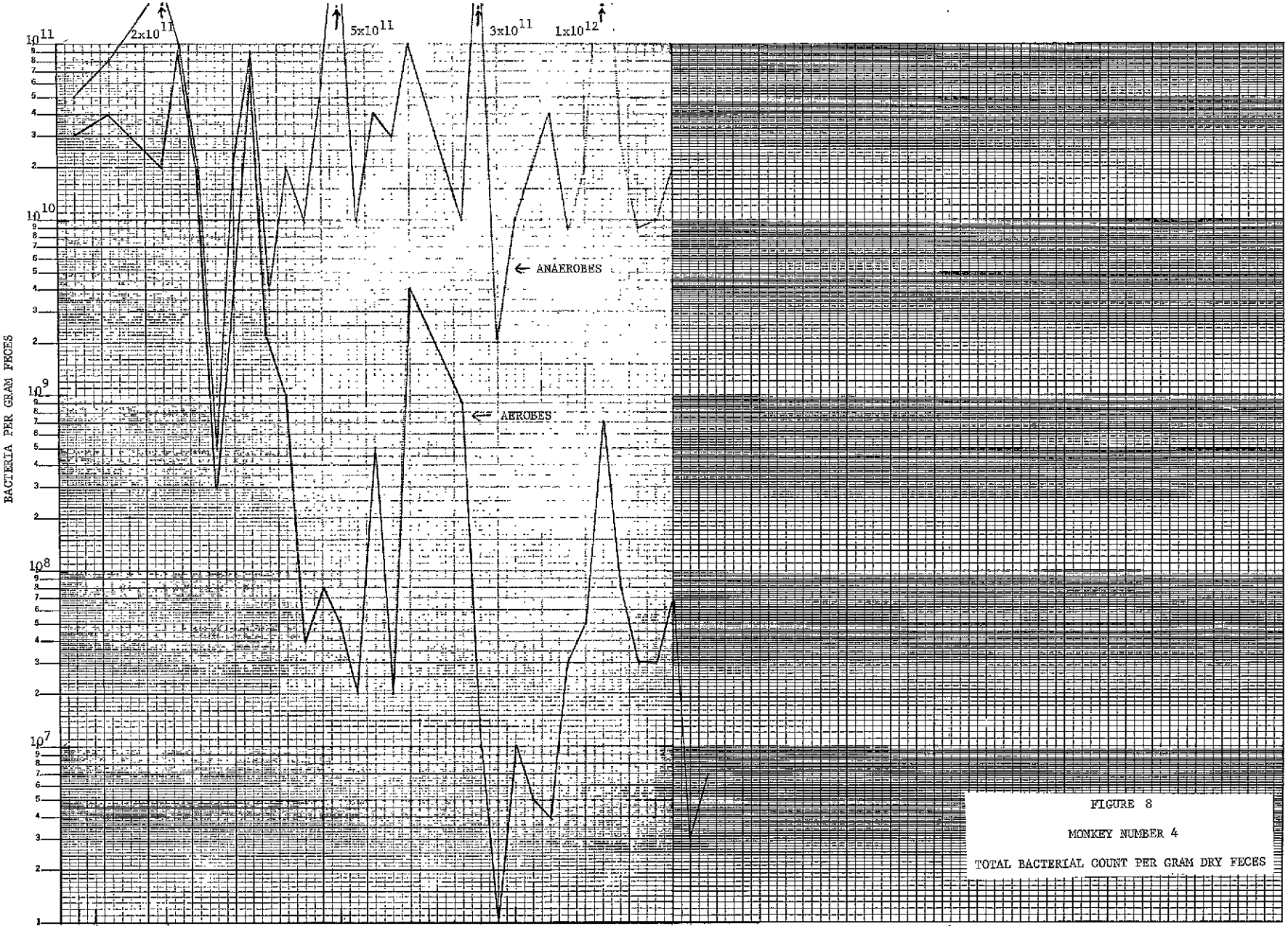


FIGURE 8

MONKEY NUMBER 4

TOTAL BACTERIAL COUNT PER GRAM DRY FECES

FOLDOUT FRAME (

FOLDOUT FRAME 2

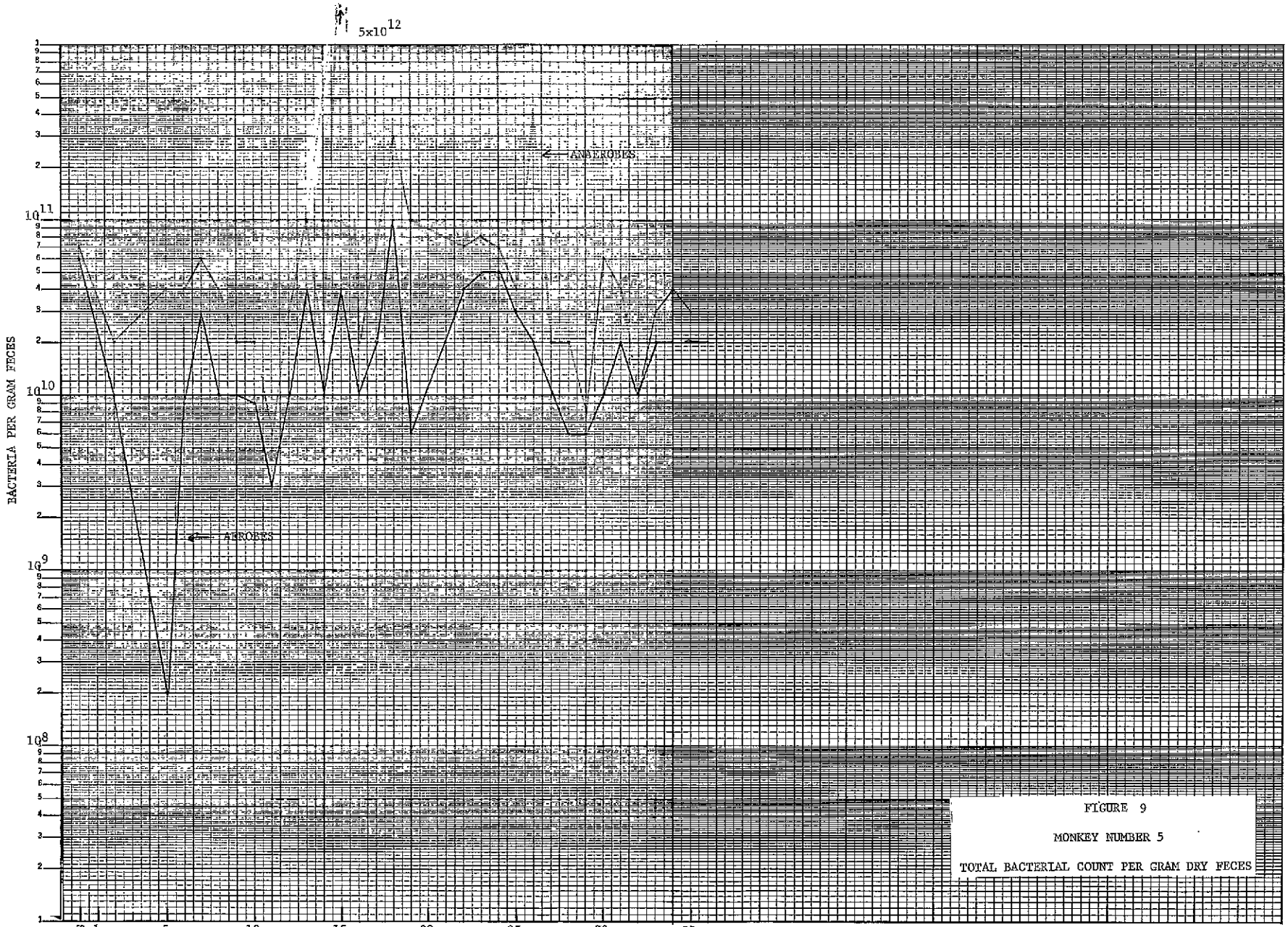


FIGURE 9

MONKEY NUMBER 5

TOTAL BACTERIAL COUNT PER GRAM DRY FECES

FOLDOUT FRAME 1

FOLDOUT FRAME 2

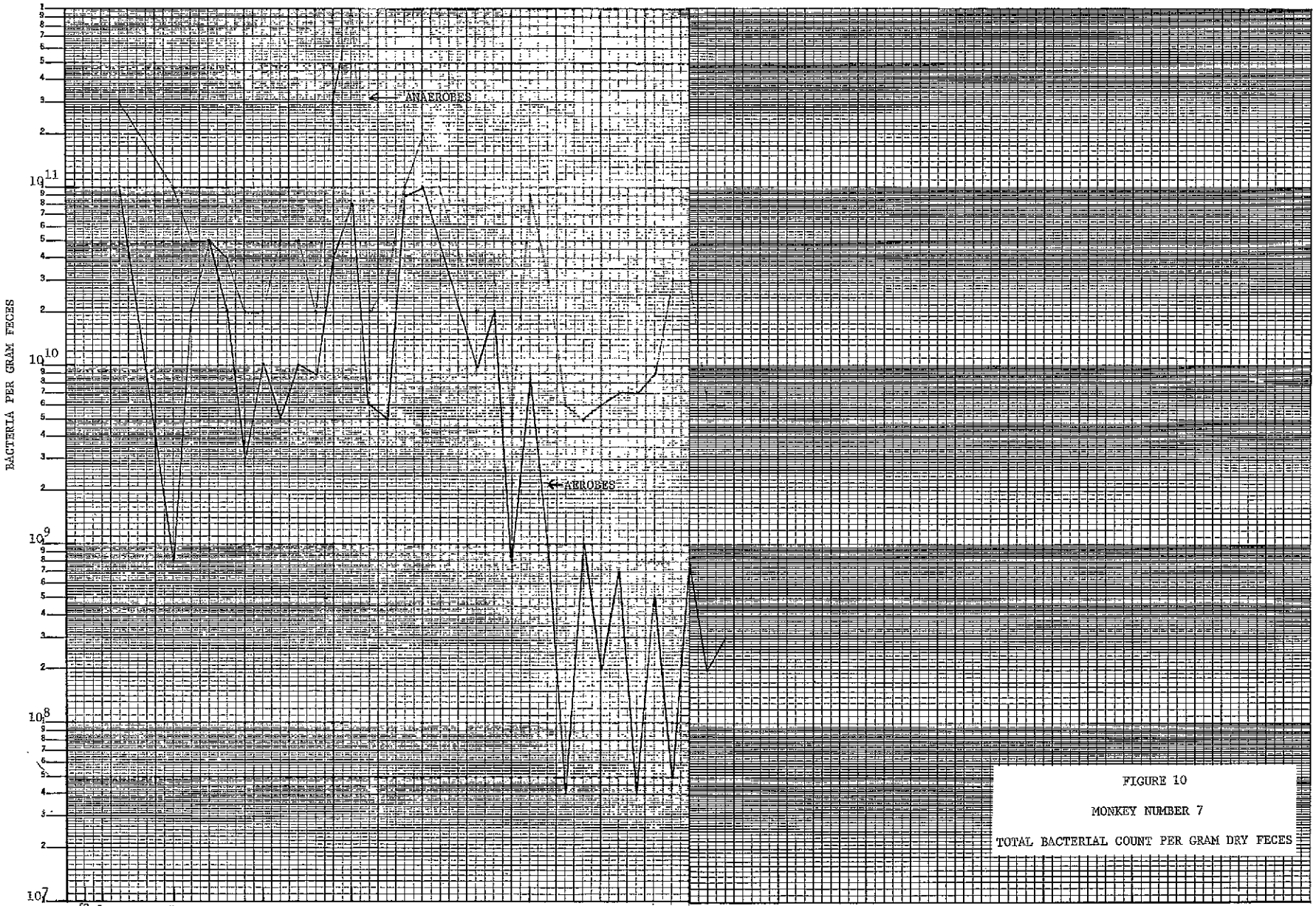


FIGURE 10

MONKEY NUMBER 7

TOTAL BACTERIAL COUNT PER GRAM DRY FECES

FOLDOUT FRAME

FOLDOUT FRAME 2

TABLE VI

MONKEY MICROFLORA DATA

TOTAL VIABLE AEROBIC AND ANAEROBIC COUNTS* PER GRAM FECES**

	TEST MONKEYS								CONTROL MONKEYS							
	3		4		5		7		2		6		8		9	
WEEK SAMPLED	AEROBES	ANAEROBES	AEROBES	ANAEROBES	AEROBES	ANAEROBES	AEROBES	ANAEROBES	AEROBES	ANAEROBES	AEROBES	ANAEROBES	AEROBES	ANAEROBES	AEROBES	ANAEROBES
START (0 WEEK) 3/31/69	4×10^{10}	1×10^{11}	2×10^{10}	5×10^{10}	6×10^{10}	7×10^{10}			1×10^{10}	3×10^{10}						
1																
2	5×10^{10}	1×10^{11}	4×10^{10}	3×10^{10}	1×10^{10}	2×10^{10}	1×10^{10}	3×10^{11}	9×10^{10}	6×10^{10}	2×10^{11}	4×10^{11}	3×10^{11}	3×10^{11}	1×10^{11}	3×10^{11}
3																
4																
5			2×10^{10}	2×10^{11}	2×10^{9}	4×10^{10}	8×10^{9}	1×10^{11}	3×10^{10}	5×10^{10}	3×10^{9}	1×10^{11}	5×10^{10}	6×10^{10}		
6	3×10^{9}	4×10^{10}	1×10^{11}	1×10^{11}	9×10^{9}	4×10^{10}	2×10^{10}	5×10^{10}			2×10^{10}	6×10^{10}			1×10^{10}	4×10^{10}
7	2×10^{9}	2×10^{10}	2×10^{10}	2×10^{10}	8×10^{10}	1×10^{10}	5×10^{10}	5×10^{10}	1×10^{10}	3×10^{10}			3×10^{10}	1×10^{11}		
8	4×10^{9}	1×10^{10}	5×10^{9}	5×10^{9}	1×10^{10}	4×10^{10}	2×10^{10}	4×10^{10}			1×10^{11}	2×10^{11}			9×10^{9}	1×10^{10}
9	4×10^{9}	1×10^{10}	4×10^{9}	2×10^{10}	1×10^{10}	2×10^{10}	3×10^{9}	2×10^{10}	5×10^{9}	8×10^{9}			7×10^{9}	1×10^{10}		
10	3×10^{9}	1×10^{10}	2×10^{10}	9×10^{9}	9×10^{9}	2×10^{10}	1×10^{10}	2×10^{10}			2×10^{10}	5×10^{10}			5×10^{9}	3×10^{10}
11	1×10^{9}	2×10^{10}	2×10^{9}	4×10^{9}	3×10^{9}	6×10^{9}	5×10^{9}	5×10^{10}	1×10^{10}	3×10^{10}			2×10^{9}	1×10^{10}		
12	2×10^{9}	2×10^{10}	1×10^{9}	2×10^{10}	1×10^{10}	3×10^{10}	1×10^{10}	5×10^{10}			1×10^{10}	1×10^{10}			7×10^{9}	1×10^{10}
13	4×10^{9}	1×10^{10}	4×10^{9}	1×10^{10}	4×10^{10}	1×10^{11}	9×10^{9}	2×10^{10}	5×10^{9}	2×10^{10}			6×10^{9}	1×10^{10}		
14	4×10^{9}	6×10^{10}	5×10^{9}	6×10^{10}	1×10^{10}	2×10^{10}	4×10^{10}	3×10^{10}			8×10^{10}	2×10^{10}			3×10^{9}	3×10^{10}
15	9×10^{9}	4×10^{10}	5×10^{9}	5×10^{11}	5×10^{10}	3×10^{12}	8×10^{10}	1×10^{12}	7×10^{9}	2×10^{10}			2×10^{9}	6×10^{9}		
16	2×10^{8}	2×10^{11}	2×10^{9}	1×10^{10}	1×10^{10}	2×10^{10}	6×10^{9}	2×10^{10}			7×10^{8}	1×10^{10}			2×10^{9}	8×10^{9}
17	3×10^{9}	1×10^{10}	5×10^{8}	4×10^{10}	2×10^{10}	7×10^{10}	5×10^{9}	3×10^{10}	2×10^{8}	1×10^{10}			5×10^{8}	4×10^{11}		
18	9×10^{8}	3×10^{10}	2×10^{8}	3×10^{10}	1×10^{11}	3×10^{11}	9×10^{10}	1×10^{11}			1×10^{10}	3×10^{11}			2×10^{10}	3×10^{10}
19	2×10^{9}	2×10^{10}	4×10^{9}	1×10^{11}	6×10^{9}	1×10^{11}	1×10^{11}	2×10^{11}	4×10^{9}	1×10^{10}			1×10^{10}	9×10^{10}		
20																
21																
22	5×10^{8}	7×10^{10}	9×10^{8}	1×10^{10}	4×10^{10}	7×10^{10}	1×10^{10}	2×10^{10}	5×10^{10}	3×10^{10}			3×10^{10}	4×10^{11}		
23	9×10^{8}	2×10^{10}	1×10^{9}	3×10^{11}	5×10^{10}	5×10^{10}	2×10^{10}	5×10^{10}			4×10^{9}	9×10^{10}			1×10^{10}	2×10^{10}
24	5×10^{9}	4×10^{12}	1×10^{10}	2×10^{9}	5×10^{10}	7×10^{10}	8×10^{8}	5×10^{9}	7×10^{10}	7×10^{11}			2×10^{11}	3×10^{12}		
25	2×10^{9}	5×10^{10}	1×10^{9}	1×10^{10}	5×10^{10}	4×10^{10}	9×10^{9}	9×10^{10}			1×10^{10}	4×10^{10}			2×10^{10}	2×10^{10}
26	4×10^{8}	1×10^{10}	5×10^{8}	2×10^{10}	2×10^{10}	4×10^{11}	1×10^{9}	3×10^{10}	2×10^{10}	3×10^{10}			8×10^{9}	1×10^{10}		
27	4×10^{8}	6×10^{9}	4×10^{9}	4×10^{10}	2×10^{10}	2×10^{10}	9×10^{9}	6×10^{9}			9×10^{9}	4×10^{10}			2×10^{10}	3×10^{10}
28	1×10^{9}	2×10^{10}	3×10^{9}	3×10^{9}	6×10^{9}	2×10^{10}	1×10^{9}	5×10^{9}	3×10^{9}	1×10^{10}			2×10^{10}	4×10^{10}		
29	3×10^{9}	2×10^{10}	5×10^{9}	2×10^{10}	6×10^{9}	2×10^{9}	2×10^{8}	2×10^{9}			7×10^{9}	1×10^{10}			2×10^{10}	3×10^{10}
30	1×10^{9}	4×10^{10}	7×10^{8}	1×10^{12}	1×10^{10}	6×10^{10}	7×10^{8}	7×10^{9}	1×10^{10}	5×10^{10}			1×10^{9}	2×10^{10}		
31	9×10^{9}	6×10^{10}	2×10^{9}	3×10^{10}	2×10^{10}	4×10^{10}	4×10^{9}	7×10^{9}			8×10^{10}	6×10^{10}			2×10^{10}	6×10^{10}
32	2×10^{9}	3×10^{9}	3×10^{9}	9×10^{9}	1×10^{10}	1×10^{10}	5×10^{8}	9×10^{9}	3×10^{10}	3×10^{10}			9×10^{9}	3×10^{10}		
33	5×10^{9}	4×10^{10}	3×10^{9}	1×10^{10}	2×10^{10}	3×10^{10}	5×10^{9}	3×10^{10}			4×10^{10}	5×10^{10}			6×10^{9}	3×10^{10}
34	6×10^{8}	2×10^{10}	7×10^{9}	2×10^{10}	2×10^{10}	4×10^{10}	2×10^{8}	3×10^{10}	5×10^{10}	9×10^{10}			3×10^{9}	2×10^{10}		
35	2×10^{9}	5×10^{10}	3×10^{8}	2×10^{10}	2×10^{10}	3×10^{10}	2×10^{8}	6×10^{9}			3×10^{10}	7×10^{10}			9×10^{9}	6×10^{10}
36	5×10^{8}	2×10^{10}	7×10^{8}	2×10^{10}	2×10^{10}	3×10^{10}	3×10^{8}	6×10^{9}	2×10^{10}	4×10^{10}			5×10^{9}	2×10^{10}		
37	6×10^{9}	2×10^{10}	3×10^{10}	2×10^{10}	4×10^{10}	5×10^{10}	7×10^{9}	3×10^{10}			5×10^{9}	4×10^{10}			3×10^{9}	4×10^{10}
38																

*Aerobes - 5% Blood Agar (BBL Prepared Plates) Duplicate Plates Incubation - 48 to 56 Hours @ 35°C

Anaerobes - 5% Blood Agar (BBL Prepared Plates) Duplicate Plates Incubation - 72 to 80 Hours @ 35°C

**Calculated on Dry Basis

FOLDOUT FRAME

FOLDOUT FRAME 2

loss of this organism (E. coli) may have an effect on the natural or acquired immunities of the astronaut. This action of E. coli may be strain specific.

The E. coli and the Lactobacillus were judged the most important members of the protective mix* in that most is known about the interactions of E. coli and Lactobacillus with other indigenous genera, both qualitatively and quantitatively. The results of Part B of this program are illustrative of the complex interactions and how changing the variable of diet alone reflects changes in the interactions.

Animal Number 7, an isolated animal, suffered from a bad skin rash acquired about a month after isolation started. Almost pure cultures (10^7 to 10^8) of microorganisms (Staphylococcus epidermidis) were recovered from the groin. He seemed normal, other than his red blotched skin and heavy dandruff. We did nothing about his condition. If his health had failed, we had planned this animal would be the prime isolated candidate for a massive dose of antibiotics. The whole course of the experiment could then have changed (at least for this animal) and his control partner. Much would have depended upon the antibiotic used to attempt cure of the S. epidermidis infection. If enough of the protective mix were removed from his microbial ecology, the possibility of runaway Candida or an antibiotic resistant Staphylococcus infection setting in were very real. For this reason, we did not give an antibiotic and the animal survived the entire period with the rash.

It must be pointed out that this potential of a "runaway" infection is exactly what could happen if the same situation (a need for antibiotics) were to occur on a long space flight. Phillips (1966), Seelig (1966), and many others have written at length on the causes and consequences of "runaways" of normal commensals. The study of the effects of antibiotic therapy during

*It must be iterated that the total sum of the effects of all the microflora make up the protective mix. Our information as to the contribution of many genera is almost nil other than the fact they are a factor.

bioconfinement is planned as a normal portion of the work proposed for 1970.

Serum Protein Studies

Attached as Table VII are the results of serum protein analysis (Electrophoresis).

The plasma proteins are the most readily accessible proteins in the body, and we followed protein metabolism of the animals by this method. The autoclaved diet is supposed to be adequate in nutritional value and the loss of vitamins had been covered by supplementary feeding of vitamin concentrates. The extent of protein damage or loss due to the sterilization process is unknown (Browning reactions normally tying up lysine).

The plasma proteins are an indication of the general health of the animal. While the list of physiological problems that are contributory to an inadequate supply of protein to the body is long, usually specific dietary deficiency may be suspected. No particular reason existed to suspect liver damage (another possibility) in both the control and experimental animals, thus our finding of low albumin content were more indicative of inadequate protein supply.

An area of concern to us was the early failure of the animals to gain weight. The diet given the animals was exactly the same as that given on a previous primate test when satisfactory growth (.1 Kg/mo) was observed. Table VIII lists the diet constituents and Tables IX and X, the vitamin supplement. We increased the animals' food ration and changed the diet of one (Number 6) of the control group to unsterilized food. We thought it possible that the sterilization may be affecting the food content in some different manner other than the vitamin loss which we compensate for, i.e., "Browning reactions".

TABLE VII

SERUM PROTEINS OF REPRESENTATIVE PRIMATES AT FISCAL WEEK 48 OF EXPERIMENT

PRIMATE	TOTAL PROTEIN gm/100 ml	ALBUMIN gms	% TOTAL PROTEIN	α_1 gms	% TOTAL PROTEIN	α_2 gms	% TOTAL PROTEIN	β gms	% TOTAL PROTEIN	γ gms	% TOTAL PROTEIN	ALBUMIN GLOBULIN RATIOS
No. 3 I-S	5.9	2.64	44.7	.52	8.8	.77	13.1	.57	9.7	1.40	23.7	.89
No. 7 I-S*	7.8	2.85	36.5	.70	9.0	.76	9.7	1.23	15.8	2.26	29.0	.67
No. 6 C-NS	7.6	3.50	46.0	.36	4.8	.60	7.9	.97	12.7	2.17	28.6	1.13
No. 9 C-S	6.3	3.12	49.5	.40	6.3	.51	8.1	.62	9.9	1.65	26.2	.98
Versatol (Human)	7.2	4.0	55.5	.32	4.4	.28	10.9	.79	11.0	1.31	18.2	1.25
Literature	7.8	4.9	62.8	GLOBULINS = 2.9								**1.67**

*Has violent skin rash - S. epidermis 10^8/gm

I = Isolated S = Sterile Diet

C = Control NS = Non-Sterile

TABLE VIII

CONSTITUENTS OF PRIMATE DIET BEFORE AUTOCLAVE STERILIZATION ROCKLAND PRIMATE DIET

Crude Protein	17.0 percent minimum
Crude Fat	5.0 percent minimum
Crude Fiber	3.0 percent maximum

Ground Yellow Corn

Dried Skimmed Milk

Dehulled Solvent Extracted Soybean Meal

Animal Fat (Preserved with Propylene Glycol, BHT, Citric Acid)

Ground Whole Wheat

Dehydrated Alfalfa Meal

Brewer's Dried Yeast

Cane Sugar

1.5 percent Calcium Carbonate

0.75 percent Salt

Vitamin B-12 Supplement

Ribolflavin Supplement

Calcium Patothenate

Niacin

Folic Acid

Pyridoxine Hydrochloride

Thiamine Hydrochloride

Vitamin A Palmitate

D-Activated Plant Sterol (Source of Vitamin D-2)

Vitamin E Supplement

Choline Chloride

Ascorbic Acid and Traces of Manganese Sulphate

Iron Carbonate

Iron Oxide

Copper Oxide

Cobalt Carbonate

Potassium Iodide

Zinc Sulphate

TABLE IX

CONTENTS OF WATER SOLUBLE VITAMIN SUPPLEMENT
GIVEN TO EACH MONKEY, EACH DAY

VITAMIN	QUANTITY
A (Palmitate) (1.8 mg)	6,000 USP units
D (30 mg)	1,200 USP units
C (Ascorbic acid)	60 mg
B_1 (As Chloride)	2 mg
B_2 (Riboflavin 5' Phosphate Sodium)	1.2 mg
B_6 (Pyridoxine Hydrochloride)	0.5 mg
B_{12} (Cyanocobalamin)	2 mg
Niacinamide	10 mg
Pantothenic Acid (As Pantothenol)	3 mg

TABLE X

DAILY VITAMIN SUPPLEMENT

VITAMIN	PROPORTION OF MINIMUM DAILY REQUIREMENT
A	4
D	3
C	6
B_1	8
B_2	2
Niacinamide	*
*MDR not established	

The above figures are for human infants. The daily ration was in all cases equal to or above the MDR for human children and adults

This change in diet increased the number of analyses for we had to consider any "new" microorganisms brought to this animal by the food and any possible differences due to change in internal conditions within that (Number 6) primate. The unsterilized food would set up a different environment for the indigenous microflora and thus a change in relative numbers probably would occur even if a change in kind is not evidenced. It is emphasized that the failure of the animals to gain weight was exactly that and was not a substantial weight loss. The animals were all quite active throughout the program. It could very well be that we attained a balance and that the animals desired just enough food to maintain their weight. Some food wastage began to appear following the diet-quantity increase. All the animals visually appeared quite normal. The hematology showed normal although our serum protein analyses indicated that protein intake could be low. Increasing the quantity of food given seemed to alleviate this condition.

Our overall expectation of growth was that the animals should have gained about a half to two-thirds of a kilogram during the isolation period. This was generally not realized. The growth curve of the one animal with an unsterilized diet and the low serum protein results of all the animals indicate that perhaps more or better protein could have been utilized in the diet unless a normal requirement of primate nutrition is the presence of certain types of gram-negative microorganisms.

Hurst (1965) studying weight gain of growing rhesus monkeys did not correlate weight with age but rather studied weight gain per month on a particular diet. His daily diet weighed from 200 to 230 grams per day depending upon the fruit fed (water percentage unknown) and contained from 223 to 117 calories per kilogram. Our original daily food portion (without fruit) averaged 125 grams

per day dry weight. Our animals, like those of Hurst, were taken from the wild, thus exact ages could not be determined. The average weight shortly after receipt in our laboratories was 3.81 kilograms (high 4.10, low 3.52 and an average of 3.7 kg at experiment start). Eight months later, the weight was 3.5 kilograms. For a period, their average weight dropped to 3.38. No animal confined or control varied much from the average. Following the shorter sterilization cycle and increased food allowance, loss of weight ceased. Previously referred to was the weight gain of the animal receiving unsterilized food (3.6 to 4.4 Kg) (and having a near "normal" flora throughout). The confined animal with the least change in total microflora (Number 5) was 3.8 kilograms, up from 3.5 kilograms.

Table XI illustrates weight changes during confinement. (Weights were measured weekly on isolated animals and on controls, bi-weekly.) Figures 11 through 18 graphically represent the individual changes. Two animals stand-out from the overall picture. The animal (Number 6) receiving unsterilized food from Week 18 immediately started gaining weight. His microfloral profile shows no loss of E. coli or Lactobacillus. Animal Number 5, although losing E. coli as his mates, hung on to Lactobacilli longer than his mates. This animal also was the only animal to have substantial numbers of Proteus internally. These two animals thus were the only animals to have high numbers of aerobic gram-negative microorganisms in their intestinal tract. There is, therefore, an indication that the cause of non-food utilization may be due to loss of gram-negative microorganisms. This point should be further studied in detail. The possibility of E. coli (and perhaps others) being able to synthesize some required element of the diet is very real. E. coli is a known vitamin and nutrient synthesizer. Smith, Beamer, Vellios and Schultz (1959)

TABLE XI

ABBREVIATED RAW DATA OF PRIMATE WEIGHTS (Kg)
RECORDED DURING EXPERIMENT

	ANIMAL NUMBER	WEEK							NOTES
		1*	4**	17	27	35	36	37	
BIO-ISOLATED	3	3.9	3.8	3.5	3.1	3.2	3.2	3.0	
	4	3.5	3.0	3.0	2.9	3.0	3.0	3.0	
	5	4.1	3.5	4.0	3.5	3.8	3.8	3.8	Has High Proteus
	7	3.6	4.0	3.5	3.0	3.0	3.0	2.9	
NON-ISOLATED	2	3.9	3.7	3.3	3.1	3.2	3.2		
	6	3.6	3.6	3.2	3.9	4.2	4.3	4.4	Normal Diet (Non-Sterile)
	8	3.9	3.9	3.4	3.3	3.4	3.4	3.4	
	9	3.9	4.1	3.8	3.4	3.5	3.5	3.5	

*Arrival Weight

**Isolation Start

FIGURE 11
CHANGES IN WEIGHT OF PRIMATE DURING EXPERIMENT
MONKEY NUMBER 2

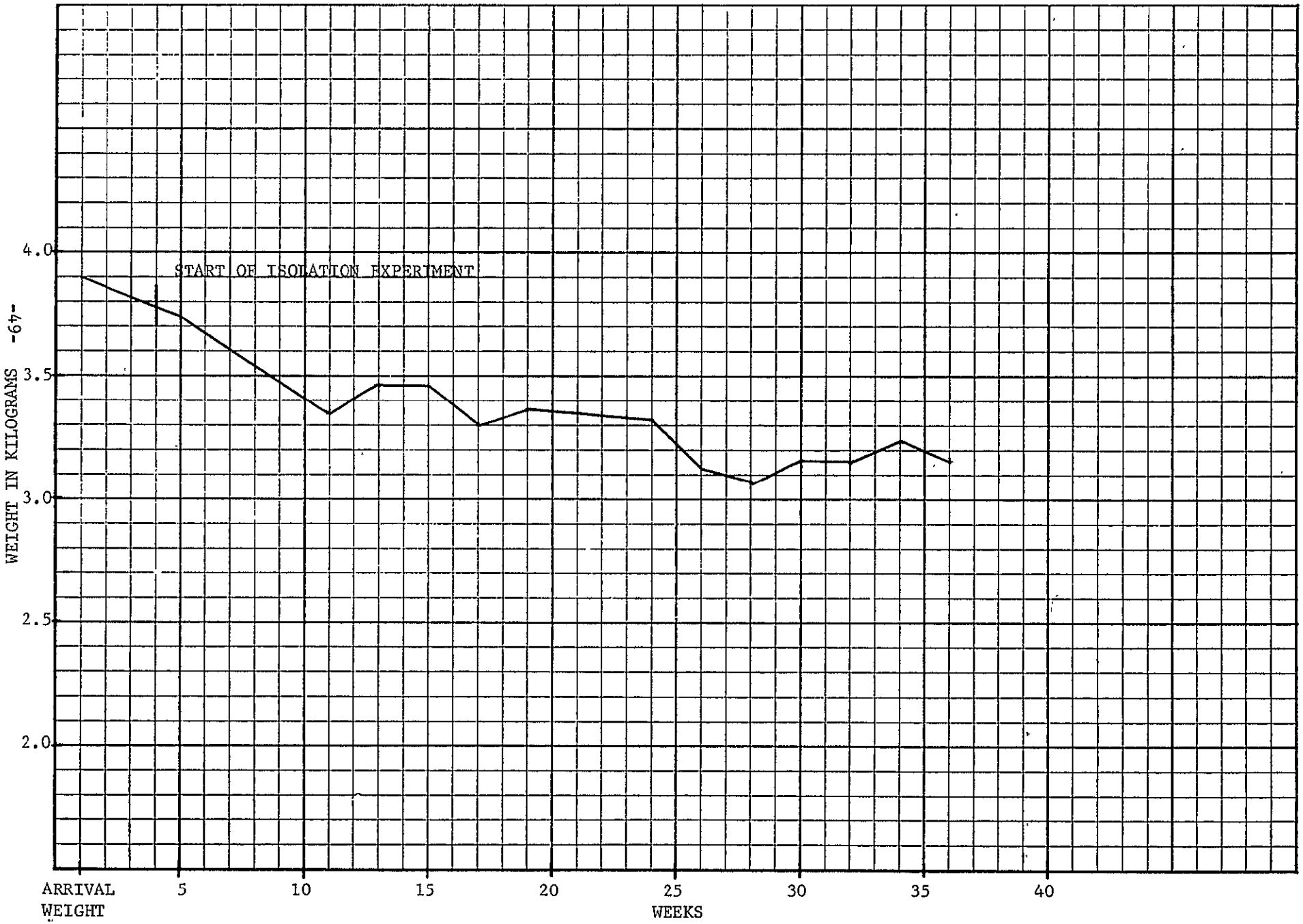


FIGURE 12
CHANGES IN WEIGHT OF PRIMATE DURING EXPERIMENT
MONKEY NUMBER 6

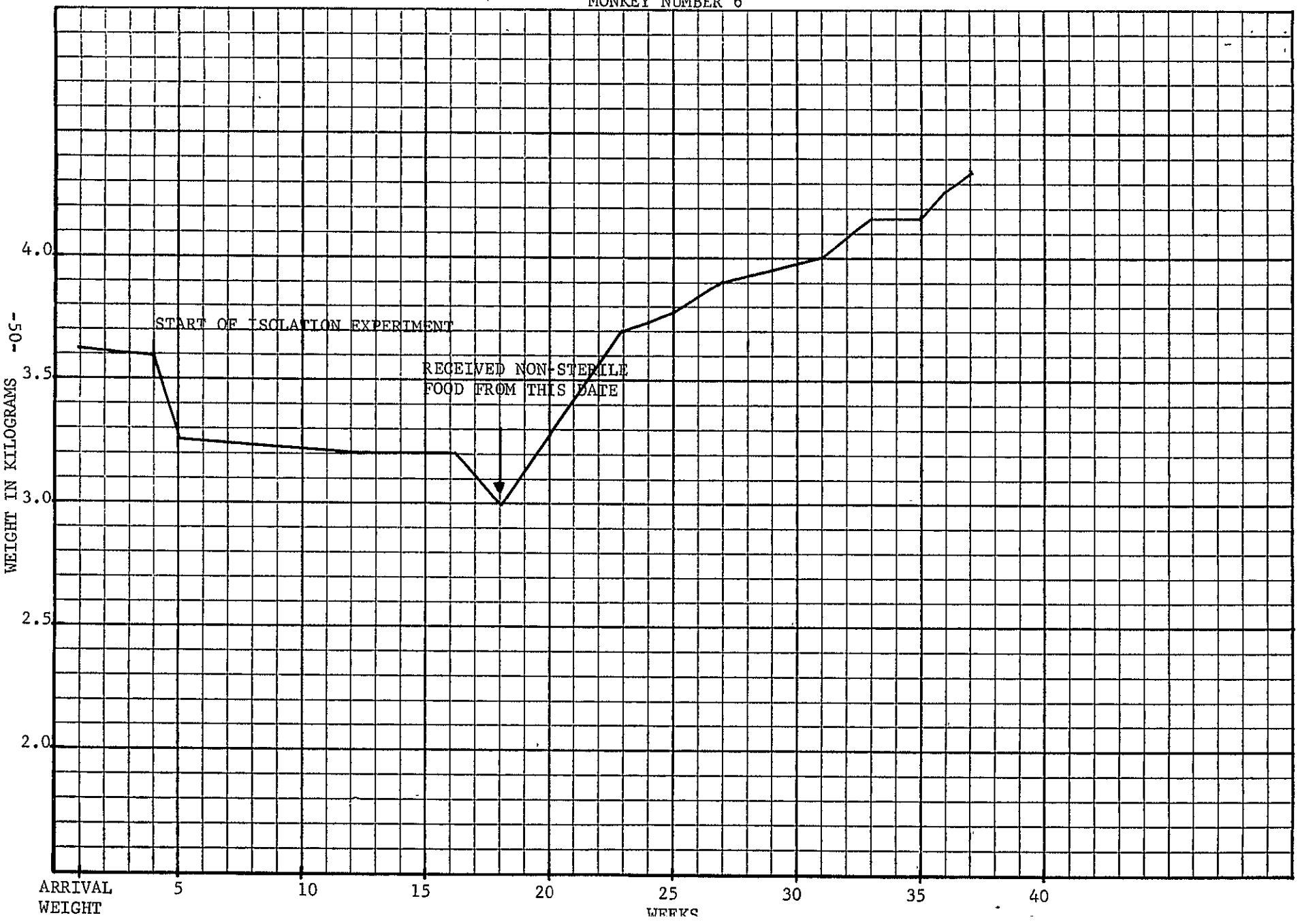


FIGURE 13
CHANGES IN WEIGHT OF PRIMATE DURING EXPERIMENT
MONKEY NUMBER 8

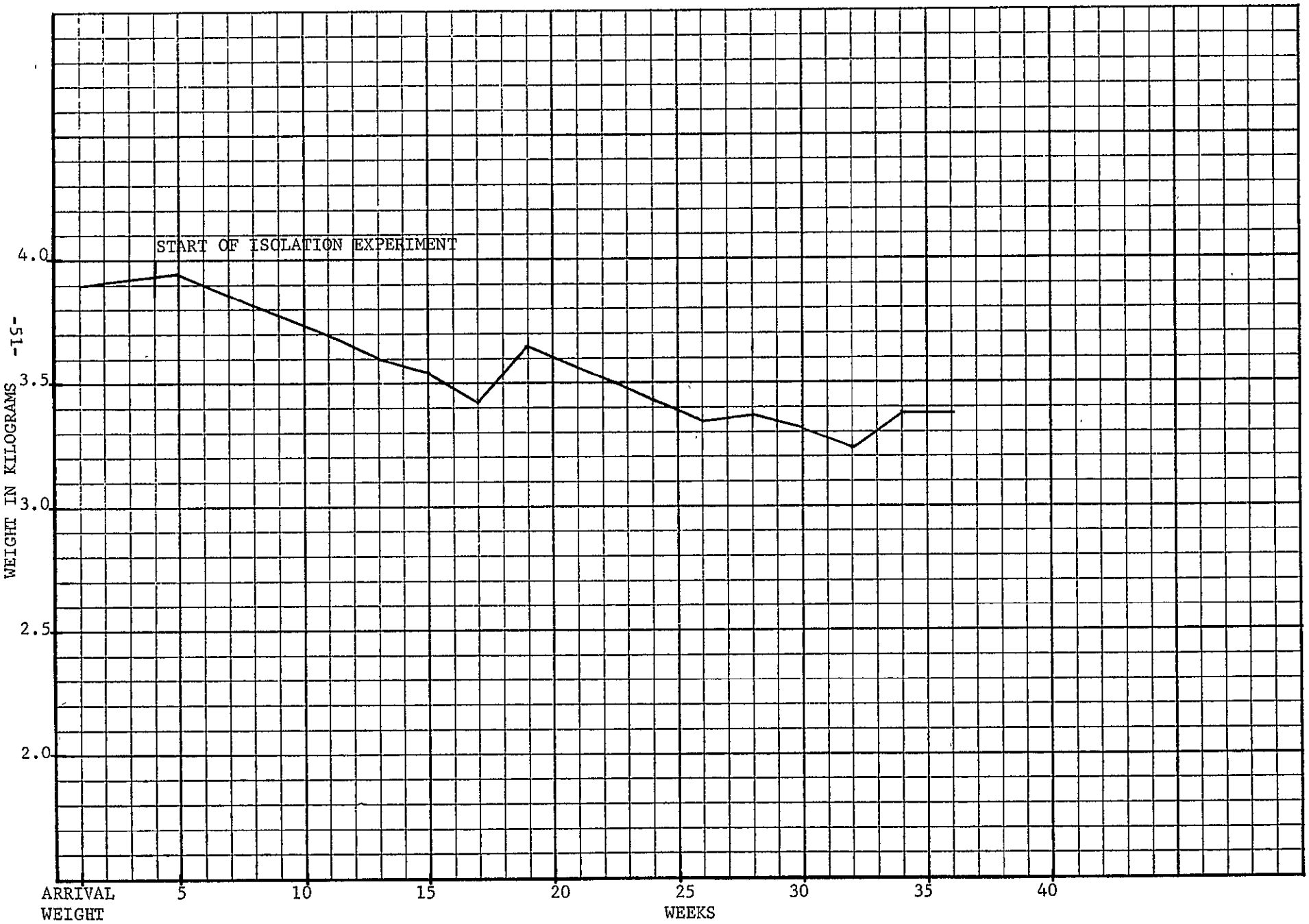


FIGURE 14
CHANGES IN WEIGHT OF PRIMATE DURING EXPERIMENT
MONKEY NUMBER 9

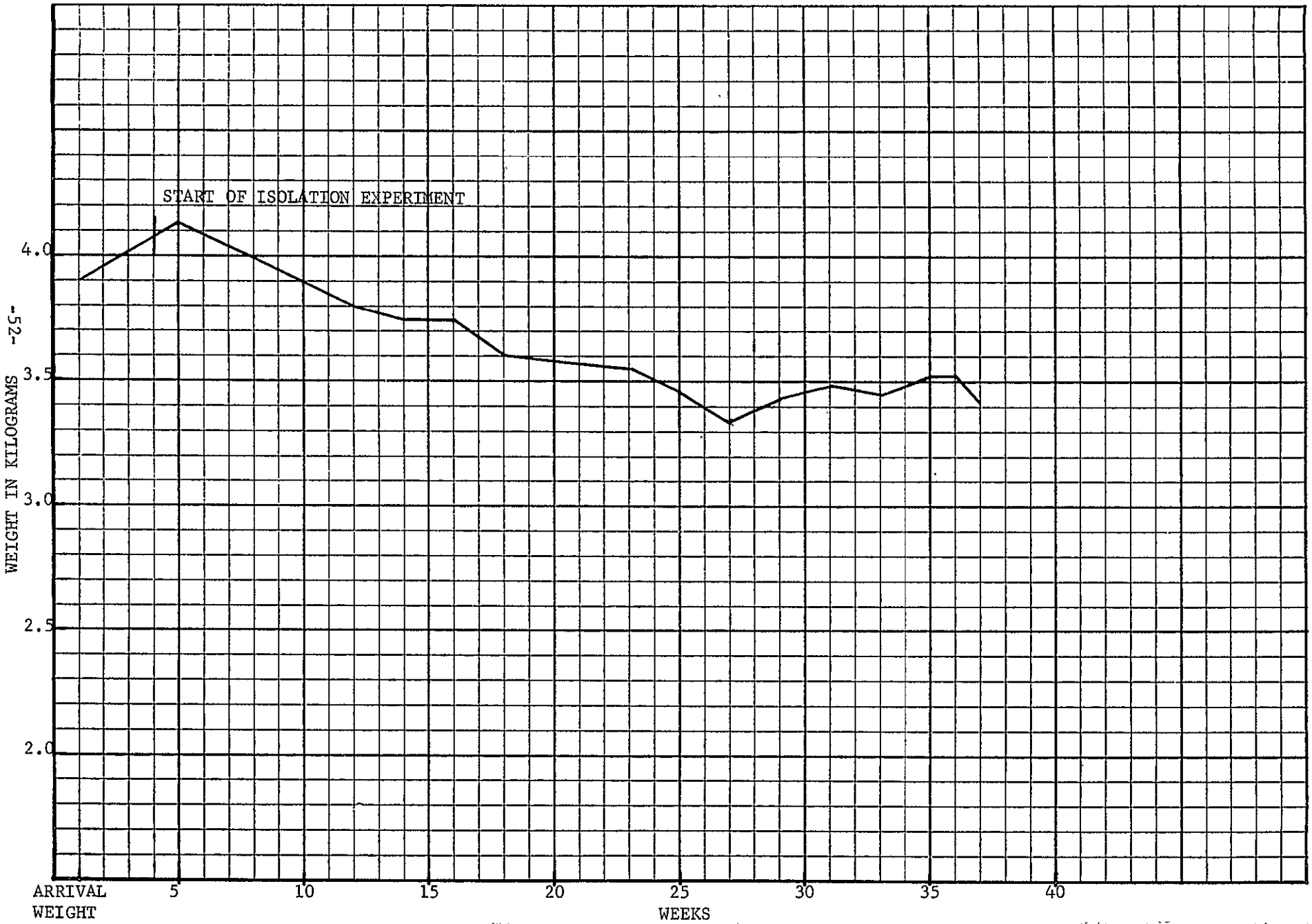


FIGURE 15
CHANGES IN WEIGHT OF PRIMATE DURING EXPERIMENT
MONKEY NUMBER 3

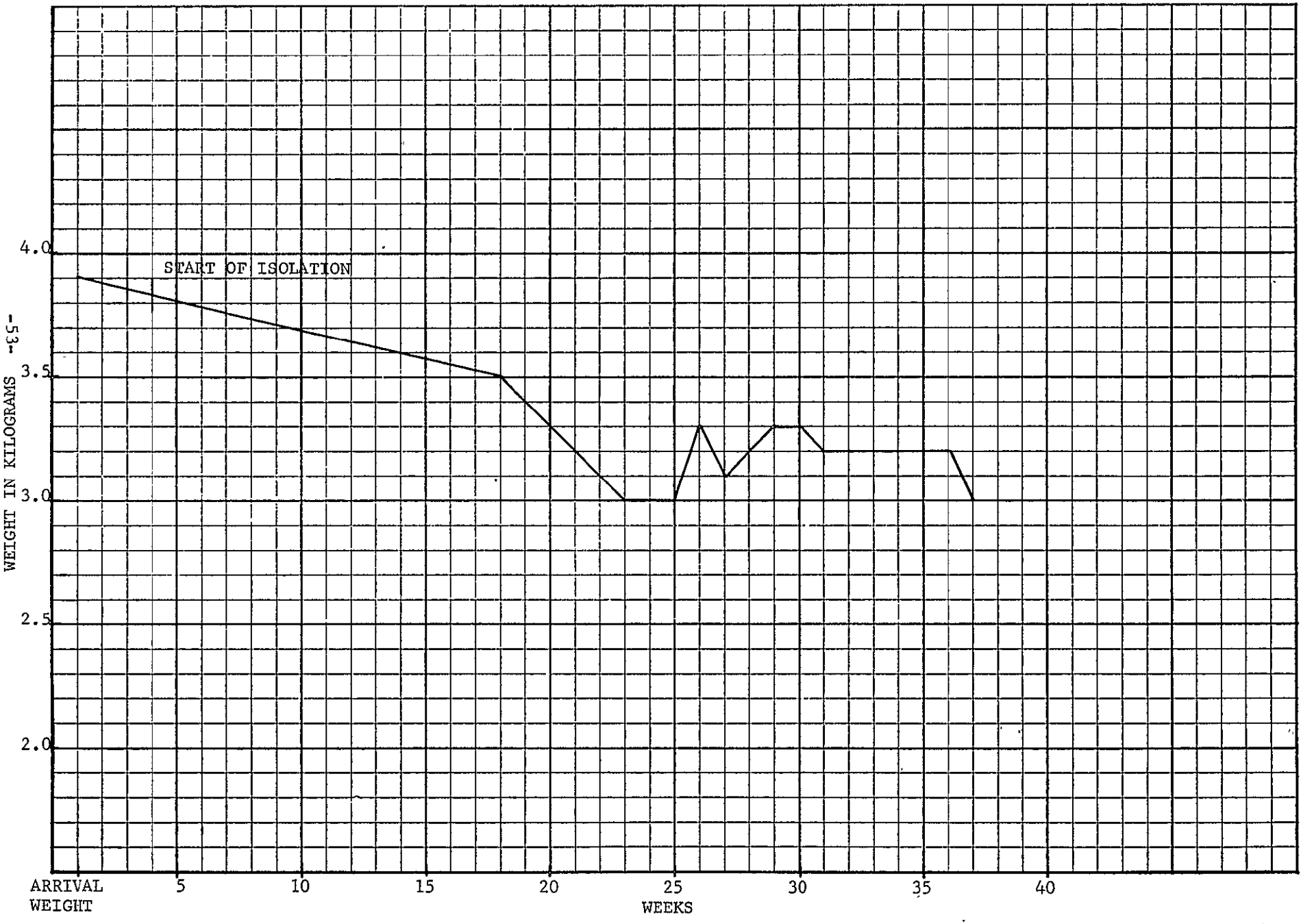


FIGURE 16
CHANGES IN WEIGHT OF PRIMATE DURING EXPERIMENT
MONKEY NUMBER 4

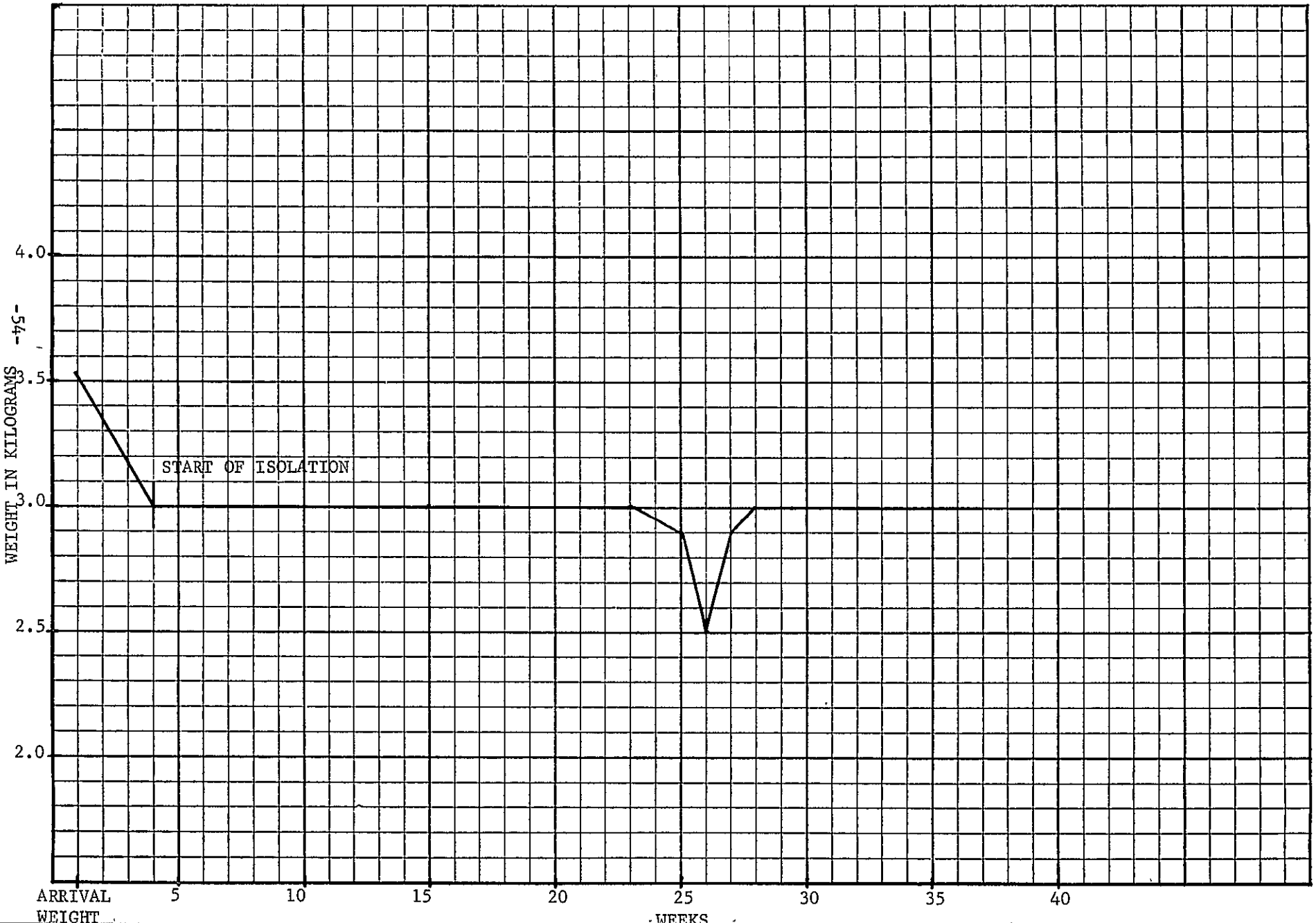
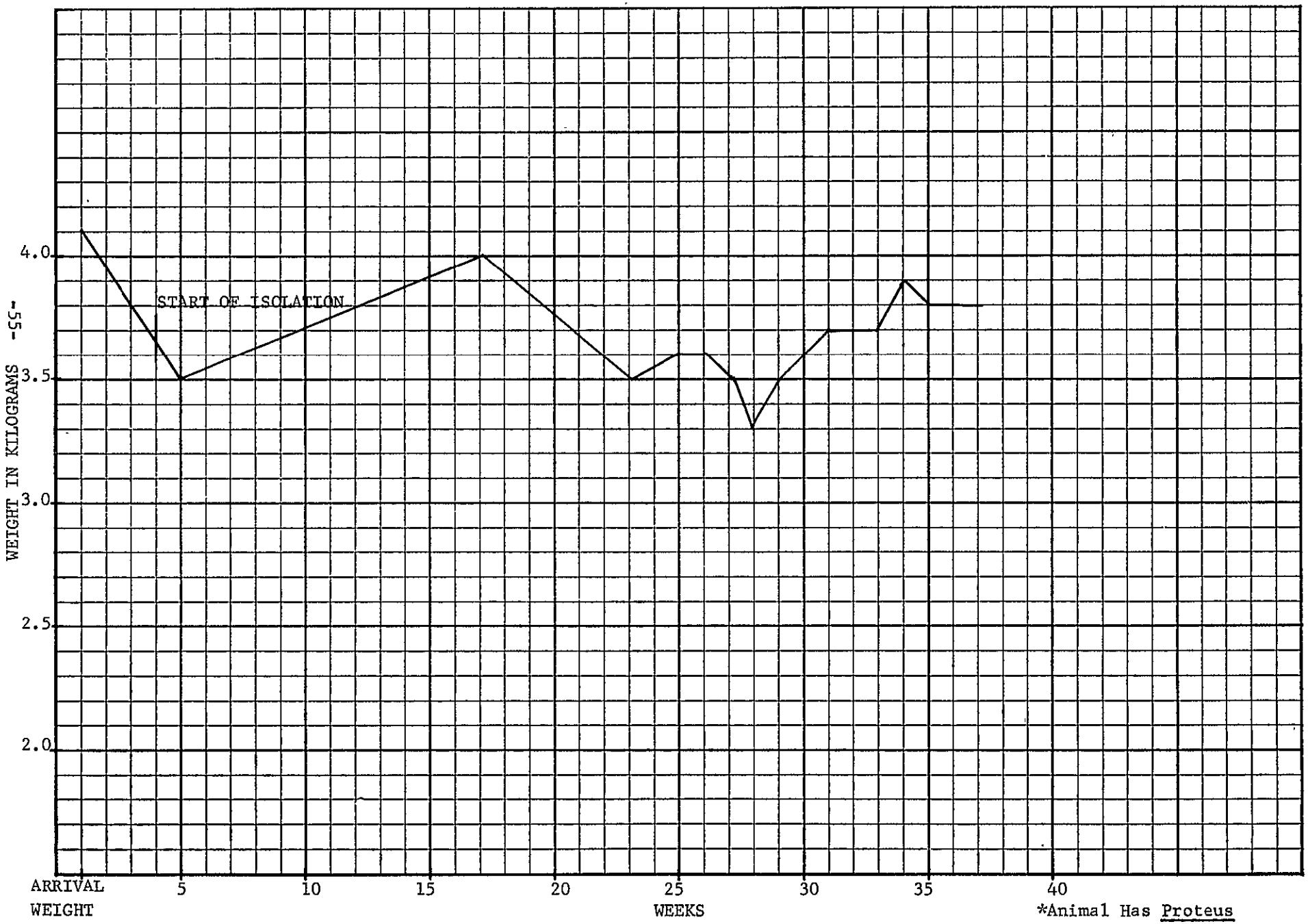
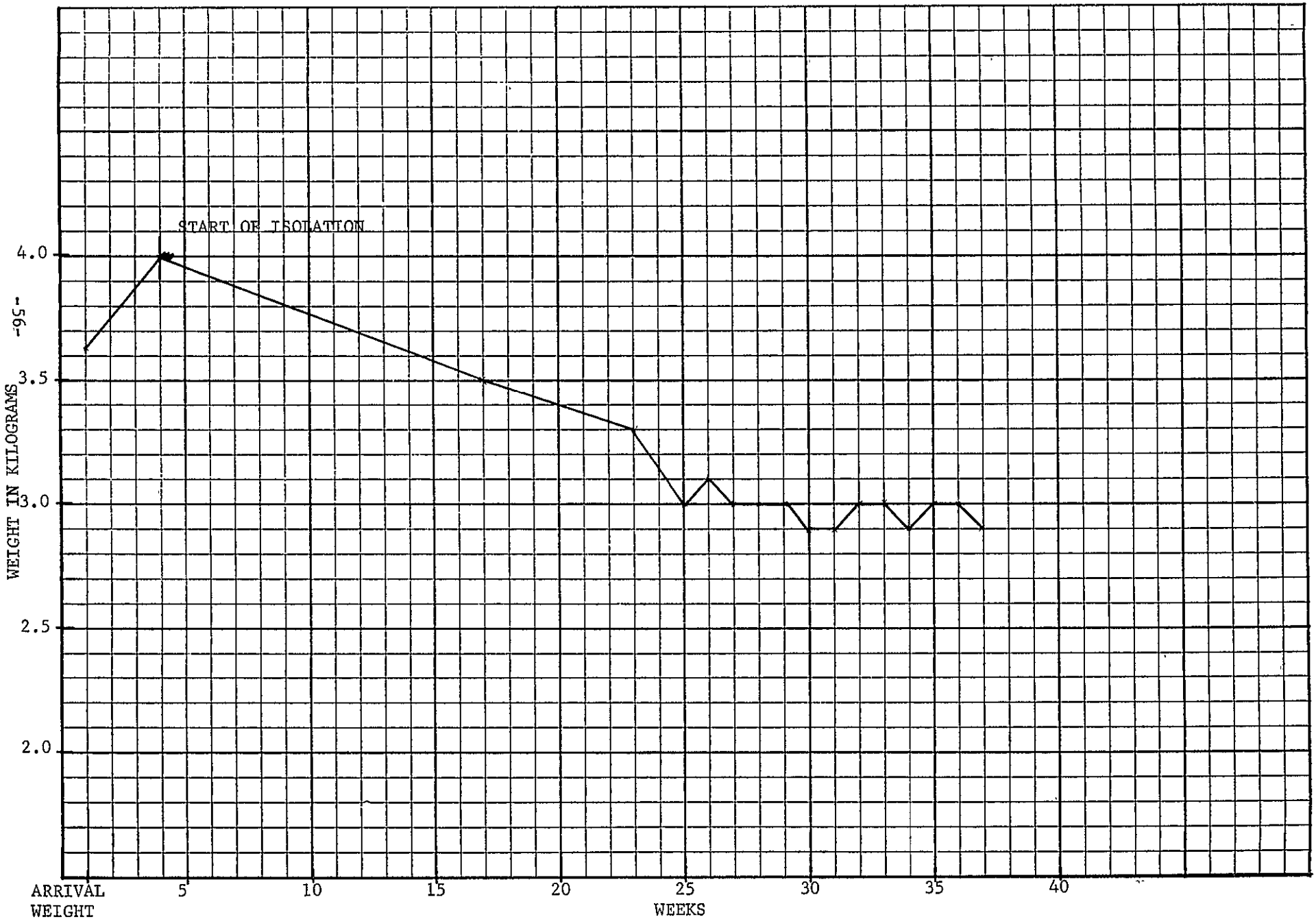


FIGURE 17
CHANGES IN WEIGHT OF PRIMATE DURING EXPERIMENT
MONKEY NUMBER 5*



*Animal Has Proteus

FIGURE 18
CHANGES IN WEIGHT OF PRIMATE DURING EXPERIMENT
MONKEY NUMBER 7



have speculated that the coliforms play a significant role in normal digestive processes and the assimilation of nutritive materials. A different diet, of course, may remove the requirement for E. coli if there truly is one. Again, before long term missions occur, the role of these microorganisms must be clarified. In Part B of this program, other rather unexpected effects of E. coli and the Apollo diet are shown. Studies on these possibilities should include synergistic and combined actions.

Table XII, microbiological profile of Animal Number 2, illustrates the complexity and number of the total isolates found in our animals. Our original plan to follow only six marker organisms quickly gave way to following the course of the predominate genera in each animal. The data, qualitative and quantitative on the eight animals are shown in Appendix B, Tables B-I through B-VIII.

Most of the organisms numerically stayed approximately the same. There was some cycling within a range of about two logs, but in no case did an overt pathogen rise to levels that appeared to endanger the animals. It is our belief that a new protective mix was gendered, and that under the conditions of the experiment, enough harmless (to the animal) commensals remained, to inhibit rise of the potential pathogens indigenous to the animals. This is no reason to believe that we were other then fortuitious! The particular diet, the general well-being of the active animals plus the continuous attention are probably factors in the animals' survival. Animal Number 7 was fortunate that his skin Staphylococci infection was evidently annoying rather than lethal. In a previous experiment (Bengson and Thomae, 1968) where a runaway rash developed (Pseudomonas aeruginosa) severe methods were called for to save

TABLE XII

ISOLATION AND IDENTIFICATION OF MICROFLORA
FOR ONE CONTROL MONKEY

Week 2-70, samples were taken from Monkey Number 2 for bacteriological examination. The sites sampled were: feces, gingiva, throat, eye and groin. Procedures for sampling and methods of analysis as per S.O.P.'s.

Tentative identification of genera as follows:

SITE	MICROORGANISM	UNIDENTIFIED TYPES		ISOLATES LOST ON SUB-CULTURING	
		AEROBES	ANAEROBES	AEROBES	ANAEROBES
Feces	Streptococci sp.	7	0	1	4
	Streptococci sp.				
	Streptococci sp.				
	Corynebacterium sp.				
	Staphylococci sp.				
	Staphylococci sp.				
	Neisseria sp.				
	Lactobacilli sp.				
	Lactobacilli sp.				
	Veillonella sp.				
	Bacteroides sp.				
	Bacteroides sp.				
	Bacteroides sp.				
Gingiva	Streptococci sp.	4	1	4	0
	Liptotrichia sp.				
	Neisseria sp.				

TABLE XII (Continued)

SITE	MICROORGANISM	UNIDENTIFIED TYPES		ISOLATES LOST ON SUB-CULTURING	
		AEROBES	ANAEROBES	AEROBES	ANAEROBES
	Streptococci sp.				
	Neisseria sp.				
	Staphylococci sp.				
	Staphylococci sp.				
	Corynebacterium sp.				
	Haemophilus sp.				
	Haemophilus sp.				
	Bacteroides sp.				
	Bacteroides sp.				
	Veillonella sp.				
	Bacteroides sp.				
	Diplococci sp.				
	Vibro sp.				
	Fusobacterium sp.				
	Fusobacterium sp.				
	Actinomyces sp.				
	Mycoplasma				
Eye	Staphylococci sp.				
	Staphylococci sp.				
Groin	Staphylococci sp.				
	Staphylococci sp.				
Throat	Similar microflora to gingiva but fewer types.				

the animal. We were forced to bathe (complete immersion and wetting) the animal in 0.1% peracetic acid to destroy the infection. This is hardly treatment to attempt with human astronauts.

The potential for disease was always present. Tables B-I through B-VIII show every animal was possessed of sufficient variety of pathogenic organisms from the start, to furnish basis for disease. So little is known about the interrelationships of overt pathogens with the other microflora of the body, not to mention the physiological and psychologic states that could contribute, that any precise statement as to why something did or did not happen is at present speculation. Empirical knowledge is all we possess.

Summary and Conclusions

Eight primates (Macaca mulatta) were studied with the objectives of determining the effects of altered ecological relationships on the host by monitoring the changes in the indigenous microflora during long-term bio-isolation. Four of the animals were supplied with sterile air, sterile food, and sterile water. Three control animals were supplied with normal air, sterile food, and sterile water and one control animal with normal air, normal (non-sterile) food, and sterile water. The animals were isolated for seven and one-half months. Initial microbial analyses of body surface areas, body cavity areas, urine and feces of each animal disclosed each animal to have an unique indigenous microflora with regards to variety, location, and numerical counts. General agreement was found only in total numbers of microflora and a few specific genera.

Bio-isolation was verified by regular sampling of the isolator interior surfaces, filters, and the circulating air systems. All materials to be introduced into the isolator were sterilized and sterility verified by test within a transfer (holding) isolator.

Following baseline isolation of microbial isolates to select the marker organisms, weekly enumeration of the selected tracer (marker) organisms was done. When isolates indicated non-tracer organisms were increasing, identification to genera was performed.

During the course of the experiment, most of the animals lost E. coli by simplification. Total number of Lactobacilli also declined. In one instance, a gram negative organism, Proteus, rose in number to partially fulfill the void left by E. coli loss. This animal was the only animal in which Proteus had been found, but the rise in numbers was not thought to be atypical but rather to be expected.

Serological and immunological studies were made on all subjects. A Standing Operating Procedure (SOP) was written to cover all phases of the Gnotobiology Laboratory operations.

An extensive bibliography has been compiled (Appendix M) in conjunction with the literature review. Statistical analyses verified the loss of microflora under the experimental conditions, was not an individual phenomena but could (98%+ certainty) be expected to occur in bio-isolation (Appendix D).

IV. RECOMMENDATIONS FOR EXPERIMENT - PART A

1. Further investigation be conducted to find suitable ways and means of "conventionalizing" personnel that have simplified or distorted their protective mix of microflora.
2. The effect of introducing "strange" microorganisms to subjects depleted of their normal flora should be studied.
3. Long term effects on natural body defenses of changed microflora should be studied.

4. Methods of achieving or holding an individual microflora mix compatible with those of an associated group should be searched out.
5. The effects of antibiotic therapy on protective mixes and the results of re-exposure to normal flora carrying individuals are unknown for other than persons with relatively high immuno defenses. The problems involved in treating individuals that are borderline normal or have delicately balanced indigenous flora or unbalanced flora should be studied.
6. The effects of different diets on the microorganism balance be further pursued. In particular, diets that tend to encourage the growth of certain microorganisms species should be investigated.
7. That as space flight time increases (per mission) that attempts to develop a more common microflora prior to flight be continued.
8. The role of particular microorganisms in relationship to nutrient utilization must be calrified.
9. Methods of identifying one or more protective mixes for individuals must be sought out.
10. Experiments on bio-isolation be conducted to extend at least one year and preferably to cover the entire period contemplated for a mission.

V. REFERENCES - PART A

1. Abrams, G. D., 1969 The normal microbial flora and resistance of the small intestine. Symposium The Germfree Animal as a Tool in Research, Leuven, Belgium.

2. Adamovich, B. A., 1969 See Burnazyan, A. I., 1969.

3. Anonymous, 1967 Schwartz Bio-research Newsletter

4. Beck, M. D., J. A. Munoz, and N. S. Scrimshaw, 1957 Studies on diarrheal disease in central america. Amer. J. of Tropical Med. Hyg., 6:62-71.

5. Bengson, M. H., and F. W. Thomae, 1965 Controlling the hazards of biological and particulate contamination within manned spacecraft. Contamination Control, 4, 11, pp. 9-12.

6. Bengson, M. H., and F. W. Thomae, 1966 Development of gnotobiotes from classic animals by isolation, In: Symposia, IX Internat. Congress for Microbiol., Moscow, USSR, pp. 399-407.

7. Bengson, M. H. and F. W. Thomae, 1968 Research in the effects of alteration of the indigenous microflora of the monkey. AMRL-TR-67-177, Aerospace Med. Research Laboratories, AMD, AFSC, Wright-Patterson AFB, Ohio.

8. Burnazyan, A. I., V. V. Parin, Y. G. Nefyodov, B. A. Adamovich, S. B. Maximimn, B. L. Goldschwend, N. M. Samsonv, and G. N. Kerikov, 1969 Year long medico-engineering experiment in a partially closed ecological system. J. Aerospace Med., 46(10), pp. 1087-1094.

9. Craven, J. and O. P. Miniats, 1969 Antagonism between Escherichia coli strains in dual infected gnotobiotic pigs. Symposium Germfree Animals as a Tool in Research, Leuven, Belgium.

10. Dubos, R, 1967 The gastrointestinal flora of the so-called normal mouse. In: The Interaction of Laboratory Animals and Associated Organisms, A. M. Brown (ed.), Carworth Europe Collected Papers, Vol. II.

11. Ducluzeau, R., and P. Raibaud, 1969 Mechanisms in the establishment of various bacterial strains in the gastro-intestinal tract and gnotoxenic animals. Symposium The Germfree Animal as a Tool in Research, Leuven, Belgium.

12. Gall, L., and P. E. Riely, 1964 Determination of aerobic and anaerobic microflora of human feces. AMRL-TR-64-107, Biomedical Lab., Aerospace Medical Research Laboratories AMD, Wright-Patterson AFB, Ohio.

13. Gorden, H. A., and L. Pesti, 1969 The effects of aging on the composition of the intestinal flora of mice housed in the open or closed environment. Symposium The Germfree Animal as a Tool in Research, Leuven, Belgium.

14. Gustafsson, B., 1968 V. Intestinal microflora and germfree life, p. 119. In: Human Ecology in Space Flight, D. H. Calloway (ed.), Volume III, New York Acad. Sci., New York, N.Y.

15. Hurst, C. A., 1965 Monthly weight increases in growing Rhesus monkeys. AMRL-TR-65-24, 6571 Aerospace Medical Laboratory, Aerospace Medical Division, Holloman Air Force Base, New Mexico.

16. Luckey, T. D., 1963 Germfree Life and Gnotobiology. Academic Press, New York, New York.

17. Luckey, T. D., 1966 Potential microbic shock in manned aerospace systems. J. Aerospace Med., 37:1223-1228.

18. Luckey, T. D., 1968 Gnotobiology and aerospace systems, pp. 317-353. In: Advances in Germfree Research and Gnotobiology, M. Miyakawa and T. D. Luckey (eds.), Chem. Rubber Press, Cleveland, Ohio.

19. Malyoth, R., and E. Sickel, 1969 Preliminary experiences with Lactobacillus bifidus in gnotobiotic life and conventionalization of pigs. Symposium Germfree Animals as a Tool in Research, Leuven, Belgium.

20. Mata, L. J., C. Carrillo, and E. Villatoro, 1969 Fecal microflora in healthy persons in a preindustrial region. Appl. Microbiol., 17:4, 596-602.

21. Mata, L. J., J. J. Urrirtia, B. Garcia, R. Fernandex, and M. Behar, 1969 Shigella infection in breast-fed Guatemalon Indian neonates. Amer. J. Dis. Child., Vol. 117:142-146.

22. Milville, G. S., W. H. Whitcomb, and R. S. Martinez, 1967 Hematology of the Macaca mulatta monkey. Lab. Animal Care, 17:No. 2, 189-198.

23. Moyer, J. E., and Y. E. Lewis, 1964 Microbiologic studies of the two-man space cabin simulation: Interchange of oral and intestinal bacteria. Document SAM-TDR-64-3, USAF School of Aerospace Medicine, Brooks Air Force Base, Texas.

24. Phillips, A., 1966 Candida albicans in the gnotobiotic animal. In: Symposia, IX International Congress for Microbiology, Moscow, USSR.

25. Seelig, M., 1966 Mechanisms by which antibiotics increase the incidence of candidasis and alter immunological defenses. Bact. Rev., 30:2, 442-559.

26. Smith, E. B., P. R. Beamer, F. Vellios, and D. M. Shultz, 1959 Principles of Human Pathology. Oxford Univ. Press, New York, p. 268.

27. Tanami, J., 1967 Antagonistic and symbiotic state of bacteria in the intestinal tract of gnotobiotic animals. In: Symposia IX International Congress for Microbiology, Moscow, USSR, pp. 299-313

28. Thomae, F. W., H. Kaplan, and M. H. Bengson, 1968 Establishment and maintenance of a dominant intestinal flora by nutritional means, pp. 366-369. In: Advances in Germfree Research and Gnotobiology, M. Miyakawa and T. D. Luckey (eds), Chemical Rubber Press, Cleveland, Ohio.

29. Van der Waaij, D., J. M. de Vries, and L. Lebberberk, 1969 Colonization resistance in the digestive tract of conventional entero-bacteriaceae-free and in antibiotic-decontaminated mice. Symposium The Germfree Animal as a Tool in Research, Leuven, Belgium.

30. Wagner, M., and T. Starr, 1968 Microbic interactions in the gnotobiotic mouse. In: Germfree Biology, Plenum Press, New York, New York.

31. Wilkens, J. R., 1967 Man, his environment and microbiological problems in long term space flight. Document NASA-TM-X-60422, National Aeronautics and Space Administration, Washington, D.C.

32. Winitz, J., 1966 Chemical diet: Clinical applications seen. J.A.M.A., 196, 6, 35.

33. Young, A. and J. Weston, 1969 A mathematical model of the effect of a predator species diversity. Jet Propulsion Laboratories, Technical Report 32-1359, Pasadena, California.

INTRODUCTION - PART B

The two hypotheses tested during this program are based on historically important concepts. The first began a century ago when Liebig and others applied the principles of organic analysis to foodstuffs with the preconceived idea that the analytical data from feeds could be used to determine the most economical system for livestock production. Tables of protein, carbohydrate and lipid composition of foods were accumulated and used widely. Discerning experimenters added mineral ash to this list beginning about 1880. About 1900, doubts arose concerning the validity of the basic concept (McCollum, 1964). During the first decade of this century, the work of Hopkins in England and Osborn and Mendel of U.S.A. showed that one misconception was that proteins were equal; the total nitrogen of feeds actually does not provide a reliable index of the biological value of proteins because it cannot reflect the content of individual essential amino acids. The next two decades confirmed as fact that the gross composition of foodstuffs does not accurately reflect biological value. Proximate analysis, it was learned, does not reveal the vitamin and trace element contents. Thereafter food tables were developed which included vitamin and mineral (including some trace element) data. It was believed this data combined with information on known requirements would allow the formulation of diets adequate for man or beast. That this too was a misconception was shown by the dramatic failures obtained from feeding U.S. Army K rations to rats and monkeys. These results are as yet unpublished to our knowledge and both the reporter (Dr. C. A. Elvehjem) and the principal investigator (Dr. H. Spector) are deceased. Others associated with the work are reluctant to provide concrete data. Disregarding this lack of reference, the fallacy of the concept was shown when attempts were made to correlate

TABLE XIII

LOCKED FLORA STUDIES

STATE*	SPECIES	FINDING	DATE AND AUTHOR
3	Dogs, Rabbits, Guinea Pigs, Pigeons	Decreased N_2 utilization and death	1895-1916, Kianizin
3	Guinea Pigs	Weight loss and death	1901, Charrin and Guillemonat
5	Guinea Pigs	Weight loss and death	1931, Reagen
3	Guinea Pigs	Survival with flora simplification	1941, Nelson
4	Rats	Survival and dramatic flora simplification	1942, Reback
6 & 7	Rats	Complete microbial sterility attained	1963, Luckey
0	Man	Cross infection	1964, Moyer and Lewis
0	Man	Increased bacteria on body	1964, Gall and Riely
0	Man	Simplification toward pathogens	1966, Boeing
7	Monkey	Microflora changes	1967, Bengson, Prince and Thomae
6	Mice	Bacteria free status	1967, Van der Waaij
7	Monkey	Simplification	1969, Bengson and Geating**

*CODE
0 = Virtually no aspetic procedures within the closed chamber.
1 = Sterile food.
2 = As 1, plus sterile air.
3 = As 2, in sterilized environment.
4 = As 3, with bacteriostatic and/or bactericidal drug administration.
5 = As 3, with frequent cleaning or transfer.
6 = Procedures of 4 and 5.
7 = Gnotobiotic conditions.

**Reference, this present report Part A.

biological activity with microbiological assay of some of the B-vitamins. The classic work of Snell (1945) showed that elements of the vitamin B_6 complex were more than one thousand times as active for assay microorganisms than for animals. The work on folic acid, vitamin B_6 and biotin (Luckey et al., 1944-46) complexes are less dramatic but confirm the idea that some members of the B-vitamin complex are more active for microorganisms than for animals.

This was the background that makes it important to evaluate the Apollo diets biologically. The diet (used in this experiment) was designed to be adequate according to calculations from food analysis, and it had been found to be adequate according to subsequent chemical and microbiological assays for individual nutrients. But was it actually adequate for rearing mammals? Are our analytical methods presently adequate to give assurance of biological value without bioassay? This is the conceptual basis for the major hypothesis tested herein.

The second hypothesis involved is: prolonged isolation will cause a simplification of the flora in the intestine. The consequences of this may include the formation of a dominant flora which will markedly alter the nutritive requirements of the host. Therefore, it was pertinent to feed Apollo diet to mice which had known microorganisms representing different parts of the intestinal flora. The base for this hypothesis has been previously reviewed (Luckey, 1968) and is summarized in Table XIII. This concept is partially validated by the disastrous effects of adding potentially pathogenic strains of bacteria from the intestinal tract to germfree animals; i.e., the resultant deaths when Escherichia coli was added to germfree guinea pigs by Tanami (1959). These and other experiments were reviewed in "Gnotobiology and Aerospace Systems" by Luckey (1968).

Isolator Problems

Of the wide variety of things which may happen to germfree animals, gnotophoric animals and astronauts, only one will be discussed in detail. Psychologic problems of claustrophobia or of agoraphobia, sociologic problems of maintaining discipline for a long period of time, physical problems for disuse of certain organs, from the inefficiency of physical operations without a toe hold, and from a variety of stresses which will arise, will not be discussed. An appropriate concept to present is the potential for microbial disturbance in prolonged manned aerospace system which has been reviewed by Luckey (1968).

Evidence from the exploratory isolation experiments which have been done to date suggests that the simple act of isolation of a single animal in a cage may have profound effect upon that animal; however, data from control animals are generally missing. If a contaminated animal is isolated in a sterile environment and provided with sterile food, water and air, a dramatic change is apparent in the microbial flora within a few months. The microflora of the intestine will simplify and often the total flora of the animal and environment may be reduced by autodisinfection to very few species of microorganisms. Usually one of the microorganisms found is a mold which does not affect the intestines, and often one of the microorganisms found in the intestines is a yeast. The bacterial species found seems to vary according to unknown variables. Reagan (1931) found only E. coli and Bacillus subtilis, with no other bacteria present in the flora of isolated guinea pigs. Nelson (1941) could only find one bacterial species and one yeast in the intestinal flora of isolated guinea pigs. The pattern of autodisinfection was confirmed by Rebeck (1942) who sometimes found two microorganisms and no yeast.

Work with J. R. Pleasants in the Lobund Laboratory indicated that germfree rats which became monocontaminated could be decontaminated. No organisms could be detected. To the extent that laboratory tests were reliable, the rats were again germfree. In these experiments, the animals were transferred daily with germicidal (0.1% peracetic acid) baths from one isolator to the other. Detergents and bacteristatic drugs were used only in the Reback and Lobund experiments. The earlier studies of Bengson, Prince and Thomae (1967) are reported elsewhere. Unfortunately, the early work of Kianizin (1916) and of Charrin and Guillemonat (1901) was done before vitamin destruction by heat sterilization was appreciated. Thus, their results showing a decrease of nitrogen utilization, weight loss and death in isolated animals may be open to this interpretation. It is interesting to note that both Schmidt (1965) and Zablotny (1928) have found hibernating animals to be resistant to infectious microorganisms. Starvation and low carbohydrate diets were shown to reduce the microflora in animals by Porter and Rettger (1940).

Evidence from the animal work cited above and that of Part A of this experiment suggests that there will be a predictable simplification of the flora of any astronaut in isolation for a prolonged period of time. The effects of more than one animal per cage and the introduction of other variables have not been studied. These locked flora animal studies suggest that there are no truly indigenous microorganisms of the intestinal tract which are not subject to change by diet or other conditions. There must be an interaction and equilibrium established between the intestinal microflora and the external environment. Luckey's (1965) review of studies on monoflora in animals suggests that any given species in the intestinal tract will populate that area to about the same density as there are total microorganisms of a mixed population.

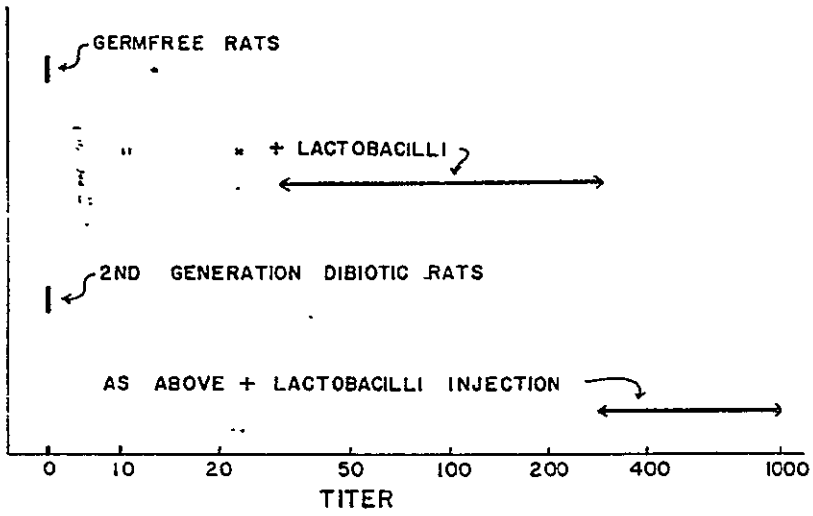
The experiments with human subjects have been less vigorously controlled, and show few consistent effects. They should be repeated, with conditions closely simulating those of astronauts on prolonged flights. Studies on the changes of microflora in men isolated in small groups gave varying results. In one experiment (Boeing Company, 1965) the limited bacteriologic data indicated a simplification of the microflora of the nose, throat and mouth with an almost pure culture of Staphylococcus aureus in the throat and nose and primarily Vincent's organism in the mouth. Moyer and Lewis (1964) found evidence of cross-infection between confined men. Examination of the fecal flora of men in group isolation by Gall and Riely (1964) showed a shift in both the anaerobic and aerobic flora. Shigella and enteropathogenic types of E. coli were frequently cultured. Candida species occurred with unusually high frequency. Unusual organisms were noted; a decrease in enterococci seemed to complement the rise seen in coliforms; and new types of cultures of anaerobic bacteria were found. It would be most interesting to have these experiments on humans repeated under strict isolation, with sterile food, water and air provided for several months. Feeding synthetic diets to humans was associated with fast, dramatic decrease in the intestinal microflora according to Winitz and co-workers (1966).

Since the change in intestinal microflora may cause discomfort and disease, better isolation conditions and other variables should be imposed in future studies. Manned aerospace systems will eventually involve periods of time which will be long enough to allow natural decay rates of bacteria on surfaces and dry foods to greatly reduce the numbers of microbic species within the environment. It is anticipated that filtered air and manufactured or condensed water will be provided for the astronauts. Much of the food will be processed

in such a manner that bacterial contamination will be low. This may be considered a modified locked-flora system with the potential that the flora will simplify. The "control" primates of Part A were subject to such conditions and E. coli was lost. The dominant species may or may not be compatible with the well-being of the host. The effects of stress, disease, medication, radiation and possible sources of re-inoculation should be systematically presented to study their effect upon intestinal microflora simplification. Studies of Tanami (1959) previously discussed in Part A suggest that certain strains of E. coli should not be allowed to become dominant, although E. coli seems to be an important member of the human "protective mix". If harmful microflora became dominant, disaster would be eminent. Administration of germicidal drugs will lead to further flora simplification, possibly to germfreeness (Van der Waijj, 1969). This suggests that a Streptococci or Lactobacilli inoculum should be routinely provided if a balance with predominantly innocuous microorganisms is to be maintained. However, if a microflora of such an innocuous microorganism is maintained, this does not seem to provide adequate stimuli to maintain our defense mechanisms. This was illustrated (Figure 19) in the experiment of Wagner (1959) in which second and third generation Lactobacilli monoflora rats did not develop antibodies against their monoflora. If the Lactobacilli were inoculated into these monoflora rats, then antibody was produced. Apparently the defense mechanisms of the mucosa membranes are more than adequate to keep out Lactobacilli with low invasive potential. This robs the body of the stimulus needed to provide other defense mechanisms. If the prolongation of the manned aerospace system included the establishment of a colony in a hostile environment where there was no recontamination, the astronauts, and particularly the second

FIGURE 19

ANTIBODY PRODUCTION - LACTOBACILLUS SP.



generation astrobabies, may not be exposed to the continuous microbial attack which must be important to the normal development of their defense mechanisms.

II. THE EXPERIMENT - PART B

Procedures

The gnotobiologic evaluation of Apollo-68 diet was carried out in the Gnotobiology Laboratories at the Valley Forge Space Center (VFSC) of the General Electric Company at King of Prussia, Pennsylvania.

Classic mice in the "open" laboratory were reared in the conventional manner. The isolated mice were reared in standard plastic isolators with strict adherence to accepted gnotobiotic procedures (Figure 20). Each isolator (20 mice) were equipped as shown in Table XIV. The procedures and protocol used were according to conventional Standing Operating Procedures with examples of the ones used, directed to this experiment, attached to Appendix K. Where applicable, the procedures of Huempfner (1967), the GF* Supply Division Instructions for Isolator Set-Up and Use and the Report of the Committee on Standardized Procedures for the Microbiological Monitoring of Gnotobiotic Animals issued by the Association for Gnotobiotics, were utilized.

Experimental Design

The original experimental design proposed a preliminary experiment with commercial** mouse food to check out the methods and personnel skills. This experiment, using Groups 11-14, is discussed in detail in Appendix L. Following this, mice fed the Apollo diet were to be maintained through three generations. The diet, environment and microbic state for each group of 20 mice is shown in Table XV. As the program developed, events and results made it expedient to deviate from the original design to a revision. This design is given in

*G.F. Supply, A Division of Standard Safety Equipment Company, 431 North Quentin Road, Palatine, Illinois, 60067

**Commercial food used was Purina Lab Chow Diet 5010C, Product of Ralston-Purina Company, St. Louis, Missouri

FIGURE 20

OPEN ISOLATOR LABORATORY CONDITIONS

FIGURE 20 (Continued)

TABLE XIV

MOUSE ISOLATOR EQUIPMENT LIST (INTERNAL)

ITEM	QUANTITY
1. Cages Polycarbonate	5
2. Lids, Stainless Steel	5
3. Water Bottles, Molded Plastic	6
4. Stainless Steel Sippers	6
5. Rubber Stoppers Size One Hole	6
Bel-Art Size #6 Solid	2
6. Triple Beam Balance, Ohaus	1
7. Forceps, 250 mm Stainless (AHT)	1
8. Surgical Scissors, 6½" (Clay Adams)	1
9. Plast-O-Mat Ribbed Plastic Floor Mat	1
10. Protective Working Gloves (Canvas)	1 Set
11. 500 ml Square Pak Water Bottles (AMSCO)	2
12. Wire Grid Gage Floors	5
13. Culture Tubes, Screw Caps	24
14. Cotton Swabs, Sterile (Swubes)	24
15. Test Tube Rack	1
16. Wide Mouth Jars Mayonaise	2
17. Rubber Tips for Forceps	3
18. Tall Form Weighing Beakers	1
19. Food Cans	2-8
20. Feeders (Small Cones)	40
21. Can Opener Wind Up Type	1
22. Sterile Dyes	
23. Tweezers, Long	1
24. Aluminum Foil, 12 x 8 Sheets	5
25. Toweling	
26. Disposal Bags, Plastic	10

TABLE XV

PART B

GROUP IDENTITY IN PROPOSED EXPERIMENTAL DESIGN

GROUP*	DIET	DIET STERILITY	ENVIRONMENT	MICROBIC STATE	MICE
1	Apollo	+	Germfree	Germfree	20
2	Apollo	+	Gnotobiotic	Coli	20
3	Apollo	+	Gnotobiotic	Lactic	20
4	Apollo	+	Gnotobiotic	Candida	20
5	Apollo	+	Gnotobiotic	Coli + Lactic	20
6	Apollo	+	Gnotobiotic	Coli + Candida	20
7	Apollo	+	Gnotobiotic	Lactic + Candida	20
8	Apollo	+	Germfree	Classic	20
9	Apollo	+	Classic	Classic	20
10	Apollo	No	Classic	Classic	20
11	Lab	+	Germfree	Germfree	20
12	Lab	+	Germfree	Classic	20
13	Lab	+	Classic	Classic	20
14	Lab	No	Classic	Classic	20

*All received sterile distilled water.

Table XVI. One design change resulted from the receipt from the vendor of monocontaminated mice on the first order for germfree mice. Following confirmation in our laboratory that the monocontaminant was Staphylococcus epidermidis, it was decided to use two additional groups of animals incorporating this microbe: Group 15 with Staphylococci alone and Group 16 with a diflora of Staphylococci plus Candida. A second change was inaugurated when it became obvious that most animals in isolation were not reproducing. New colonies of germfree mice were purchased and used with one modification to the environment: filter paper squares were added as bedding following the food efficiency determination (Figure 21).

A preliminary experiment (Groups 11-14 inclusive) is presented in Appendix L in the form that it was given at the AALAS Meeting by H. Kaplan. The preliminary work assured us that the major experiment (this contract) was feasible and that the routines developed were satisfactory for vigorous germfree and gnotobiotic experiments. Figure 22 graphically illustrates the results.

All animals were of the ICR strain and purchased from a single source: Charles River Breeding Laboratory, Wilmington, Massachusetts. Table XVII provides information on the receipt, condition and use of animals from the commercial source. This table does not reflect groups of animals begun from young weaned during the course of this experiment. Each group began with 20 mice divided into four cages with three males and two females placed together. The polycarbonate cages had stainless 3/8" wire screen floors and tops. These were changed weekly in all groups excepting 8 and 26 where they were changed daily. Food and deionized or distilled water (resistance > 1 million ohms) were provided from glass containers ad libitum. A

TABLE XVI

PART B

FINAL EXPERIMENTAL DESIGN

GROUP IDENTIFICATION	DIET AND STERILITY	ENVIRONMENT	MICROBIC STATE
1	A+	GF	GF
2	A+	Gn	E. coli
3	A+	Gn	Lactobacillus
4	A+	Gn	Candida
5	A+	Gn	Coli + Lactic
6	A+	Gn	Coli + Candida
7	A+	Gn	Lactic + Candida
8	A+	Iso	Classic
9	A+	C	Classic
10	A-	C	Classic
11	L+	GF	GF
12	L+	Iso	Classic
13	L+	C	Classic
14	L-	C	Classic
*15	A+	Gn	Staph.
*16	A+	Gn	Staph. + Candida
17	A+	Gn	Bacterioides
18 as No. 1	A+	GF	GF
19 as No. 1	A+	GF	GF
20 as No. 2	A+	Gn	E. coli
21 as No. 2	A+	Gn	E. coli
22 as No. 3	A+	Gn	Lactobacillus
23 as No. 3	A+	Gn	Lactobacillus
24 as No. 4	A+	Gn	Candida
25 as No. 5	A+	Gn	Coli + Lactic
26 as No. 5	A+	Gn	Coli + Lactic
27 as No. 6	A+	Gn	Coli + Candida
28 as No. 6	A+	Gn	Coli + Candida
29 as No. 7	A+	Gn	Lactics + Candida
30 as No. 7	A+	Gn	Lactics + Candida
31 from No. 8	A+	Iso	Classic
32 from No. 31	A+	Iso	Classic
33 from No. 9	A+	C	Classic
34 from No. 10	A-	C	Classic
35 from No. 33	A+	C	Classic
36 from No. 34	A-	C	Classic
37 as No. 11	L+	GF	GF
38 Cont. No. 2	A+	Gn	E. coli + Staph.
39 Cont. No. 3	A+	Gn	Lactic + Staph.
40 Cont. No. 5	A+	Gn	E. coli + Lactic + Staph.
41 Cont. No. 6	A+	Gn	E. coli + Candida + Staph.
42 Cont. No. 7	A+	Gn	Lactic + Candida + Staph.

KEY:

Diet A = Apollo
L = Laboratory Mouse Diet (Purina Autoclavable No. 5010C)

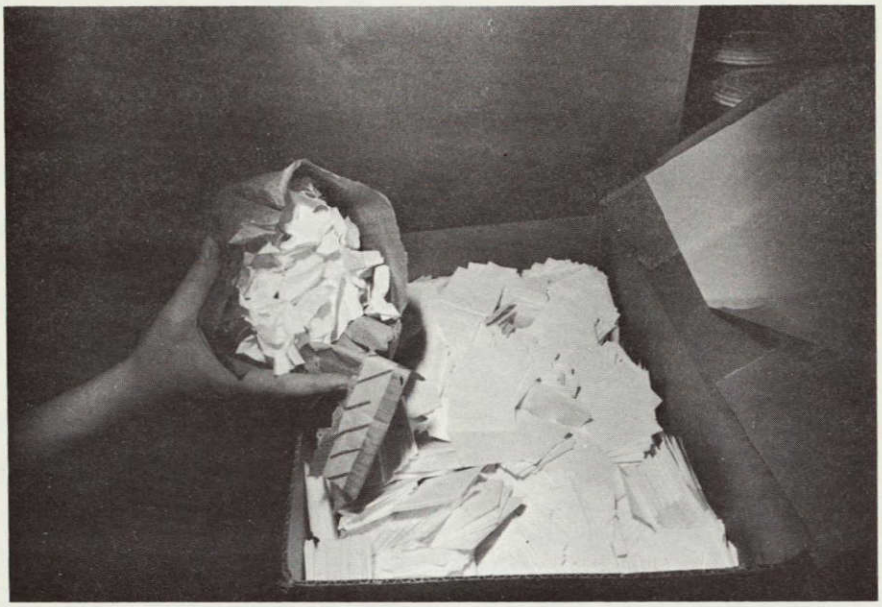
Sterility + = Sterile
- = Non-sterile

Environment GF = Germfree
Gn = Gnotobiotic
C = Classic
Iso = Classic Animals in isolation with germfree procedures

*Arrived contaminated with Staphylococcus epidermidis

FIGURE 21

FILTER PAPER BEDDING AND ITS USE



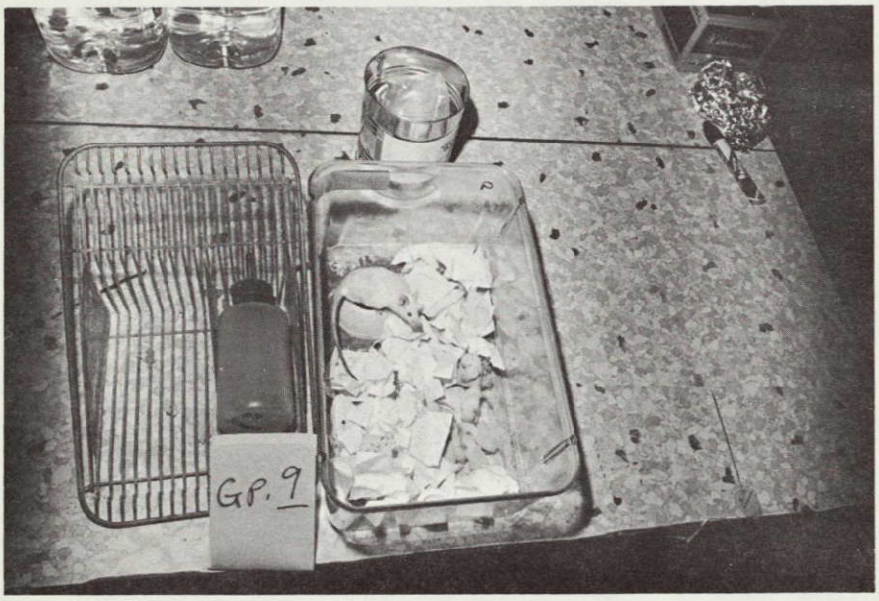
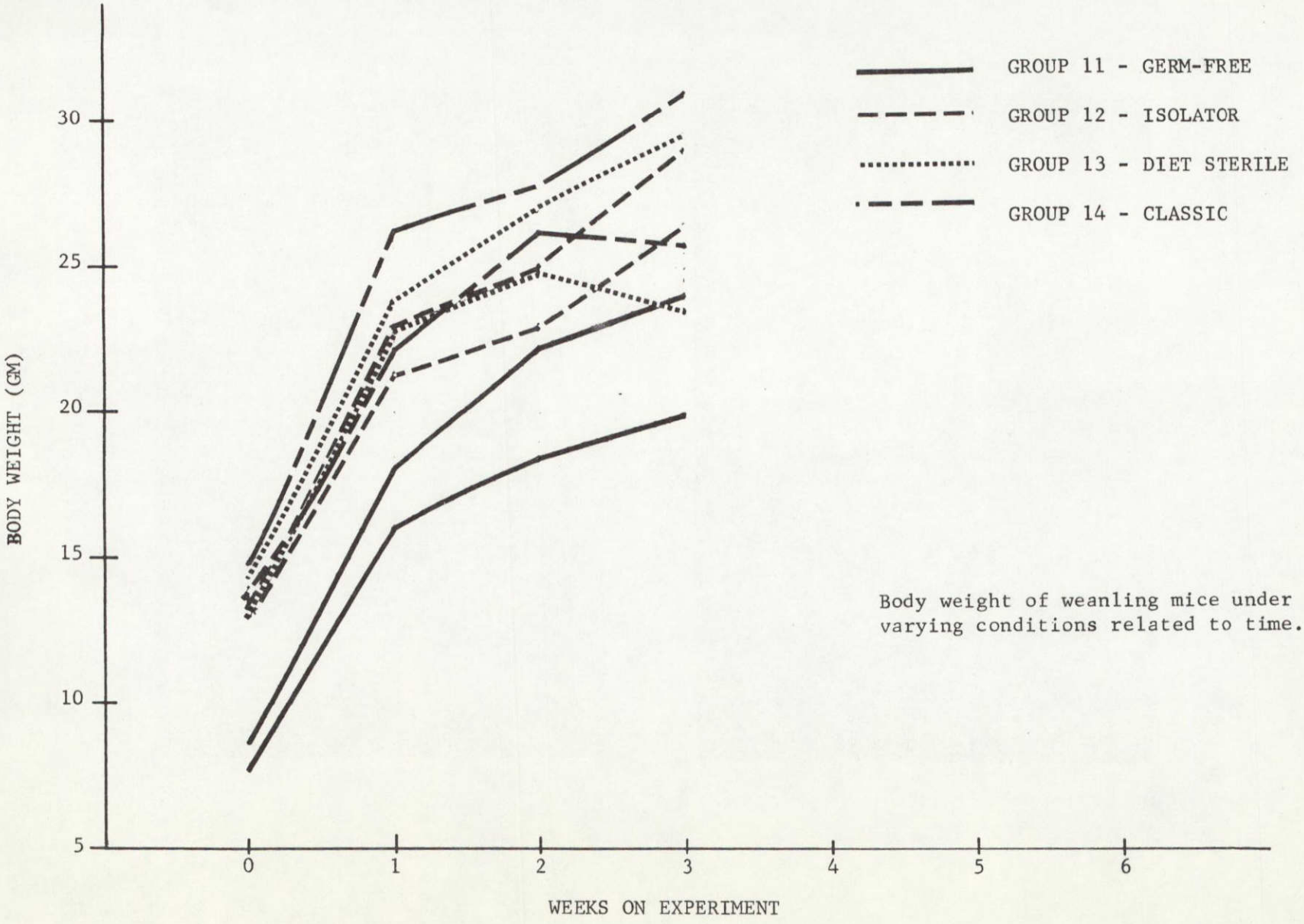


FIGURE 22

RESULTS OF PRELIMINARY FOOD EFFICIENCY EXPERIMENT



Body weight of weanling mice under varying conditions related to time.

TABLE XVII

ACQUISITION, APPARENT CONDITION AND DISTRIBUTION OF CD-1 MICE AT TIME OF RECEIPT

DATE MICE RECEIVED	NUMBER	SEX	APPARENT CONDITION	TYPE	DISTRIBUTION GROUPS 3 FEMALES/2 MALES	USE
3/6/69	36	Female	Good	Classic	12 and 14	Control studies using Classic Diet-Sterile and Unsterile
	24	Male	Good	Classic	12 and 14	
	12	Female	Good	Germfree	11 and 13	
	8	Male	Good	Germfree	11 and 13	
5/19/69	120	Female	Good	Classic	8, 9, and 10	First Generation on Apollo Diet
	80	Male	Good	Classic	8, 9, and 10	
	86	Female	Good	Germfree*	1 through 7**	
	58	Male	Good	Germfree*	1 through 7**	
6/10/69	86	Female	Poor	Germfree	1 through 7	Restart of mice due to contaminant in mice received 5/19/69
	58	Male	Poor	Germfree	1 through 7	
8/29/69	45	Female	Fair	Germfree	17, 18, 19, 20, 22, 25, 27, and 29	To avoid total loss of Groups 1, 2, 3, 5, 6, and 7 plus start of Group 17.
	15	Male	Fair	Germfree		
10/6/69	86	Female	Good	Germfree	19, 21, 23, 24, 26, 28, and 30	Restart of Group 1 through 7 in lieu of progeny
	56	Male	Good	Germfree		

*Group received contaminated with Staphylococcus epidermidis.

**Groups 15 and 16 added to experimental design. All other mice destroyed.

12-hour light cycle was followed. The temperature was maintained at 26 ±4°C. The Air flow was ±1.5 cfm and the laboratory relative humidity was 50%.

Food Efficiency

Food efficiency data were obtained during a 5-day period. Weighed quantities of food were placed in conical feeders which were placed on 15 cm petri plates. Aluminum foil was placed under the 3/8" wire mesh floor. This provided adequate provisions for the minimizing of waste and the collection of wasted food with excreta. This waste was collected, dried, weighed and an estimate made of the fecal and food contribution to the whole. The food lost plus that which remained in the feeder were deducted from the total food intake. The total food consumption and the weight increment of the mice provided the data needed to obtain the food efficiency.

$$\text{Food efficiency} = \frac{\text{gm gain} \times 100}{\text{gm food}}.$$

The Apollo-68 diet preparation, its constituent parts and other pertinent information are to be found in Appendix E. The Radiation Sterilization is discussed in Appendix F.

III. DISCUSSION OF THE EXPERIMENTAL RESULTS - PART B

Data from the many parameters measured to provide an overall judgement of the biologic and gnotobiologic value of the Apollo diet are discussed below. Classically, the growth data (Table XVIII) provide one of the most reliable indices. Here the averages for each sex is presented separately. It is noted that at some period of the growth the females were as large or larger than the males in about half of the groups; therefore, a single average value for the growth of each group is given in the last column. It should be noted

TABLE XVIII

SUMMARY OF MOUSE GROWTH DATA

GROUP	1969 DAY-MONTH	AGE DAYS	MALE WEIGHT, GM			FEMALE WEIGHT, GM			REMARKS
			NUMBER	AVERAGE	RANGE	NUMBER	AVERAGE	RANGE	
1	10-6	20	8	8.5	6.4-10.7	12	7.7	6.1-9.5	
	19-6	29	8	13.0	9.8-18.5	12	12.0	9.8-14.1	
	24-6	34	8	16.5	11.9-20.2	12	15.5	11.8-18.8	
	22-7	63	5	17.8	14.8-21.6				Autopsy
	6-8	77	2	15.3	14.6-15.9	3	25.4	24.6-26.3	
2	10-6	20	8	7.5	6.6-9.3	12	6.7	6.1-8.2	
	19-6	29	7	12.6	10.4-16.4	11	10.7	9.1-13.4	
	24-6	34	7	16.2	14.1-19.9	11	14.1	11.5-17.3	
	22-7	63	5	19.6	17.7-22.7				Autopsy
3	10-6	20	8	8.2	6.8-9.9	12	7.3	6.0-9.2	
	19-6	29	8	12.4	10.8-14.5	11	10.8	10.1-13.3	
	24-6	34	8	15.5	13.0-16.6	11	13.9	12.5-16.0	
	22-7	63	5	17.5	14.7-20.4				Autopsy
4	10-6	20	8	8.8	7.5-11.4	12	8.6	6.3-11.7	
	19-6	29	8	12.3	9.5-17.4	12	12.3	10.0-16.5	
	24-6	34	8	14.1	12.2-19.4	12	14.7	10.5-18.2	
	22-7	63	5	14.6	13.2-16.4				Autopsy
	6-8	77				5	17.9	14.0-20.8	
5	10-6	20	8	9.2	7.2-11.6	12	8.0	7.1-9.2	
	19-6	29	8	12.3	9.4-16.3	10	11.4	10.6-12.8	
	24-6	34	8	17.0	13.0-21.3	10	15.4	13.2-17.0	
	22-7	63	2	20.9	18.0-23.8				Autopsy
6	10-6	20	8	7.4	6.2-8.8	12	8.2	5.5-9.5	
	19-6	29	6	11.3	8.0-13.3	11	11.7	9.6-16.4	
	24-6	34	6	15.6	12.9-18.9	11	14.3	10.9-14.6	
	22-7	63	2	15.9	13.7-18.0				Autopsy
	6-8	77	2	19.5	17.0-21.9				
7	10-6	20	6	8.4	7.0-9.4	12	7.4	5.2-9.3	
	19-6	29	5	12.9	10.9-13.7	10	11.1	7.0-13.0	
	24-6	34	5	13.4	11.6-14.1	10	14.3	12.6-17.4	
	22-7	63	4	15.5	11.6-20.8				Autopsy
8	20-5	22	8	11.1	10.5-11.6	12	10.1	9.0-11.3	
	26-5	28	8	13.0	10.7-15.5	12	11.2	10.0-12.5	
	30-5	32	8	15.7	12.3-19.5	12	14.8	12.6-17.2	
	9-6	42	8	20.4	15.2-25.9	11	17.1	15.1-18.7	
	12-6	46	5	25.4	17.9-23.6	9	17.2	13.8-20.3	
	27-6	60	5	20.0	15.1-24.6				Autopsy

TABLE XVIII (Continued)

GROUP	1969 DAY-MONTH	AGE DAYS	MALE WEIGHT, GM			FEMALE WEIGHT, GM			REMARKS
			NUMBER	AVERAGE	RANGE	NUMBER	AVERAGE	RANGE	
9	20-5	22	8	10.9	10.2-11.5	12	9.7	9.1-10.8	
	26-5	28	8	13.0	11.8-15.4	12	12.0	11.3-14.3	
	30-5	32	8	18.7	16.0-20.9	12	18.4	15.4-20.1	
	9-6	42	8	24.4	18.1-28.1	12	22.3	18.6-25.8	
	13-6	46	8	22.1	16.8-27.8	11	20.8	19.1-23.4	
	27-6	60	4	28.0	25.1-31.6	1	23.8		Autopsy
	20-7	83±2				3	32.8	29.7-36.0	Female at weaning
10	20-5	22	8	11.2	9.9-11.3	12	10.2	9.2-11.9	
	26-5	28	8	13.0	11.5-13.8	12	12.3	10.3-14.2	
	30-5	32	8	20.2	19.4-23.4	12	18.5	16.1-20.6	
	9-6	42	8	22.4	19.4-26.9	12	22.0	19.4-25.7	
	13-6	46	8	22.4	21.1-24.8	11	20.5	17.0-23.9	
	27-6	60	5	27.6	25.2-29.4				Autopsy
11*									
12*									
13*									
14*									
15	20-5	21	8	11.1	8.7-13.2	12	8.1	7.1-9.0	
	26-5	27	8	12.8	10.8-15.0	12	10.5	8.7-12.2	
	30-5	31	8	17.7	14.9-20.5	12	14.5	12.6-17.6	
	9-6	41	7	22.6	19.0-26.9	12	17.4	14.6-24.3	
	13-6	48	6	24.5	20.1-28.8	12	17.7	14.6-22.5	
	27-6	62	5	25.5	16.8-31.8				Autopsy
	6-8	102	1			1	25.4		
16	20-5	21	8	9.2	7.5-11.7	12	8.5	7.0-10.4	
	26-5	27	8	10.9	10.0-12.8	12	9.7	8.1-11.2	
	30-5	31	8	14.1	10.9-16.3	12	12.9	9.7-15.3	
	9-6	41	8	19.1	16.4-23.2	12	15.4	11.3-19.5	
	13-6	48	8	20.5	17.2-24.4	11	13.5	11.4-18.7	
	27-6	62	5	23.5	21.9-26.6				Autopsy
	6-8	102				8	20.1	14.3-26.1	Sex ?
	17-8	113	3	15.3	14.2-17.5				Sex ? at death
	24±8	120	2	15.2	14.6-15.7				Dead
17	29-8		4	10.7	9.3-12.8	14	12.4	8.5-17.4	
	8-9					14	16.7	10.3-21.5	Not sexed
	12-9		3	18.3	16.1-20.5	11	17.7	12.0-20.8	
	21-11		1	25.6		3	30.5	25.8-35.4	Autopsy

TABLE XVIII (Continued)

GROUP	1969 DAY-MONTH	AGE DAYS	MALE WEIGHT, GM			FEMALE WEIGHT, GM			REMARKS
			NUMBER	AVERAGE	RANGE	NUMBER	AVERAGE	RANGE	
18 1	29-8	25	1	10		3	17.3	16.0-18.9	
19 1	6-10	30	11	15.6	12.9-20.1	8	13.1	10.2-17.0	
	10-10	34	9	17.1	12.9-20.9	8	14.5	11.5-17.4	
	10-12	95	5	28.4	26.2-31.1				
20	29-8	25	2	10.9	9.6-12.1	6	11.0	8.4-15.5	
	2-9	29	2	13.3	12.6-14.0				
21	6-10	30	8	15.0	12.7-19.0	12	14.0	10.6-19.3	
	10-10	34	8	16.0	12.9-21.3	12	13.4	9.3-17.8	
	10-12	96	5	23.5	21.4-25.4				
22	29-8	25	1	9.5		3	12.1	8.8-14.5	
23	6-10	30	8	14.4	10.0-17.6	12	16.6	9.5-21.7	
	10-10	34	8	15.4	10.0-18.1	12	18.4	10.9-24.7	
	10-12	96	5	31.5	24.3-36.7				
24	6-10	30	8	13.7	9.5-18.0	11	12.9	10.2-18.4	
	10-10	34	8	15.2	13.3-16.9	11	18.3	14.5-24.8	
	11-12	96	3	27.2	23.6-32.5				
25	29-8	25	2	10.4	8.4-12.3	5	14.8	12.6-16.8	
26	6-10	30	8	14.6	10.5-17.2	11	18.1	12.9-19.5	
	10-10	34	8	16.0	13.3-18.8	11	17.2	14.0-20.4	
	11-12	96	5	32.2	27.1-38.2				
27	29-8	25	2	11.9	11.9-11.9	4	12.5	9.3-15.3	
28	6-10	30	7	14.0	10.4-18.4	10	16.0	11.8-20.5	
	10-10	34	7	15.4	12.4-17.4	9	18.0	13.4-25.2	
	11-12	96	5	33.1	30.0-36.2				Autopsy
29	29-8	25	2	9.6	9.3-9.8	5	11.5	9.7-15.7	
30	6-10	30	8	12.9	9.4-15.3	10	17.1	13.0-22.4	
	10-10	34	7	13.6	9.3-16.5	8	16.8	14.0-22.2	
	11-12	96	5	31.7	29-32.8				Autopsy
31	4-9	20	3	10.5	9.2-11.2				
	8-9	24	3	10.3	10.5-11.9				
	12-9	28	3	16.0	14.2-17.2				
	27-10	Ca74	4	25.4	16.7-36.1	1	20.3		

TABLE XVIII (Continued)

GROUP		1969 DAY-MONTH	AGE DAYS	MALE WEIGHT, GM NUMBER	MALE WEIGHT, GM AVERAGE	MALE WEIGHT, GM RANGE	FEMALE WEIGHT, GM NUMBER	FEMALE WEIGHT, GM AVERAGE	FEMALE WEIGHT, GM RANGE	REMARKS
32		18-11	22?	10	11.1	10.3-12.4	10	11.0	8.1-13.3	
		21-11	25	10	18.3	7.8-23.3	9	14.1	9.0-22.7	
		28-12	32	2	22.1	22.0-22.2				Autopsy
33		11-8	24	10	8.6	7.8-9.1				Sex ?
		18-8	31	8	13.6	12.8-14.7	2	10.8	10.5-11.0	
	a	23-8	36	8	15.0	14.1-16.3	2	12.3	12.0-12.6	
	b	2-9	24	5	7.6	1.2-8.0	7	6.9	6.3-7.5	
		8-9	30	7	9.2	7.4-10.1	4	7.8	7.2-8.3	
		12-9	34	7	11.7	9.5-13.0	4	10.9	10.0-12.2	
		27-10	90±10	4	22.9	20.1-26.2	1	22.8		Autopsy
34		11-8	22	13	6.7	5.5-9.8				Sex ?
		18-8	39	4	9.6	9.0-10.0	6	10.3	9.5-11.1	
	a	23-8	34	4	13.3	12.7-14.3	6	12.9	9.8-14.9	
	b	2-9	21	4	9.0	8.3-10.0	7	7.8	6.6-9.1	
		8-9	27	4	11.6	10.6-13.7	7	10.1	8.5-12.4	
		12-9	31	4	14.5	12.9-17.0	7	12.9	10.9-15.1	
		27-10	90±10	4	26.9	21.8-32.8				Autopsy
35		24-12	19	11	5.9	4.4-6.7				Mixed Sex
		31-12	26	2	22.1	20.0-24.1	3	21.3	20.0-23.8	
36		24-12	19	11	5.9	4.4-6.7				As No. 35
		31-12	26	4	33.2	29.3-38.3	1	25.2		
37		21-11		4	26.4	25.6-27.8				Autopsy
38 Cont. 2		20-5	20	8	10.1	7.9-13.5	12	8.6	7.1-10.0	Run 5 Days
39 Cont. 3		20-5	20	8	10.7	8.3-11.9	12	8.7	7.0-11.0	Run 5 Days
40 Cont. 5		20-5	20	8	9.1	7.3-10.5	12	9.6	9.1-11.5	Run 5 Days
41 Cont. 6		20-5	20	8	10.1	8.7-11.8	12	9.7	7.7-12.3	Run 5 Days
42 Cont. 7		20-5	20	8	9.4	8.0-11.9	12	9.2	6.8-10.9	Run 5 Days

*Groups 11 to 14 used in Food Efficiency Studies.

that the growth rate data was taken early in the life of each group in order that a maximal value could be obtained before the beginning of the decreased growth rate at maturity. The food efficiency data (Table XIX) also was taken during the early period of each experiment; it usually started within one week after the weaned mice were fed Apollo diet. Each value for food efficiency was obtained from one cage containing three females and two males. These two parameters are summarized in Table XX to give a view of food utilization under different microbic conditions. These values obtained involving classic mice showed considerable variation when the experiment was reported but the values obtained were generally high. Monognotophoric mice with Candida and Staphylococcus gave growth rates equivalent to those obtained for the classic mice. Bacteroides monoassociated mice had a good growth rate greater than that of the other gnotobiotic mice. The only group which had a low growth rate were the diassociated mice carrying Candida plus Lactobacillus. This group also showed a lower food efficiency than most groups. Lowered food efficiency was also noted in germfree coli monoassociated and Lactobacillus monoassociated mice. Surprisingly, the poorest food efficiency was found in the Bacteroides monoassociated mice whose growth and general appearance were excellent. The lowest value (5.6) may correlate with later deaths from E. coli. The best food efficiency was obtained with classic mice fed non-sterile diet and the Staphylococcus monoassociated mice. Since this latter group showed the greatest alopecia, there appears to be no correlation between food utilization and appearance nor was there good correlation between growth rate and food efficiency for this group.

The quantitative data available at autopsy is given in Table XXI and summarized in Table XXII. Each of the parameters shows a difference between

TABLE XIX

MICE FOOD EFFICIENCY DATA

GROUP		BODY WEIGHT gm START	BODY WEIGHT gm END	BODY WEIGHT gm CHANGE	FOOD gm START	FOOD gm WASTE	FOOD gm END*	FOOD gm USED	GM GAIN/GM FOOD x 100	AVERAGE
1	A	63.6	75.1	11.5	111.3	52.1	50.9	60.4	19.0	
	B	56.8	74.4	17.6	96.0	65.3	63.7	32.3	54.5	32.0
	C	57.7	71.9	14.2	115.6	56.0	54.6	61.0	23.3	
	D	66.0	88.0	22.0	114.5	44.0	42.9	70.5	31.2	
2	A	31.5	45.9	14.4	101.7	58.1	56.7	54.0	32.0	
	B	60.2	75.7	15.5	111.4	60.8	59.3	52.1	29.8	30.0
	C	57.7	71.2	13.5	112.9	59.7	58.2	54.7	24.7	
	D	57.4	75.9	18.5	106.6	52.6	51.3	55.3	33.4	
3	A	57.8	74.4	16.6	102.1	52.2	50.9	51.2	32.4	
	B	58.7	72.3	13.6	110.1	58.7	57.2	52.9	25.7	29.0
	C	42.8	54.7	11.9	116.9	76.8	74.9	42.0	28.3	
	D	58.4	74.0	15.6	101.5	50.1	48.9	52.6	29.7	
4	A	73.0	84.9	11.9	102.7	54.4	53.0	49.7	23.9	
	B	63.6	73.6	10.0	98.2	57.4	65.0	42.2	32.7	25.2
	C	55.9	64.8	8.9	102.5	58.6	57.1	54.4	19.6	
	D	53.9	69.4	15.5	105.8	61.0	59.5	46.3	33.5	
5	A	39.0	52.4	13.4	113.1	64.5	62.9	50.2	26.7	
	B	57.2	79.9	22.7	107.1	42.9	41.8	65.3	34.8	30.4
	C	58.2	76.9	18.7	123.2	60.8	59.3	63.9	29.3	
	D	57.9	81.0	23.1	107.1	32.8	32.0	75.1	30.8	
6	A	44.0	60.0	16.0	109.7	57.7	56.0	53.7	29.8	
	B	52.7	64.5	11.8	101.6	49.2	47.7	53.9	21.0	24.1
	C	61.9	69.4	7.5	106.8	58.8	57.0	49.8	15.1	
	D	39.2	53.9	14.7	107.8	59.9	58.0	49.8	29.5	
7	A	46.7	41.6	5.1	106.7	66.8	64.8	41.9	12.2	
	B	49.4	54.9	5.5	103.3	63.1	61.2	42.1	13.1	19.4
	C	34.8	49.7	14.9	109.6	(60.)	60	49.6	30.0	
	D	44.9	56.6	11.7	104.6	53.5	51.9	52.7	22.2	
8	A	53.6	71.2	17.6	110.8	66.5	66.5	44.3	39.7	
	B	63.9	84.3	20.4	104.7	49.1	49.1	55.6	36.7	34.0
	C	55.2	66.1	10.9	128.0	85.1	85.1	42.9	25.4	
	D	59.8	81.6	21.8	111.2	39.7	47.6	63.6	34.3	

*END = Waste corrected for fecal contamination. Where data could not be obtained 3% of the waste was deducted.

TABLE XIX (Continued)

GROUP		BODY WEIGHT GM			FOOD GM				GM GAIN/GM FOOD x 100	AVERAGE
		START	END	CHANGE	START	WASTE	END	USED		
9	A	63.8	95.1	31.3	108.8	32.6	32	77	40.6	38.9
	B	57.1	83.2	26.1	112.3	41.7	41	71	36.7	
	C	64.1	94.9	30.8	119.4	41.8	41	78	39.5	
	D	66.2	96.5	30.3	111.6	35.1	34	78	38.8	
10	A	56.1	94.1	38.0	103.8	31.8	31	72	52.7	46.9
	B	56.9	93.1	36.2	116.3	41.1	40	76	47.6	
	C	68.4	96.6	28.2	105.9	32.7	32	74	38.1	
	D	62.3	99.6	37.3	118.2	42.9	42	76	49.1	
11	A	60.4	76.1	15.7	99.8	46.0	40.0	53.8	29.2	23.2
	B	83.4	100.6	17.2	173.9	94.2	77.1	80.6	21.4	
	C	92.0	106.2	14.2	102.2	21.2	13.6	75.0	19.0	
	D	84.1	55.4	----	94.4	80.6	73.4	3.8	----	
12	A	108.3	120.3	12.0	128.5	51.7	43.7	76.8	15.6	13.9
	B	108.3	105.6	2.7	177.6	90.0	82.0	87.6	----	
	C	108.8	121.7	12.9	172.6	80.3	72.3	92.3	14.0	
	D	116.2	126.7	10.5	157.9	71.8	63.8	86.1	12.1	
13	A	111.8	134.1	22.3	199.2	99.4	92.4	99.4	22.4	17.7
	B	11.4	128.8	17.4	160.7	55.4	54.9	95.4	18.2	
	C	103.1	118.9	15.8	183.5	96.6	89.4	86.9	18.2	
	D	119.6	130.5	10.9	168.5	77.3	69.2	91.2	12.0	
14	A	123.2	130.8	7.6	129.6	42.7	34.0	86.9	8.7	9.3
	B	119.8	127.5	7.7	111.7	23.4	16.4	88.3	8.7	
	C	115.9	122.3	6.4	120.0	30.8	21.6	89.2	7.2	
	D	95.5	104.1	8.6	121.7	52.6	46.6	69.1	12.4	
15	A	53.9	73.2	19.3	112.1	71.4	69	43	44.9	42.4
	B	55.9	79.1	23.2	113.6	50.9	49	64	36.3	
	C	54.7	78.7	24.0	100.2	59.9	56.9	43.3	55.4	
	D	63.0	84.8	21.8	119.2	54.7	53	66	33.1	
16	A	49.7	66.2	16.5	104.3	72.9	69.9	34.4	48.0	36.1
	B	50.9	74.5	23.6	118.7	57.5	55	64	15.0	
	C	54.1	61.6	7.5	129.7	81.4	80	50	15.0	
	D	41.9	64.5	22.6	103.8	58.0	57	51	44.4	
17	A	67.7	68.1	0.4	80.4	43.1	42	38	1.1	11.5 (15.0)
	B	52.0	55.7	3.7	80.4	46.8	46	34	10.9	
	C	45.4	50.4	5.0	82.0	52.8	52	30	16.7	
	D	68.4	75.2	6.8	83.5	44.8	44	39	17.4	
19	A	74.0	80.8	6.8	80	37.2	36.2	43.8	15.5	
	B	69.3	78.1	8.8	80	36.5	43.5	20.3	18.7	
	C*	35.6	42.6	7.0	80	53.3	52.8	27.2	25.7	
	D	63.5	68.0	4.5	80	46.7	45.4	34.6	13.2	

* Only 3 animals carried to end.

Group 20 not applicable

TABLE XIX (Continued)

GROUP		BODY WEIGHT GM START	BODY WEIGHT GM END	BODY WEIGHT GM CHANGE	FOOD GM START	FOOD GM WASTE	FOOD GM END	FOOD GM USED	GM GAIN/GM FOOD x 100	AVERAGE
	A	67.4	67.0	-.4	80	50	49	31	0	
21	B	78.7	81.1	2.4	80	48	47	33	7.2	5.6
	C	66.0	64.7	-1.3	80	61	60	20	0	
	D	69.9	76.2	6.3	80	40	39	41	15.4	
	A	74.1	79.0	4.9	80	33.3	33	47	10.4	
23	B	87.3	90.8	3.5	80	38.1	37	43	8.1	20.0
	C	78.0	88.5	10.5	80	36.8	36	44	23.8	
	D	75.2	85.5	10.3	80	43.5	43	37	37.8	
	A	77.5	91.3	13.8	80	41.0	40	40	34.5	
24	B	73.9	55.1	18.8	80	53.4	52	28	----	40.4
	C	64.0	78.1	14.1	80	38.6	38	42	33.4	
	D	58.9	81.2	22.3	80	38.8	38	42	53.2	
	A	86.2	79.3	----	80					
26	B	86.8	85.8	----	80	No Record		Spilled		
	C	74.8	71.5	----	80					
	D	60.2	67.2	7.0	80					
	A	65.4	75.3	9.9	80	58.7	58	22	45.1	
28	B	70.2	86.2	16.0	80	50.4	49	31	51.7	35.5
	C	62.3	44.4	---*	80	25.0	25	55	----	
	D	59.1	63.7	4.6	80	33.4	33	47	9.8	
	A	83.5	41.2	---*	80	73.5	----	----	----	
30	B	80.1	80.6	.5	80	44.2	43	37	1.4	27.1
	C	55.8	61.6	5.8	80	69.4	69	11	52.7	
	D	54.7	46.7	---*	80	50.7	50	30	----	
31	A	33.9	48.1	14.2	84.1	41.3		43	33.0	33
	A	56.0	53.4	---*	60	39.5	39	21	----	
32**	B	55.6	47.9	neg.	60	40.6	25	25	---	---
	C	54.3	105,5	51.5	60	27.0	21	39	?	
	D	55.5	114.7	59.2	60	26.4	24	36	?	
	A	66.2	71.8	5.6	100	42.0	41	59	9.5	
33	B	64.4	72.2	7.8	100	37.2	36	64	12.2	
	C	48.4	68.2	19.8	80		32***	48	41.2	
	D	47.3	57.1	9.8	80		32***	48	29.4	
	A	50.4	59.8	9.4	100	42.1	41	59	15.9	
34	B	49.9	70.8	20.9	100	42.8	42	42	58	36.1
	C	56.7	71.5	14.8	80		32***	48	30.8	
	D	60.6	77.0	16.4	80		32***	48	34.2	

* One died so no data are valid for cage C.

** 3 days only.

*** Lost in processing - Assumed 40 for estimate (40 x 4/5 = 32 since only ran 4 days).

TABLE XX

APOLLO DIET FOOD UTILIZATION

GROUP NUMBER	STATE	GROWTH RATES	AVERAGE	FOOD EFFICIENCY	AVERAGE
1, 19	Germfree	.57, .37	.47	32.0, 18.3	25.2
2, 21	Gn, Coli	.58, .25	.46	30.0, 5.6	17.3
3, 23	Gn, Lactic	.50, .35	.48	29.0, 20.0	24.5
4, 24	Gn, Candida	.41, .89	.65	25.2, 40.4	32.8
5, 26	Gn, Coli + Lactic	.55, .35	.45	30.4	30.4
6, 28	Gn, Coli + Candida	.51, .44	.48	24.1, 35.5	29.8
7, 30	Gn, Lactic + Candida	.43, .18	.31	19.4, 27.1	23.3
8, 31, 32	Classic, Isolation	.47, .69, 1.07	.74	34.0, 33.0	33.5
9, 33 a.b., 35	Classic, Food Sterile	.83, .42, .41, 2.3*	.55	38.9, 20.8	29.8
10, 34 a.b., 36	Classic, Non-Sterile Food	.87, .54, .53, 3.3*	.64	46.9, 36.1	41.5
15	Gn, Staph.	.65	.65	42.4	42.4
16	Gn, Staph. + Candida	.47	.47	36.1	36.1
17	Gn, Bacteroides	.54	.54	15.0	15.0

*Not used in the average value.

TABLE XXI

AUTOPSY DATA

GROUP	AGE DAYS	CECUM, gm		Hb, %		WBC/mm^3		REMARKS
		AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	
1	63	3.9	2.4-5.7	11.1	10.4-11.7	2990	1430-4070	
2	62	3.3	2.3-4.4	6.7	5.4-8.7	2008	440-5280	
3	63	3.7	3.1-4.8	10.5	7.7-12.8	3610	1760-5610	
4	63	3.0	2.2-4.7	9.9	6.7-11.9	4303	1925-6490	
5	63	1.9	1.8-2.0	5.8	5.6-5.9	1925	1430-2420	
6	63	2.8	1.5-4.0	7.9	6.7-9.0	4565	3740-5390	
7	63	3.0	1.6-5.5	9.7	7.7-12.9	2227	1100-2970	
8	60	0.3	0.2-0.4	13.5	12.1-14.2	2200	1430-3630	
9	60	0.5	0.3-0.7	13.9	13.3-14.2	4246	2530-6710	
10	60	0.4	0.2-0.6	10.1	5.9-13.2	4312	2640-5280	
11*								
12*								
13*								
14*								
15		3.0	2.2-3.3	12.5	11.8-13.0	5874	1320-12,760	
16		3.2	2.6-4.0	12.3	11.4-13.2	6864	2750-8690	
17		1.1	0.9-1.3	15.6	14.2-17.4	2273	1430-3630	3 Females Pregnant
18								
19		1.4	0.9-2.4	13.9	12.5-15.3	5555	2750-7480	
20								
21		1.9	1.2-2.6	15.2	13.9-17.0	1625	660-2420	4 Only
22								
23		2.1	1.3-2.7	13.5	13.2-15.6	2222	1210-3630	
24		1.0	0.6-1.7	14.4	12.8-16.0	5573	4070-8140	
25								
26		1.7	1.4-2.3	15.5	13.6-17.0	2222	1430-3080	
27								
28		1.4	0.9-1.8	15.7	14.2-16.8	2585	880-3630	
29								
30		2.5	1.8-3.5	14.0	12.2-15.0	3384	1980-4730	
31		0.6	0.6-0.8	13.6	12.9-15.3	2127	1430-2530	
32	41	0.6	0.5-0.6	16.3	15.0-17.5	2070	1610-2530	
33	90±10	0.5	0.4-0.6	10.1	3.8-14.6	2250	990-3410	
34	90±10	0.5	0.4-0.6	10.8	8.7-13.0	4195	880-3830	
35	28	0.4	0.3-0.5	16.1	15.3-17.0	6792	5170-7810	
36	28	0.9	0.9-1.0	15.6	15.0-16.3	3476	2090-6490	
37		0.8	0.3-1.1	15.9	15.6-16.0	3527	2310-4420	

*Not Autopsied (Food Efficiency Study)

TABLE XXII

AUTOPSY DATA SUMMARIZED - PART B

GROUP	STATE	CECUM, gm	Hb, %	WBC/mm^3
1, 19	GF	3.9, 1.4	11.1, 13.9	2990, 5555
2, 20	Coli	3.3, 1.9	6.7, 15.2	2008, 1625
3, 23	Lactic	3.7, 2.1	10.5, 13.5	3610, 2222
4, 24	Candida	3.0, 1.0	9.9, 14.4	4303, 5573
5, 26	Coli + Lactic	1.9, 1.7	5.8, 15.5	1925, 2222
6, 28	Coli + Candida	2.8, 1.4	7.9, 15.7	4565, 2585
7, 30	Lactic + Candida	3.0, 2.5	9.7, 14.0	2227, 3384
8, 31, 32	Classic, Isolation	0.3, 0.6, 0.6	13.5, 13.6, 16.3	2200, 2127, 2070
9, 33, 35	Classic, Sterile Diet	0.5, 0.5, 0.4	13.9, 10.1, 16.1	4246, 2250, 6792
10, 34, 36	Classic, Non-Sterile Diet	0.4, 0.5, 0.9	10.1, 10.8, 15.6	4312, 4195, 3476
15	Staph	3.0	12.5	5874
16	Staph + Candida	3.2	12.3	6864
17	Bacteroides	1.1	15.6	2273
37	GF Lab Diet	0.8	15.9	3572

classic and germfree mice. The enlarged cecum of germfree animals seemed to be somewhat alleviated in the second experiment when filter paper bedding was used. The mice which were monoassociated with Bacteroides showed a good reduction in cecum size in the first experiment. The Coli diassociated mice (with either Lactobacillus or Candida) seemed to have smaller ceca than other gnotobiotic mice (excepting Group 17 as noted above). The effect of diet on cecum size can be noted by comparing the germfree mice fed laboratory chow (Group 37) to those fed Apollo diet. Presumably, the reduction is due in part to the fiber content of the diet. This has been reported to reduce the cecum size in germfree rats (Luckey, 1963).

The hemoglobin values obtained in the first experiment were generally less than those found in the last experiment for each group. Mice monoassociated with Coli or diassociated with Coli plus Lactobacillus or Candida were found to have very low hemoglobin values in the first experiment. These seemed to be alleviated when the filter paper bedding was used in the second experiment. The hemoglobin of Bacteroides monoassociated mice was comparable to the best obtained under any condition. The hemoglobin of classic mice changed from 12 $\pm$2 gm per 100 ml of blood in the first two generations to 16 $\pm$4% in the third generation. The reason is not understood. However, in general, the third generation mice showed better characteristics than did first or second generation mice. This phenomenon was seen previously in the experiments of Luckey, et al. (1955) with mice fed a radiation sterilized, syntype diet.

The Apollo diet, it contents, preparation and handling is discussed in detail in Appendix E and the radiation sterilization of this diet in Appendix F.

Identification of the Gnotobiotes

The mice in Groups 2 through 7 and Groups 15 through 17 were all either mono or dignotobiotes. See Table XXIII.

The mono and dignotobiote groups were, with the exception of Groups 15 and 16, established by inoculation with organisms previously isolated from mice. The specific groups and bacteria strains used are also presented in Table XXIII.

The bacteria were grown in media as recommended by the ATCC or in the case of the Bacteroides by Dr. Carl Abramson (Pennsylvania State University) prior to inoculation into the animal per os.

The Bacteria

1. E. coli was grown in Trypticase soy broth after isolation on MacConkeys medium from the feces of monobiotes maintained in our laboratory. Incubation was aerobic at 37°C for 24 hours.
2. Lactobacillus leichmannii was isolated in pure culture on Bacto B_{12} inoculum broth from a freeze dried culture purchased from ATCC. Incubation was at 37°C for 24 hours under anaerobic conditions.
3. Candida albicans was isolated in pure culture from a lyophilized stock obtained from ATCC on Mycophil broth. Incubation was at room temperature on a shaker for 24 hours.
4. Staphylococcus epidermidis was a contaminant of the original group of mice received as "germfree".
5. Bacteroides sp. is an indeterminant strain supplied by Dr. Carl Abramson of Pennsylvania State University and purported to have been originally isolated from a mouse. Subculture was made upon receipt into Fluid Thioglycollate and blood agar plates incubated anaerobically for 48 hours at 24°C.

TABLE XXIII

IDENTIFICATION OF BACTERIAL SPECIES USED TO ESTABLISH GNOTOBIOTIC CLASSIFICATIONS

MOUSE GROUP	CLASSIFICATION	BACTERIAL SPECIES
2	Monobiote	*Escherichia coli* ATCC #15144
3	Monobiote	*Lactobacillus leichmannii* ATCC #7830
4	Monobiote	*Candida albicans* ATCC #10231
5	Dibiote	*E. coli* + *L. leichmannii*
6	Dibiote	*E. coli* + *C. albicans*
7	Dibiote	*L. leichmannii* + *C. albicans*
15	Monobiote	*Staphylococcus epidermidis* (contaminant)
16	Dibiote	*S. epidermidis* + *C. albicans*
17	Monobiote	*Bacteroides* sp. (Abramson, 1969)

Cultures were checked for purity, prior to use, by subculturing them into differential media and/or microscopic examination of wet and gram-stained slides.

The cultures were inoculated per os by diluting the original cultures one to ten in sterile 0.85% saline and adding approximately 5 ml of this solution directly to the water, food and mouths of the mice.

Establishment of Bacteria

Confirmation of the establishment of the organisms in the animal was made by culture of fecal swabs (from random representatives) of each cage on media and under conditions suitable for the specific organism. Verification was made by gram stain slides of the isolated cultures.

Fecal pellets, having an average weight of 0.0265 gm, were analyzed to approximate total counts of the specific organisms after establishment. Analyses were by plate counts on specific media for the various strains. The pellets were taken, aseptically and serial dilutions made in sterile physiological saline. Mixing by mashing and ultrasonics assured a representative mix.

The total counts per 0.0265 gm samples are given in Table XXIV.

Maturation Rate

Details concerning maturation rate is presented in several sections of the report. These include: growth rate, body size at maturity, and breeding. Development of young born during this project and other items of maturation are presented below. Other facets of the topic are to be found in the autopsy and histology data. The weekly recorded observations are listed in Table XXV.

Many details about the maturation of each group have been summarized in Table XXVI. This information is distilled further (Table XXVII) to provide

TABLE XXIV

CONFIRMATION COUNTS OF BACTERIAL SPECIES INTRODUCED

MOUSE GROUP	ORGANISM	COUNT/0.0265 gm SAMPLE	ADJUSTED COUNT/GRAM $\frac{\text{COUNT} \times 1.0 \text{ gm}}{0.0265 \text{ gm}}$ = ADJUSTED COUNT
1	Axenic	No Growth	
2	*E. coli*	3.2×10^{10}	1.2×10^{12}
3	*L. leichmannii*	1.5×10^{4}	5.7×10^{6}
4	*C. albicans*	5.7×10^{8}	2.1×10^{10}
5	*E. coli*	1.5×10^{9}	5.7×10^{11}
	L. leichmannii	1.3×10^{4}	4.9×10^{6}
6	*E. coli*	4.5×10^{10}	1.7×10^{12}
	C. albicans	8.4×10^{7}	3.2×10^{9}
7	*C. albicans*	5.4×10^{7}	2.0×10^{9}
	L. leichmannii	1.3×10^{4}	4.9×10^{6}
15	*S. epidermidis*	5.4×10^{9}	2.0×10^{11}
16	*S. epidermidis*	9.9×10^{9}	3.7×10^{11}
	C. albicans	4.3×10^{7}	1.6×10^{9}
17	*Bacteroides* sp.	2.2×10^{8}	8.8×10^{9}

TABLE XXV

WEEKLY RECORDED OBSERVATIONS

ITEMIZED LIST RECORDED EACH WEEK BY A TRAINED OBSERVER (Dr. Terry Hand)

1. Size
2. Health
3. Posture
4. Response to Irritation
5. Fur Coat
6. Skin
7. Ears
8. Eyes
9. Nares
10. Tail and Extremities
11. Rectal-Genital Area
12. General Body

TABLE XXVI

MATURATION

GROUP	DEATH[1]	HAIR[2]	SKIN[3]	GENERAL APPEARANCE
1	4	2-3	1-2	Scrawny at start; 2 weeks, large abdomens.
2	11	3	1	Looked ill until 3 months when lone survivor grew new hair.
3	9	4	2	Fair at start; at 1 month hypoactive and looked ill, abdomen distended.
4	9	3-4	3	Fair at start; 1 month, all look ill; later extremely sluggish.
5	16	3	1	Fair at start; 1 month, all look ill to the end, no cecal enlargement noted.
6	16	3	3	Very poor at start; eat well, hypoactive, all subnormal large abdomens, anemia, ill.
7	13	2	2	Scrawny at start; look ill at 1 month, bulging abdomens, anemia.
8	11	3-5	2-4	Good at start; 1 week look wet; after 2 months, looked better.
9	2	0-1	0	Good at start; active and good, fur best after 2 months, breeding OK.
10	1	0-1	0	Good at start; active and good throughout; breeding OK.
15	9	3	4	OK at start; 3 weeks big abdomen, look ill, look moribund.
16	1	4-5	1-2	Start OK; fur bad in 2 weeks, big abdomen at 3 weeks, all ill, little more meat.
17[4]	4	0	0	Start good; appearance best of any in isolation to date.
18	1	1	0	OK throughout; good appearance and movement.
19	9	3	1	As 18.
20	12	0	0	As 18, with large abdomens at 6 weeks.
21	2	2	0	As 18, with anemia in some.
22	6	0	0	OK at start; short experiment.
23	9	2	0	As 18.
24	5	2	0	As 18, with ear bleeding on one at one time (fighting ?).
25	3	0	1	As 18.
26	7	2	0	OK at start; fur looks woolly, animals look ill.
27	3	0	0	As 26, with better fur coat.
28	0	1	0	As 18, with fuzzy fur coat.
29	9	0	0	Underweight at start, development OK.
30	13	2-3	0-1	Very active group - all OK.
31	8	0	0	Looked good throughout.
32	18	0	0	OK until catastrophic deaths at 2 weeks (18 of 20 died).
33	0	0	0	OK at start; looked anemic at 2 weeks otherwise better than 34, breeding OK.
34	7	2-3	0	OK at start; some look ill at 2 months, breeding OK.
35	0	0	0	Good throughout, short experiment.
36[5]	0	0	0	As 36.

(1) The number dead at 60 days of age (prior to autopsy) is based upon 20 mice per group; where a group had fewer animals, a correction was applied to give comparable data.
(2) Hair loss scale 0 (normal) to 5 (completely nude).
(3) Skin erythema scale 0 none and 4 severe erythema with edema.
(4) Filter paper bedding was added to this and all subsequent groups reported herein.
(5) Group 38-42 inclusive looked good at the start. All were terminated at 1 week when the contamination was confirmed. Therefore, they are not present in detail

TABLE XXVII

MATURATION - APPEARANCE

GROUP[1]	CATEGORY	DEATHS		APPEARANCE		GENERAL
		NO./20	AVERAGE	FUR[2]	SKIN[3]	
1, 19	GF	4, 9	7	3	1	Poor and OK at start, large abdomens.
2, 20	Gn Coli	11, 12	11	0-3	0	OK and ill with large abdomen.
3, 23	Gn Lactic	9, 9	9	2-4	1	OK at start, some ill, large abdomen
4, 24	Gn Candida	9, 5	7	2-4	2	OK at start, one group ill.
5, 26	Gn Coli + Lactic	16, 7	12	2-3	1	OK at start look ill later.
6, 28	Gn Coli + Candida	16, 0	8	2	2	Fair at start, ill later, large abdomen.
7, 30	Gn Lactic + Candida	13, 13	13	2	1	One poor at start and ill, one OK, large abdomen.
8, 31, 32	Classic, Isolation	11, 8, 18	12	0-5	0-4	All good at start, sproadic illness and death.
9, 33	Classic, Sterile Diet	2, 0	1	0	0	All good throughout, breeding good.
10, 34	Classic, Non-Sterile Diet	1, 7	4	0-3	0	All OK at start, some ill later, breeding fair.
15	Gn Staph.	9	9	3	4	OK at start, big abdomen, moribund.
16	Gn Staph. + Candida	1	1	4-5	2	Start OK, big abdomen, look ill.
17	Gn Bacteroides	4	4	1	0	Best of any in isolation.

(1) Other groups were not maintained long enough to receive comparable consideration, i.e., 40 days for the evaluation of the death rate.
(2) Fur rating scale is 0-5 where 0 indicates a normal quantity of hair and 5 is given for nude animals.
(3) Skin rating scale is 0-4 where 0 indicates a normal skin and 4 is much erythema with edema.

a more easily grasped concept of the effect of different environmental conditions and microfloras upon mice fed the sterile Apollo diet. Only one category (Groups 10, 34, and 36) received the non-sterile diet.

Survival to maturity is indicated as the number of deaths corrected from groups of 20 mice each at 60 days (which is prior to autopsy). The average of the groups in each category has been used for comparative consideration. Usually only one, or possibly no, animals would be expected to die during this time period if the animals were healthy at the start, the diet were adequate, the environment good and infections did not occur. This was actually the case with Group Number 9, the classic mice fed the radiation sterilized diet. Comparable animals fed non-sterile diet showed more than the average mortality. The difference is probably not in the nutrient content of the diet as much as in the bacterial content, which even though low could contribute as many as 10^3 microorganisms per gram of food fed. The only other mice fed the radiation-sterilized diet, Groups 8, 31, and 32, were placed in isolation and treated as gnotobiotes. Here 60% of the mice died during the first 40 days of the experiment (60 days of age minus the 20 days at weaning before the experiment started). All other mice were received as gnotobiotic and were not strictly comparable dispite the fact they were the same strain and obtained from the same supplier. The animals were definitely less vigorous and smaller. Therefore, the germfree animals with 7 dead form a new base for comparing the other categories. The E. coli monognotophoric mice gave increased deaths, and, particularly in the first series, di-association with Candida or Lactobacillus did not alleviate this deadly action of E. coli. The di-association of Candida plus Lactobacillus seemed to be a detrimental combination since two-thirds of the mice died in

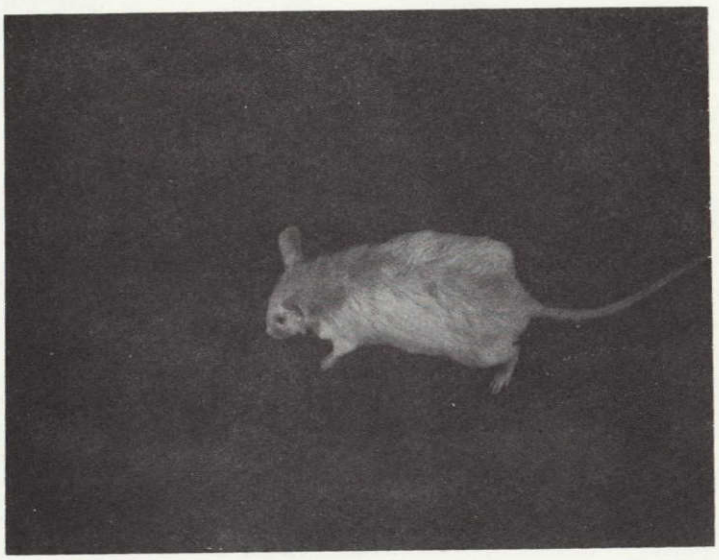
each experiment. Bacteroides alone or the diflora of Candida plus Staphylococcus produced lower number of deaths than was found for germfree mice. This indicates a protective factor was negated by these floras. In view of the low B-vitamin content of the diet, the intestinal synthesis of nutrients may be a factor here.

The appearance of the animals may be compared in Tables XXVI and XXVII and from the photographs in Figures 23 to 33. The only consistently good animals were those fed radiation-sterilized diet in the open laboratory; those in isolation were among the worst of all. Mice fed non-sterile Apollo diet in the open laboratory (Groups 10, 34 and 36) looked good most of the time; however, they occasionally had rough fur and sometimes loss of fur was noted. The breeding in this group was not as consistently active as that found in Groups 9 and 33. Consistently, the group which appeared to be the worst was Number 16, gnotophoric mice carrying both Candida and Staphylococcus. Other groups looked seriously ill much of the time in the first experiment and occasionally ill during the second series. The picture of abundantly healthy mice with eyes bright, glossy fur and good skin color was rarely, if ever, seen in any group. This may be attributed to any of the serious shortcomings for the diet noted in the composition section. Monognotophoric mice associated with Bacteroides looked good most of the time, but their fur was often matty and not glossy.

The Alopecia observed in the mice in several isolators is rated by severity in Table XXVIII. Table XXVIII describes the observed severity, the diet fed and the microbiological status of the mice. It appears that the presence of Candida alone is sufficient to forestall this condition. In other animals with a normal microflora, the hair and skin seems normal regardless of

FIGURE 23

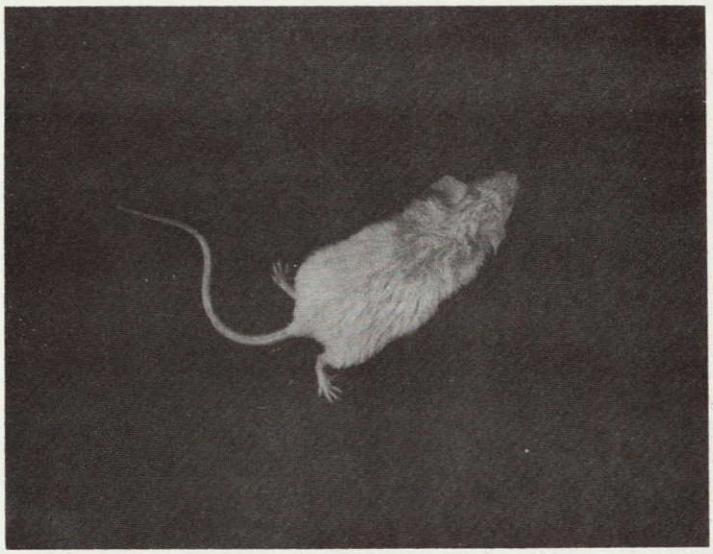
APPEARANCE OF TYPICAL MICE



CATEGORY GF (GROUPS 1, 18, 19)

FIGURE 24

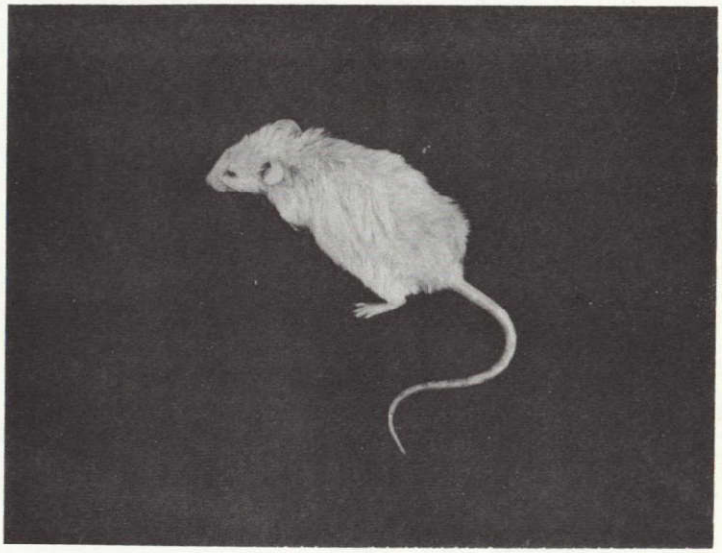
APPEARANCE OF TYPICAL MICE



Gn. E. coli (GROUPS 2, 20, 21)

FIGURE 25

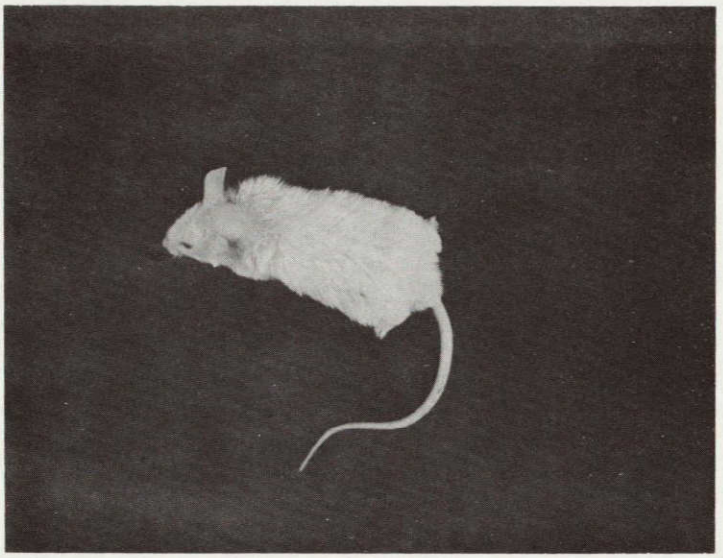
APPEARANCE OF TYPICAL MICE



Gn. Lactobacillus (GROUPS 3, 22, 23)

FIGURE 26

APPEARANCE OF TYPICAL MICE



Gn. Candida (GROUPS 4, 24)

FIGURE 27

APPEARANCE OF TYPICAL MICE

Gn. E. coli + Lactobacillus (GROUPS 5, 25, 26)

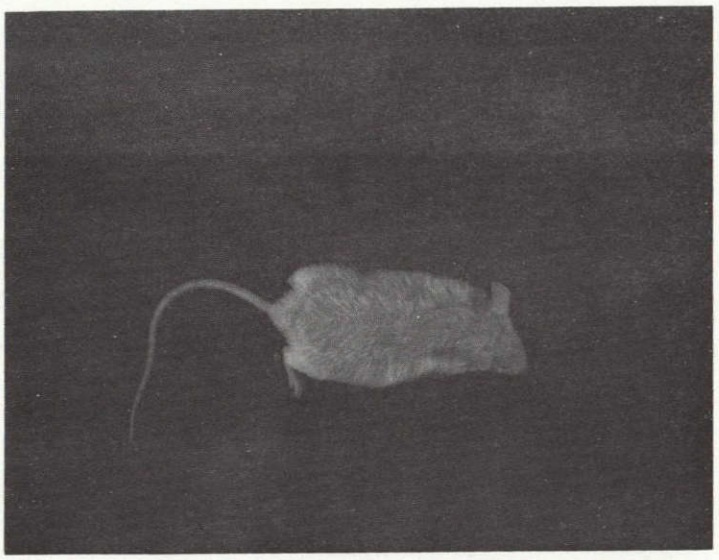
FIGURE 28

APPEARANCE OF TYPICAL MICE

Gn. E. coli + Candida (GROUPS 6, 27, 28)

FIGURE 29

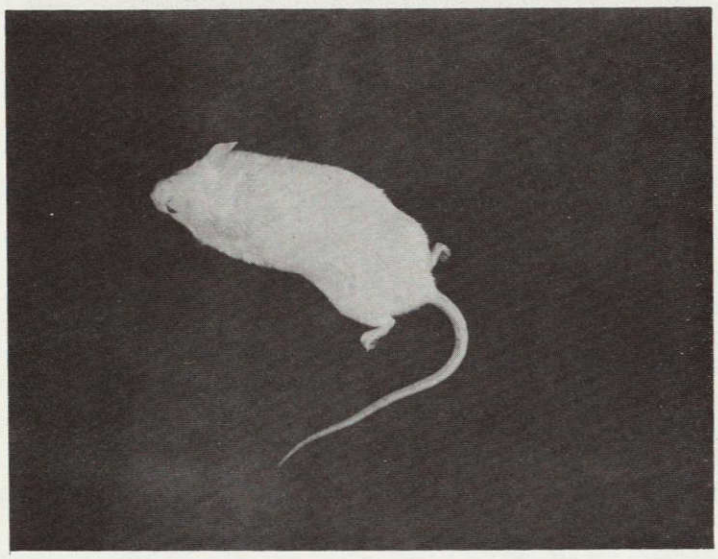
APPEARANCE OF TYPICAL MICE



Gn. Lactobacillus + Candida (GROUPS 7, 29, 30)

FIGURE 30

APPEARANCE OF TYPICAL MICE



Gn. Bacteroides (GROUP 17)

FIGURE 31

APPEARANCE OF TYPICAL MICE

GF MICE IN ISOLATION (GROUPS - PURINA LAB CHOW 5010C)

FIGURE 32

APPEARANCE OF TYPICAL MICE

CLASSIC MICE, OPEN LAB. STERILE DIET (GROUPS 9, 33, 35)

FIGURE 33

APPEARANCE OF TYPICAL MICE

CLASSIC MICE, OPEN LAB. NON-STERILE DIET (GROUPS 10, 34, 36)

TABLE XXVIII

COMPARISON OF HAIR CONDITION OF MICE
UNDER DIFFERING MICROFLORAL REGIMES AND WITH DIFFERING DIETS

GROUP DESIGNATOR	REGIME	DIET	HAIR CONDITION
Germfree	Isolator	AS	Bald +++
Germfree	Isolator	NS	Normal
Classic	Open Colony	AS	Normal
Classic	Open Colony	N	Normal
Classic	Open Colony	A	Normal
Classic	Isolator	AS	Normal
Monognotophoric (Lactobacillus)	Isolator	AS	Bald ++
Monognotophoric (E. coli)	Isolator	AS	Bald +
Monognotophoric (Bacteroides)	Isolator	AS	Normal
Monognotophoric (Candida)	Isolator	AS	Normal
Dignotophoric (Lactobacillus and Candida)	Isolator	AS	Normal
Dignotophoric (E. coli and Candida)	Isolator	AS	Normal
Dignotophoric (Lactobacillus and E. coli)	Isolator	AS	Bald +

A = Apollo Diet
N = 5010C
S = Sterile

Bald +++ = Very Severe
Bald ++ = Severe
Bald + = Moderate
Normal = No trace of hair loss

which diet (Apollo or 5010C) is fed and the sterilizable 5010C mouse diet seems entirely adequate. Where germfree or gnotobiotic mice received the Apollo diet, the mice became bald unless Candida or Bacteroides are present. These observations were made within 30 days of start of the feeding of the Apollo-68 ration.

Reproduction and Lactation

Reproduction and lactation was obtained in only seven of the groups (Table XXIX). Only one of the germfree mice had a litter and it did not survive to test lactation. Since three of the Bacteroides monoassociated mice had litters, the gnotobiotic conditions were compatible with reproduction. The lactation was very poor in this group. This may be attributed partially to the diet as well as the environmental conditions being marginal. Surprisingly, when one considers their poor appearance, the classic mice in isolation (Group Number 8) did reproduce and weaned several young. Equally good performance was noted in the classic mice fed the radiation sterilized diet (Group Number 9). Here 35 mice were weaned for six litters. Some of these were used for Groups 33 and 34 in order to continue the study into the third generation. First generation mice fed the non-sterile Apollo diet (Group 10) reproduced and lactated less well than those fed the irradiated diet. However, the second generation mice performed as well whether the diet was irradiated or not (compare Groups Number 33 with Number 34).

These data suggest that the Apollo diet used is adequate under classic conditions, but is inadequate under a variety of conditions in isolation where the flora is not "normal". The results from gnotophoric mice were slightly better in the second experiment than the first; due possibly to the reduced humidity and filter paper bedding.

TABLE XXIX

REPRODUCTION AND LACTATION DATA

GROUP	STATE	PREGNANT*	LITTERS	LACTATION	
				DAYS	NO. WEANED
1, 19	GF	1, 0	1, 0	0	0
2, 20	Coli	0			
3, 23	Lactic	0			
4, 24	Candida	0			
5, 26	Coli + Lactic	0, 1	0		
6, 28	Coli + Candida	0			
7, 30	Lactic + Candida	0, 1	0		
8, 31	Classic, Isolation	6, 1	5, 0	21	37
9, 33	Classic, Sterile Diet	6, 3	6, 3	21	35, 12
10, 34	Classic, Non-Sterile Diet	2, 5	2, 4	21	14, 12
15	Staph	0	0		
16	Staph + Candida	0	0		
17	Bacteroides	4	3	2	0

*Includes Autopsy Findings

Maturation Rate of Young

Litter Number 3 of Group 8 was born on August 14, 1969. Six survived five days during which the mother was not noted for her care of the young. At five days of age, the ears were free and the young seemed to move little. At ten days of age, the eyes were still closed, white fuzz appeared on the skin and they were crawling weakly and with the typical unsteady gait of animals in this stage of development. They had a good appearance at 21 and 34 days. The weaning weights of the three survivors were 11.0 and 9.2 gm. These are quite satisfactory.

Litter Number 5 of Group 8 was born on September 12, 1969. Eight young survived. At four days, they were quite inactive, eyes and ears were not open and they appeared to be well cared for. At seven days, a white fuzz was observable. Four survived to weaning and looked quite normal.

At maturity, the above mice became Experiment Group Number 31. They subsequently gave birth to two 3rd generation litters. The first of these on October 17, 1969 contained 12 young and the second had 14 young born October 20. Both litters developed well, showed white fuzz at the end of seven days and were weaned on November 11, at the age of two weeks. They looked well, with good fur and posture, their eyes were open and appeared to be within normal limits of development. Their weights at one month of age were 11.1 for males and 11.0 for the females. Further data on their development is seen under the heading Group Number 32.

Three of the five litters born in Group Number 9 on July 18-21 were weaned on August 11, 1969. The records show development at 5 $\pm$2 days was normal. They seemed active and at seven days a fine coat of fur had started. At weaning, they were active, alert and showed every sign of good development and

maturation. The fur coat was complete and intact, the eyes showed luster and normal aperture, the ears were somewhat yellow and looked anemic, the skin was within normal limits. The extremities and tails were within normal limits, the anal-genital areas were clear with well developed scrotems and the general body development looked good for this age group. The averaged weights at weaning were 8.6 gm for the mixed sexes. They appeared well at 35 days of age excepting anemia. Groups Number 33 and 34 were comprised of these animals.

Two litters from dams in Group Number 9 did not survive. The sixth litter born on October 9, 1969 comprised 12 animals. At eight days, they appeared to be in good health with both ears and eyes just opening. White fuzz covered them and they were able to crawl. This is better development than was noted in comparable animals in isolation (Groups 8 and 31). At 13 days, the eyes were open, the fur was good, the general development was rated as good. The animals were moving about well and nibbling at material in the cage. These (Litter 6, Group 9) were also used for Group Number 33 data. Their weaning weights were 7.6 for males and 6.7 for females at 24 days. This is somewhat lower than expected.

The first litter from Group Number 10 died within two days. The second litter, born October 12, 1969, comprised ten young. At five days of age, they appeared to be having a good development. The ears were up with the eyes still closed. A trace of white fuzz could be seen. At ten days, the eyes were still closed, the mice had a normal fur coat, would crawl actively and showed normal development and maturation. These mice were weaned at 21 days. Their average weaning weights were 9.0 for males and 7.8 for females. This is acceptable. These mice were used as Group Number 34; thus later information on their development is shown by that number.

The second generation classic mice in the open laboratory reproduced well. Six litters were weaned from December 1-3, 1969. The growth, development and maturation was acceptable. These third generation animals were used for Groups Number 35 and 36. Detailed information for these mice is found under discussion of various aspects of those groups.

Maturation Rate - Reproduction

Data on reproduction as a component of maturation is presented in Table XXX. It is noted that all groups reared to maturity in the open laboratory did reproduce lactate and produce viable off-spring at weaning. This is a critical part of the evaluation of the Apollo-68 diet; to allow the third generation mice to be fed the diet whether or not it was radiation sterilized. The reproduction performance of classic mice in isolation (Groups 8 and 31) is evidence of the viability of the species under sub-marginal conditions. The animals looked ill, particularly Group 8, the diet was partially deficient in several nutrients and the environment was suboptimal. The reproductive performance of gnotobiotes under the conditions of the experiment was minimal. The best was noted in the Bacteroides monognotophoric mice; even here the best of the three litters cast survived only two days. It appears that in isolation the conditions (diet plus environment) used approached the limit beyond which reproduction and maturation to give continuity to this species was impossible.

Body Size

The body size data are summarized in Table XXXI. The data taken at autopsy at 60-63 days provides a uniform group of young males in the first experiment. It is apparent that all groups in isolators excepting Group 17 were stunted when compared to Groups 9 and 10 which were reared in the open

TABLE XXX

MATURATION - REPRODUCTION

GROUP	CATEGORY	LITTERS (3)	LACTATION, DAYS	NUMBER WEANED
1	GF (1)	1	-	-
26	Coli + Lactic (1)	0	-	-
30	Lactic + Candida (1)	0	-	-
17	Bacteroides (1)	2	2	None
8	Classic - Isolation (1)	6	21	7
31	Classic - Isolation	3	21	20
9	Classic - γ Diet (1)	6	21	35
33	Classic - γ Diet (1)	4	21	11
10	Classic Control (2)	2	21	11
34	Classic Control (2)	7	21	12

(1) The Apollo diet fed these mice was sterilized by γ radiation.

(2) The Apollo diet fed these mice was not sterilized.

(3) Mice in all categories listed were found to be pregnant. Those with no litters were confirmed by autopsy.

TABLE XXXI

BODY SIZE DATA

GROUP	STATE	EXPERIMENT A				EXPERIMENT B	
		AUTOPSY		LATER		AUTOPSY DATA	
		AGE*	AVERAGE*	AGE	AVERAGE	AGE	AVERAGE
1, 19	GF	63	17.8	15.3	77	95	28.4
2, 20	Coli	63	19.6			96	23.5
3, 23	Lactic	63	17.5			96	31.5
4, 24	Candida	63	14.6	17.9	77	96	27.2
5, 26	Coli + Lactic	63	20.9			96	23.2
6, 28	Coli + Candida	63	15.9	19.5	77	96	33.1
7, 30	Lactic + Candida	63	15.5			96	31.7
8,31,32	Classic, Isolation	60	20.0			74	25.4
9,33,35	Classic, Sterile Diet	60	28.0	32.8	83	90±	22.9
10,34,36	Classic, Non-Sterile Diet	60	27.6			90±	26.9
15	Staph	62	25.5	25.4	102		
16	Staph + Candida	62	23.5	20.1	102		
17	Bacteroides	(105)	29.3				

*Age in days and body size in gm.

aboratory. It should be noted that Groups 1-10 were begun on June 10, roups 15-16 on May 20, and Groups 17 on August 29. Since these separate ssembleges of mice were involved, it may be unwise to consider Groups 15-17 n exactly the same category with Groups 1-10. The mice of Groups 17 were lso older at autopsy. The status of these groups did not change as later ata became available. Thus it was probably less a factor of age than either n intrinsic factor in the mice, the way they were handled or a slight change unknown) in their environment. Given the conditions as they were for Groups 1-10, t is noted that the most stunted were those with Candida while mice mono-ssociated with Coli or di-associated with Coli plus Lactobacillus were heavier han germfree mice. This represents microbial antagonism (by Candida) and robably the alleviation of dietary shortcomings by Coli. In both conditions, he presence of Lactobacillus as a diflora of Candida plus Coli gave body size ata equivalent to Candida, while that of Candida plus Staphylococcus gave ata comparable to the Staphylococcus monoflora. Thus the microbial dominance n this first experiment is clearly Staphylococcus > Candida > Coli > Lactobacillus. "The worst is greater than the best.") This is the type of information which is uch needed. In the second experiment, the diet was the same, the mice were ifferent assemblege of the same strain from the same source and the conditions ere altered alightly. The temperature was never low (as it was at the eginning of the first experiment), the humidity was probably lower as the experi-ent was begun on October 6, 1969 (the first experiment was run during summer nd the second during fall), and filter paper bedding was provided. The esults of body size were quite different; there was no pattern as seen in the first xperiment. The results can be appreciated readily by rearranging the groups n order of body size at maturity:

BODY SIZE IN GRAMS	FLORA
33	Coli + Candida
32	Coli + Lactobacilli
32	Lactobacilli + Candida
31	Lactobacilli
28	Germfree
27	Classic (Non-Treated Food)
27	Candida
25	Classic in Isolation
23	Coli
23	Classic (γ-Food)

Under these conditions, Coli gave an adverse effect which was converted into a positive effect by either Candida or Lactobacillus; Candida showed nor harmful effect and Lactobacillus was helpful in all categories. The differences between the results in the two experiments cannot be explained.

Serum Protein Analyses

Tables XXXII and XXXIII give the results of our serum protein study of the mice. The gnotobiotic animals and the germfree animals show remarkable similarity to the conventionals. The albumin to globulin ratios tend to indicate protein is present in sufficient amount bearing out our original data using food efficiency studies as a guide. Table XXXIII compares our results with another investigator (Phillips, 1967) who also used C. albicans as a challenge organism. His results are reported as averages of mice. The data from the animals in our experiment uses pooled blood samples from four mice in each group. All animals in our experiment were fed the Apollo diet.

TABLE XXXII. SERUM PROTEINS IN CONVENTIONAL, GERMFREE, AND GNOTOBIOTIC MICE FED APOLLO DIET

SAMPLE GROUP			TOTAL PROTEIN WEIGHT (gms)	ALBUMINS		GLOBULINS												ALBUMIN/GLOBULIN RATIO
						α_1		α_2		β_T		β_1		β_2		γ		
DESIGNATOR		NUMBER		(gms)	%	(gms)	%	(gms)	%	(gms)	%	(gms)	%	(gms)	%	(gms)	%	
CONTROLS	Versatol	1***	7.1	3.73	52.6	.38	5.3	.80	11.3	.75	10.5	-	-	-	-	1.44	20.3	1.11
	Versatol	2***	7.2	4.10	57.0	.38	5.3	.71	9.8	.76	10.6	-	-	-	-	1.25	17.3	1.32
	Versatol	3***	7.2	4.07	56.5	.34	4.8	.79	11.0	.70	9.7	-	-	-	-	1.3	18.0	1.30
Germfree		1	5.8	3.12	53.9	.48	8.3	.32	5.5	**1.50	25.9	.93	16.1	.57	9.8	.38	6.5	1.18
E. coli		2	5.6	3.23	57.5	.40	7.2	.35	6.3	1.27	22.6	.55	9.7	.73	13.0	.35	6.3	1.36
Lactobacillus		3	4.9	2.77	56.5	.40	8.2	.35	7.2	1.04	21.2	.46	9.4	.58	11.8	.34	6.9	1.30
Candida		4	6.1	3.58	58.5	.44	7.2	.32	5.3	**1.38	22.6	.69	11.3	.69	11.3	.38	6.2	1.42
E. coli + Lactobacillus		5	5.0	2.76	55.2	.40	8.0	.40	8.0	1.09	21.8	.34	6.8	.74	14.8	.35	7.0	1.23
E. coli + Candida		6	6.6	4.13	62.5	.41	6.3	.41	6.3	1.24	18.6	NON SEPARABLE				.41	6.3	1.68
Lactobacillus + Candida		7	5.0	2.96	59.0	.46	9.2	.30	6.0	1.02	20.4	.35	6.8	.69	13.5	.36	7.2	1.39
Classic in GFE		8	4.8	2.60	55.4	.46	9.6	.23	4.8	**1.10	23.0	.57	12.0	.53	11.0	.35	7.2	1.18
Classic, Conv. E., Sterile Diet		9	5.2	3.01	57.9	.49	9.5	.28	5.3	**1.09	21.0	.54	10.5	.55	10.5	.33	6.3	1.38
Classic, Conv. E., Non-Sterile Diet		10	4.6	2.51	54.5	.51	11.1	.25	5.5	**.97	21.1	.51	11.0	.55	12.0	.36	7.8	1.25
Staph.		15	5.1	2.91	57.0	.55	10.7	.38	7.5	*.93	18.3	.35	6.8	.58	11.4	.33	6.5	1.33
Staph. + Candida		16	5.3	2.88	54.4	.59	11.1	.41	7.8	*1.01	18.9	.48	7.2	.56	11.7	.41	7.8	1.19

TABLE XXXII (Continued)

SAMPLE GROUP		TOTAL PROTEIN WEIGHT	ALBUMINS		GLOBULINS												ALBUMIN/GLOBULIN
					α 1		α 2		β T		β 1		β 2		γ		
DESIGNATOR	NUMBER	(gms)	(gms)	%	(gms)	%	(gms)	%	(gms)	%	(gms)	%	(gms)	%	(gms)	%	RATIO
Bacteroides Sp.	17	4.8	2.61	54.3	.57	12.0	.40	8.4	.87	18.1	-	-	-	-	.35	7.2	1.83
Germfree	19	2.9	1.43	49.4	.31	10.9	.32	10.9	.60	20.6	NON SEPARABLE				.24	8.2	0.97
E. coli	21	4.8	2.79	58.1	.46	9.7	.31	6.4	.93	19.4	.36	7.5	.57	11.8	.31	6.4	1.38
Lactobacillus	23	4.2	2.39	56.9	.44	10.4	.29	7.0	.74	17.5	NON SEPARABLE				.34	8.2	1.32
Candida	24	4.1	1.58	38.5	.50	12.8	.59	14.3	1.12	27.2	-	-	-	-	.30	7.2	0.62
E. coli + Lactobacillus	26	4.0	2.39	59.8	.49	12.2	.25	6.1	.63	15.8	-	-	-	-	.24	6.1	1.49
E. coli + Candida	28	7.0	2.76	39.4	.31	4.5	.58	8.3	2.87	41.0	2.13	30.4	.74	10.6	.48	6.8	0.65
Lactobacillus + Candida	30	5.3	3.04	57.4	.57	10.7	.31	5.8	.97	18.4	-	-	-	-	.41	7.7	1.35
Second Generation from Group 8	31	4.3	2.25	52.4	.51	11.9	.25	5.9	.87	20.3	.36	8.3	.51	12.0	.41	9.5	1.10
Third Generation from Group 8	32	4.6	2.45	53.3	.49	10.7	.24	5.2	1.00	21.8	.46	10.0	.54	11.8	.39	8.5	1.14
Second Generation from Group 9	33	3.7	2.08	56.3	.42	11.2	.23	6.3	.65	17.5	-	-	-	-	.32	8.7	1.28
Second Generation from Group 10	34	4.6	2.59	56.2	.51	11.2	.31	6.7	.83	18.0	-	-	-	-	.36	7.9	1.28
Third Generation from Group 9	35	4.0	2.39	59.8	.40	10.0	.25	6.1	.63	15.8	-	-	-	-	.33	8.2	1.48
Third Generation from Group 10	36	4.6	2.54	55.1	.51	11.2	.28	6.1	.91	19.8	-	-	-	-	.36	7.9	1.23
Conventional Germfree	37	5.4	2.73	50.5	.56	10.3	.39	7.2	1.28	23.8	.39	7.2	.89	16.6	.44	8.2	1.02

*Specimens showing prominent double peak in β globulins
**Specimens with marked homogenous double peak in β region
***Control #1 run with Groups 1-7
***Control #2 run with Groups 8-10, 15, and 16
***Control #3 run with Groups 17, 19, 21, 23, 24, 26, 28, 30-37

All groups except #37 on Apollo Diet

TABLE XXXIII

SERUM PROTEINS IN CONVENTIONAL AND GERM-FREE MICE AND IN THE GERM-FREE MOUSE AFTER ASSOCIATION WITH CANDIDA ALBICANS

	GROUP						
	1	2	3	4	5	6	7
	GERM-FREE MICE	GERM-FREE MICE	CVN MICE	CVN MICE	CVN MICE (STERILE DIET)	MICE WITH C. Albicans	MICE WITH C. Albicans
ALBUMIN	57.4	53.9	38.3	54.5	57.9	53.0	58.5
α_1 GLOBULIN	10.4	8.3	8.0	11.1	9.5	9.0	7.2
α_2 GLOBULIN	17.1	5.5	6.2	5.5	5.3	9.4	5.3
β GLOBULIN	14.9	25.9	35.8	21.1	21.0	23.3	22.6
γ GLOBULIN	0.5	6.5	11.7	7.8	6.3	6.0	6.2
TOTAL PROTEIN (g/100 ml)	6.2	5.8	6.3	4.6	5.2	4.7	6.1

GROUPS 1, 3, and 6 - Results of Phillips, A. W. (1967).

GROUPS 2, 4, 5, and 7 - Results of this experiment

Groups 1 and 2, comparison of the work of Phillips and this experiment, using germfree mice, differ primarily in the β and γ globulin fractions. Other investigations have shown germfree animals are low in β globulins and particularly low in γ globulins when compared with conventional mice. It would appear, therefore, that the Apollo diet tested contains sufficient antigens to activate the bodily defenses of the germfree mice. Not taken into account are the strain differences. Phillips used ND4 mice from Manor Farms, reared as a colony in Phillips' laboratory. We used CRL-CD1 from Charles River Laboratory. The previous feeding history of the animals may have included antigens in the diet. The results are comparable. Our standard autoclaved Purina Lab Chow (Diet 5010C) probably contain antigens in sufficient number to activate the bodily defenses. It has been demonstrated that a function of the microflora is to activate bodily defenses (the synthesis of gamma-globulin and other immuno-globulins) and thus provide a defense against infection. This stimulus can also be dead microorganisms as well as other antigens in the diet. Wostman (1965) has shown even highly purified diets can serve as such a stimulus. The Apollo diet was known to contain substantial numbers of microorganisms before sterilization and the bacterial analyses of the Apollo diet showed high numbers of S. fecalis in the diet as received from the original vendor. They were, of course, killed, but the antigenic properties could have remained.

Table XXXIV compares the protein fractionation data of CRL-CD1 mice. The only difference known to us is the diet. The mice fed the Apollo diet have a somewhat lesser total protein with a percentage depression appearing primarily in the α_2 fraction and a far higher percentage appearing in the β globulins. All other fractions are comparable. As yet, no reason is clear for the higher β fractions in the Apollo diet mouse.

TABLE XXXIV

PROTEIN FRACTIONATION

(CRL MICE)

	MOUSE NUMBER	TOTAL PROTEIN	PERCENT TOTAL PROTEIN					ALBUMIN GLOBULIN RATIOS
			ALBUMIN	α_1	α_2	β	γ	
*Germ-Free 10 Week Old Male Mice	121	7.23	58.9	8.02	15.76	9.26	8.02	1.3
	122	6.64	60.09	9.33	14.90	9.78	5.87	1.5
	123	7.16	57.54	8.65	17.17	8.51	8.10	1.4
	124	7.08	59.32	9.18	16.94	8.19	6.35	1.4
	125	7.19	54.10	9.17	15.99	8.34	12.37	1.2
Mice** Apollo Diet	Germ-Free	5.8	53.9	8.3	5.5	25.9	9.8	1.18

*Calculated from data furnished by Charles River Laboratories Courtesy Foster, H.L. (1969).

**Pooled blood sample from four mice.

Reticuloendothelial System and Phagocytic Activity Studies in Gnotobiotic Mice Using Apollo-68 Diet

"There is no significant difference between strains of mice in their rate of carbon clearance except as related to the relative combined weights of liver and spleen" (Doll, 1962). Thorbecke and Benacerroff (1959) earlier had found that the combined weight of liver and spleen is significantly lower in germfree than in normal control mice. There may be strain differences, however, in the overall immunological defenses. The aforementioned studies of Phillips (1967) and those of Wostman (1952) have shown that some strains of germfree mice have appreciably lower γ globulins when compared with conventionals. The α_2 fractions and the β fractions are also generally low in these germfree mice. When the mice were exposed to normal microflora, these fractions all increased within two weeks of exposure. In that the total serum proteins, except in certain pathological and malnutrition cases, seemed to be about the same, this increase was marked by a concurrent decrease in serum albumin.

Bauer and Horowitz (1964) investigating the relationship of phagocytosis and stress have discussed some of the changes occurring in the immunological competence when germfree animals have access to antigens in their diet. In our discussion of the serum protein findings of this experiment, this was covered in some length. Challenge with antigenically inert colloidal carbon removes to some extent the influences of the antigenic stimuli (Doll, 1962).

We have compared the K and α values of our strain of germfree mice and their conventional counterparts in Table XXXV. The carbon clearance rates in the Notre Dame strain, when germfree and gnotobiotic animals are used, are shown as literature values. Our data correlates well and reinforces the concept

TABLE XXXV

PHAGOCYTIC INDICES OF GERMFREE, GNOTOBIOTIC AND CONVENTIONAL ANIMALS FED THE APOLLO DIET

GROUP		ANIMAL'S WEIGHT (gms)	WEIGHT OF LIVER AND SPLEEN (gms)	WEIGHT OF LIVER AND SPLEEN/100 gm MOUSE	K	α	REMARKS
NUMBER	DESIGNATOR						
1	Germfree	22.8	0.9555	4.19	.009	4.93	
2	E. coli	10.8 14.2	0.5700 0.6215	5.28 4.38	.006 .021	3.46 6.29	Average 4.87
3	Lactobacillus	31.9	1.5068	4.72	.034	6.82	
4	Candida	19.0	0.8369	4.41	.024	6.51	
5	E. coli and Lactobacillus	29.0 22.8	1.3062 0.9555	4.51 4.19	.029 .014	6.76 5.66	Average 6.21
6	E. coli and Candida	20.1 16.2	0.9768 0.9375	4.86 5.80	.029 .025	6.29 5.02	Average 5.63
7	Lactobacillus and Candida	28.7	1.0597	3.69	.041	9.65	
8	Classic in G.F.E.	30.2 31.0	1.5181 1.5462	5.02 4.99	.033 .018	6.36 5.29	Average 5.82
9	Classic, Conv. E. Sterile Diet	31.2 35.1	1.7618 1.9577	5.65 5.59	.012 .044	4.09 6.25	Average 5.17
10	Classic. Conv. E. Non-Sterile Diet	37.0 30.0	2.0033 1.3546	5.44 4.52	.022 .008	5.12 4.40	Average 4.76
15	Staphylococcus	26.9	1.3723	5.12	.007	3.67	
16	Staphylococcus and Candida	26.2 24.1	1.3521 1.0255	5.15 4.25	.003 .004	2.81 3.71	Average 3.26
Literature	Germfree			4.68*	.0051-.0119	4.04*	*These values represent average from Doll (1962)
	Conventional				.0095 to .0116	3.99*	

of Bauer and Horowitz, that "microbial flora stimulates the immunologic functions but does not increase phagocytic functions of lymphatic tissue, and in healthy animals, living in a microbial environment, immunologic events derive mainly from the flora".

To this concept, we iterate the effect of antigenic stimulus from most probably diet and certainly from ingestion of heat killed bacteria.

Phagocytic Index data for the complete experiment is found in Appendix I.

Interferon Data for the complete experiment is to be found in Appendix H. This data is negative and indicates the mice had not been challenged prior to analyses.

Complete hematology data for the mice is to be found in Appendix G. Data was obtained at time of autopsy.

IV. SUMMARY OF PART B - BIOLOGICAL EVALUATION OF AN APOLLO DIET

A research effort, primarily concerned with an evaluation of the Apollo-68 diet and its effects on the indigenous microflora (particularly the digestive tract) and the general well being of the body was performed. Physiological and pathological effects due to feeding germfree mice sterile, inoculated, and nominal (untreated) space foods were studied and compared.

Forty-two colonies of classical and germfree mice were studied and evaluated during the course of this experiment. The colonies were evaluated using morphologic, hematologic, and biochemical examinations including the following: growth rate; food utilization efficiency; body size, maturation rate, reproduction and lactation; autopsy with histology of appropriate tissues; immunologic defense mechanisms (antibody response, phagocytic index, interferon, and properidin); and hematology (hemoglobin, total white blood cells, differential white blood cell count, serum protein, and gamma globulins). The germfree mice were fed Apollo-type diets as follows: sterilized; inoculated with a B-vitamin

synthesizing bacteria; inoculated with a B-vitamin requiring bacteria; inoculated with a yeast; variations of the above; and addition of other microorganisms such as Staphylococcus epidermidis. Classical mice served as controls and were fed sterilized and non-sterilized Apollo-type diets as well as commercial mouse diets.

The formula for the Apollo-68 diet was compiled from actual food consumption data of four pre-flight members of the Apollo astronaut team in the fall of 1968. This diet was prepared by the Whirlpool Corporation in comminuted form and sterilized at Brookhaven National Laboratory with 5 million rads gamma radiation. The mice accepted this diet readily. The food utilization efficiency seemed to be somewhat depressed by the presence of Candida albicans either alone or when in combination with E. coli or L. leichmannii. When the extremes of the data are compared, it was noted that a two-fold difference in food efficiency may result due to differences in the indigenous or inoculated microflora of the G.I. tract. The data demonstrates that Apollo-68 diet is adequate for classic mice into the third generation when growth, food utilization efficiency, general appearance, and reproduction are evaluated. This is true whether the diet was sterilized or not.

The results under different gnotobiotic conditions suggest that microbic stress may be playing an important role in nutritional efficiency and in the evaluation of the diets as well as in the general well being of the subjects eating the diet. In the first 50 days, the results of the experiments indicated a dramatic effect due to the presence of different "indigenous" microorganisms on the well being of the mice. Mice carrying S. epidermidis, E. coli, Lactobacillus with E. coli, Candida with E. coli, Lactobacillus with

Candida, or the classic animals in isolation survived poorly. Two-thirds of all animals in these groups expired, the exception being only 50% of the classic animals in isolation died. The survival of other gnotophoric groups was relatively good. One could conclude from this experiment that it is not desirable to have E. coli alone or with Candida or Lactobacillus as a predominant microorganism. Although Candida did not appear to affect the mortality; the presence of E. coli and Candida resulted in a lower mortality that the S. epidermidis. In turn, it was noted that this was the only group which carried Candida and the only group which had acceptable food utilization efficiency. Therefore, the Staphylococcus-Candida interaction appears to be beneficial to the animal while the Candida-Lactobacillus combination was lethal to a greater extent than either organism alone.

In the initial feeding experiment with Apollo diet, hair loss was observed in most groups reared in isolators while none of those reared without isolation showed apparent alopecia. The most serious hair loss to the point of being almost completely hairless, was observed in both the S. epidermidis gnotophoric mice and the classic mice in isolators. A second experiment was performed with many possible factors which could stress the animals eliminated. Cellulose filter paper was placed in the cages for bedding. This bedding may have given the mice a sense of security since they could hide. In the second experiment, hairlessness was not observed: some degree of alopecia was found on the backs of the germfree animals and on those carrying the Lactobacillus species and E. coli only as well as those harboring both E. coli and Lactobacillus. Under these conditions, alopecia was apparently prevented by the addition of a Bacteroides or Candida species and of course by the variety of indigenous microorganisms of classic mice in isolation.

The above observations indicate that the diet is adequate for classic mice under normal conditions, but it appears to be only a marginal diet inadequate under a variety of stressful environmental conditions.

V. RECOMMENDATIONS - PART B

1. Specific problems in the control of the microflora should be studied. These include:
   a. use of experimental marginal and abundant diets to control microflora;
   b. the effects of stress as part of an evaluation of any astronaut diet;
   c. a search for the exact reasons for the *E. coli* caused deaths and for the denudation and alopecia observed.
2. A computer program should be initiated to afford some elements of prediction when considering the interactions of microflora and astronaut diets.
3. A continuously updated literature search and evaluation should be planned and the information placed into the computer program recommended above (2).
4. Periodic symposia for knowledgeable scientists working in the area of microbial ecology and human nutrition should be organized.
5. An experiment, similar in design to the diagrammed in Table XXXVI should be started to further define effects of marginal diets under space flight conditions.

TABLE XXXVI

EXPERIMENTAL DESIGN

GROUP	DIET*	DIET STERILITY	ENVIRONMENT AND MICROBIAL INOCULUM**		
			CLASSIC	GNOTOBIOTIC	OTHER
1	Lab Chow	No	+		
2-3-4	Lab Chow	Yes	(2) +	(3) +(Germfree)	(4) +(Classic-Isolated)(a)
4	Abundant	No	+		
6-7-8	Abundant	Yes	(6) +	(7) +(Germfree)	(8) +(Classic-Isolated)
9	Marginal	No	+		
10-11-12	Marginal	Yes	(10)+	(11) +(Germfree)	(12) +(Classic-Isolated)
13	Marginal	Yes		+(*E. coli*)	
14	Complete	Yes		+(*E. coli*)	
15	Marginal	Yes		+(*E. coli* + *S. faecalis*)	
16	Marginal	Yes		+(*E. coli* + *Bacteroides sp.*)	
17	Marginal	Yes		+(*E. coli* + Human strain *E. coli*)	
18	Marginal	Yes		+(*E. coli* + Human strain *E. coli*)(b)(c)	
19	Marginal	Yes		+(*E. coli* + *Lactobacillus casei*)	
20	Marginal	Yes		+(*E. coli* + *Lactobacillus casei*)(b)(c)	
21	Marginal	Yes		+(*E. coli* + specific phage)(c)	
22	Marginal	Yes	+ (with *E. coli* toxins)		

N.B. All microorganisms will be mouse adapted unless otherwise stated.

(a) Classic animals placed in complete microbial isolation with only sterile food, water, bedding, and air provided.

(b) Prior establishment of the second bacteria.

(c) Repeated or continuous inoculation (i.e., incorporate into sterile diet).

*Diet to be fed to animals during test.

**Classic - naturally occurring indigenous microflora

Gnotobiotic - refers to the intentional inclusion of specific microorganisms

VI. REFERENCES - PART B

1. Bauer, H., and R. E. Horowitz, 1964 Immunologic competence and phagocytosis in germfree animals with and without stress. Journ. Amer. Med. Assoc., 187, pp. 217-718.

2. Bengson, M. H., A. E. Prince, and F. W. Thomae, 1968 A study of the significance of the microfloral changes occurring during long term space flights, In: Advances in Germfree Research and Gnotobiology, M. Miyakawa and T. D. Luckey (eds.), Chem. Rubber Press, Cleveland, Ohio.

3. Boeing Airplane Company, 1965 Manned environmental system assessment. NASA-CR 134.

4. Charrin, M., and M. Guillemonat, 1901 Influence de la sterilization des milieux habités, de l'air respire', et des aliments ingere's sur l'organisme animal. Compt. Rend. Acad. Sci., 132:1074-1076.

5. Doll, J. P., 1962 Rate of carbon clearance in three strains of germfree mice. Amer. J. Physiol., 203:291-295.

6. Gall, L. S., and P. E. Riely, 1964 Determination of aerobic and anaerobic microflora of human feces. AMRL-TR-64-107, Republic Aviation Corporation, Long Island, New York.

7. Heumpfner, H. R., 1967 Laboratory standard operating procedures germfree animal techniques. Dept. of Community Medicine, University of Kentucky College of Medicine, Lexington, Kentucky.

8. Kianizin, I., 1916 The effect of higher animals of the sterilization of the inhabited medium, the air and the food. J. Physiol., 50:391-396.

9. Luckey, T. D., 1963 Germfree Life and Gnotobiology. Academic Press, New York, New York.

10. Luckey, T. D., 1965 Gnotobiologic evidence for functions of the microflora. Ernahrungsforschung, 10:192-250.

11. Luckey, T. D., 1968 Gnotobiology and aerospace systems, In: Advances in Germfree Research and Gnotobiology, M. Miyakawa and T. D. Luckey (eds.), Chem. Rubber Press, Cleveland, Ohio.

12. Luckey, T. D., G. M. Briggs, C. A. Elvehjem, and E. G. Hart, 1943 Activity of pyridoxine derivatives in chick nutrition. Proc. Soc. Exptl. Biol. Med., 58:340-344.

13. Luckey, T. D., G. M. Briggs, and C. A. Elvehjem, 1944 The use of Streptococcus lactis R for the measurement of "folic acid. J. Biol. Chem., 153:157-167.

14. Luckey, T. D., P. R. Moore, and C. A. Elvehjem, 1946 A differential microbiological assay for O-heterobiotin. Proc. Soc. Exptl. Biol. Med., 61:97-100.

15. Luckey, T. D., P. R. Moore, C. A. Elvehjem, and E. B. Hart, 1946 Biological activity and metabolism of d, l-O-heterobiotin in the chick. Proc. Soc. Exptl. Biol. Med., 61:185-187.

16. Luckey, T. D., P. R. Moore, C. A. Elvehjem, and E. B. Hart, 1946 Effect of diet on the response of chicks to folic acid. Proc. Soc. Exptl. Biol. Med., 62:307.

17. Luckey, T. D., J. L. Tepley, and C. A. Elvehjem, 1944 The measurement of folic acid. Science, 100:201-202.

18. McCollum, E. V., 1964 From Kansas Farm Boy to Scientist. University Kansas Press, Lawrence, Kansas.

19. Moyer, J. E., and Y. E. Lewis, 1964 Microbiologic studies of the two man space cabin simulation: Interchange of oral and intestinal bacteria. SAM-TDR 64-3. USAF School of Aerospace Medicine, Brooks Air Force Base, Texas.

20. Nelson, R. C., 1931 Progressive changes in the flora of the intestinal tract of guinea pigs from birth to maturity. M.A. Thesis, Univ. of Notre Dame, Notre Dame, Indiana.

21. Phillips, A. W., 1967 Effects of association of Candida albicans with the germfree mouse on its serum proteins, In: Advances in Germfree Research and Gnotobiology, M. Miyakawa and T. D. Luckey (eds.), Chem. Rubber Press, Cleveland, Ohio.

22. Porter, J. R., and L. F. Rettger, 1940 Influence of diet on distribution of bacteria in stomach, small intestine and cecum of white rats. J. Infect. Dis., 66:104-110.

23. Reagan, M. J., 1931 The effect of sterile food on the bacterial flora of the intestines in guinea pigs. B. S. Thesis, University of Notre Dame, Notre Dame, Indiana.

24. Rebach, J. F., 1942 Studies on the intestinal flora of the white rat. M.S. Thesis, Univ. of Notre Dame, Notre Dame, Indiana.

25. Schmidt, J. P., 1965 Response of hibernating animals to physical, parasitic and infectious agents. Hibernation Conference, Toronto, Canada.

26. Snell, E. E., 1945 The vitamin B_6 group: IV. Evidence for the occurrance of pyridoxamine and pyridoxal in natural products. J. Biol. Chem., 157:491-505.

27. Tanami, J., 1959 Studies on germfree animals. J. Chiba Med. Soc., 35:1-24.

28. Thorbecke, G. J., and B. Benaecerroff, 1959 Some immunological and functional aspects of lymphoid tissues of germfree animals: II. Studies on phagocytosis. Ann. N. Y. Acad. Sci., 78:247-254.

29. Van der Waaij, D., 1968 Production of bacteria free mice by antibiotic decontamination, In: Advances in Germfree Research and Gnotobiology, M. Miyakawa and T. D. Luckey (eds.), Chem. Rubber Press, Cleveland, Ohio.

30. Van der Waaij, D., J. J. deVries, and J. Lekberkeck, 1969 Colonization resistance in the digestive tract of conventional, entero bacteriaclae-free and in antibiotic decontaminated mice. Adv. Study Institute Symposium, Germfree Animal as a Tool in Research, Leuven, Belgium.

31. Wagner, M., 1959 Serologic aspects of germfree life. Ann. N. Y. Acad. Sci., 78:261-271.

32. Winitz, M., R. F. Adams, D. A. Seedman, and J. Graff, 1966 Regulation of intestinal flora patterns with chemical diets. Fed Proc. Abstract, 25:343.

33. Wostman, B. S., 1968 Defense mechanisms in germfree animals, In: The Germfree Animal in Research, M. A. Coates (ed.), Acad. Press, New York, New York.

34. Wostman, B. S., G. B. Olson, and J. R. Pleasants, 1965 Serum proteins of germfree rats fed water soluble diets. Nature, 206:1056-1057.

35. Zablotny, D., 1928 Infection and immunity in hibernating animals. Zentralblatt Bacteriol. I. Abt., 106:397-398.

GENERAL ELECTRIC
SPACE DIVISION
SPACE SYSTEMS ORGANIZATION